



Glandular trichomes from the leaves of *Artemisia tridentata vaseyana* (Rydb.) Beetle : ontogeny, ultrastructure, and terpenoid production  
by Francoise Djibode

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biological Sciences  
Montana State University  
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**Abstract:**

Terpenoids are produced by glandular trichomes of leaves of the mountain big sagebrush, *Artemisia tridentata vaseyana* (Rydb.) Beetle. Sagebrush possesses two types of leaves, summer ephemeral and winter persistent. I hypothesized that the peltate glandular trichomes of the two types of leaves were different and have a different pattern of producing terpenoids. Scanning electron microscopy and transmission electron microscopy were used to show the different types of trichomes of sagebrush leaves. Gas chromatography and mass spectroscopy were used for qualitative and quantitative analysis of the terpenoids in ephemeral leaves. Attempts to extract a fragment of the cyclase gene for study of the differential expression of cyclase mRNA in clipped versus control ephemeral leaves did not give satisfactory results.

Sagebrush has three types of trichomes: (1) "T"-shaped cover trichomes which blanket the leaves, giving them a silvery look. (2) Ten-cell peltate glandular trichomes on ephemeral leaves which are oblong in shape, and consist of one pair of stalk cells, one pair of secreting-support cells, and three pairs of secreting cells. This gland produces highly pressurized volatile terpenoids in the secretory head of the mature trichome and has four times the surface area as the round shaped trichome found on the persistent leaf. (3) Round trichomes on persistent leaves which have six pairs of cells consisting of one pair of stalk cells, one pair of support cells, and four pairs of secretory cells. These glands secrete volatile and non-volatile terpenoid compounds and are susceptible to shedding in extreme environmental conditions.

In three months, trichomes of the summer leaves reach their optimum production of volatiles, which makes this leaf type suitable for studies of alternatives for methyl bromide. The major terpenoid constituents of ephemeral leaves are camphor, eucarvone, eucalyptol (1, 8-cineole), and camphene. Camphor and eucarvone are natural pesticides, herbicides and fungicides. The increase of camphor (70%), eucarvone (66%), and  $\alpha$ - and  $\beta$ -pinene 15 days after pruning, shows that *A. t. vaseyana* reacts to pruning of its branches. A mixture of camphor, eucarvone, eucalyptol, and  $\alpha$ -pinene may be effective in controlling insect pests.

GLANDULAR TRICHOMES FROM THE LEAVES OF *ARTEMISIA TRIDENTATA*  
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# APPROVAL

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This dissertation has been read by each member of the dissertation committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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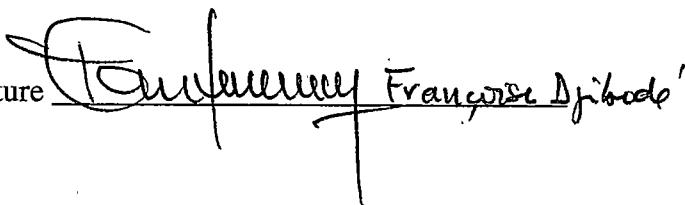
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## ABSTRACT

Terpenoids are produced by glandular trichomes of leaves of the mountain big sagebrush, *Artemisia tridentata vaseyana* (Rydb.) Beetle. Sagebrush possesses two types of leaves, summer ephemeral and winter persistent. I hypothesized that the peltate glandular trichomes of the two types of leaves were different and have a different pattern of producing terpenoids. Scanning electron microscopy and transmission electron microscopy were used to show the different types of trichomes of sagebrush leaves. Gas chromatography and mass spectroscopy were used for qualitative and quantitative analysis of the terpenoids in ephemeral leaves. Attempts to extract a fragment of the cyclase gene for study of the differential expression of cyclase mRNA in clipped versus control ephemeral leaves did not give satisfactory results.

Sagebrush has three types of trichomes: (1) "T"-shaped cover trichomes which blanket the leaves, giving them a silvery look. (2) Ten-cell peltate glandular trichomes on ephemeral leaves which are oblong in shape, and consist of one pair of stalk cells, one pair of secreting-support cells, and three pairs of secreting cells. This gland produces highly pressurized volatile terpenoids in the secretory head of the mature trichome and has four times the surface area as the round shaped trichome found on the persistent leaf. (3) Round trichomes on persistent leaves which have six pairs of cells consisting of one pair of stalk cells, one pair of support cells, and four pairs of secretory cells. These glands secrete volatile and non-volatile terpenoid compounds and are susceptible to shedding in extreme environmental conditions.

In three months, trichomes of the summer leaves reach their optimum production of volatiles, which makes this leaf type suitable for studies of alternatives for methyl bromide. The major terpenoid constituents of ephemeral leaves are camphor, eucarvone, eucalyptol (1, 8-cineole), and camphene. Camphor and eucarvone are natural pesticides, herbicides and fungicides. The increase of camphor (70%), eucarvone (66%), and  $\alpha$ - and  $\beta$ -pinene 15 days after pruning, shows that *A. t. vaseyana* reacts to pruning of its branches. A mixture of camphor, eucarvone, eucalyptol, and  $\alpha$ -pinene may be effective in controlling insect pests.

## CHAPTER 1

## INTRODUCTION

*Artemisia tridentata* McArthur encompasses five subspecies including mountain big sagebrush *Artemisia tridentata* ssp. *vaseyana* (Rydb.) Beetle (Beetle, 1960; McArthur, 1979; Goodrich *et al.*, 1985). *Artemisia tridentata tridentata* Nutt., *A. tridentata vaseyana*, *A. tridentata wyomingensis* Beetle and Young, *A. tridentata lahotan* (Brunner) Winward, and *A. tridentata spiciformis* (Osterhout) are all members of the subspecies (Beetle, 1960; McArthur, 1979). The subgenus is natural with *A. tridentata* at its core but its limits are still under investigation because classical taxonomic methods do not reveal the evolutionary relationships among subspecies within the *Tridentatae* (Wilt *et al.*, 1992). *Artemisia t. vaseyana* was chosen for this study because of its low level of 2-methyl-2-propenal content compared to *A. t. tridentata*. Two-methyl-2-propenal is an extremely volatile irritant and bitter terpenoid which plays an important role in sagebrush choice by deterring browsing by wild mammals (Scholls *et al.*, 1977; Bray *et al.*, 1991). *Artemisia t. vaseyana* is the preferred taxon browsed during winter by mule deer because of its low content of 2-methyl-2-propenal (Personius *et al.*, 1987; Wambolt *et al.*, 1991; Weber *et al.*, 1994; Wambolt, 1996). All subspecies of *A. tridentata* exhibit two distinct types of stem growth: vegetative-perennial and reproductive-annual (DePuit and Caldwell, 1973). Two types of leaves are formed on the vegetative shoots during the course of the year. The primary new leaves which develop along the main branches at the beginning of spring



are large and typically sharply trilobed. As growth continues, new short lateral branchlets grow in August from the upper axil of the existing primary leaves. The branchlets support large quantities of smaller, less distinctly trilobed leaves which persist through the next winter, long after the large initial leaves are shed (Diettert, 1938; Goodwin, 1956; DePuit and Caldwell, 1973). The large primary leaves are shed from the end of June to September, depending on the environmental conditions and the developmental stage of the plant. The smaller leaves persist until the next spring when leaf growth for the new season commences, but they never persist for two entire growing seasons. The primary leaves are commonly called "ephemeral leaves", while the smaller leaves are termed "persistent leaves".

In the genus *Artemisia*, glandular trichomes are the secretion sites of the plant's natural products including terpenoids, coumarins and flavonoids (Buttkus *et al.*, 1977; Kelsey and Shafizadeh, 1980; Ascensão and Pais, 1982; Kelsey *et al.*, 1982; Duke and Paul, 1993). These substances have long been of interest to phytochemists because of their importance as flavoring, perfumery and pharmaceutical agents; they are also used for their pesticidal and herbicidal properties (Duke and Paul, 1993; Weaver *et al.*, 1995).

A number of glandular trichome secretions are thought to have a role in plant defense because of their toxicity, their deterrence to herbivorous insects, and their anti-fungal and anti-bacterial activity (Stipanovic, 1983; Kelsey *et al.*, 1984; Duke *et al.*, 1988). Contents of sagebrush trichomes have been extracted, identified and quantified (Kelsey *et al.*, 1982; Cedarleaf *et al.*, 1983). According to Cedarleaf *et al.* (1983), monoterpenoid content of the leaves is the highest in July (4.18% of dried leaves) when

only the large leaves are present; monoterpenoids thereafter decline in winter and reach the lowest level (0.97% of dried leaf in weight) in May of the following year.

Monoterpenes (Welch and McArthur, 1981) and sesquiterpene lactones (Kelsey and Shafizadeh, 1979, 1980) are the major secondary metabolic products in sagebrush, with lower quantities of coumarins (Shafizadeh and Melnikoff, 1970; Brown *et al.*, 1975) and flavonoids (Rodriguez *et al.*, 1972). Most of the studies have been focused on the terpenoid content of the persistent leaves, because the sagebrush leaves are used by wild animals as food during winter time when only persistent leaves are available (Longhurst *et al.*, 1969; Scholl *et al.*, 1977; Welch and McArthur, 1986; Bray *et al.*, 1991). Several studies have shown that high levels or composition of various proportions of terpenoids reduced rumen microbial activity of browsing species (elk, mule deer, and pronghorn antelope) (Personius *et al.*, 1987; Bray *et al.*, 1991).

Monoterpenoids have been demonstrated to act as toxins and feeding deterrents to a large variety of insects. They thus appear to have important roles in protecting plants from insect attack (Zalkow *et al.*, 1979; Saxena and Basit, 1982). However, some studies describe cases in which herbivorous mammals or insects appear to be immune to the noxious effects of terpenoids; and these substances then serve as attractants, feeding stimulants, or oviposition cues in these organisms (Radwan and Crouch, 1978; Hanson *et al.*, 1986).

Chemical changes induced by herbivory could be active responses to discourage further herbivory (Green and Ryan, 1972; Carroll and Hoffman, 1980; Edwards and Wratten, 1982; Schultz and Baldwin, 1982; Fowler and Lawton, 1985). Preliminary

evidence suggested that these chemical changes inhibited herbivore growth and development, and decreased herbivore population growth, thus reducing herbivore pressure on the plant. There are fewer reports of induced defenses among terpenoids than among other classes of secondary metabolites, especially in leaf tissue. This may be due to the existence of physiological constraints on terpenoid biosynthesis in mature leaves. Terpenoid biosynthesis is generally restricted to trichomes that are active early in leaf development (Fahn, 1979). Thus, evidence for the induced responses of terpenoids to herbivory should be sought not only in leaves present at the time of damage but also in leaves initiated immediately after damage has occurred (Coleman and Jones, 1991; Tallamy and Raupp, 1991). Another study suggested that sagebrush responded to any leaf herbivory by leaf-chewing arthropods by altering the chemical composition of leaves (Wiens *et al.*, 1991).

#### Sagebrush Origin, Systematics and Evolution

*Artemisia* L. is one of the genera of the Tribe (ensemble of genera) Anthemideae (Family Asteraceae) which consists of over 400 species including wormwood, sagebrushes, mugworts, sageworts, worseed and tarragon (Beetle, 1960; McArthur, 1979; Duke *et al.*, 1988). *Artemisia* is the most specialized and advanced genus in the Anthemideae. A number of characteristics are indicative of the advanced evolutionary position of *Artemisia* in that tribe. The inflorescences are reduced to small capitula consisting of two to twenty, mostly discoid and perfect florets. The arrangement of the capitula in elongate, narrow panicles characterizes *Artemisia* relative to other genera in the

Anthemideae. The taxa are all wind-pollinated and the elongation of the inflorescence is an effective means of exposing pollen and stigma to wind movement (Carlquist, 1966). Wind pollination (Thorne, 1973) in members of this genus is good evidence that the woody members are a specialization compared to taxa from the herbaceous ancestors in the same genus which are devoid of woodiness (Carlquist, 1962).

Progenitors of *Artemisia* probably evolved in the mesic, temperate habitats of mid-eastern Asia during the late Miocene to early Pliocene. *Artemisia* of the Eurasian subgenus *Sephiridium* (Bess.) Rouy occurs in the area immediately east of the Caspian Sea, where there are as many as 35 species (Person, 1974). Krasheninnikov (1946) had defined a Chino-nipponese region as the area with the highest species diversity, including the herbaceous members, out of the six principal centers of Eurasian *Artemisia*. The North-American members of *Artemisia*, including the sagebrushes, are likely derivatives of the subgenus *Dracunculus* (Bess.) plants that bridged Beringia from Asia to North America. Early systematic treatment combined all woody North American species of *Artemisia* with the Old World woody members of the subgenus *Sephiridium*. Recent chemical (Kelsey and Shafizadeh, 1979), cytological and chromosomal karyotype studies (Moss, 1940; Giessman and Irwin, 1974; McArthur *et al.*, 1981) support the segregation of North American taxa as the subgenus *Tridentatae*.

*Artemisia* subgenus *Tridentatae* is the most successful group of shrubs within the cold desert region of western North America. This group covers an estimated 68 million hectares, primarily in the region known as the Great Basin. *Artemisia t. vaseyana* extends from 31° N parallel in Baja California, north into British Columbia, south and east through

the Great Basin and the Rocky Mountains, and into the southwestern states of Arizona and New Mexico (Beetle, 1960). It is primarily encountered below 1750 m elevation, and in a zone of precipitation from 150 mm to 500 mm per year (Beetle, 1960).

The subgenus *Tridentatae* is composed of 11 species of differing geographic distribution (McArthur and Plummer, 1978) and taxonomic importance (McArthur, 1979). They are: *Artemisia arbuscula* Nutt., *A. argillosa* Beetle, *A. bigelovii* Gray, *A. cana* Pursh, *A. rothrockii* Gray, *A. tridentata* Nutt., *A. tripartita* Rydb., *A. pygmaea* Gray and *A. rigida* (Nutt.) Gray (Beetle, 1960; McArthur, 1979). Four are further divided into subspecies: *A. arbuscula* (2 subspecies), *A. cana* (3 subspecies), *A. tridentata* (5 subspecies), *A. tripartita* (2 subspecies). Few species of the genus *Artemisia* and no other *Tridentatae* occur in South America (McArthur, 1979).

#### Development and Longevity of the Sagebrush Leaves

Shrub success in its range may be attributed in large part to the dimorphic development of its leaves. DePuit and Caldwell (1973) suggested that the ability of the plant to maintain part of the leaves through the winter enables it to begin growth and utilize water in the early spring. Development and maintenance of large ephemeral leaves during optimal growing conditions may also increase photosynthetic potential by reducing mesophyll resistance (DePuit and Caldwell, 1973). Confusion exists regarding the longevity of ephemeral and persistent leaves. On the basin big sagebrush, *A. t. tridentata*, growing east of the Rocky Mountains, mature leaves remaining on the plant over winter are discarded soon after spring growth resumes (Diettert, 1938; Branson *et al.*, 1976). On

three subspecies of big sagebrush growing in the Great Basin (*A. t. tridentata*, *A. t. vaseyana*, and *A. t. wyomingensis*), persistent leaves remain green on the plant throughout the subsequent growing season. These leaves senesce during initiation of summer drought, concurrent with abscission of the large ephemeral leaves (Miller *et al.*, 1986). Miller and Schultz (1987) studied the ontogeny, development, and longevity of both kinds of leaves from *A. t. wyomingensis* for two years. The study concluded that the big sagebrush is a semi-evergreen shrub, which maintains a portion of its leaves through the winter. Longevity of the persistent leaves is in the range of 12 to 13 months, with no leaves persisting through two winters (Miller and Schultz, 1987). Winter-persistent leaves did not reinitiate elongation the subsequent spring. The large ephemeral leaves are the first to develop early in the spring from small leaf buds at the apex of the branches. Ephemeral leaf elongation begins in early spring, forming tight clusters or fascicles at stem apices prior to stem elongation. When branches begin to elongate, ephemeral leaves are alternately positioned along the branch. These early ephemeral leaves are the largest leaves on the plant (Diettert, 1938; Miller and Schultz, 1987). As spring progresses and the early ephemeral leaves are near maturity, a small cluster of leaves begins to develop in the axil of each ephemeral leaf. These fascicles contain both ephemeral and persistent leaves. Lateral leaf fascicles are properly termed "short shoots". Each short shoot fascicle is subtended by a long shoot and one large lateral leaf (Miller and Schultz, 1987). Later developing ephemeral leaves are smaller than the early ephemeral leaves, but larger than the fully expanded persistent leaves (Miller and Schultz, 1987). Ephemeral leaves on the reproductive stem are not lobed and have no short shoot fascicle in their axes. At the

onset of drought, both the persistent leaves of the previous season and the large early ephemeral leaves of the current year begin to senesce. Once the water potential in the wettest soil drops below -0.2 MPA, ephemeral leaves begin to senesce and long shoot elongation stops. This generally occurs at the end of July through August (Miller and Schultz, 1987). This pattern of growth is encountered in all taxa belonging to the *Tridentatae* subspecies.

No reports have been published showing the difference between the glandular trichomes of the large leaves and the small leaves, which appear to be physiologically and anatomically different, and might explain reduced terpenoid content of small leaves that serve as animal forage during the winter. There are no published reports showing terpenoid content in sagebrush ephemeral leaves after clipping of the branches. Likewise there are no reported investigations of the genetic control of the induced response of the monoterpenoid content of the leaves of a greenhouse-grown *A. t. vaseyana* plant.

#### Objectives of the Study

The objective of this study is to investigate the possible use of volatile terpenoids from the mountain big sagebrush (*A. t. vaseyana*) as alternatives to methyl bromide in agriculture. Methyl bromide is an effective herbicide, nematocide, insecticide and fungicide that has been used commercially for soil fumigation and quarantine (post-harvest) purposes for most of the twentieth century (Ragsdale and Wheeler, 1995). Considerable evidence has been accumulated that methyl bromide is a potent compound that contributes to the depletion of the ozone layer, and is scheduled to be phased out in the U.S.A. by

2005 under the Clean Air Act (United States Environmental Protection Agency, 1993).

Over 90% of the earth's ozone is present in the stratosphere that extends from 16 to 160 km above the earth's surface. Stratospheric ozone provides a protective layer for the earth's surface by absorbing ultraviolet radiation from the sun, thus moderating the climate on our planet (World Meteorological Organization, 1994). Atmospheric pollutants such as chlorine (photolysis product of chlorofluorocarbons) and bromine break down stratospheric ozone molecules by reacting with them chemically (Molina and Rowland, 1974). At least seven organic bromine compounds have been identified in the atmosphere (Penkett *et al.*, 1985). Bromoform emitted by ocean sources is the largest contributor to atmospheric bromine, but has a short life due to photolysis (Penkett *et al.*, 1985). Methyl bromide is the major carrier of bromine to the stratosphere (Penkett *et al.*, 1985). It breaks down to form bromine which not only reacts with the ozone molecule, but it is also 50 times more reactive than chlorine in depleting ozone by combining with reservoir chlorine species, freeing the chlorine to react with additional ozone (World Meteorological Organization, 1994; United Nations Environment Programme, 1995; Cox *et al.*, 1995). It has become important to discontinue methyl bromide production and find alternative compounds for use in agriculture.

### Hypotheses

I hypothesized that:

- 1) The glandular trichomes of the two types of leaves have different anatomy and physiology.



- 2) The ephemeral leaves respond to clipping and defoliation by increasing the quantity and quality of their terpenoid content.
- 3) Terpenoid production by the sagebrush is under genetic control, and is stimulated by clipping or defoliation.

In this dissertation I examine the ontogeny and ultrastructure of the glandular trichomes from both ephemeral and persistent leaves from greenhouse grown plants of *A. t. vaseyana*. The terpenoids produced by ephemeral and persistent leaves were analyzed. I also address the evolutionary significance of the plant in terms of herbivory by mammals and insects. The study of volatile compounds produced by the ephemeral leaves after clipping of the branches was also done for the ultimate goal of using analogs of the volatile compounds emitted by ephemeral leaves as an alternative to methyl bromide for the control of post-harvest and soil borne pests. An attempt was made to isolate a fragment of terpenoid synthase from the large leaves of the sagebrush plant. Such a gene fragment can be used to test the differential expression of terpene produced in response to clipping of branches.

## CHAPTER 2

TRICHOMES OF THE LEAVES OF *ARTEMISIA TRIDENTATA VASEYANA*:  
ONTOGENY AND ULTRASTRUCTUREIntroduction

Glandular trichomes which secrete lipid material have been described as occurring on leaves and flower parts of a large number of genera in the plant families Asteraceae, Lamiaceae, and Geraniaceae (Haberlandt, 1914; Metcalfe and Chalk, 1950; Cutter, 1969). The glandular trichomes of the genus *Artemisia* from the Asteraceae are the accumulation sites of a variety of terpenoids. The major composition of the terpenoids secreted in the leaves from the sagebrush plant are monoterpenoids and sesquiterpenoids. There is considerable interest in the exploitation of the terpenoids secreted by the sagebrush as herbicidal and anti-herbivore agents. Sagebrush is a perennial plant which possesses two types of leaves: the ephemeral leaves which are present only in summer during a period of increased terpenoid production, and the persistent leaves which are mostly present in winter and show a monthly decrease in the production of terpenoids (Cedarleaf *et al.*, 1983).

Previous Studies of Trichomes from the Genus *Artemisia*

The non-glandular trichomes of the genus *Artemisia* are uniseriate, with a multicellular nucleated stalk topped by an enucleate unicellular "arm" which is 400-500  $\mu\text{m}$  long and present in both adaxial and abaxial surfaces of the leaf (Diettert, 1938).

There is an interspecific variation in non-glandular trichome density, from lower density in *A. nova* and *A. rothrockii* to no cover of trichomes in *A. pygmaea*, a taxon which compensates its lack of protection from the trichome layer by an increased cuticular thickness (Shultz, 1983). The two-armed, stalked trichomes were termed "cover trichomes" by Vesque and Viet (1981), due to the manner in which these trichomes blanket the leaf surface. The presence of these air-filled trichomes appears to be significant in an arid climate (Shultz, 1983). The transpirational rates were measured from pubescent and glabrous surfaces by Ehleringer *et al.* (1976). They concluded that a hairy coat is more effective in reducing transpiration in the presence of wind, and is also more effective than a heavy cuticle in reducing water loss in windy environments where ground water is abundant. The effect of leaf pubescence on photosynthesis was also investigated by Ehleringer *et al.* (1976) and Ehleringer and Mooney (1978); they concluded that pubescence modifies the heat load of the plant by increasing light reflectance, thus maintaining leaf temperature at a thermal optimum for photosynthesis.

Glandular trichomes from *A. tridentatae* have been studied using light microscopes (Diettert, 1938; Kelsey and Shafizadeh, 1980). The ten-celled peltate gland from the genus *Artemisia* is composed of one pair of stalk cells; and four pairs of cells filled with secretory material (Diettert, 1938). No distinction was made as to whether the leaves were ephemeral or persistent but if it was 10-celled, it must only have been from the ephemeral leaves. The biseriate peltate glandular trichome of *Artemisia nova* is filled with sesquiterpene lactone enclosed in a membranous sac (Kelsey and Shafizadeh, 1980). The gland from *A. nova* is typically 25 to 30  $\mu\text{m}$  wide in transection. Differentiation of

epidermal cells into trichomes begins when the foliar primordium has reached a length of 80  $\mu\text{m}$  (Diettert, 1938). Glandular trichomes are found throughout the plant, on young stems, leaves, inflorescence bracts, corollas, and achenes of *A. nova* (Slone and Kelsey, 1985).

### Secretion of Terpenoids

Terpenoids are biosynthesized *in situ* by the glandular trichomes. Gland cells frequently display ultrastructural features (active chloroplasts or plastids) that suggest active metabolism of gland products (Fahn, 1979). Direct evidence for the biosynthetic capabilities of gland cells has become available. Studies with tobacco (*Nicotiana tabacum* L.), the glandular trichomes of which secrete principally divane-type diterpenes and sucrose esters, have demonstrated that gland cells incorporate basic metabolic precursors into both of these types of glandular substances (Keene and Wagner, 1985; Kandra and Wagner, 1988). Spearmint (*Mentha spicata* L.) plants accumulate an essential oil containing principally monoterpenes in their glandular trichomes. The enzymes of monoterpene biosynthesis were shown to be located only in the cells of the glandular trichomes and not in the remainder of the leaf (Gershenson *et al.*, 1989).

Cell cultures of *Artemisia annua* L. failed to produce artemisinin, a compound which has potent antimalarial activity (Duke *et al.*, 1994). The lack of artemisinin biosynthesis in cell culture is due to the fact that the sesquiterpene lactone artemisinin is produced in the glandular trichome, not in other parts of the plant (Duke *et al.*, 1994). Glandless leaves of *A. annua* could not produce artemisinin (Duke *et al.*, 1994). Thus,

different techniques have been developed to obtain highly-purified preparations of glandular trichomes, for the *in vivo* study of the different pathways of terpenoid synthesis in the whole intact glands (Slone and Kelsey, 1985; Gershenson *et al.*, 1987).

The objective of this part of the study was to examine the ontogeny and pattern of volatile production, to show the differences between the peltate glandular trichomes of the ephemeral and the persistent leaves of *A. t. vaseyana*, and to discuss the implication for pesticide research.

### Materials and Methods

#### Plant Material

Seeds of *A. t. vaseyana* were collected in November, 1995, from the study site in the Gallatin Valley, at an altitude of 1550 m on a southwestern facing slope in T2S, R4SW, approximately 8 km northeast of Bozeman, Montana. The seeds from a plant tagged #3 showed the highest germination rate (90-97%) and were retained for further experiments. Seeds from plant #3 vernalized at 4 °C for 10 days in a wetted Whatman Paper No. 3 were sown on 24 January and 23 June, 1996, one per cell in a 72-cell seed bed (No. 1206 insert for No. 1020 flat 11" x 22" T. O. plastics, Belden Plastics, St. Paul, MN), filled with pre-wetted Aqua 2000 potting media (one part of Bozeman silt loam, one part of peat moss, one part of washed concrete sand, and 1lb per cubic yard of Aqua Gro 2000G). The ensemble was covered with a clear plastic dome. The plants were watered with tap water once every two days after seedling establishment. In addition, they were watered twice per week with a dilute solution of Peter's 20-10-20 general purpose

fertilizer (0.25 g/l). The seedlings were transplanted after two months into 60-cubic-inch pots (Stuewe & Sons, Inc., Corvallis, OR) where they were grown for one year. One-year-old plants were transplanted into 8-inch pots (AZALEA Pot, Belden Plastics, St. Paul, MN) for further growth. Leaves from mature plants (30 to 90 days for the ephemeral leaves; 30 to 365 days old for persistent leaves) were sampled and prepared for microscopy studies.

### Leaf Size

Leaf Size Measurement. Leaves from 24 plants, grown from 24 January to 21 October, 1996, were harvested. One ephemeral leaf and one persistent leaf from each plant were harvested from the topmost section and the base of each harvested branch, respectively. A total of 48 ephemeral and 48 persistent leaves was measured with a ruler. Leaf length (including petiole), petiole length, and leaf width (at the widest point) were measured to compare the ephemeral and the persistent leaves.

Leaf Surface Area Measurement. Twenty-seven ephemeral and 29 persistent leaves were randomly selected from four plants. Their surface area was measured using Carl Zidas's (1985 Carl Zeis Inc. Heidelberg, Germany) thin pad. The leaves were immobilized on the console of the thin pad using clear tape. The mean area per leaf type was computed.

The length and the width of the transmission electron microscope print of one mature glandular trichome from an ephemeral leaf and one print from the mature trichome

of a persistent leaf were measured three times with Carl Zidas's thin pad. The thin pad was set with the magnification of each print, and the surface area of each type of trichome was automatically shown on the paper printed out from the printer of the thin pad. These measurements of the two types of trichomes permitted comparison of the area of the two types of mature peltate trichomes.

### Size of Peltate Glandular Trichomes

Determining Trichome Number on Ephemeral Leaves. Three-month-old ephemeral leaves harvested from many plants were cut into 1 mm squares and placed in a rigid plastic container (50 ml polypropylene centrifuge tube with screw cap), capped loosely, and frozen quickly in liquid nitrogen ( $-190^{\circ}\text{C}$ ). The tube was loosely capped to prevent shattering by the temperature differential and to allow some liquid nitrogen to flow into the tube and freeze the leaf tissues. The tube was removed from the liquid nitrogen tank and 3 cm<sup>3</sup> of finely powdered dry ice (prepared by wrapping a piece in clean paper towels and crushing with a hammer) were added to the tube. The tube was loosely capped and vortexed using a model G-560 Vortex-Genie 2 mixer (Scientific Industries, Bohemia, NY) at a maximum speed of 3200 rpm for 1 min (Yerger *et al.*, 1991). The thick cover trichomes were sheared off the surface of the leaf tissues and the number of peltate glandular trichomes from the 27 samples were counted using a dissecting microscope (x 600). One sample was photographed to show the organization of the trichomes on the surface of the leaf (Figure 1).



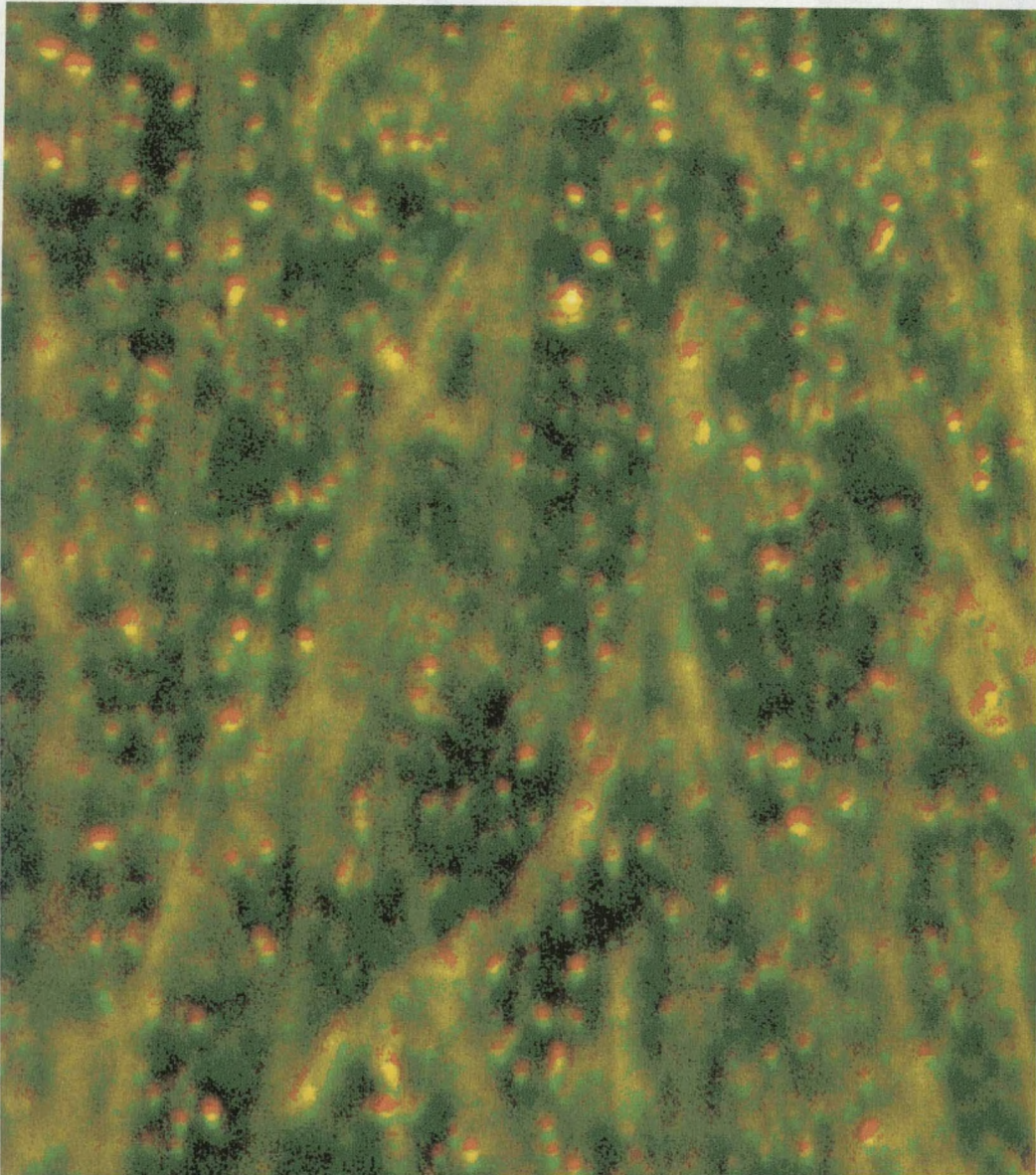


Figure 1: Light micrograph of non-stained surface of the ephemeral leaf after removal of the cover trichomes (750 x). Green = leaf surface, yellow = remaining cover trichomes, red dots = glandular trichome with secretory head.

Determining Trichome Number on Persistent Leaves. The above procedure did not reveal the organization of the peltate trichomes on the surface of the persistent leaves.



The cover trichomes on the small leaves were removed after keeping the leaves at approximately 95% humidity (taped glass petri dish containing a pile of 2 x 59 cm<sup>2</sup> wetted germination papers) at 4 °C (in the refrigerator) for three days. The cover trichomes were removed by gently pulling off the clear tape applied on the surface of the leaf. The surface with the fewest number of cover trichomes remaining was cut from four leaves with a circular clipper 1 mm in diameter. The number of peltate glandular trichomes was counted per 0.785 mm<sup>2</sup> through a dissecting microscope for all thirty samples obtained. One of the four samples photographed under the dissecting microscope is cropped and is shown as an example (Figure 2).



Figure 2: Non-stained surface of persistent leaf denuded of cover trichomes, showing the peltate glandular trichomes (1,200 x). The dark green color observed on the surface of the leaf may be due to the dark spots left by broken glandular trichomes (see procedure). GT = glandular trichome, BGT = broken glandular trichome.

### Ultrastructural Studies

Scanning Electron Microscopy (SEM). Five developing ephemeral leaves were harvested at 30, 32, 34, 36, 38 and 40 days old. The two blades of each leaf were longitudinally cut from the central vein and each sample was cut into two before being fixed overnight in 2.5% glutaraldehyde in 0.1 M cacodylate buffer. Five developing persistent leaves were harvested at 30, 32, 34, 36, 38 and 40 days old. The entire leaf was fixed overnight in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (Pease, 1964). After washing with distilled water, and serially dehydrating in ethanol, the tissues were mounted on aluminium specimen stubs with silver conducting paint, coated with 20 nm of gold-palladium in a sputter coater (Anatech Hummer X). Fixed specimens were observed and photographed with the scanning electron microscope (JEOL, JSM-640) at 15kV.

Transmission Electron Microscopy (TEM). Five mature persistent leaves were sampled when they were 90 days, 120 days, and 12-13 months old. One mature ephemeral leaf was sampled at 30, 40, 60, 70, and 90 days old. This sampling was repeated every five weeks for two years. They were cut into 1 mm x 1 mm squares and fixed overnight in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (Pease, 1964). The tissues were then rinsed for 45 min in the buffer, and stored overnight in the sucrose buffer and postfixed for one hour in 1% OsO<sub>4</sub> in 0.5 M cacodylate buffer. The tissues were then rinsed in distilled water, dehydrated in an alcohol series, and embedded in Spurr's resin (Pease, 1964). Thin-section samples, obtained with a diamond knife on an ultramicrotome

(Reichert-Jung Ultracut E), were stained for five min in methanol saturated uranyl acetate, and poststained with Reynolds's lead citrate for three min (Reynolds, 1963). The sections were observed and photographed in a transmission electron microscope (Zeiss EM 10CR) at 60 kV.

## Results

### Leaf Composition of *A. t. vaseyana* Plant

A greenhouse-grown plant of *A. t. vaseyana* had both ephemeral and persistent leaves (Figure 3). The ephemeral leaves grew from April to July when they began to senesce, while the persistent leaves began development in July and were present throughout two winters.

### Differences in the Types of Leaves

The mean length (petiole included) of the 48 ephemeral leaves was 196 mm ( $\pm 38$  mm), and the mean length of the 48 persistent leaves was 145 mm ( $\pm 38$  mm). The mean width of the 48 ephemeral leaves was 58 mm ( $\pm 3$  mm) as compared to 29 mm ( $\pm 5$  mm) for the 48 persistent leaves. The mean length of the petiole was 75 mm ( $\pm 13$  mm) for the 48 ephemeral leaves, and was 53 mm ( $\pm 5$  mm) for the 48 persistent leaves. The mean area (measured with Zidas's thin pad) of the 27 ephemeral leaves was 210.87 mm<sup>2</sup> ( $\pm 37.67$ ) while the mean area of the 29 persistent leaves was 142.78 mm<sup>2</sup> ( $\pm 41.55$ ). The persistent leaves had only 67.71% of the area of the ephemeral leaves.



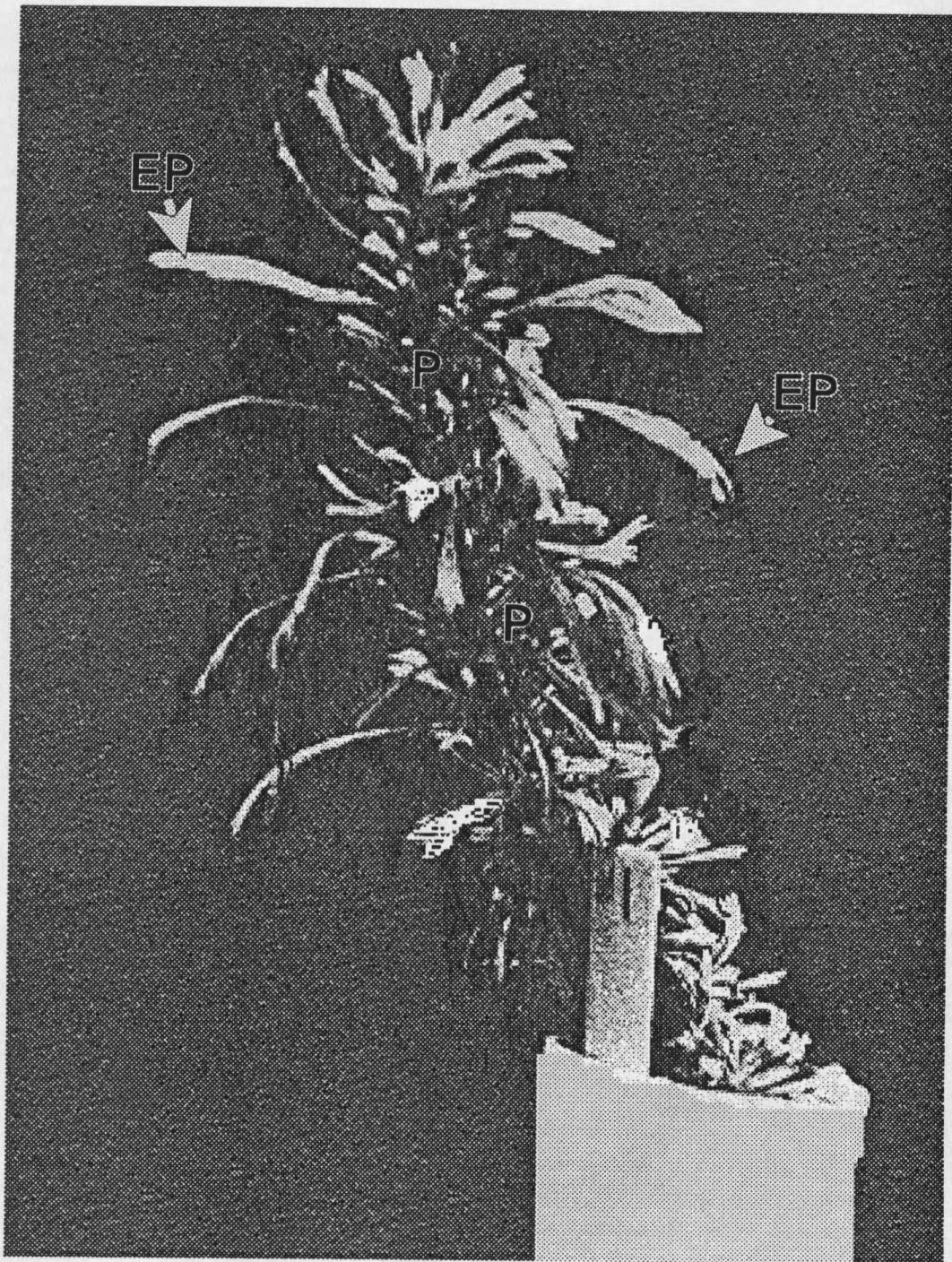


Figure 3: Ten-month-old greenhouse-grown plant of *Artemisia tridentata vaseyana*. The ephemeral leaves (EP) and the persistent leaves (P) were both present.

### Glands

As reported by Shultz (1983), veins were the only locations on the leaves that were examined where glands did not develop. The number of glands is the same on the upper and lower side of the leaf. The number of glands averaged  $87 (\pm 34)$  per  $\text{mm}^2$  on the ephemeral leaves while the persistent leaves had  $110 (\pm 34)$  per  $\text{mm}^2$ . The peltate gland from the ephemeral leaf had an average surface area of  $1.81 \times 10^{-3} \text{ mm}^2$  and a perimeter of  $15.38 \times 10^{-2} \text{ mm}$ . The peltate trichome from the small persistent leaf had an average area of  $4.277 \times 10^{-4} \text{ mm}^2$ , and a perimeter of  $7.605 \times 10^{-2} \text{ mm}$ . The glandular trichome of the ephemeral leaf had twice the perimeter, and four times the area of the glandular trichome of the persistent leaf. Glands from the persistent leaves were not easily studied because of their smaller size and their propensity to shed when the leaves were fixed for study.

### Cover and Glandular Trichomes of Ephemeral Leaves of *A. t. vaseyana*

The adaxial surface of the leaf is covered with filamentous trichomes that give the leaves a silvery appearance; the glandular trichomes are under these cover trichomes. Numerous mature oblong glands may be seen among the filamentous cover trichomes (Figure 4). The enlarged peltate glandular trichome shows the top two cells of the gland and cuticular sac (Figure 5). Transmission electron microscopy of the immature stage of the cover trichome shows a "T"-shaped gland with expanded arms with enucleate cells filled with a clear substance (Figure 6).

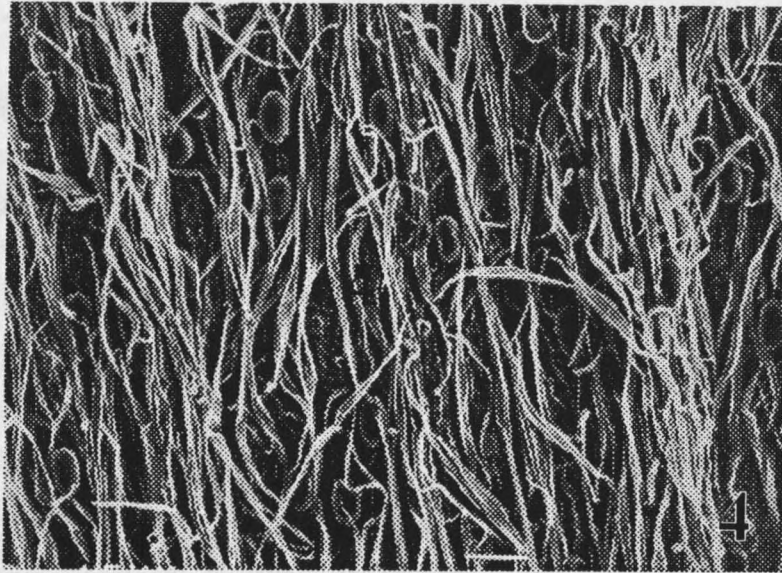


Figure 4: SEM of adaxial side of a mature ephemeral leaf of *Artemisia tridentata vaseyana* (400 x). The leaf is covered with fully developed peltate trichomes and cover trichomes. Scale bar = 50 $\mu$ m.

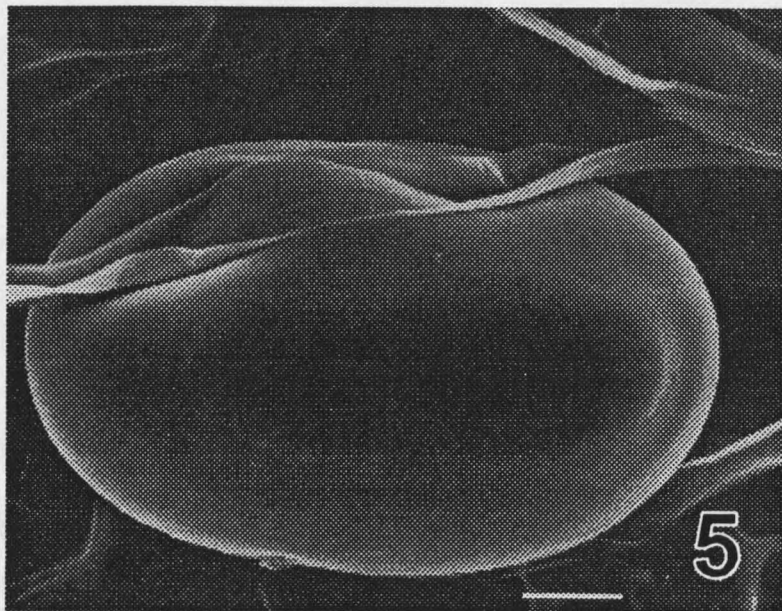


Figure 5: SEM of an enlarged gland from an ephemeral leaf of *Artemisia tridentata vaseyana* (2,400 x). The gland has an oblong shape, two cells (dark center) and bubble-like cuticular sac. Scale bar = 50 $\mu$ m.



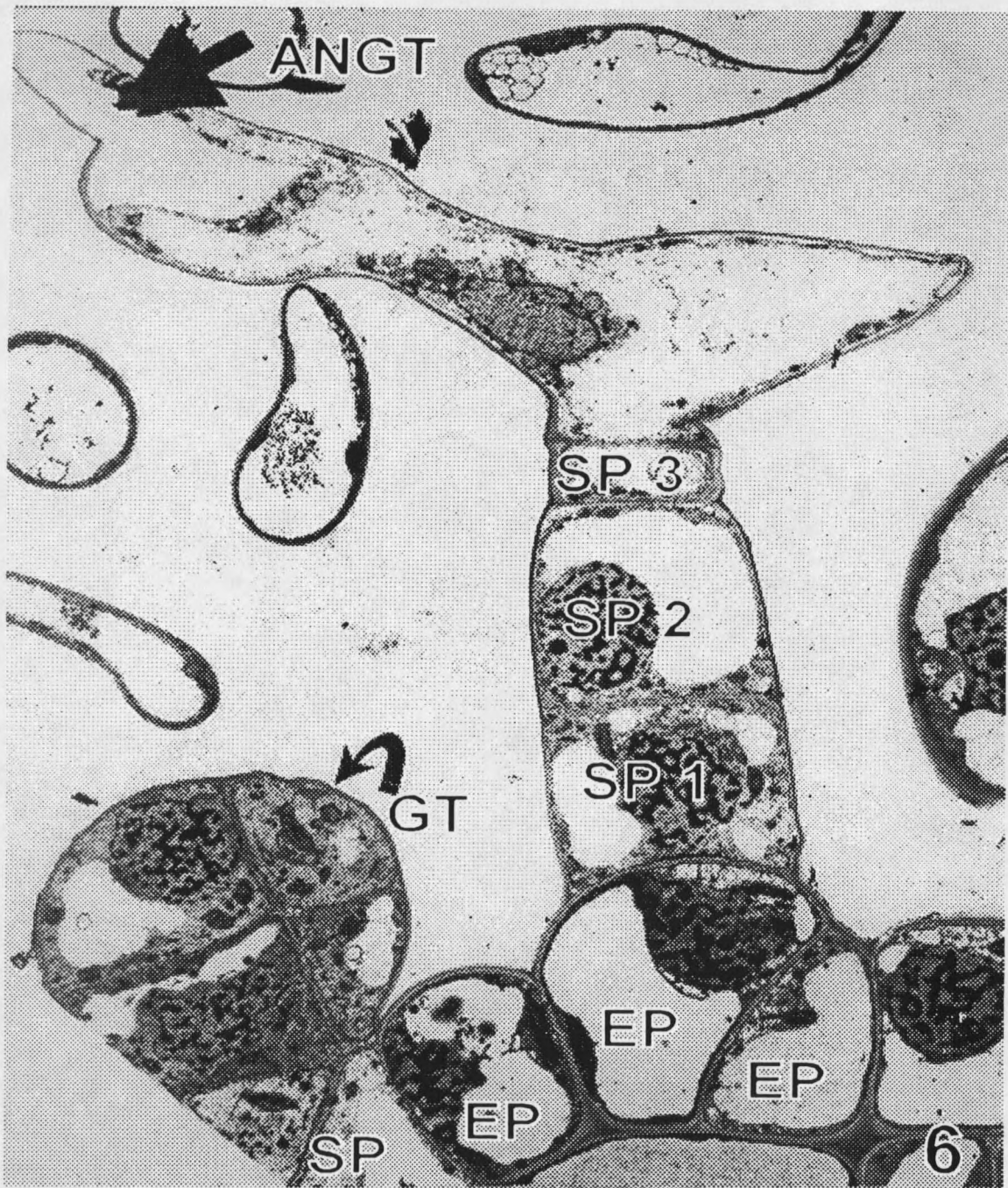


Figure 6: Trichomes from ephemeral leaves of *Artemisia tridentata vaseyana* (6,000 x). The nonglandular trichome has a base of 2 epidermal cells, and has 3 support cells and air-filled arms. ANGT = arm of non glandular trichome, EP = epidermal cell, GT = glandular trichome, SP = support cell.

During development of the glandular trichome, the earliest stage is one cell. The epidermal cell enlarges and protrudes above the leaf surface (Figure 7). It thereafter divides anticlinally into a two-cell trichome (Figure 8). The two cells may be at an early mitotic stage, with a dividing line on the same level as the epidermal cell (dark arrow); this cell may be at the beginning of a periclinal division that produces the four-celled stage. The two cells divide periclinally into four cells making a six-celled trichome composed of two stalk cells and two pairs of gland cells (Figure 9).

The top pair of cells of the four-celled trichome divide periclinally giving an eight-celled trichome which is composed of a pair of stalk cells and three pairs of gland cells (Figure 10). The top cell pair of the eight-celled trichome has already developed a reticulated cell wall, typical of transfer cells. The function of reticulated cell walls is to increase the surface area for rapid nutrient transfer.

The apical cells of the eight-celled trichome divide to give a ten-celled trichome which is composed of a pair of support cells adjacent to the epidermal cell of the leaf, followed by a pair of stalk cells with secreting chloroplasts (Figure 11). Above the stalk cells are two pair of secreting cells which contain numerous chloroplasts, and the top pair of cells which has numerous mitochondria and no chloroplasts (Figure 12).

The enlargement of one of the chloroplasts from the left side cell of the first secreting pair of cells shows electron dense substances appearing at the end of the grana membranes of the chloroplast (Figure 13). The electron-dense spots may be the monoterpenoids and were also observed outside mitochondria (Figure 12). The darker



spots in the outer cytoplasm between the chloroplasts and cell walls may be the monoterpenoids.

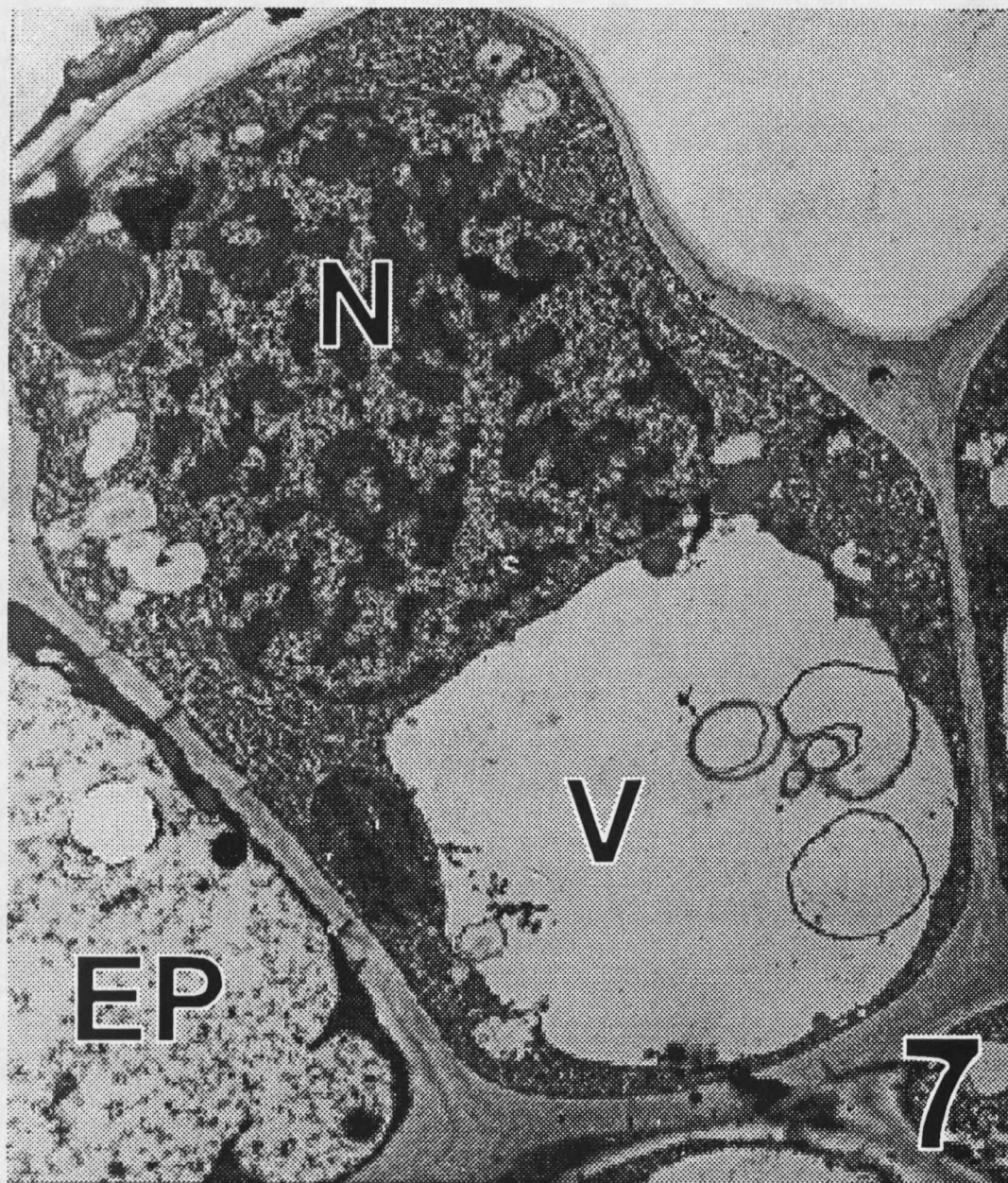


Figure 7: TEM of one-celled stage of development of peltate glands of an *Artemisia tridentata vaseyana* ephemeral leaf (13,600 x). EP = epidermal cell, N = nucleus, V = vacuole.

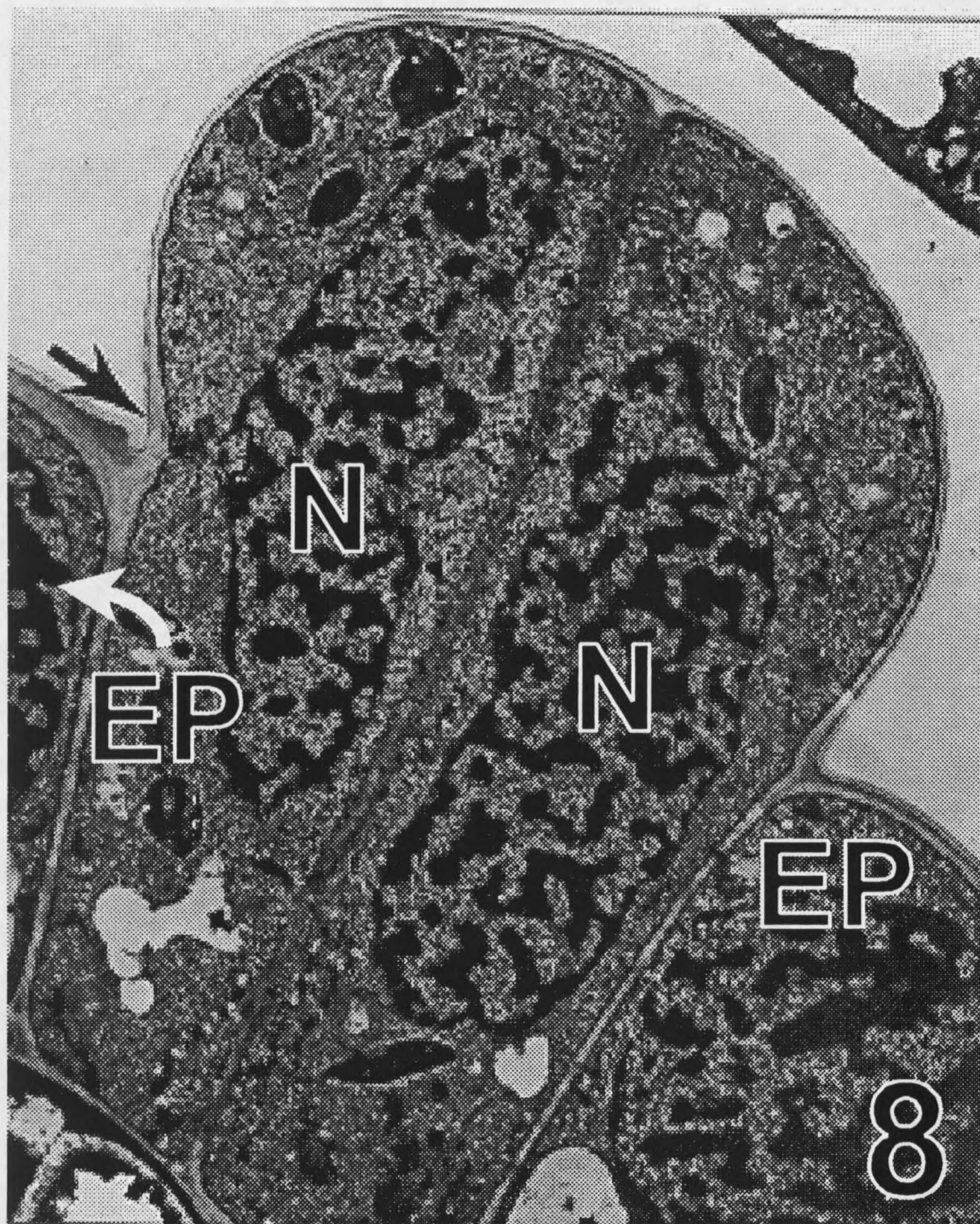


Figure 8: TEM of two-celled stage of development of peltate glands of an *Artemisia tridentata vaseyana* ephemeral leaf (13,600 x). EP = epidermal cell, N = nucleus, dark arrow = indication of early mitotic stage..



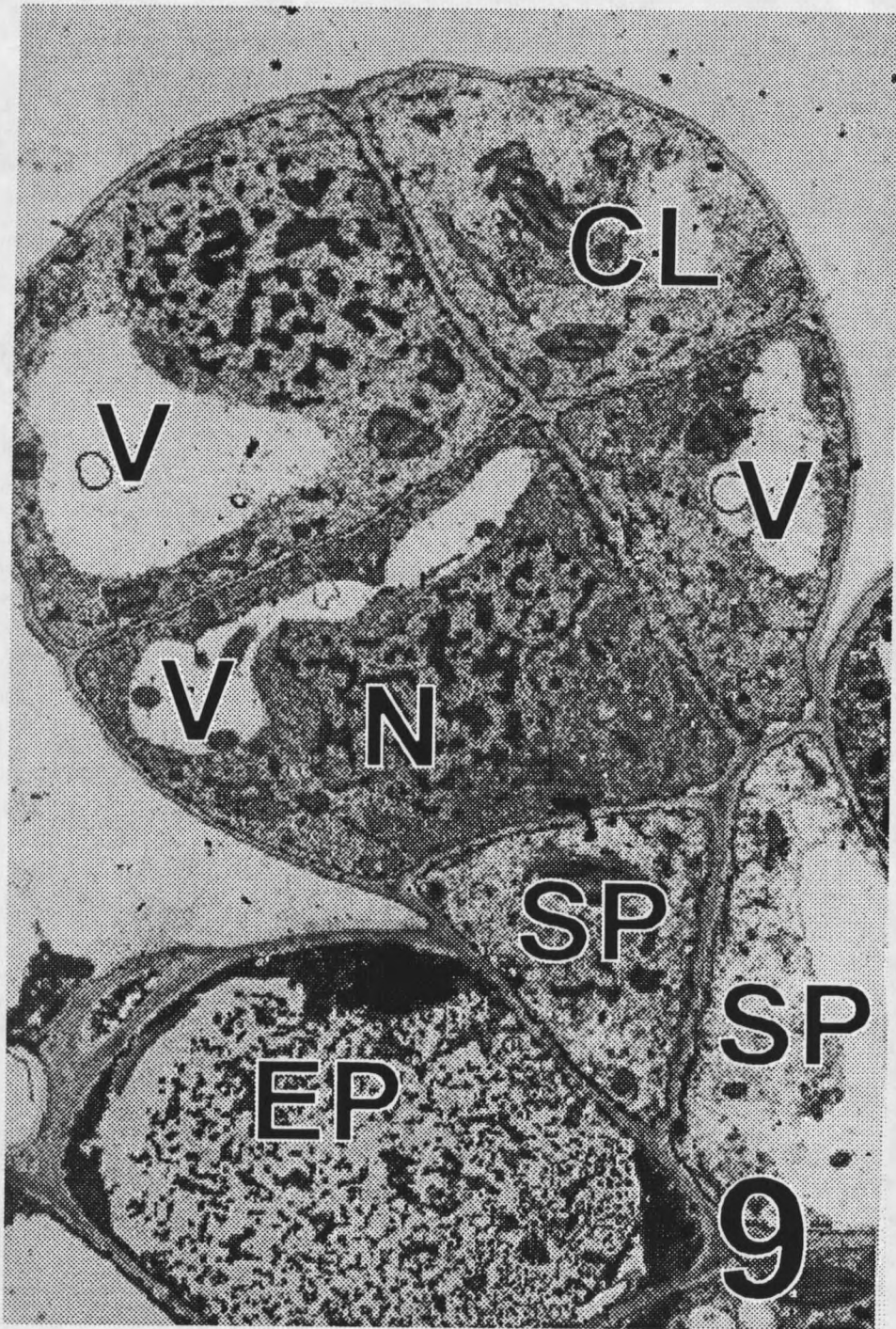


Figure 9: TEM of six-celled stage of development of peltate glands of an *Artemisia tridentata vaseyana* ephemeral leaf (12,000 x). EP = epidermal cell, CL = chloroplast, N = nucleus, SP = support cell, V = vacuole.

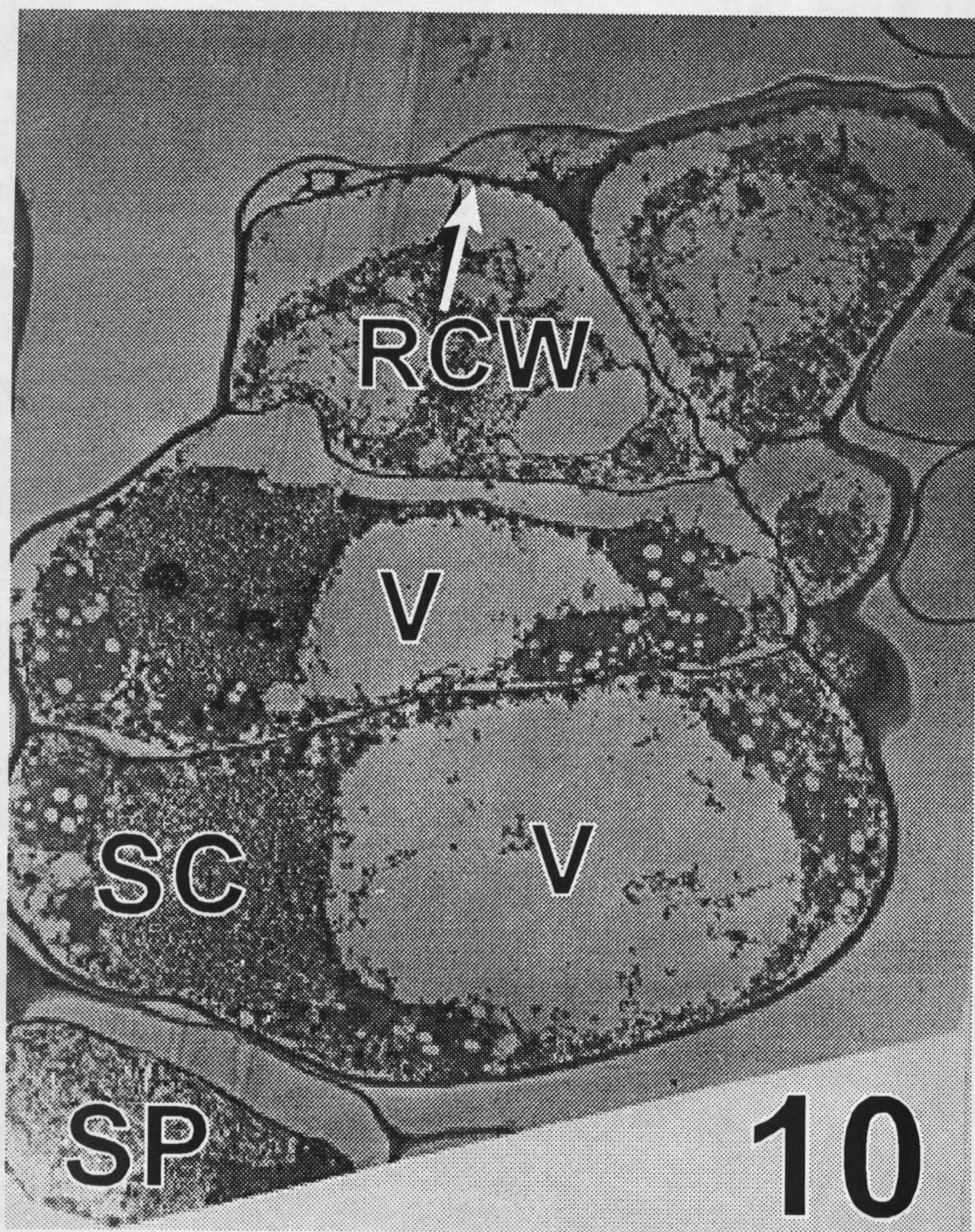


Figure 10: TEM of eight-celled stage of development of peltate glands of an *Artemisia tridentata vaseyana* ephemeral leaf (12,000 x). RCW = reticulated cell wall, SC = stalk cell, SP = support cell, V = vacuole.



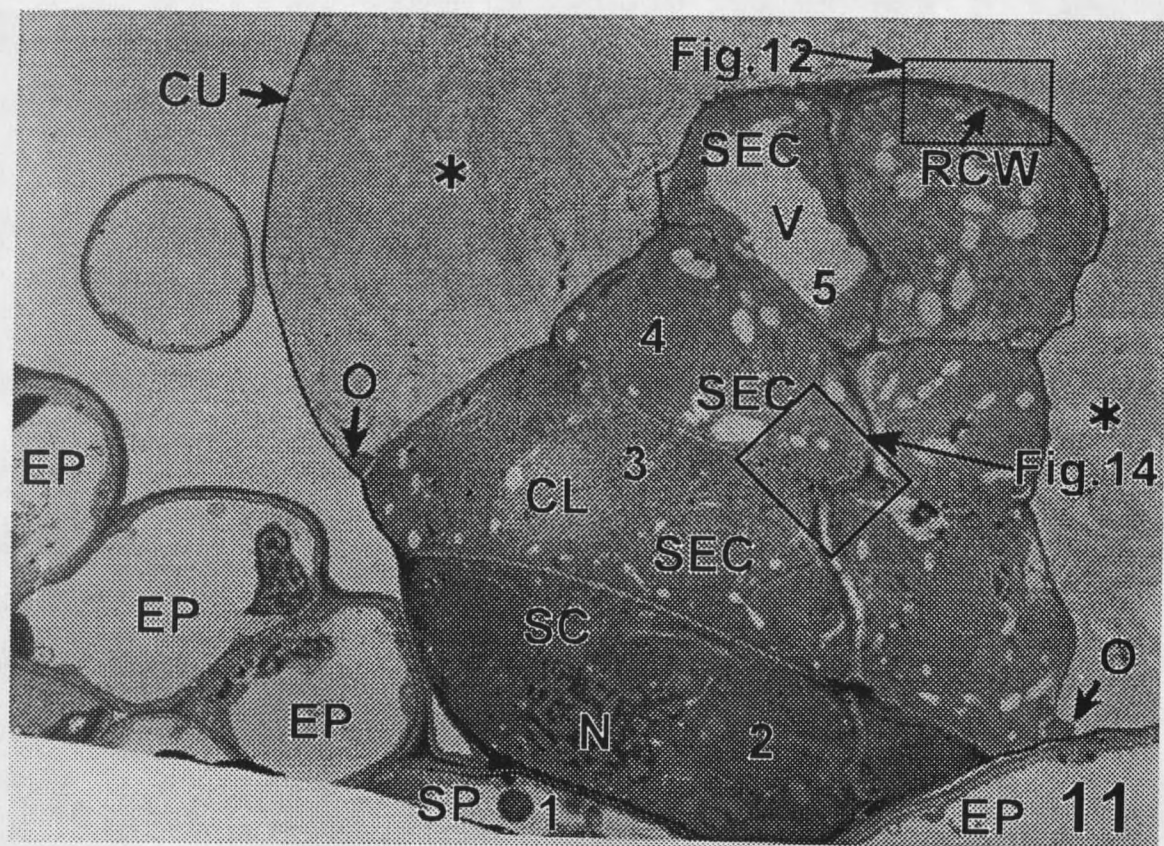


Figure 11: TEM of 10-celled stage gland of an *Artemisia tridentata vaseyana* ephemeral leaf (3,225 x). The cuticular sac is completely expanded. CL = chloroplast (enlarged in Figure 12), CU = cuticle, EP = epidermal cell, N = nucleus, O = oily secretion, RCW = reticulated cell wall, SC = stalk cell, SEC = secreting cell, SP = support cell, V = vacuole, \* = sub-cuticular space containing volatile compounds, 1 = first pair of cells (support cells which are at the same level as the leaf epidermal cells), 2 = second pair of cells (stalk cells), 3 = third pair of cells (first pair of secreting cells), 4 = fourth pair of cells (second pair of secreting cells), 5 = fifth pair of cells (topmost secretion cells).

The terpenoids crossed the cell walls of the adjacent secretory cells to reach the topmost secretory cells (Figures 14-15). The enlargement of the left and right sides of the uppermost secretory cell (Figure 16) showed that the osmiophilic secretions first accumulated inside the plasma membrane, then crossed into the cell wall and forced the

cuticle to detach from the non-reticulated cell wall (Figures 17-18). Presumably these substances separated the cuticle from the cell wall to form the subcuticular space in which the volatile terpenoids accumulated.

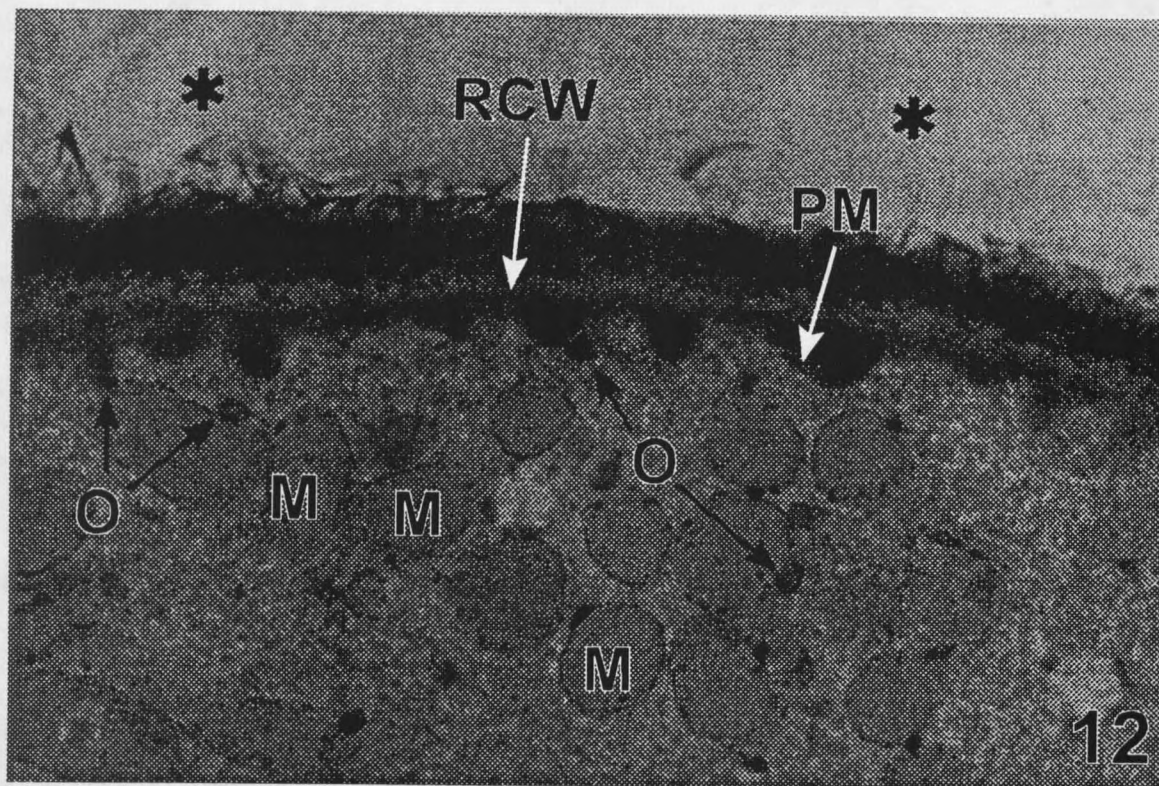


Figure 12: Enlargement of the topmost secretion cell (Figure 11) of the ten-celled glandular trichome. M = mitochondrion, O = oily secretion, PM = plasma membrane, RCW = reticulated cell wall, \* = volatile.

The enlargement of the top part of the uppermost cell (Figure 16), shows a reticulated cell wall adjacent to the developing sub-cuticular bubble (Figures 19-20). As the secretion accumulated outside the gland cells, the sub-cuticular space rose and appeared as a balloon filled with volatiles. Terpenoids produced by mitochondria and chloroplasts were presumably transported between secreting cells by rough endoplasmic



reticulum (Figure 21-22). Terpenoids were also presumably deposited into the reticulated cell walls by rough endoplasmic reticulum (Figures 19-24).



Figure 13: Higher magnification of the chloroplast from ten-celled glandular trichome. Chloroplast from Figure 11 (11,975 x). M = mitochondria, O = oily secretion, T = thylakoid membranes.

The expansion of the sub-cuticular space was apparently due to the progressive accumulation of terpenoid secretions (Figures 11 and 16). The sub-cuticular bubble developed progressively and reached its full size when the cuticle was completely separated from the stalk cells (Figures 11,16, and 25). The structure above the cells is the

sub-cuticular space that contains the volatile secretions; the cuticle became detached from the secretory cells during the development of the mature gland from the ephemeral leaves. According to Slone and Kelsey (1983) monoterpenoids are manufactured in and secreted by the chloroplasts of the four secreting cells of *A. t. vaseyana*.

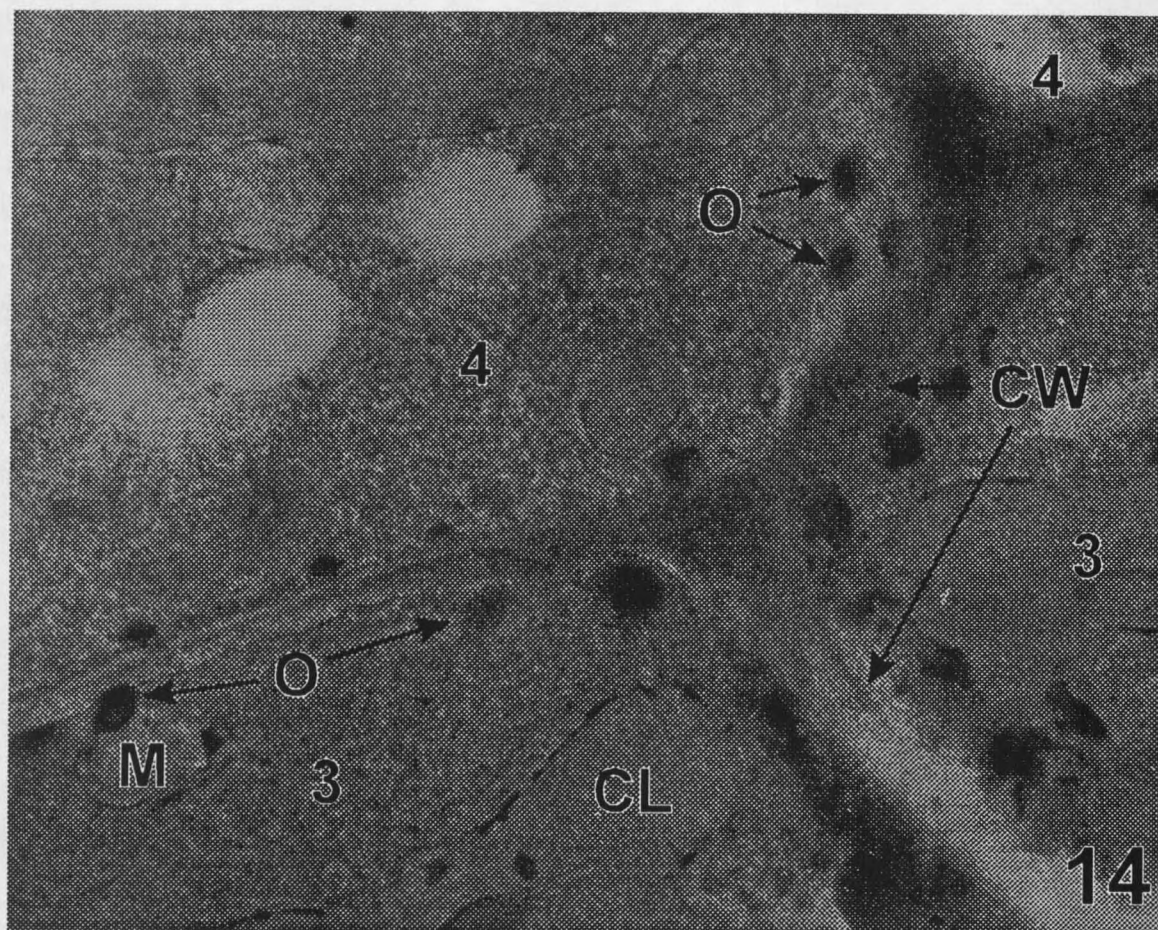


Figure 14: Enlargement of part of the ten-celled trichome from Figure 11 (91,300 x).

Oily substances are gathering into the cell wall of adjacent cells. CL = chloroplast, CW = cell wall, M = mitochondrion, O = oily substance, 3 = first pair of secretory cells, 4 = second pair of secretory cells.



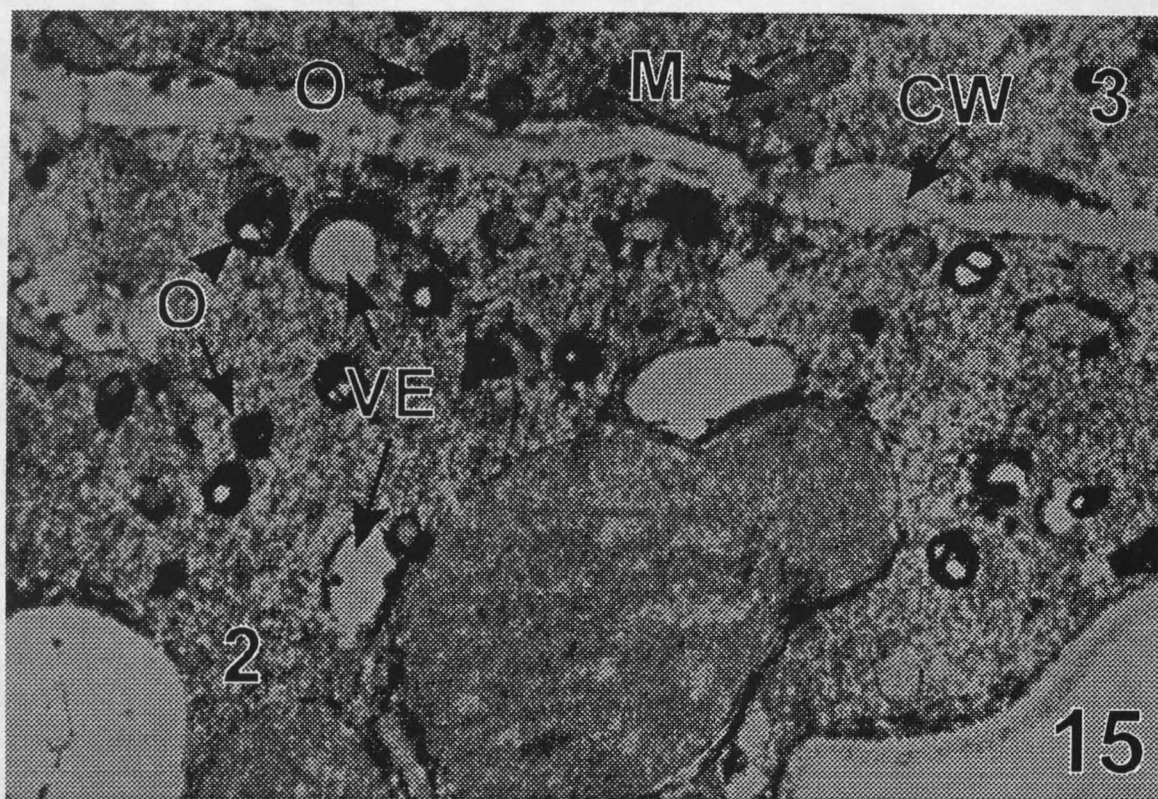


Figure 15: Enlargement of the stalk cell of a ten-celled trichome (18,600 x). Vesicles (probably mitochondria) are filled with dark substances. Vesicles are probably depositing oily substance into the cell wall. CW = cell wall, M = mitochondrion, O = oily substance, VE = vesicle, 2 = stalk cells, 3 = first pair of secretory cells.

#### Peltate Glandular Trichomes of Small Persistent Leaves

The scanning electron micrographs of the persistent leaves show round peltate glandular trichomes (Figure 26). Some of the glands were made of clusters of cells, however, numerous unicellular immature glands can also be observed on the surface of the leaf (Figure 27). One gland is superficially smoothly round, without a visible cluster of cells; its sub-cuticular space might be filled with terpenoids (Figure 28). The mature and

immature stages of these peltate glands are easily shed, and can be observed in the depression made by the filamentous (cover) trichome (Figure 29).

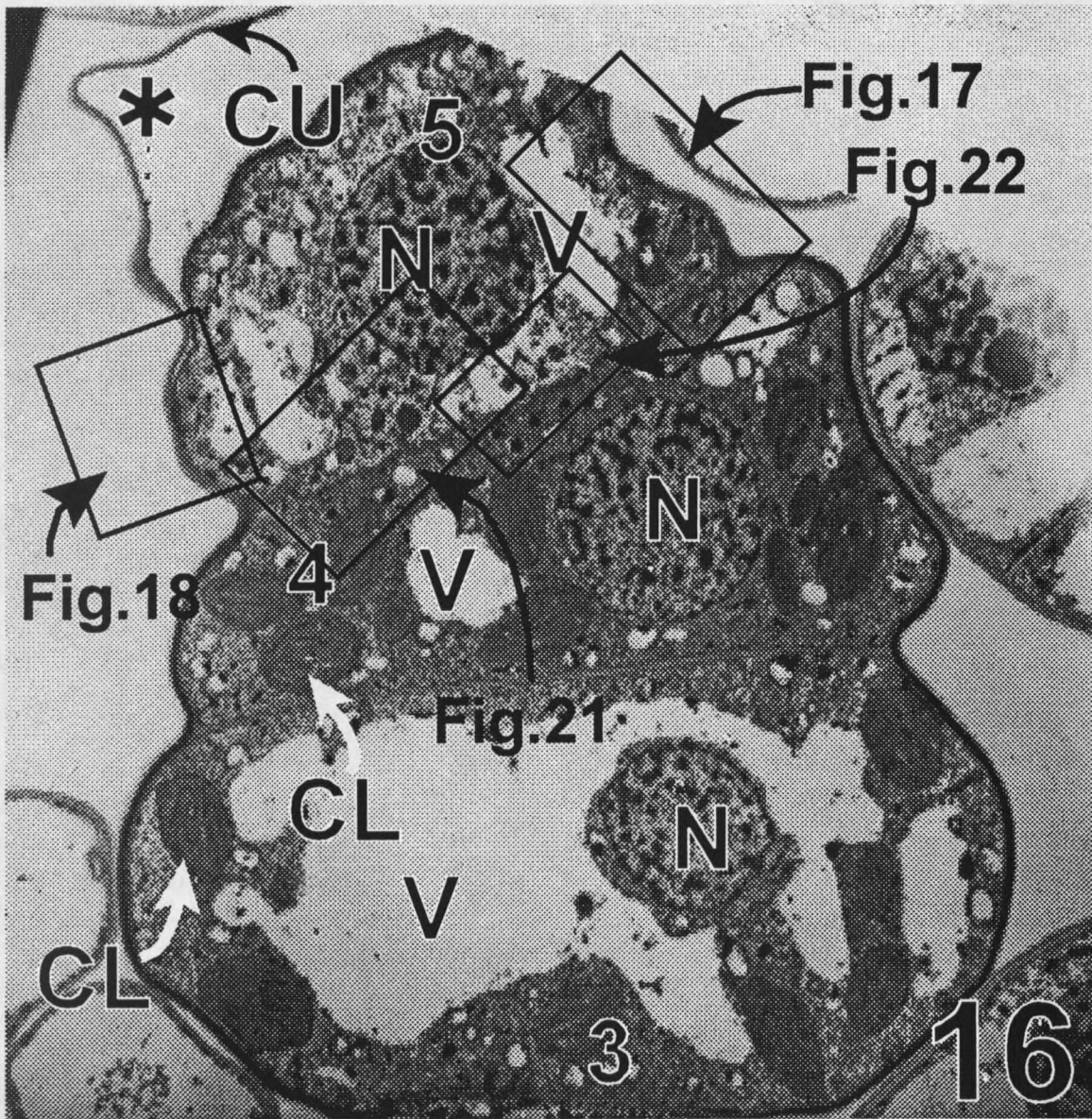


Figure 16: TEM of three cells from a mature ten-celled gland. Side view with developing sub-cuticular bubble (11,975 x). CL = chloroplast, CU = cuticle, N = nucleus, V = vacuole, 3 = first secretory cell, 4 = second secretory cell, 5 = secretory cell, \* = volatile.



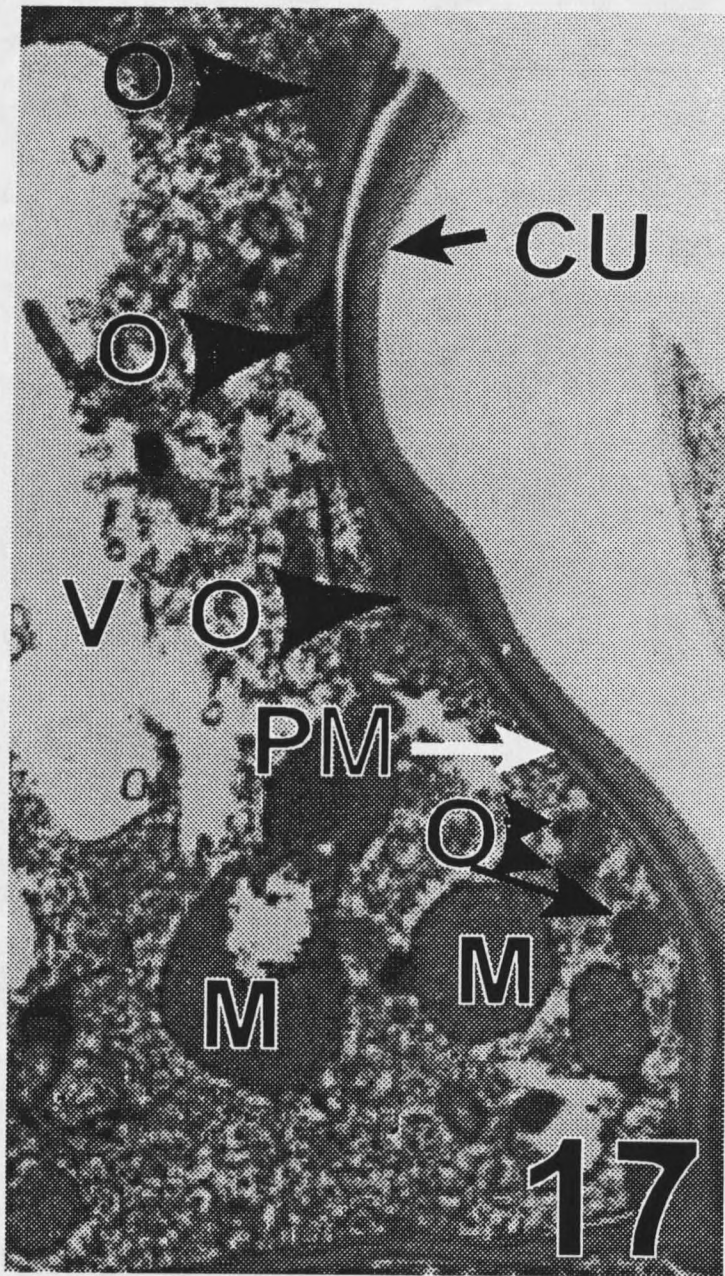


Figure 17: Enlargement of the right side of Figure 16 (38,000 x). Oily substances accumulate first behind the plasma membrane, then cross the membrane and cell wall, and the cuticle becomes detached from the non-reticulated cell wall. CU = cuticle, M = mitochondrion, O = oily substance, PM = plasma membrane, V = vacuole.

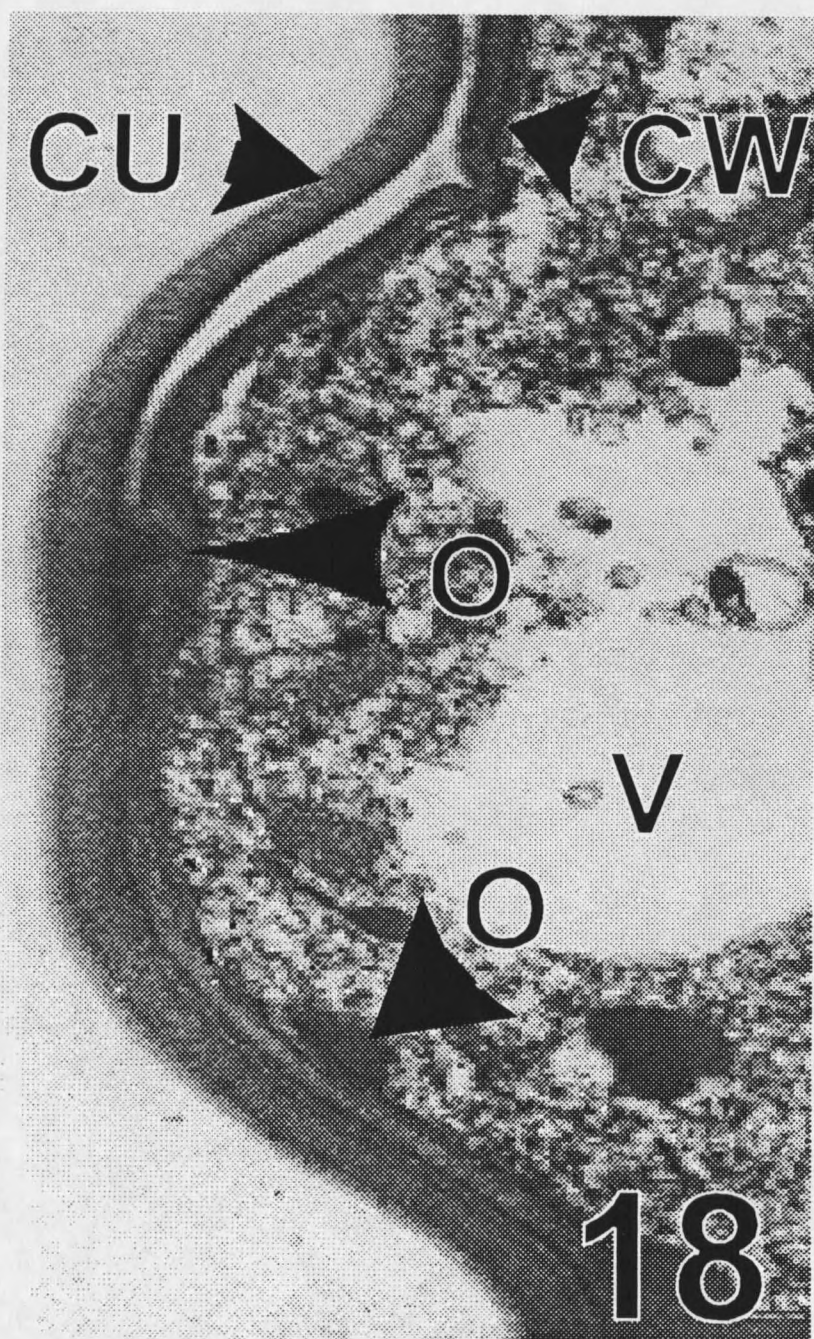


Figure 18: Enlargement of the left side of Figure 16 (38,000 x). The oily substances are lifting the cuticle from the non-reticulated cell wall. CU = cuticle, CW = cell wall, M = mitochondrion, O = oily substance, PM = plasma membrane, V = vacuole.



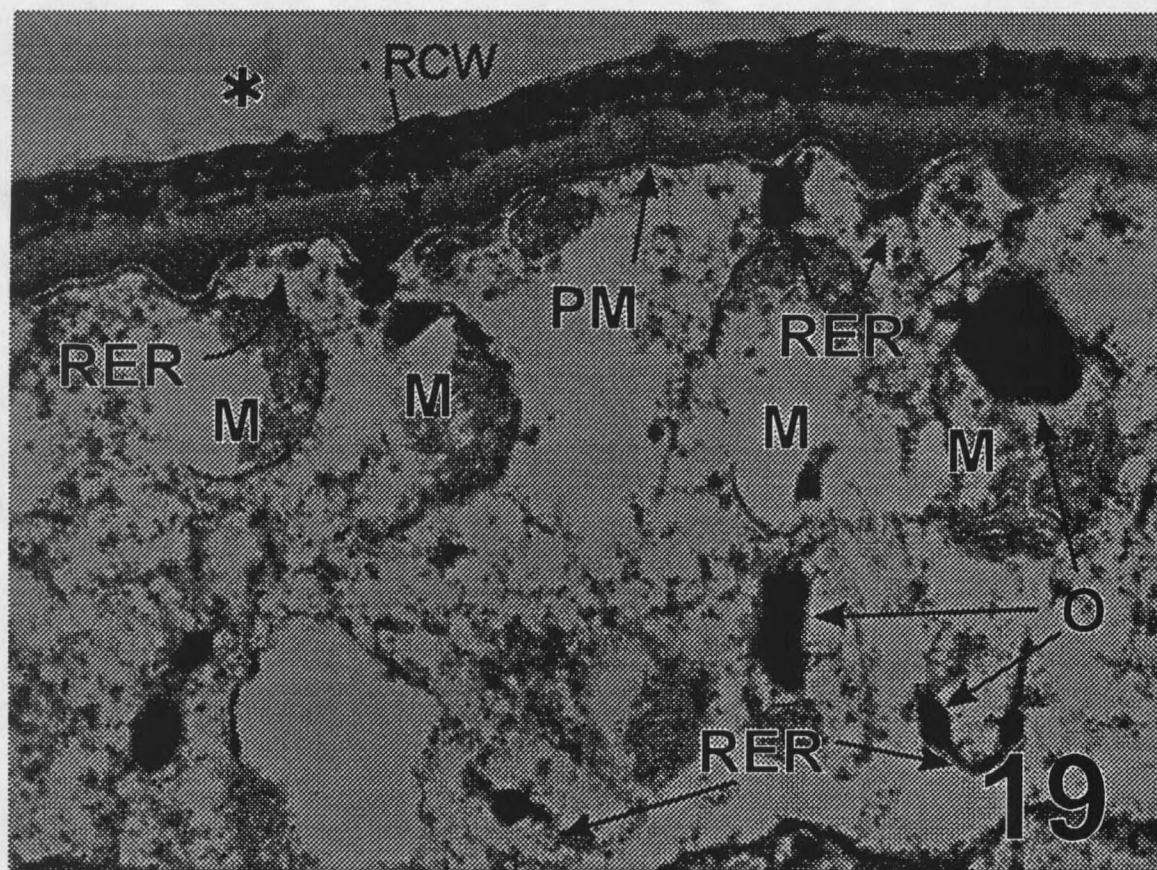


Figure 19: Enlargement of top of Figure 16 (93,000 x). Osmiophilic material can be seen in mitochondria and in the rough endoplasmic reticulum. They are transported into the cell wall by fusion between the plasma membrane and the rough endoplasmic reticulum. M = mitochondrion, O = oily substance, RCW = reticulated cell wall, RER = rough endoplasmic reticulum, \* = volatiles.

Mature trichomes of the persistent leaves have six pairs of cells. The earliest stage in gland development is one cell with a wider support cell on the epidermal cell (Figure 30). The cell undergoes periclinal and anticlinal divisions to become a four-celled gland, including an asymmetric pair of support cells (Figure 31). The latter divided giving 3 pairs of cells including two pairs of support cells, and a pair of gland cells (Figure 32). The

pairs of gland cells divide giving an eight-celled gland which comprises two pairs of gland cells, a pair of base cells and a pair of support cells (Figure 33). The mature trichome has six pairs of cells: one pair of support cells at the base, one pair of stalk cells, three pairs of secreting cells with chloroplasts; and the uppermost pair of secreting cells, show only mitochondria and vacuoles (Figure 34). The vacuoles of the four top cells were very large (Figure 34). Partial enlargement of one of the topmost cells shows a developing reticulated cell wall, which suggests that the secreting cell might also be transporting volatile into the sub-cuticular space (Figure 35).

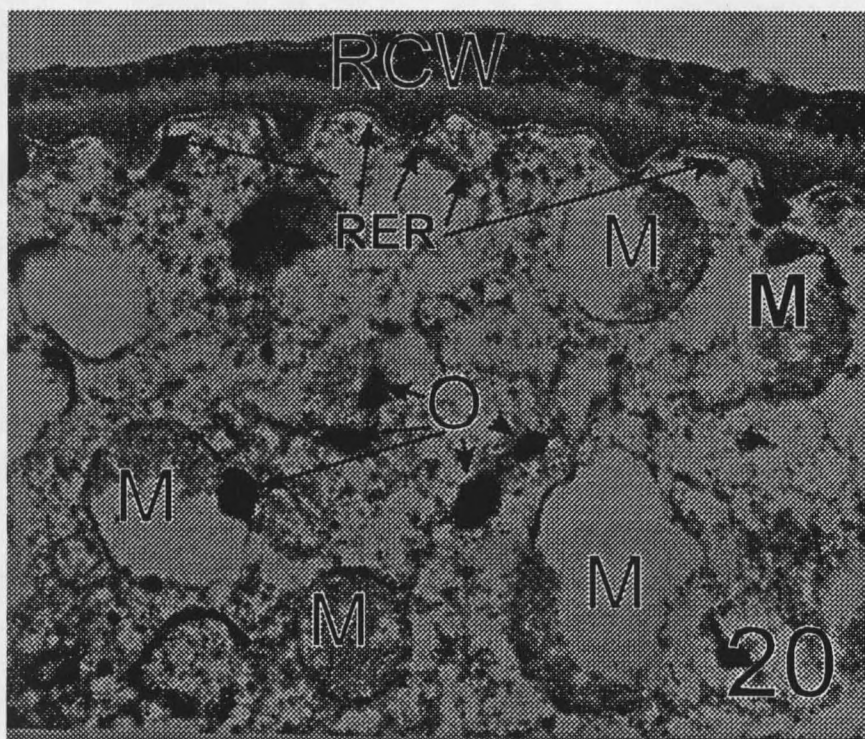


Figure 20: Enlargement of top of Figure 16 (93,000 x). Osmiophilic material is present in mitochondria and in the rough endoplasmic reticulum. It is transported into the cell wall by fusion between the plasma membrane and the rough endoplasmic reticulum. M = mitochondrion, O = oily substance, RCW = reticulated cell wall, RER = rough endoplasmic reticulum, \* = volatiles.



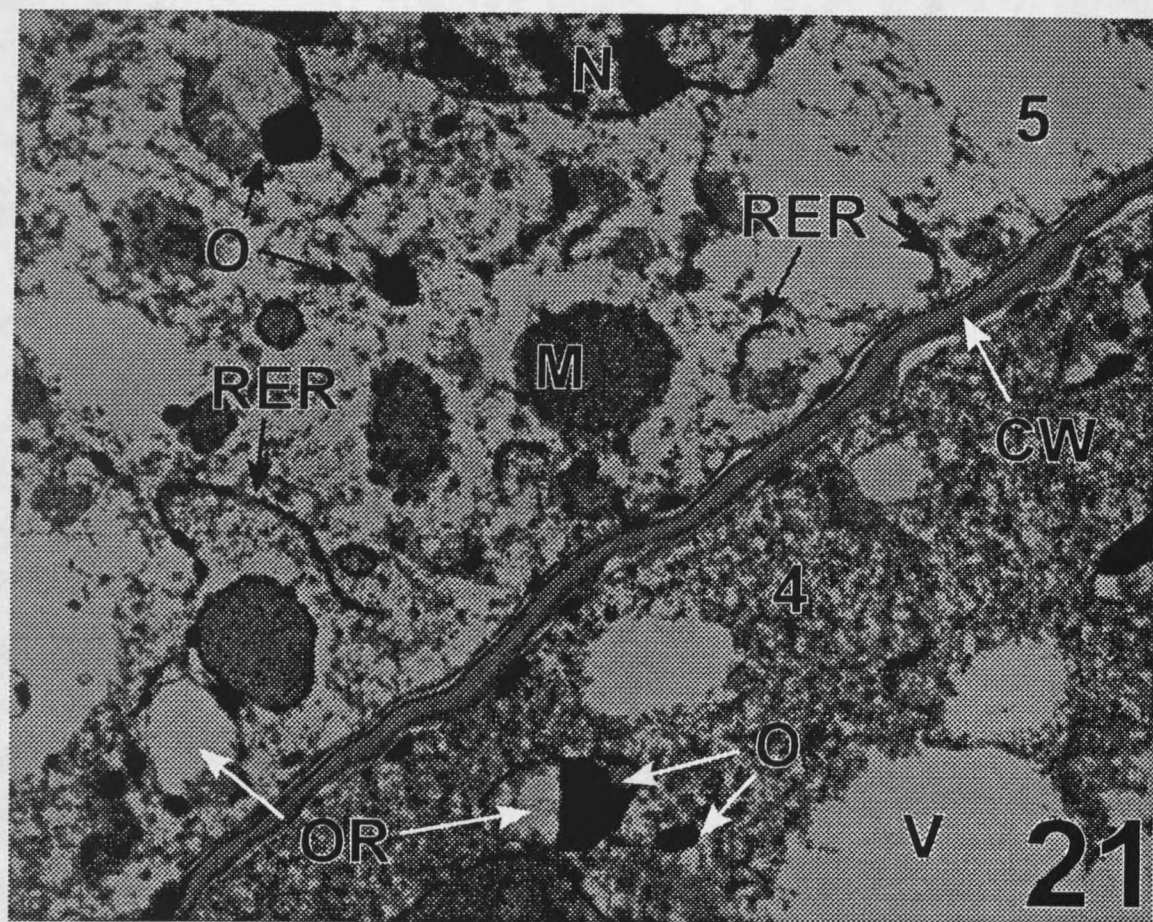


Figure 21: Enlargement of part of Figure 16 (93,000 x). Osmiophilic materials are shown between the plasma membrane and the cell wall of adjacent cells. The black spot is also observed inside the organelle. CW = cell wall, M = mitochondrion, N = nucleus, O = oily substance, OR = organelle, PM = plasma membrane, RER = rough endoplasmic reticulum, V = vacuole, 4 = second pair of secretory cells, 5 = topmost secretion cells.

My experience with trichomes of the persistent leaves was that they were easily shed. One enlarged stalk cell showed an intercellular space on its left side perhaps contributing to its susceptibility to breaking off the epidermis (Figure 34). The instability of these glands was probably due to the disproportion between the stalk cell and the

support cell, and was the probable explanation for the breaking off observed. The enlargement of one of the topmost cells showed a reticulated cell wall, which suggested that the secreting cell might be transporting volatiles into the sub-cuticular space (Figure 36).

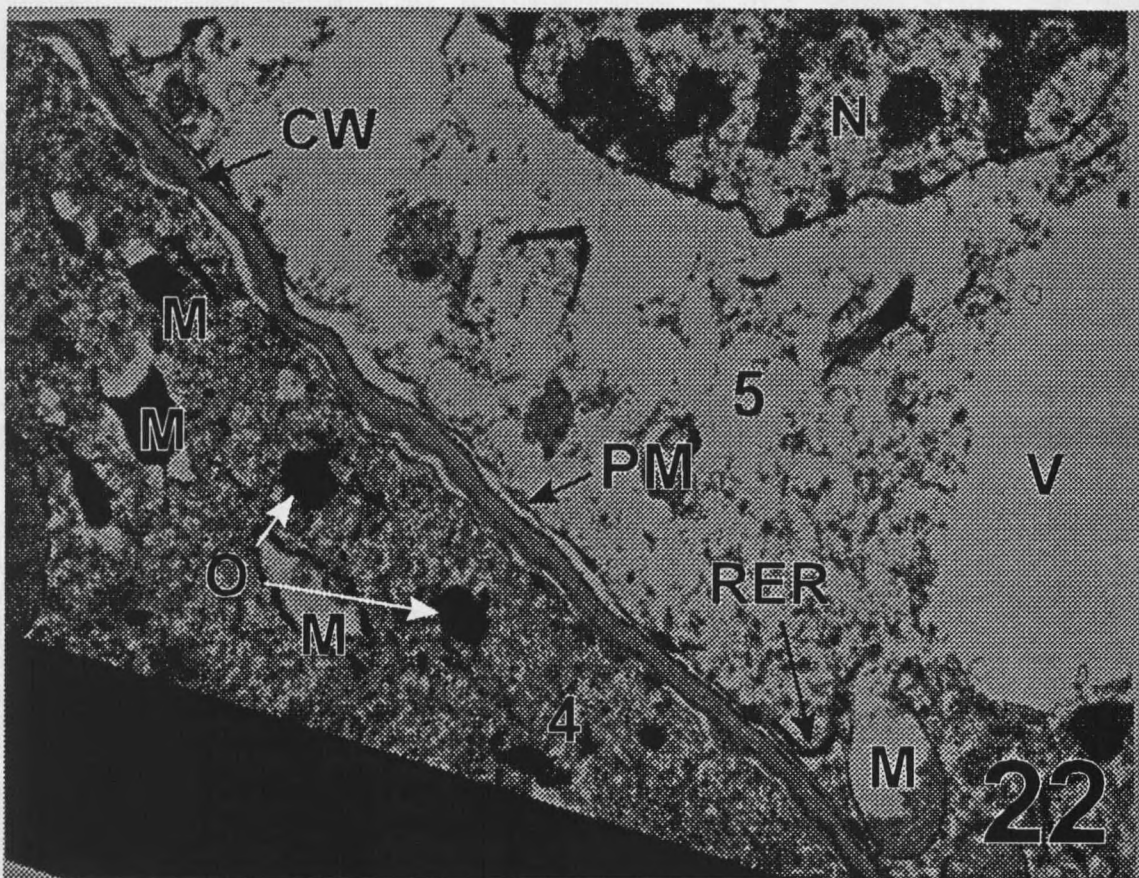


Figure 22: Enlargement of part of Figure 16 (93,000 x). Osmiophilic material can be seen within the cell wall and in the mitochondria. CW = cell wall, M = mitochondrion, N = nucleus, O = oily substance, OR = organelle, PM = plasma membrane, RER = rough endoplasmic reticulum, V = vacuole, 4 = second pair of secretory cells, 5 = topmost secretory cells.



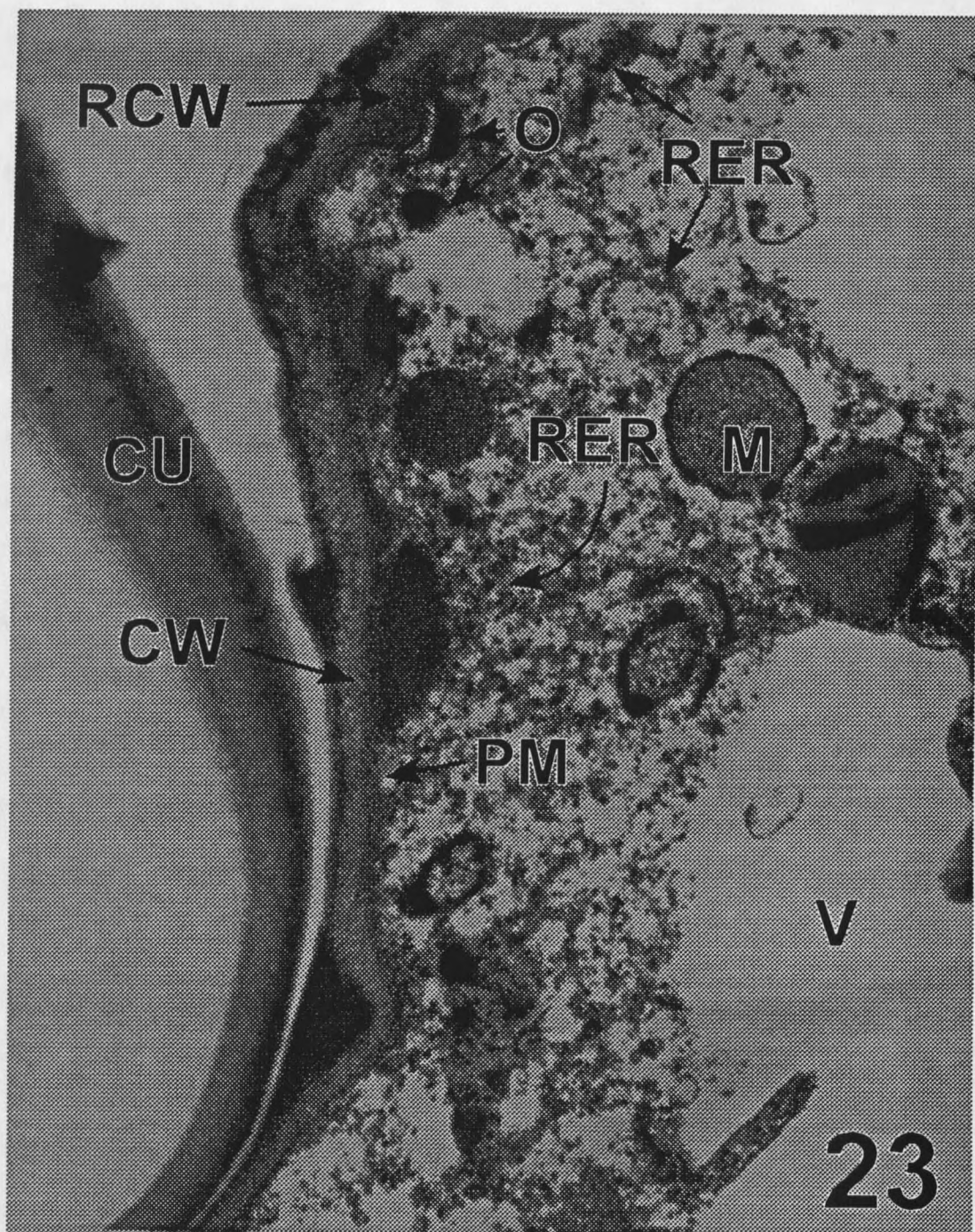


Figure 23: Enlargement of part of Figure 16 (93,000 x). Oily droplets are present adjacent to the plasma membrane and within the cell wall. CU = cuticle, CW = cell wall, M = mitochondrion, O = oily substance, PM = plasma membrane, RCW = reticulated cell wall, RER = rough endoplasmic reticulum, V = vacuole.

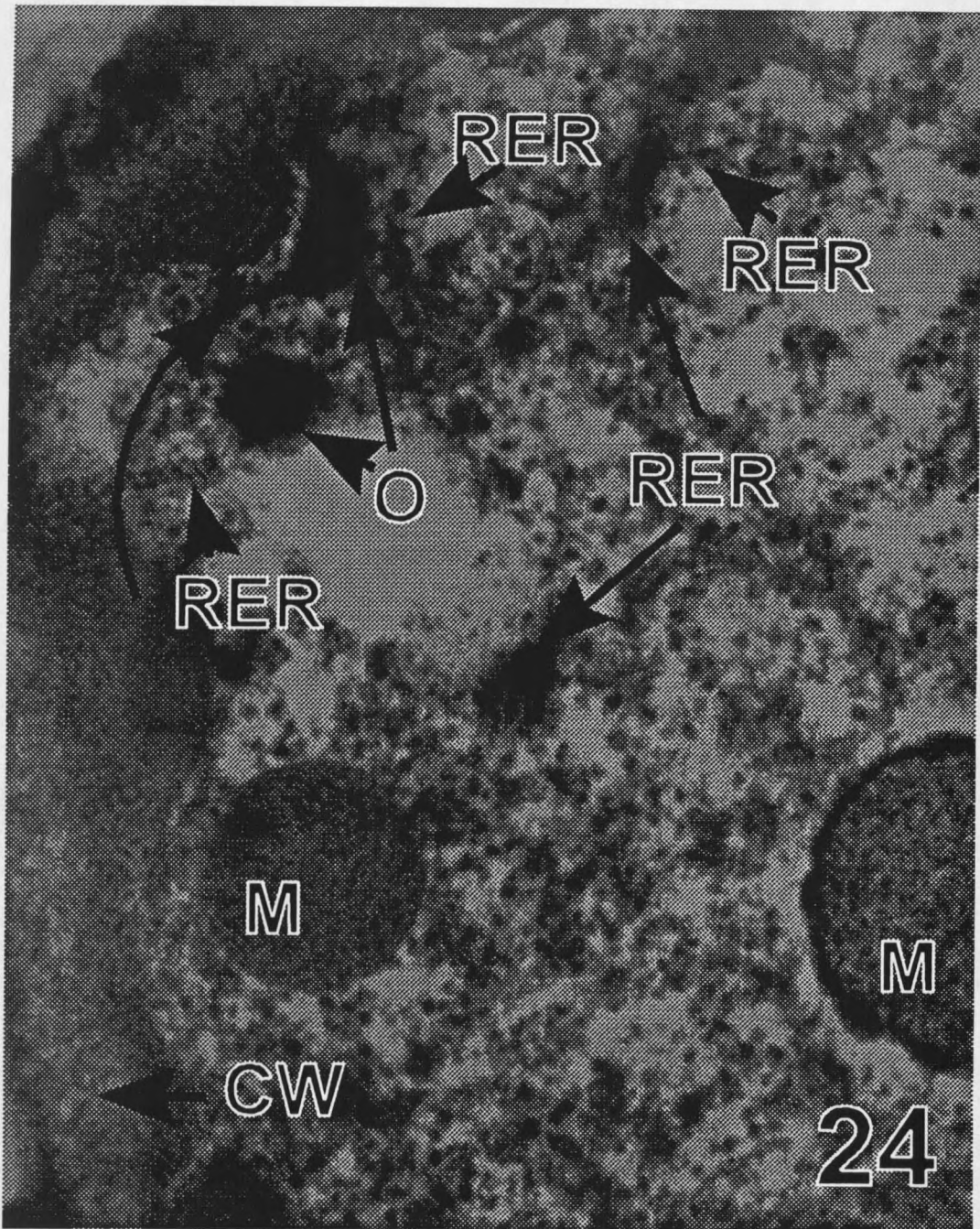


Figure 24: Enlargement of part of Figure 16 (93,000 x). Rough endoplasmic reticulum contains dark spots and is apparently transporting osmiophilic material. CW = cell wall, M = mitochondrion, O = oily substance, RER = rough endoplasmic reticulum.



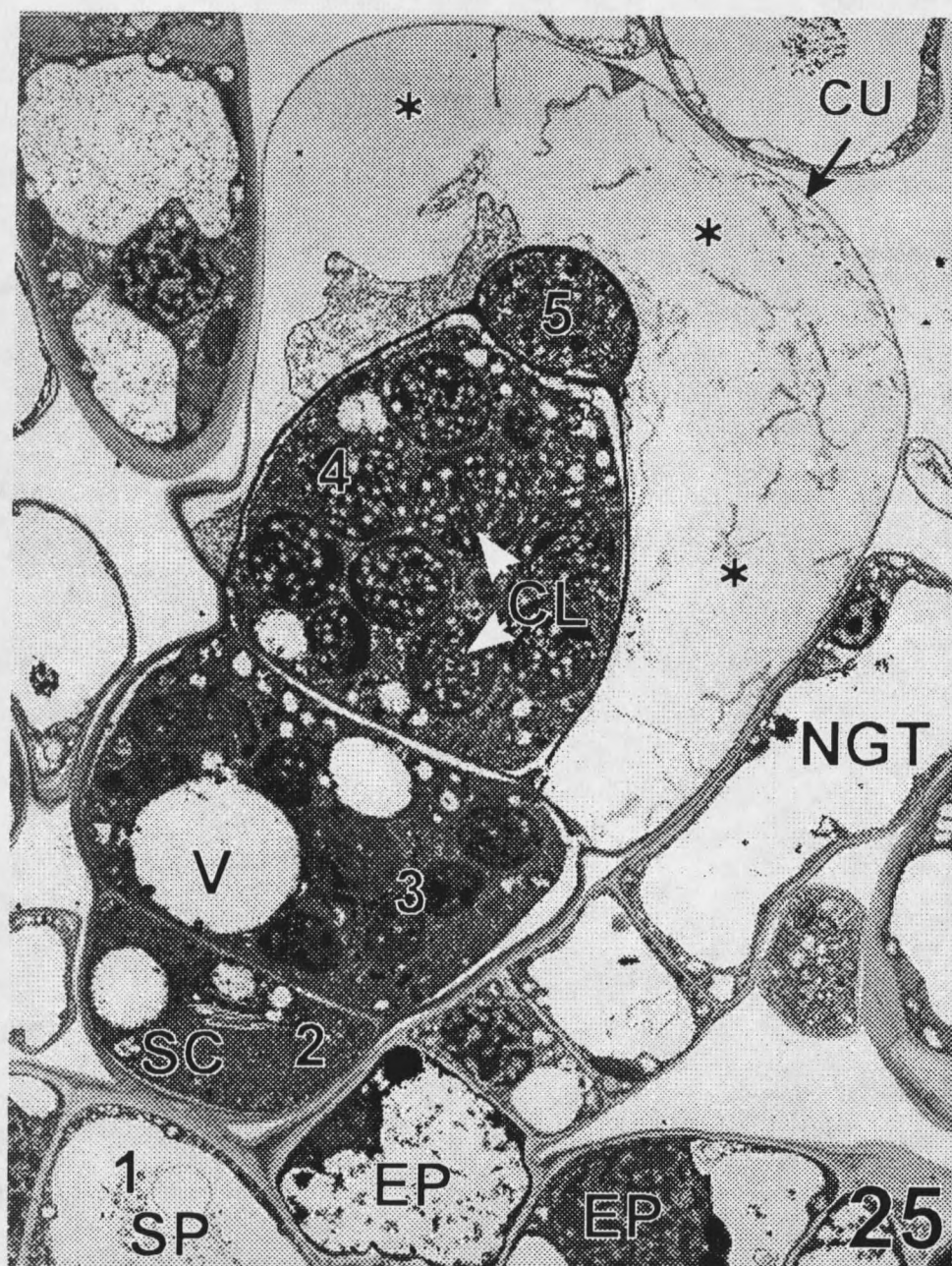


Figure 25: TEM of a mature ten-celled gland developing secretory cell. Side view (the second set of cells were out of the cutting plane) (3750 x). The cuticle becomes detached to form the sub-cuticular space containing the volatiles (\*). CL = chloroplast, CU = cuticle, EP = epidermal cell, NGT = non glandular trichome, SC = stalk cell, SP = support cell, V = vacuole, 1 = first pair of support cells, 2 = second pair of stalk cells, 3 = first pair of secretory cells, 4 = second pair of secretory cells, 5 = uppermost pair of secretory cells.



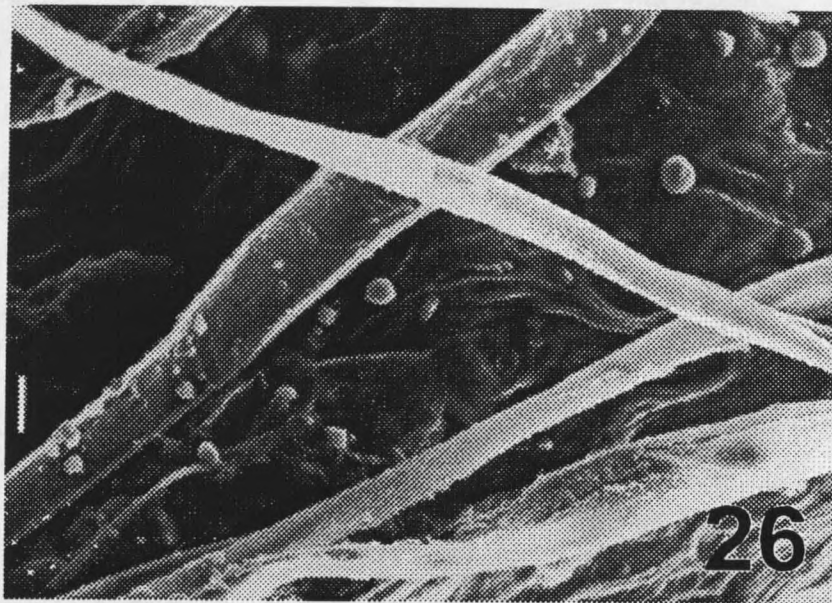


Figure 26: SEM of numerous round glandular trichomes with large filamentous trichomes from persistent leaves of *Artemisia tridentata vaseyana* (800 x).

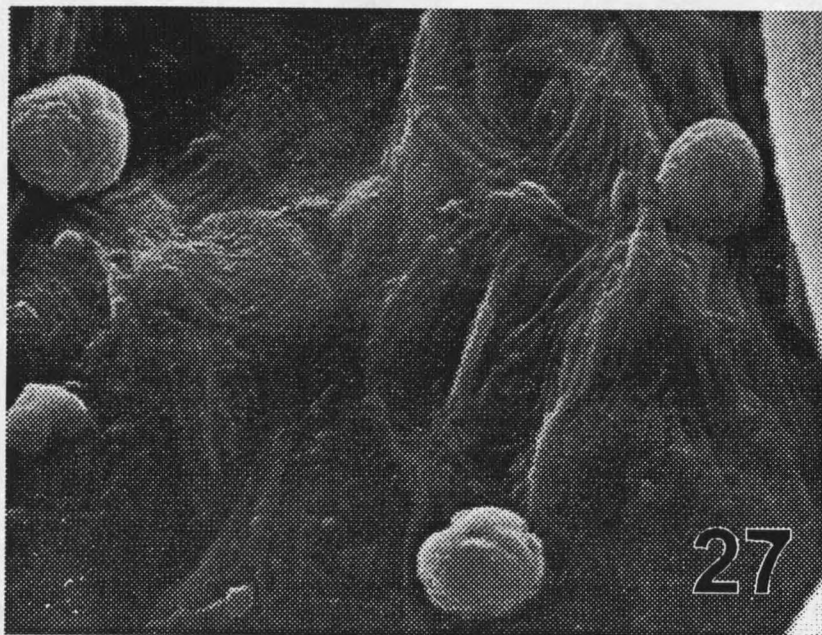


Figure 27: SEM of round-shaped glands from persistent leaves of *Artemisia tridentata vaseyana* showing cluster of cells (1,600x).

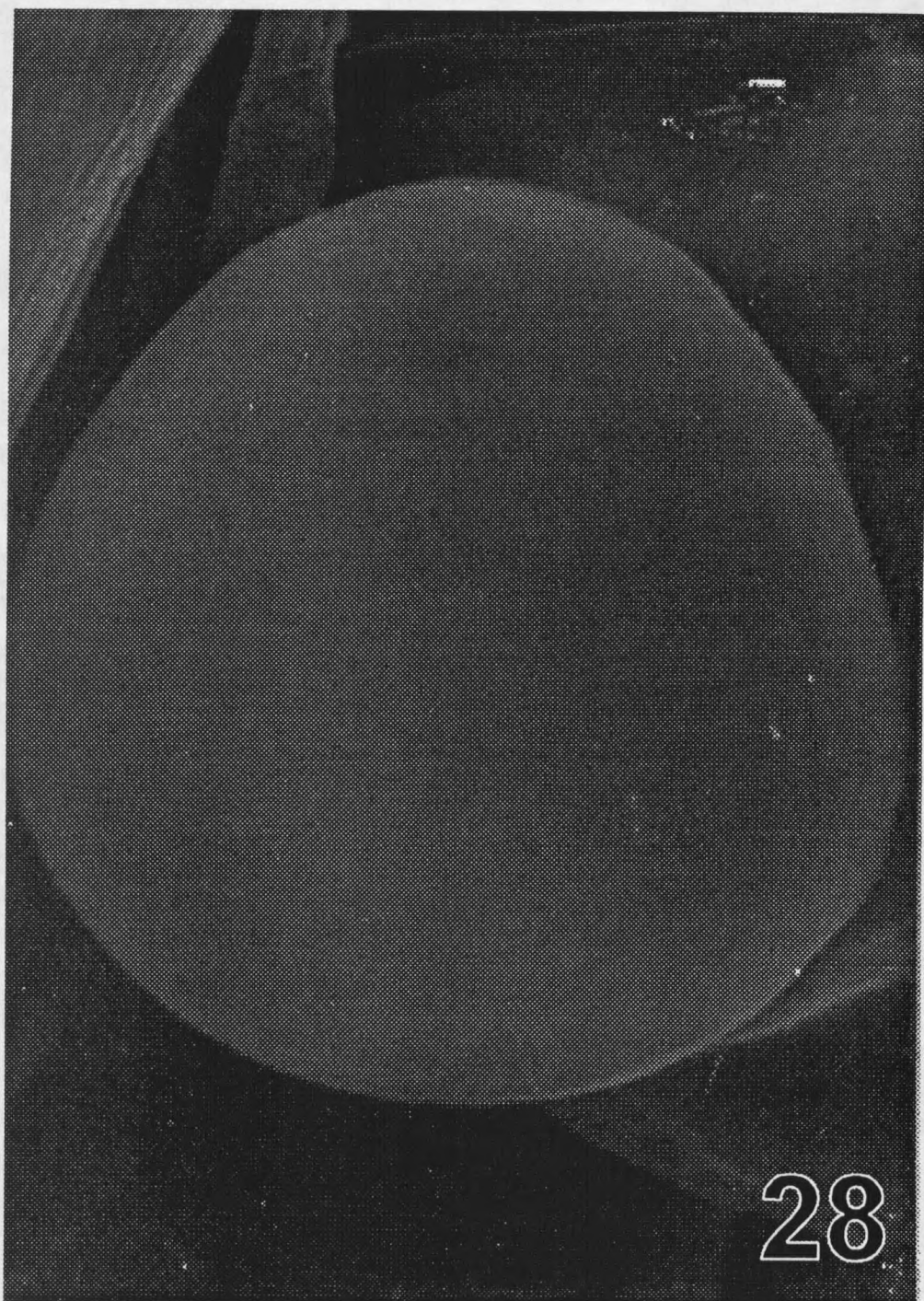


Figure 28: SEM of smooth round gland from persistent leaves of *Artemisia tridentata vaseyana* probably filled with secretion (3,800 x).





Figure 29: SEM of cover trichome and shed immature and mature glands from persistent leaves of *Artemisia tridentata vaseyana* (3,600 x). The peltate gland of persistent leaves has a long peduncle (white arrow).

The dark spots observed in the thylakoid membranes of the plastids were probably terpenoid compounds secreted by plastidal thylakoid membranes (Figures 37 and 38).

These compounds were presumably transported from the secretion site to the adjacent cell

wall through peripheral endoplasmic reticulum (Figure 38). These compounds presumably crossed the cell wall to accumulate in the topmost cells of the trichome (Figure 38).

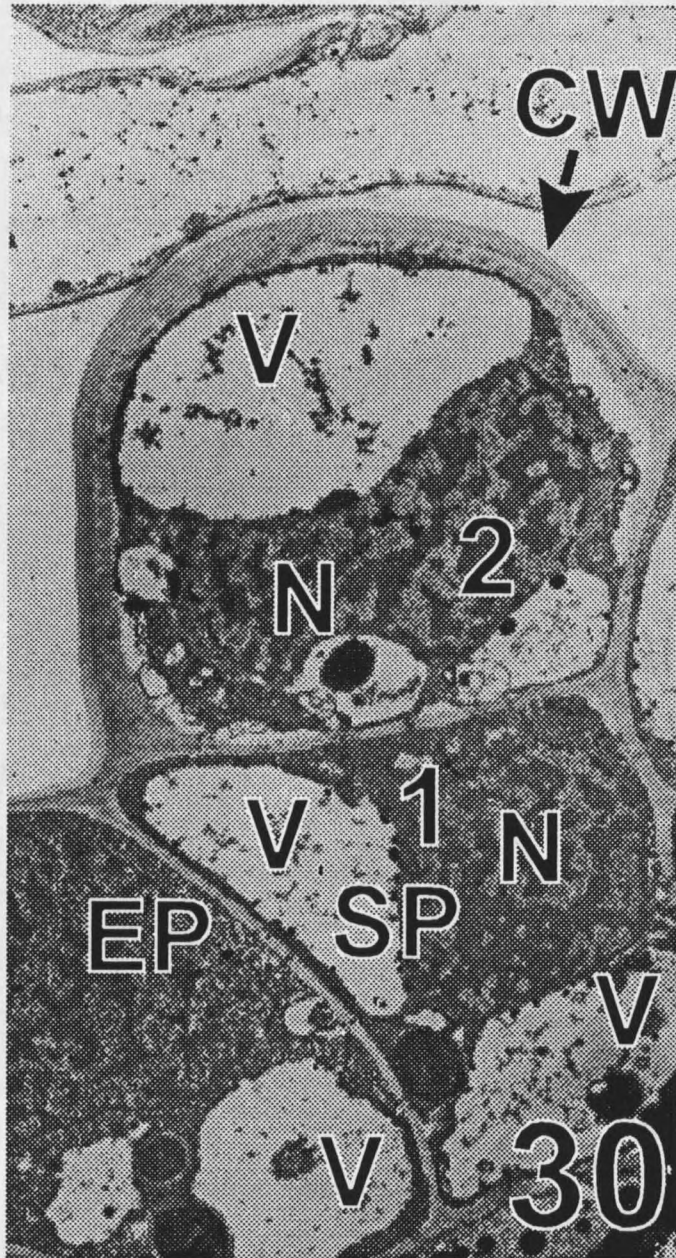


Figure 30: TEM of one-celled stage of peltate gland from persistent leaves of *Artemisia tridentata vaseyana* (12,000 x). CW = cell wall, EP = epidermal cell, N = nucleus, SP = support cell, V = vacuole, 1 = support cell, 2 = stalk cell.



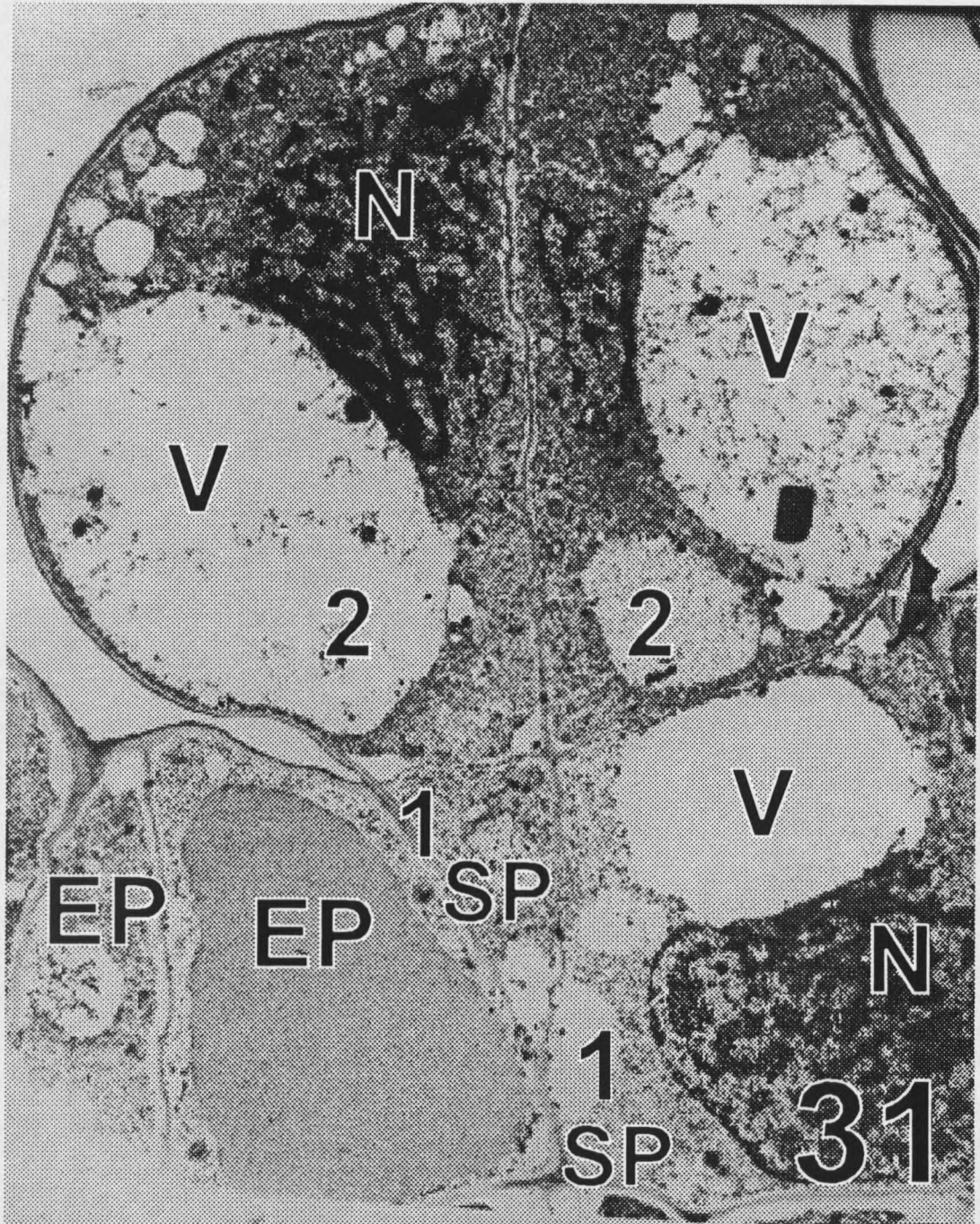


Figure 31: TEM of four-celled stage of peltate gland from persistent leaves of *Artemisia tridentata vaseyana* (12,000 x). Periclinal and anticlinal divisions have occurred, showing a pair of gland cells and an uneven view of a pair of support cells. EP = epidermal cell, N = nucleus, SP = support cell, V = vacuole, 1 = support cell, 2 = stalk cell.

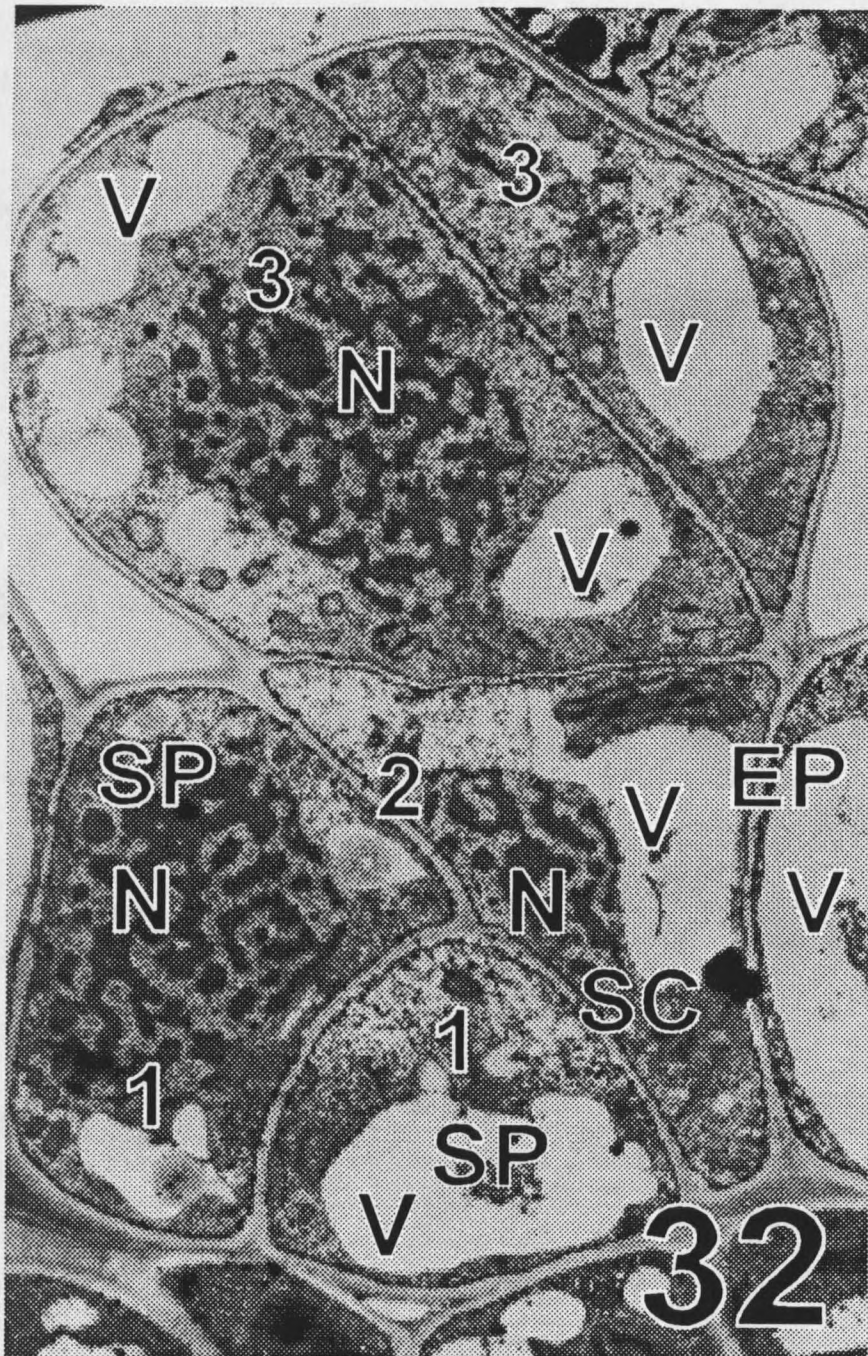


Figure 32: TEM of immature stages of peltate glands from persistent leaves of *Artemisia tridentata vaseyana*. The wider of the support pair of cells has undergone mitosis generating a pedunculate cell (12,000 x). EP = epidermal cell, N = nucleus, SC = stalk cell, SP = support cell, V = vacuole, 1 = support cell, 2 = stalk cell, 3 = gland cell.



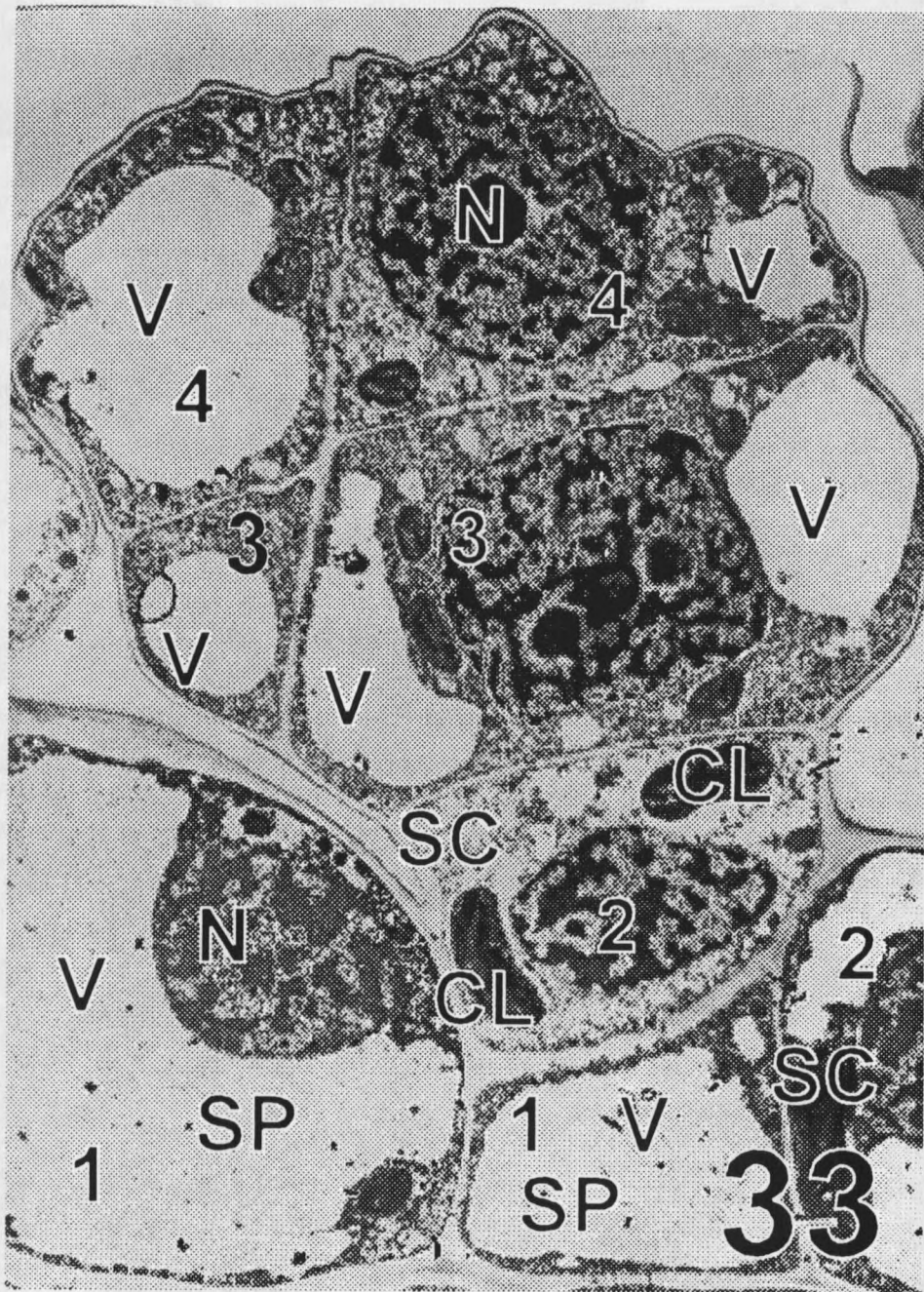


Figure 33: TEM of eight-celled stage of peltate gland from persistent leaves of *Artemisia tridentata vaseyana* (12,000 x). The gland consists of two pairs of gland cells, one pair of support cells, and one pair of stalk cells. The pedunculate cell of Figure 32 divides into two, and becomes wider at its base, generating a very unstable gland. CL = chloroplast, N = nucleus, SC = stalk cell, SP = support cell, V = vacuole, 1 = support cell, 2 = stalk cell, 3 = gland cell, 4 = gland cell.

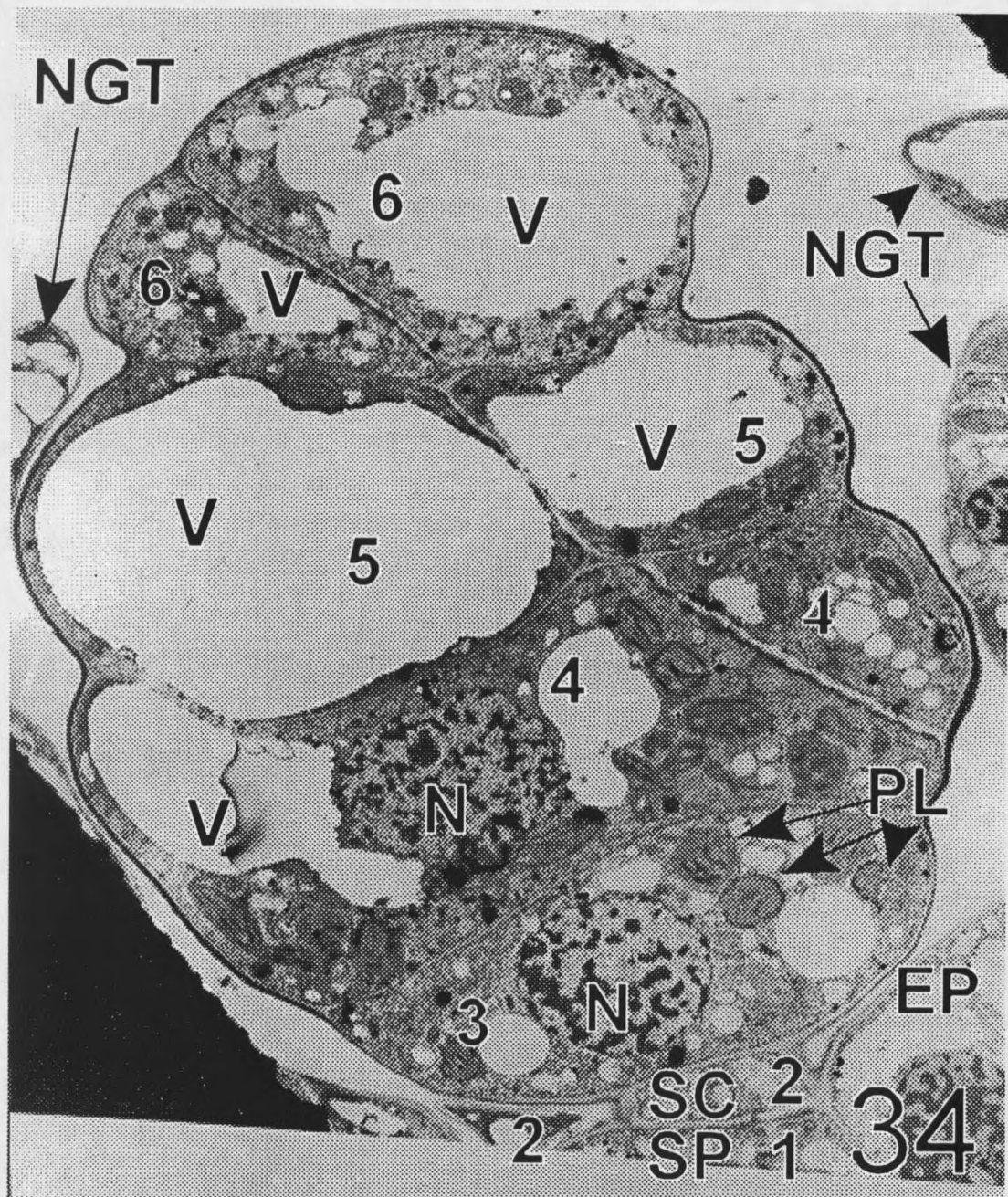


Figure 34: TEM of mature twelve-celled peltate gland from a persistent leaf of *Artemisia tridentata vaseyana* (8,750 x). EP = epidermal cell, N = nucleus, NGT = non glandular trichome, PL = plastids, SC = stalk cell, SP = support cell, V = vacuole, 1 = pair of support cells, 2 = pair of stalk cells, 3 = first pair of secreting cells, 4 = second pair of secreting cells, 5 = third pair of secreting cells, 6 = the topmost pair of secreting cells.



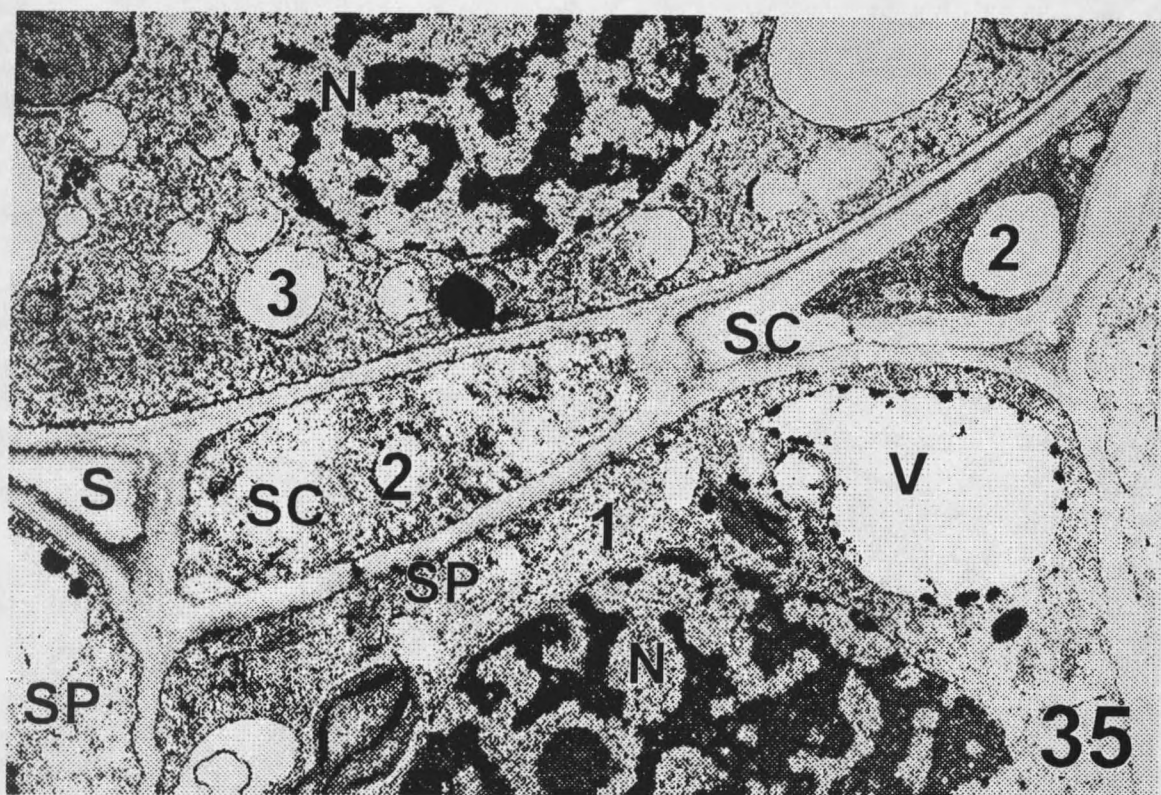


Figure 35: TEM of mature peltate gland from persistent leaf of *Artemisia tridentata vaseyana* (17,500 x). Basal part of the twelve-celled trichome from Figure 34. N = nucleus, S = space SC = stalk cell, SP = support cell, V = vacuole, 1 = pair of support cells, 2 = pair of stalk cells, 3 = first pair of secreting cells.

The compounds were presumably transported from the secretion site to the adjacent cell wall associated with peripheral smooth endoplasmic reticulum (Figure 37) and were presumably transported by smooth endoplasmic reticulum into the topmost cells of the mature trichome (Figure 37). Several organelles labeled OR (Figures 37-38) are not clearly mitochondria or vesicles but frequently have small lipid bodies around their periphery. The lipid bodies may be the terpenoids moving from cell to cell. Organelles were aligned along or were surrounded by smooth endoplasmic reticulum and I assumed

that these organelles participated in the production of terpenoid compounds because dark substances were observed outside these organelles (Figure 39).

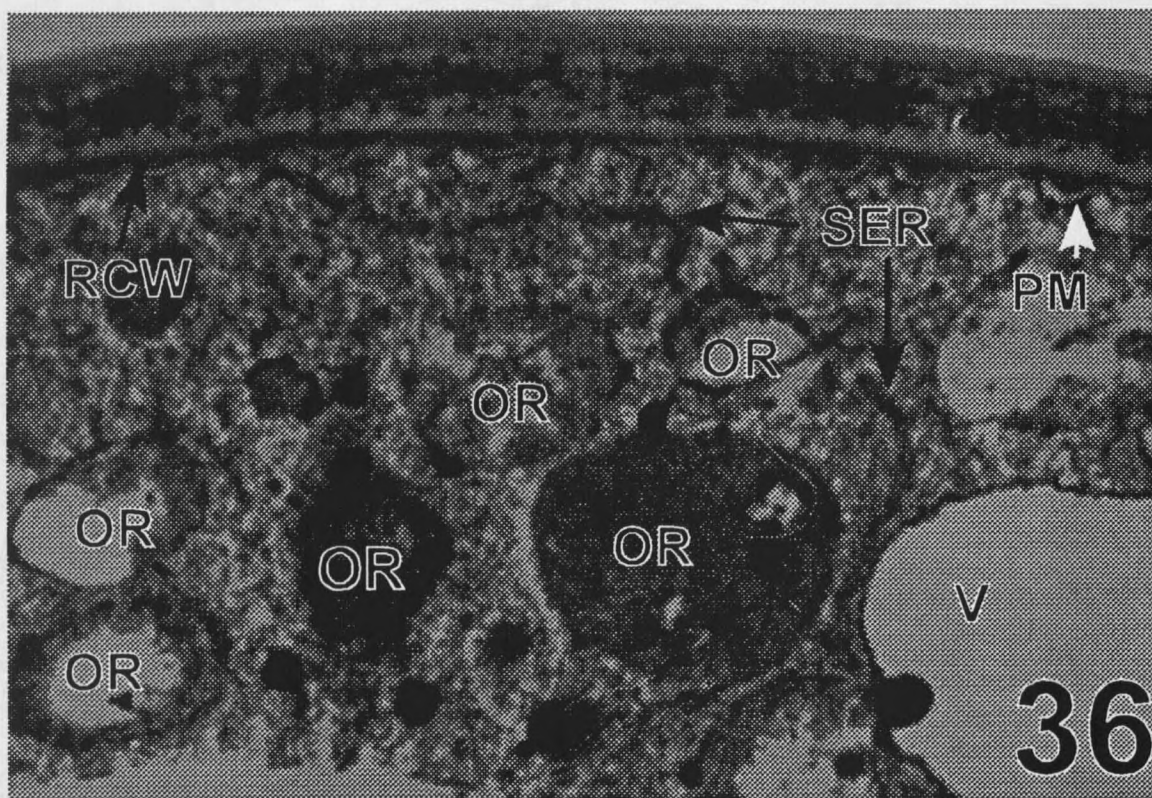


Figure 36: Enlargement of the right top cell of the twelve-celled trichome of Figure 34 (17,500 x). The endoplasmic reticulum appears to be transporting osmiophilic material from the secreting cell to the adjacent cell wall. OR = organelle, PM = plasma membrane, SER = smooth endoplasmic reticulum, V = vacuole.

A side view of the mature gland (Figure 40) showed six aligned cells with a slower or lesser development of the sub-cuticular space compared to the mature trichome of the ephemeral leaves. Terpenoids in the sub-cuticular space were probably a mixture of non-volatile and volatile (Figure 41).



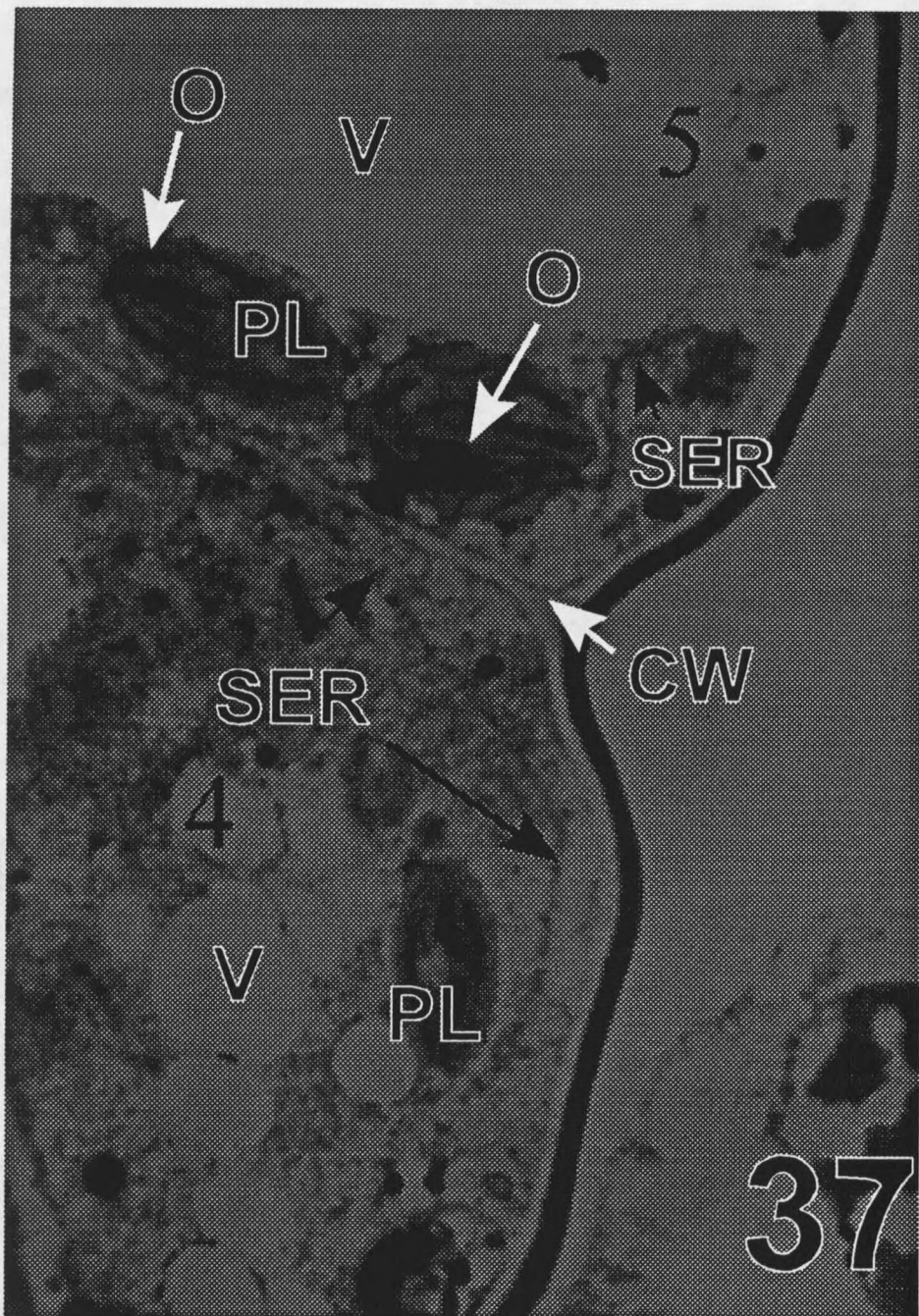


Figure 37: Enlargement of part of Figure 34 (31,850 x). CW = cell wall, O = oily substance, PL = plastid, SER = smooth endoplasmic reticulum, V = vacuole, 4 = second pair of secreting cells, 5 = third pair of secreting cells.

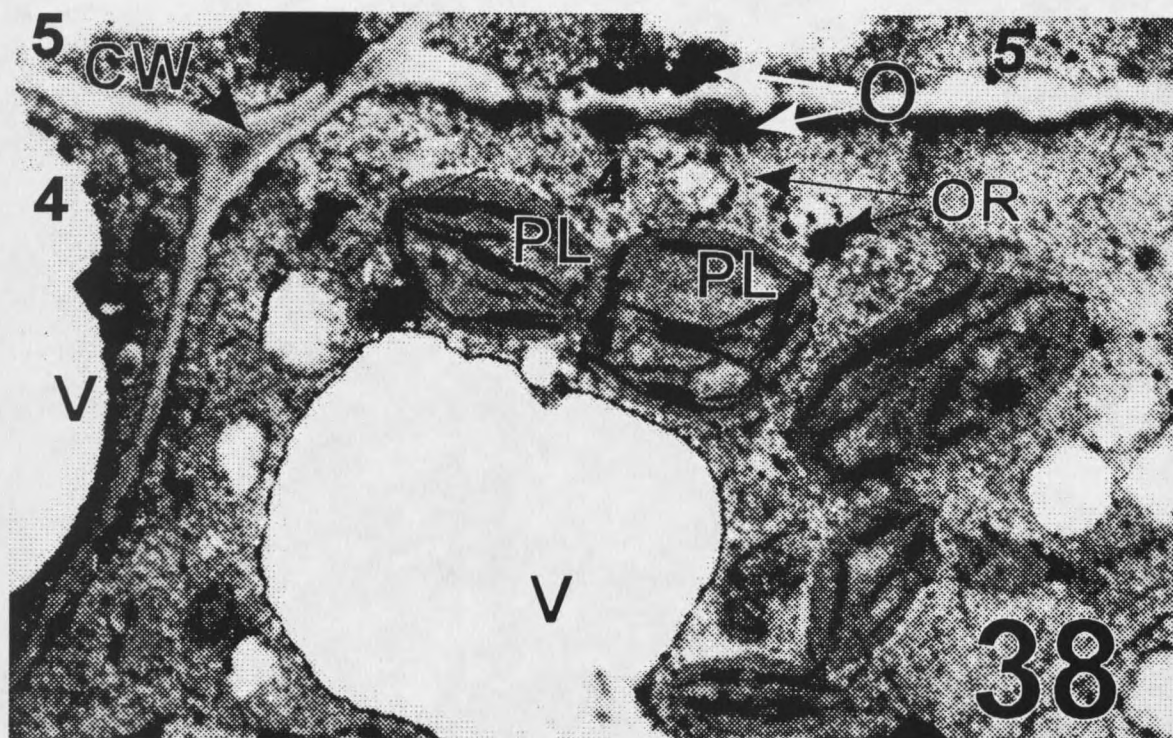


Figure 38: Enlargement of part of Figure 34 (31,850 x). Dark substances are apparent in the cell wall. CW = cell wall, O = oily substance, OR = organelle, PL = plastid, V = vacuole, 4 = second pair of secreting cells, 5 = third pair of secreting cells.

#### Comparisons Between Peltate Trichomes from Ephemeral and Persistent Leaves

The twelve-celled glandular trichome of the persistent leaves was physiologically different from the ten-celled trichome of the ephemeral leaves because: 1) dark spots considered as terpenoids were presumably produced by plastids (with few thylakoid membranes) in the twelve-celled trichome, but were secreted by chloroplasts in the ten-celled trichome; 2) terpenoids were presumably transported into the topmost cells of each type of trichome through the smooth endoplasmic reticulum in the twelve-celled trichome and was presumably transported by rough endoplasmic reticulum in the ten-celled



trichome (Figure 23); 3) twelve-celled trichomes of the persistent leaves presumably produced volatile and non-volatile terpenoids (Figure 41) while ten-celled trichomes presumably produced volatile in the sub-cuticular space (Figures 11, 16 and 25).

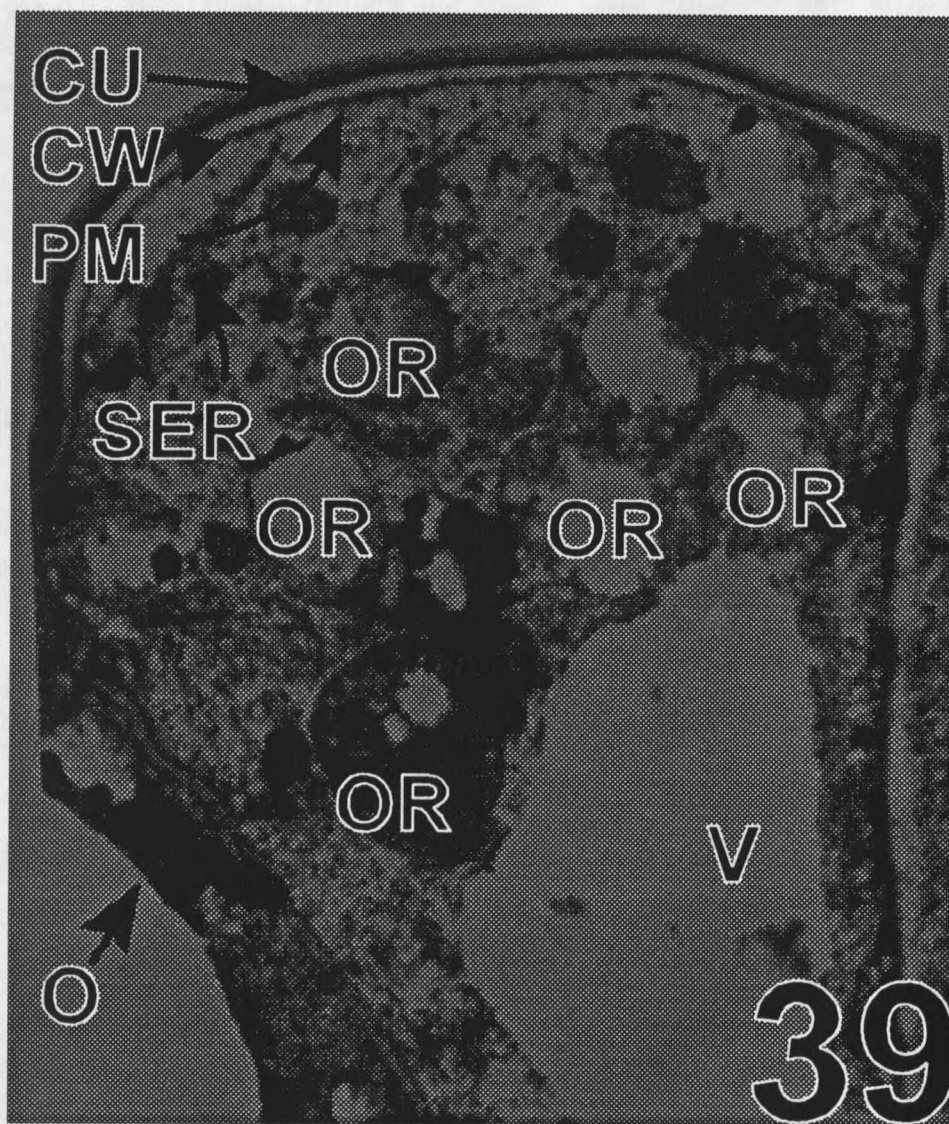


Figure 39: Enlargement of part of the topmost cell of Figure 34 (31,850 x). Dark substances are presumably being transported from the topmost cell into the sub-cuticular space by the smooth endoplasmic reticulum tubule. CU = cuticle, CW = cell wall, O = oily substance, OR = organelle, PM = plasma membrane, SER = smooth endoplasmic reticulum, V = vacuole.

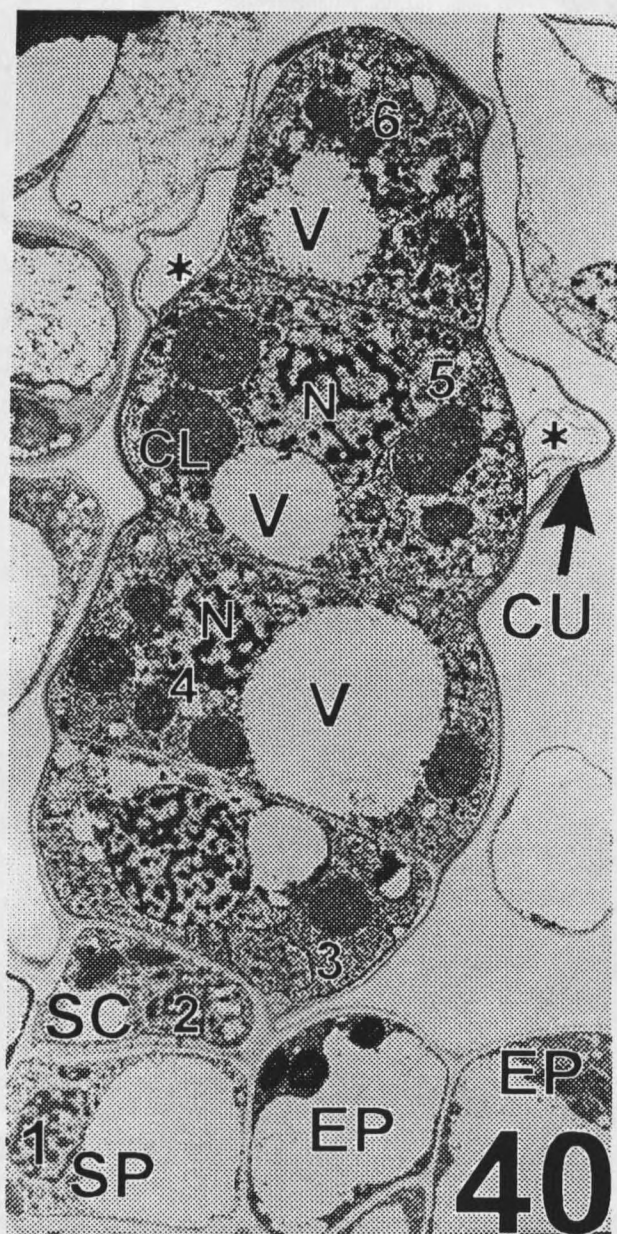


Figure 40: TEM of 12-celled peltate trichome of persistent leaves of *Artemisia tridentata vaseyana* (8.750 x). Side view outside the front plane. Uniseriate six-cell, with support cells sturdier than the pair of stalk cells. CL = chloroplast, CU = cuticle, EP = epidermal cell, N = nucleus, SC = stalk cell, SP = support cell, V = vacuole, \* = volatile, 1 = pair of support cells, 2 = pair of stalk cells, 3 = first pair of secretory cells, 4 = second pair of secretory cells, 5 = uppermost pair of secretory cells, 6 = pair of excretory cells.



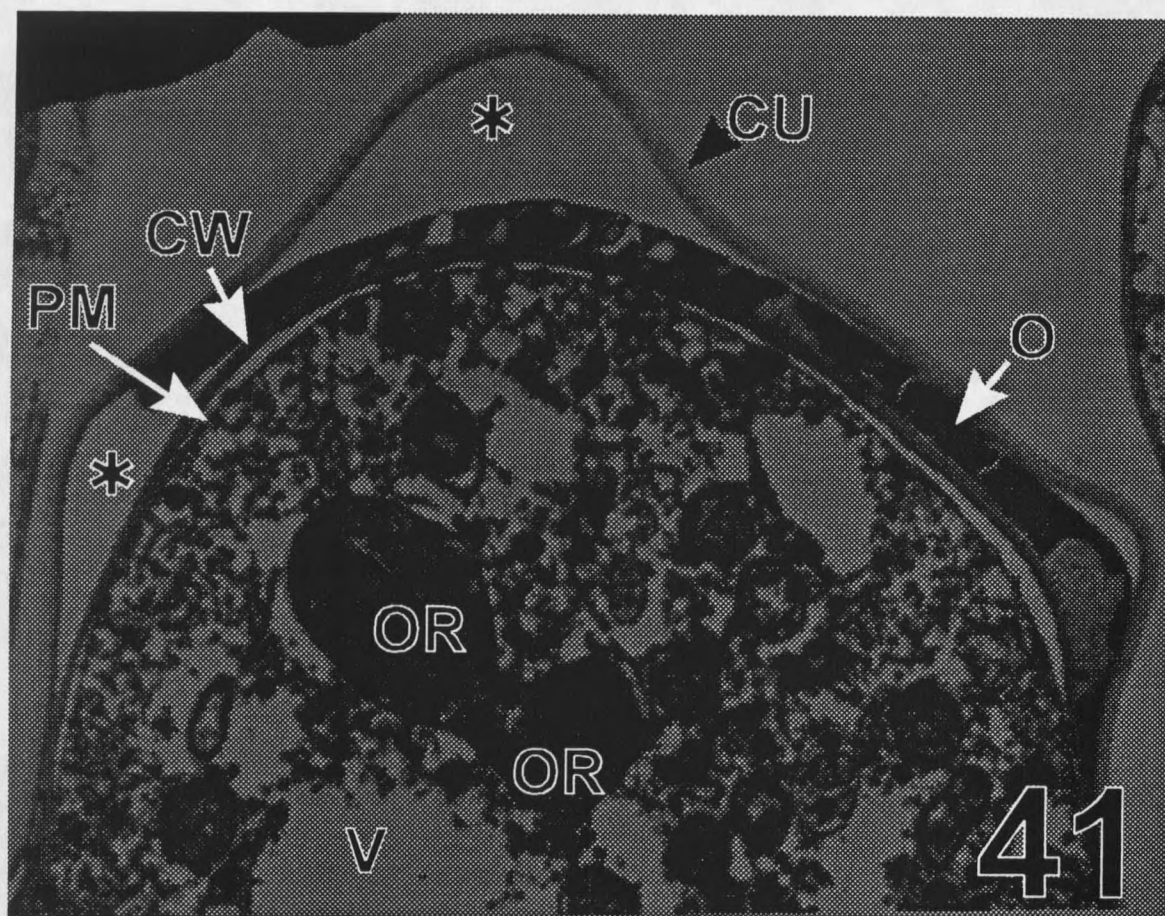


Figure 41: Enlarged part of the topmost cell of Figure 40 (17,500 x). The presence of oily substances is apparent outside the cell wall. CU = cuticle, CW = cell wall, O = oily substance, OR = organelle, PM = plasma membrane, V = vacuole, \* = volatile.

### Discussion

#### Types of Trichomes Found on the Leaves of *Artemisia tridentata vaseyana*

*A. t. vaseyana* possesses three types of trichomes on its leaves. The ephemeral summer leaves and the persistent small leaves have uniseriate two-armed stalked cover trichomes which cover the leaves and two types of peltate glandular trichomes: oblong-

shaped ten-celled trichomes on the ephemeral leaves; and smaller round-shaped twelve-celled trichomes on the persistent leaves.

#### Peltate Glandular Trichomes of Ephemeral Leaves

The glandular trichomes from the ephemeral leaf of *A. t. vaseyana* were similar to those described on the herbaceous annual plants of *Artemisia* from the Old World (Ascensão and Pais, 1982; Cappelletti *et al.*, 1986; Duke and Paul, 1993). The ten-celled trichome of *A. t. vaseyana* is oblong-shaped as has also been reported for the annual wormwood *A. annua* (Slone and Kelsey, 1983; Duke and Paul, 1993), *Artemisia campestris* ssp. *maritima* (Ascensão and Pais, 1987), and *Artemisia umbelliformis* Lam. (Cappelletti *et al.*, 1986). The ten-celled trichome of the ephemeral leaves is not easily shed which was perhaps related to the broader base of the support cell (Figure 11).

The two-celled pair above the stalk cell are also secreting cells of the ten-celled glandular trichome and contained numerous chloroplasts (Figures 11-12). There is evidence that chloroplast thylakoid membranes are involved in the production of gland terpenoids (Nielson *et al.*, 1991). The ultrastructure of trichome glands from the mutant, glandular but non-secreting tobacco T.I. 1406 is similar to that of secreting types except for the absence of normal chloroplasts (Nielson *et al.*, 1991). Interestingly, transfer of a single gene via plant breeding restores secretion and normal chloroplasts in this mutant. Chloroplasts of the gland cells of tobacco were highly involved in converting photosynthate into terpenoids. The transport system from the secretion site to the cell wall was presumably done by the rough endoplasmic reticulum. Terpenoids later reached the



topmost cells from where they could be transferred into the sub-cuticular space presumably by rough endoplasmic reticulum fusing with the plasma membrane (Figures 21-22).

Osmiophilic materials are also produced by the chloroplasts of the glandular trichomes of *A. annua* but the authors could not distinguish within cell or between cells movement of the terpenoids produced in the chloroplasts (Duke and Paul, 1993). The oily substances in this study are accumulated between the cell wall and the cuticle on the side part of the topmost cells, and seem to force the cuticle from the cell wall allowing the development of the sub-cuticular space (Figures 16-17). Terpenoids crossing the reticulate top part of the topmost cells were presumably deposited by the rough endoplasmic reticulum and seem to appear as volatile in the sub-cuticular bubble (Figures 11, 16 and 25). The transformation process that occurs when lipophilic terpenoids are transformed into volatile just by crossing the cell wall is not clear. Unzelman and Healey (1974) suggested that lipophilic secretions could be transported through the cell to the cell wall by the continuity of the cytoplasmic membrane system with the plasma membrane; this was observed in the ten-celled trichome. The inner sides of the cell wall of the apical cell pair of the ten-celled gland are reticulated (Figures 16, 19 and 20). Vermeer and Peterson (1979) suggested that such wall ingrowths were transfer cells and I think they were meant to transfer the secreted products into the head cell between the cuticular space and the cell wall. Cell wall reticulations are characteristic of a number of secretory structures (Gunning, 1977). The same reticulated secretory cell walls of the apical cell pair were found in *A. annua* glandular trichomes (Duke and Paul, 1993). This reticulated cell wall is already present in the last immature stage of the ten-celled glandular trichomes of *A. t. vaseyana* ephemeral leaves (Figure 10)

suggesting that this trichome is ready for a quick transfer of the terpenoids produced due to the short life span of the mature leaves.

#### Peltate Glandular Trichomes of Persistent Leaves

The twelve-cell trichome of the persistent leaves possesses one pair of support cells, one pair of stalk cells, three pairs of secretory cells containing plastids (partially surrounded by smooth endoplasmic reticulum) with osmiophilic material, and one pair of secretory cells with mitochondria and no plastids (Figures 34, 37 and 38). Plastids have been designated as sites of lipid synthesis in several secretory cells of *Chrysanthemum* and in *Poncirus* fruits (Heinrich, 1970; Vermeer and Peterson, 1979). Smooth endoplasmic reticulum also occurs in the cytoplasm of the twelve-celled trichome. In addition to the plastids and the endoplasmic reticulum, osmiophilic material has also been observed attached to organelles or vesicles (Figures 36 and 39). Fahn and Evert (1974) observed the smooth endoplasmic reticulum transporting osmiophilic material when studying the ultrastructure of secretory ducts of *Rhus glabra* L. Similar ultrastructure was observed in *Pinus halepensis* L. (Fahn and Benayoun, 1976). The endoplasmic reticulum, in addition to taking part in the synthesis of lipophilic substances, may also take part in intracellular transport of this material (Fahn and Benayoun, 1976). Terpenoids produced are presumably transported in the uppermost pair of cells and to the subcuticular space by smooth endoplasmic reticulum (Figures 37, 38 and 39). The smooth endoplasmic reticulum presumably fused with the plasma membrane, delivering the secreted product into the sub-cuticular space (Figures 35 and 39). Fusion of membranes of the endoplasmic reticulum, which carries oil droplets,

with the plasmalemma has also been suggested by Bosabalidis and Tsekos (1982) for the secretory cells of the essential-oil cavities of *Citrus deliciosa* Tenore, and in the osmophores of the orchid (Pridgeon and Stern, 1983). The substance present in the developing sub-cuticular space is a mixture of volatile and non-volatile terpenoids (Figure 30). The fourth and the fifth pair of cells of the twelve-celled trichome are highly vacuolated (Figure 26). Duke and Paul (1993) reported similar vacuolated gland cells in *A. annua*.

The sagebrush plant in a quiescent stage in winter (Depuit and Cadwell, 1973) has the lowest stock of endogenous photosynthate. The plant cannot allocate energy toward the catabolism of terpenoids from the glandular trichomes in winter time. No plants evolve to keep unnecessary organs (Gershenson, 1994). The mountain big sagebrush has therefore developed a twelve-celled trichome that can easily be shed under harsh environmental conditions, such as winter blizzards and storms. The capability of these trichomes to fall off was detected when I was working on the persistent leaves (Figure 29). The plant is released from the burden of sustaining the trichomes after they have finished their task of protecting the plant during the period of seed production (September to November). The sub-cuticular space of the twelve-celled trichome has a mixture of volatile and non-volatile terpenoids (Figure 41). I suggest that lower monoterpenoid content in the rumen of sagebrush-feeding mammals is perhaps due to the shedding of the twelve-cell glandular trichomes from the persistent leaves in winter storms and during browsing which are conditions similar to the processing of the leaves for transmission electron microscopy studies.

### Implications for Pesticide Research

Terpenoids stored in the sub-cuticular space of the ten-celled trichomes are toxic or impalatable volatiles that deter invertebrate herbivory during summer. Seasonally high monoterpenoid levels in the summer growing season, and the availability of alternative food make these leaves less vulnerable to browsing by mammals than during winter. The ten-celled peltate gland of ephemeral summer leaves seems to protect *A. t. vaseyana* from insect herbivorous pests during that period. Of the nine species of insects registered on sagebrush only three were present on sagebrush during summer time. Leaf chewers, such as the beetle *Trirhabda pilosa* Blake Chrysomelidae, the moth *Apterona helix* Siebold, and the phloem-feeding aphid *Obtusicauda coweni* Hunter, are present on sagebrush in summer (Messina *et al.*, 1996). However, all these insects seem to infest only weakened stands (Pringle, 1960). The others are over-wintering case builder flies mostly present on persistent leaves (Messina *et al.*, 1996). Many insects possess metabolic mechanisms for detoxifying toxic compounds and can feed on monoterpene rich plants without ill effects. Specialist insects often employ the terpenoids of their host plants as an aid to host location, or use them as precursors of pheromones (Dickens and Boldt, 1985; Blust and Hopkins, 1987).

Methyl bromide is scheduled to phase out by the year 2010, with a reduction of 50% of the worldwide quantity produced from the year 2005, because of its contribution to the depletion of the ozone layer (Ristaino and Thomas, 1997). This compound originates from the ocean and was used for half a century to control soil-borne pests by vegetable



growers in the USA, and as a post harvest fumigant in high value crops such as dried nuts, flowers and dried grains (Ristaino and Thomas, 1997). There is a pressing need to find alternative chemicals or methods to replace methyl bromide, a business worth \$1.5 billion annually. The different constituents of sagebrush have been tested for their insecticidal, herbicidal, nematocidal, antimicrobial, and antifungal capabilities (Duke *et al.*, 1988). The high level of ready-made volatile terpenoids might make the study of the structural activity of constitutive compounds easier.

The ephemeral leaves of sagebrush contain monoterpene compounds such as eucalyptol (cineole), camphor, eucarvone,  $\alpha$ -pinene,  $\beta$ -pinene and camphene. Cineole deters feeding and oviposition of some species of mosquitos, and has herbicidal activities (Klocke *et al.*, 1987). Camphor or toxaphene, the chlorinated analog, was used as a pesticide, and was at one time the most-used pesticide in the USA (Duke *et al.*, 1988). Eucarvone is similar to 3-carene, a monoterpene which is combined with  $\alpha$ -pinene and  $\beta$ -pinene, to protect the pine tree against bark beetles and their pathogenic fungi. The ephemeral leaves of sagebrush could be used for pest control. The ephemeral leaves do not shed their trichomes, and produce volatile compounds in a very short period of time. The ephemeral leaves are fully mature in June, when grown in the greenhouse, and it is a period where most of their glandular trichomes are filled with volatiles. The ephemeral leaves are a better choice to use as compared to the persistent leaves. The utilization of the ephemeral leaves in a defined period of June of each growing season will limit the variability of terpenoid content often encountered when using the persistent leaves.

## CHAPTER 3

TERPENOIDS PRODUCED BY THE EPHEMERAL LEAVES OF  
*ARTEMESIA TRIDENTATA VASEYANA* AFTER CLIPPING  
AND DEFOLIATION OF ITS BRANCHESIntroduction

Plants have evolved elegant systems to defend themselves against the pathogens and herbivores that attempt to consume them. When leaves of some plants are consumed, the plants manage to render themselves unpalatable or indigestible to herbivores. For example, defensive proteins (protease inhibitors) were demonstrated to be synthesized in tomato and potato leaves throughout the plant a few hours after being chewed by insects (Pena-Cortes *et al.*, 1995). Another plant defense strategy is to increase constitutive chemicals. Phenolic constituents of oak trees increase when they are attacked by larvae of the Lepidoptera (Feeny, 1968). Many conifer species respond to bark beetle attack by secreting oleoresin at the wound site (Nebecker *et al.*, 1993). Oleoresin, composed of roughly equal quantities of monoterpenes (turpentine) and diterpenoid resin acids (rosin) (Lewinsohn *et al.*, 1993), is toxic to both beetles and their pathogenic fungal associates (Nebecker *et al.*, 1993). The pathogenic fungi are very detrimental to pine trees and help the bark beetles overcome the defenses of the pine trees (Lewinsohn *et al.*, 1993). Many species of bark beetles are specifically attracted to the monoterpenoid compounds emitted from their conifer hosts and can utilize components of the host oleoresin in the synthesis of pheromones for promoting aggregation. The volatiles produced may also serve as

attractants for beetle predators and parasitoids (Gijzen *et al.*, 1993). Constitutive oleoresin is synthesized in the epithelial cells of secretory structures, whereas induced oleoresin originates in non-specialized cells adjacent to the site of injury.

Plant genotype is a major determinant of the quality and quantity of secondary compounds and of the anatomical defenses that affect herbivores (Berenbaum, 1981). Leaf age is an important modifier of plant-insect interactions and different ages may respond very differently to damage. Leaf dimorphism due to mesomorphic-xeromorphic conditions is a habit common to the shrubs in arid areas (Margaris, 1975; Westman, 1981). The big mountain sagebrush, *A. t. vaseyana*, has also adapted this strategy to produce two types of leaves, a larger but finer summer leaf (ephemeral) which is slowly replaced by the same shape of leaf, but much smaller and thicker (persistent). The onset of drier conditions in July triggers the development of the persistent leaves and the senescence of the ephemeral leaves (Shultz, 1983).

Biochemical and physiological changes occur when young leaves are damaged because processes of leaf expansion and development are severely compromised. Plants that usually produce constitutive secondary compounds react to such damage by increasing either the defensive compounds already produced, and/or by making more potent compounds to repel attackers (Raffa, 1991; Lewinsohn *et al.*, 1991).

Considering the importance of terpenoids in plant defense, there are significantly fewer reports of induced defenses with terpenoids than other classes of secondary metabolites, especially in leaf tissue (Gershenson and Croteau, 1991). This trend may be partially due to the existence of physiological constraints on terpenoid biosynthesis in

mature leaves (Loomis, 1967; Gershenson and Croteau, 1991). Terpenoid biosynthesis is restricted to specialized cells (trichomes or secretory cells) that are usually only active early in leaf development while terpenoid synthesis in mature leaves uses internal substrate pool reserves from the photosynthesis dark reaction carried out in the trichomes or secretory cells (Loomis, 1967). No published study has evaluated the array of terpenoids produced by greenhouse-grown sagebrush plants or terpenoids produced after clipping the plants. Such a study would permit a more accurate evaluation of the constitutive components already present and compounds induced during the interactions between herbivores and sagebrush plants.

Ultrastructural studies of the glandular trichomes of *A. t. vaseyana* (Chapter 2) have shown that the ephemeral summer leaves produce apparently volatile terpenoids (mono- and sesquiterpenoids), while the persistent winter leaves from the same plant appear to produce a mixture of volatile and non-volatile terpenoids (mono-, sesqui- and polyterpenoids). The glandular trichomes of persistent leaves are very susceptible to shedding, and much of the terpenoids produced could be lost. Therefore, the ephemeral leaves that produce volatiles should be used preferentially in studies of volatile chemicals that are used as pest repellents, fumigants and as alternatives to the volatile compound methyl bromide.

The objective of this part of the study was to assess the response of mature ephemeral leaves of *A. t. vaseyana* when subjected to clipping and defoliation as an imitation of browsing by herbivores and to determine the effects of clipping on survival of



the plants. The compounds produced due to clipping or defoliation of the branches could possibly be utilized as pesticides for insect control.

### Materials and Methods

#### Plant Production

Seeds from plant #3 vernalized at 4 °C for 10 days in a wetted Whatman Paper No. 3 were sown on 24 January and 23 June, 1996, one per individual cell in a 72-cell seed bed (No.1206 insert for No.1020 flat 11" x 22" T. O. Plastics, made by Belden Plastics, St. Paul, MN) which was filled with pre-wetted Aqua 2000 potting media (1 part of Bozeman silt loam, 1 part of peat moss, 1 part of washed concrete sand, and 1 lb per cubic yard of Aqua Gro 2000G pasteurized at 80 °C for 45 mins). The ensemble was covered with a clear plastic dome. The plants were watered with tap water once every two days after seedling establishment. In addition, they were watered twice per week with a dilute solution of Peter's 20-10-20 general purpose fertilizer (0.25 g/l). The seedlings were transplanted after two months either into 60-cubic-inch deep black pots (Stuewe & Sons, Inc., Corvallis, OR) or into 10-cubic-inch small white pots (Stuewe & Sons, Inc., Corvallis, OR), where they were grown for one year in the greenhouse. One-year-old plants were transplanted into AZ 8-inch pots (AZALEA Pot, Belden Plastics, St. Paul, MN) for further growth.

On 1 September, 1996, five of the plants grown on 24 January, 1996, were transplanted into 16-cubic-foot pots (Polytainer No. 4, Nursery Supply Inc., Tustin, CA) to compare the size of the greenhouse-grown plants to field-grown plants. The plants

sown on 23 June, 1996, were transferred into 10-cubic-inch pots or 60-cubic-inch pots on 1 September, 1996. Each of these plants had grown one straight stem during the first year of their development. They were transplanted one more time in April of 1997 into AZ 8-inch pots for the clipping experiment.

#### Control Samples of Leaves Analyzed Prior to Clipping Experiments

Same Plant at Two Different Ages and After Clipping. The five plants sown on 24 January, 1996, which were in the 16-cubic-foot pots, had developed many branches by the second year. Two of their branches (15 cm each) were clipped the first time on 30 November, 1996, and the second time on 20 January, 1997, when both types of leaves were present. The quantity of the fresh leaves to be extracted was fixed at  $3.50 \text{ g} \pm 0.05$ . Both ephemeral and persistent leaves from the clipped branches were extracted for chemical analysis. Each type of leaf was extracted when three-months old in order to compare the chemical composition of each type of leaf. The following is a description of each sample:

- A: persistent leaves from a fully-developed plant which resembled a field-grown plant were clipped the first time on 30 November, 1996, and the leaves extracted immediately (10-month-old plant).
- E<sub>1</sub>: persistent leaves from the same set of plants as in A, but clipped the first time on 30 September, 1996, and clipped a second time on 20 January, 1997 (12-month-old plant).

-E<sub>2</sub>: ephemeral leaves which grew after the first clipping of 30 September, 1996,  
were clipped a second time on 20 January, 1997, (12-month-old plant).

-E<sub>3</sub>: ephemeral leaves, second sample, from a different plant (12-month-old plant)  
treated the same as the one in E<sub>2</sub>.

Sixteen-Month-Old Plant in Sixteen-Cubic-Foot Container. The large and small leaves of the containerized plants were similar to those of field-grown plants. The large leaves had regrown after the clipping of 20 January, 1997, and the branches had varied physiological leaf growth. The samples were collected on 19 April, 1997, from the same plant that was previously clipped on 20 January, 1997. The following is a description of each sample:

-K: ephemeral leaves from a branch without persistent leaves (3.50 g)

-L<sub>1</sub>: ephemeral leaves from a branch with persistent leaves (3.50 g).

-L<sub>2</sub>: persistent leaves from the same plant and from the same branches where the ephemeral leaves of sample L<sub>1</sub> were harvested (3.50g)

Seven-Month-Old Plants in Small Containers. Plants sown on 23 June, 1996, and transplanted into 60-cubic-inch pots on 1 September, 1996, had both types of leaves sampled on 20 January, 1997, when the senescent ephemeral leaves and the persistent leaves were fully developed. The following is a description of the leaves sampled:

-F<sub>1</sub>: ephemeral leaves which were completely mature (3.59 g of fresh leaves)

-F<sub>2</sub>: persistent leaves which were fully developed (3.59 g of fresh leaves)

-F<sub>3</sub>: persistent leaves from the crown of each branch (3.55 g of fresh leaves).

### Plant Material and Methods of the Clipping and Defoliation Experiment

The remaining plants were transferred into AZ 8-inch pots on 2-4 April, 1997.

The plants from the 10-cubic-inch pots had their number followed by "Y" and the ones transferred from the 60-cubic-inch pots had their number followed by "R" (Table 1).

Table 1: Randomized block of *Artemisia tridentata vaseyana* plants used for clipping and defoliation experiments.

| North                          |                                |     |     |         |
|--------------------------------|--------------------------------|-----|-----|---------|
| Plant Row                      |                                |     |     |         |
| 1                              | 2                              | 3   | 4   | 5       |
| Defoliated                     | Observation of Senescence Time |     |     | Clipped |
| 23 <sup>a</sup> R <sup>b</sup> | 3R                             | 15R | 20R | 11Y     |
| 24Y <sup>c</sup>               | 10Y                            | 6Y  | 14R | 4R      |
| 16Y                            | 5R                             | 12R | 1Y  | 19Y     |
| 18R                            | 4Y                             | 9R  | 7Y  | 13Y     |
| 8Y                             | 17Y                            | 9Y  | 2R  | 11R     |
| 20Y                            | 14Y                            | 22R | 12Y | 7R      |

South (Alley)

<sup>a</sup> plant identification number

<sup>b</sup> plant transplanted from a 60-cubic-inch pot (R following its number)

<sup>c</sup> plant transplanted from a 10-cubic-inch pot (Y following its number)

The plants in AZ 8-inch pots were randomly arranged in six rows of six pots on the bench of a greenhouse growth room in the Plant Growth Center at Montana State University. The pots were separated by 0.25 m to prevent the plants from touching each other. Some of the small branches started to develop and the large leaves appeared three weeks after transplanting and they were fully developed by 15 June, 1997. The first



experiment of clipping and defoliation of ephemeral leaves began 20 June, 1997, before the onset of the persistent leaves. Only current-year mature ephemeral leaves, which were present from the start of the experiment, were extracted in the clipping and defoliation experiments (Figure 42). Samples of fresh ephemeral leaves (3.50 g) from three plants were air-dried for ten days in the dry room of the Plant Growth Center and weighed respectively 0.79, 0.80, and 0.81 g. The mean of 0.80 g of dried leaves was used to calculate the quantity of terpenoid obtained in 15 ml of extract during the clipping and defoliation experiment (Duke *et al.*, 1994).

The plants were aligned in five rows on the east side bench of greenhouse No. 78. Both the clipping and defoliation experiments used plants from one row of pots which were separated by three rows of plants that were to be used later for the experiment on small leaves. The clipping experiment used the set of plants labeled 20R, 14R, 1Y, 7Y, 2R and 12Y, while the defoliation experiment was carried out on the set of plants labeled 23R, 24Y, 16Y, 18R, 8Y and 20Y. A set of six plants was used per experiment to avoid leaf shortage for the remainder of the experiment. The experiment was conducted every day from 1:00 pm to 3:00 pm (Nicholas, 1973). The plants were moved from the greenhouse bench to the weighing room at noon and the experiment began at 1:00 pm when the fungus flies and the aphids, seeking refuge on the plants during their displacement to the weighing room, were gone (Table 1).

The longevity of the plants used in the experiment was monitored until December of 1998, and the mortality rate of the clipped plants was calculated in comparison to that of the unclipped plants.

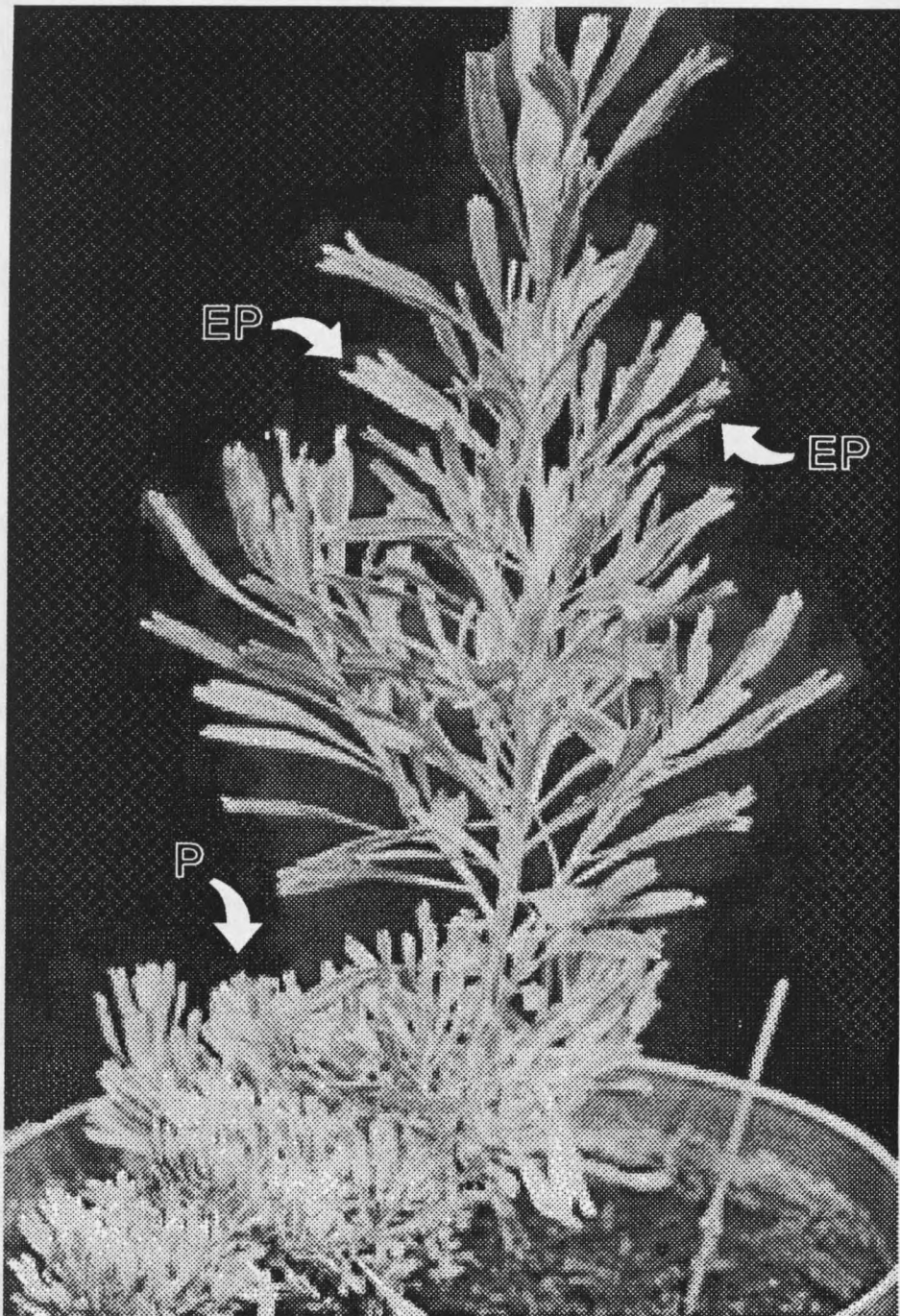


Figure 42: Second-year growth pattern of the *Artemisia tridentata vaseyana* plant. Defoliation and clipping experiments were performed on the current season's growth of ephemeral leaves (EP). Persistent leaves of the previous year (P) were not used in the clipping experiment.

Clipping experiment. The leader branches were clipped from the top toward the bottom with a pair of scissors. The scissors were cleaned with balls of cotton wetted with absolute ethanol (HP grade), and wiped dry with another cotton ball before clipping the next plant. The branches at the same level were clipped daily so that the plants were flat on top to simulate the shape of the plant after browsing by wild mammals (Figure 43). Of the leaves collected from clipped branches on 20 June, 1997, only those from below the crown were extracted. The leaves from the crown were not extracted because they were not ephemeral leaves.

The leaves from the branches clipped in the beginning of the experiment (20 June, 1997 = Day 1) were extracted as a baseline for the compounds produced by plants which had not experienced any clipping effects. A lag time of 48 hours was given to the plants to react to the first clipping. The leaves, from the same branches of the set of plants clipped on 20 June, 1997, were clipped again on 22, 23, 24, 25, 26 and 27 June, 1997, to determine the cumulative effects of continual clipping. The daily clipping of the branches and extraction of the leaves was spaced to three days (June 30), four days (July 4), five days (July 9), ten days (July 19), and 54 days from the previous clipping (September 14) to investigate the effect of spaced clipping on terpenoid production. The total weights of branches and leaves harvested during the clipping experiments were also recorded. The quantity of leaves harvested per plant and per treatment for the clipping experiment varied from 0.14 to 2.42 g (Table 2). The total quantity of leaves harvested per plant for the clipping experiment varied from 5.29 to 13.61 g. The ephemeral leaves were sparsely

distributed on the branches of each plant and could not sustain a long term experiment.

Each leaf weighed from 0.15 to 0.20 g and the number of leaves harvested per plant

during each treatment varied from two to four leaves.

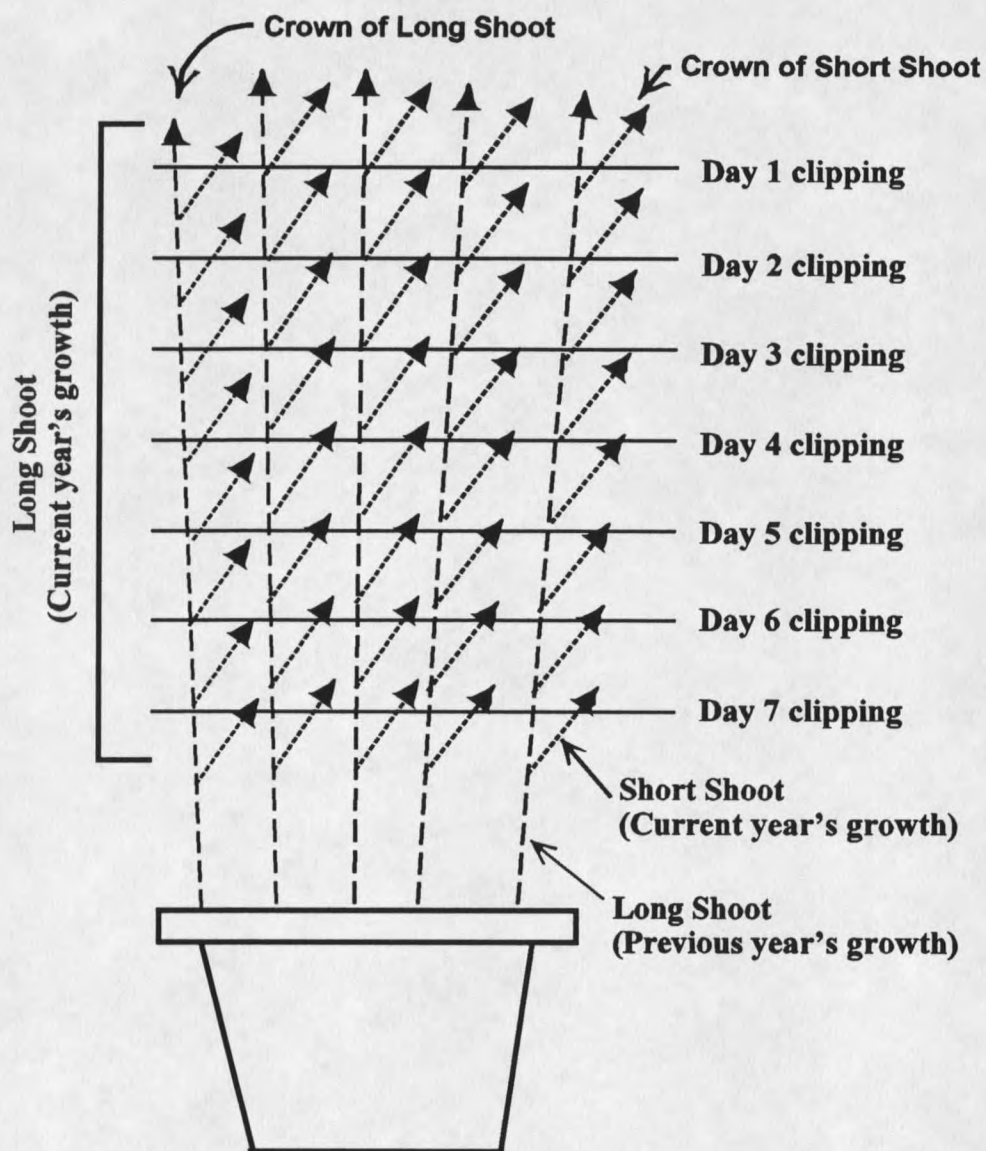


Figure 43: Diagram of the clipping experiment on the branches bearing the ephemeral leaves of *Artemisia tridentata vaseyana*. Ephemeral leaves from both short and long shoots were extracted.



Table 2: Fresh ephemeral leaves of *Artemisia tridentata vaseyana* harvested per plant and per treatment for the clipping experiment.

| Sample Code     | Date     | Weight of Fresh Ephemeral Leaves (g) |                 |      |      |       |       | Total per Treatment |
|-----------------|----------|--------------------------------------|-----------------|------|------|-------|-------|---------------------|
|                 |          | 2 <sup>a</sup> R <sup>b</sup>        | 7Y <sup>c</sup> | 12Y  | 20R  | 1Y    | 14R   |                     |
| S               | 20 June  | 0.70                                 | 0.80            | 0.43 | 0.43 | 0.82  | 0.60  | 3.78                |
| S1              | 22 June  | 0.93                                 | 0.47            | 0.60 | 0.43 | 0.62  | 0.88  | 4.17                |
| S2              | 23 June  | 1.23                                 | 0.47            | 0.53 | 0.40 | 0.55  | 1.32  | 4.50                |
| S3              | 24 June  | 0.79                                 | 0.84            | 0.62 | 0.50 | 0.68  | 1.37  | 4.80                |
| S4              | 25 June  | 1.12                                 | 0.83            | 0.67 | 0.77 | 0.58  | 1.26  | 5.23                |
| S5              | 26 June  | 1.02                                 | 1.00            | 0.52 | 0.44 | 0.95  | 1.01  | 4.94                |
| S6              | 27 June  | 1.84                                 | 0.94            | 0.70 | 0.27 | 1.25  | 1.47  | 6.47                |
| S7              | 30 June  | 1.10                                 | 0.86            | 0.71 | 0.49 | 1.28  | 0.88  | 5.32                |
| S8              | 4 July   | 1.06                                 | 0.66            | 0.81 | 0.14 | 0.75  | 0.90  | 4.32                |
| S9              | 9 July   | 0.77                                 | 0.91            | 0.40 | 0.25 | 1.33  | 0.70  | 4.36                |
| S10             | 20 July  | 0.80                                 | 0.46            | 0.63 | 0.93 | 1.13  | 1.18  | 5.13                |
| S11             | 14 Sept. | -                                    | -               | -    | -    | 2.42  | 2.04  | 4.45                |
| Total per Plant |          | 11.29                                | 8.24            | 6.62 | 5.29 | 12.36 | 13.61 |                     |

<sup>a</sup> plant identification number

<sup>b</sup> R tagged to plants transplanted from 60-cubic-inch pots

<sup>c</sup> Y tagged to plants transplanted from 10-cubic-inch pots

The weight of the branches clipped per plant and per treatment varied from 0.12 to 3.27 g (Table 3). The newly grown branches were light in weight because they did not have secondary woody development. Only branches from the current year were harvested for the clipping experiment.

Table 3: Branch tissue of *Artemisia tridentata vaseyana* harvested per plant during the clipping experiment.

| Date            | Sample Code | Branch Weight with Fresh Ephemeral Leaves (g) |                 |      |      |      |       |                     |
|-----------------|-------------|-----------------------------------------------|-----------------|------|------|------|-------|---------------------|
|                 |             | 2 <sup>a</sup> R <sup>b</sup>                 | 7Y <sup>c</sup> | 12Y  | 20R  | 1Y   | 14R   | Total per Treatment |
| 20 June         | S           | 0.31                                          | 0.31            | 0.13 | 0.22 | 0.29 | 0.39  | 1.65                |
| 22 June         | S1          | 0.51                                          | 0.31            | 0.52 | 0.22 | 0.22 | 0.47  | 2.25                |
| 23 June         | S2          | 0.53                                          | 0.28            | 0.18 | 0.19 | 0.20 | 0.74  | 2.12                |
| 24 June         | S3          | 0.43                                          | 0.54            | 0.23 | 0.14 | 0.29 | 1.18  | 2.81                |
| 25 June         | S4          | 0.76                                          | 0.44            | 0.24 | 0.19 | 0.31 | 0.76  | 2.70                |
| 26 June         | S5          | 0.50                                          | 0.51            | 0.14 | 0.10 | 0.39 | 0.60  | 2.24                |
| 27 June         | S6          | 1.24                                          | 0.59            | 0.17 | 0.15 | 0.58 | 0.61  | 3.34                |
| 30 June         | S7          | 0.75                                          | 0.65            | 0.47 | 0.16 | 0.55 | 0.65  | 3.23                |
| 4 July          | S8          | 0.49                                          | 0.51            | 0.37 | 0.12 | 0.20 | 0.76  | 2.45                |
| 9 July          | S9          | 1.08                                          | 0.87            | 0.18 | 0.19 | 0.62 | 0.71  | 3.65                |
| 20 July         | S10         | 1.78                                          | 0.28            | 0.43 | 0.42 | 0.48 | 0.67  | 4.06                |
| 14 Sept.        | S11         | -                                             | -               | -    | -    | 2.42 | 3.27  | 5.69                |
| Total per Plant |             | 8.38                                          | 5.29            | 3.06 | 2.10 | 6.55 | 10.81 |                     |

<sup>a</sup> plant identification number

<sup>b</sup> R tagged to plants transplanted from 60-cubic-inch pots

<sup>c</sup> Y tagged to plants transplanted from 10-cubic-inch pots

Defoliation Experiment. The defoliation experiment also began 20 June, 1997.

The ephemeral leaves were removed from below the crown of the leader branches, in a top to bottom direction (Figure 44). The leaves were hand-picked, with the petiole removed from the branch, one at a time. It is another type of clipping which did not require

removal of the branches with a pair of scissors, but the leaves were removed in multiple spots at the insertion point of the petiole of each leaf on the branch.

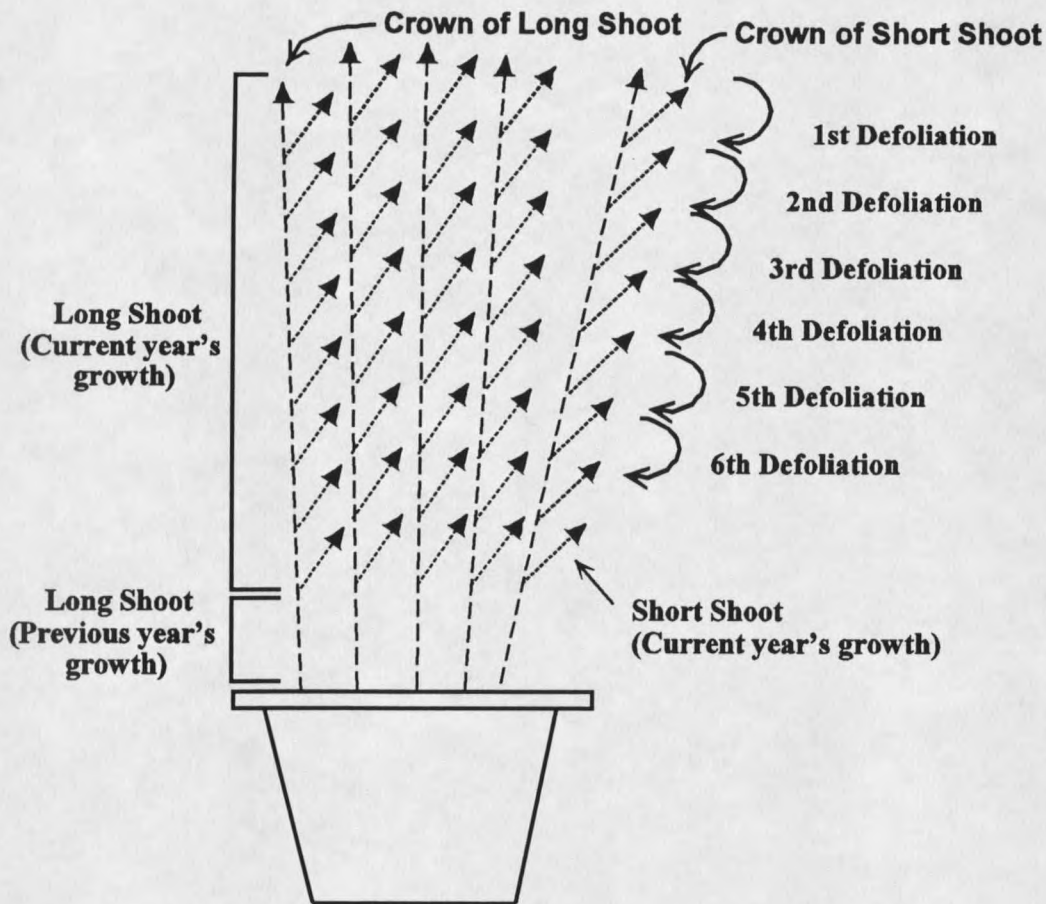


Figure 44: Diagram of the ephemeral leaf defoliation from the branches of *Artemisia tridentata vaseyana*. Ephemeral leaves from both short and long shoots were extracted.

The total weight of leaves harvested during the defoliation experiment was also recorded. The weight of leaves harvested per plant and during each experiment varied from 0.13 to 1.15 g (Table 4). The weight of leaves harvested per plant during the period of the experiment varied from 3.41 to 7.88 g.

Table 4: Fresh ephemeral leaves of *Artemisia tridentata vaseyana* harvested per plant and per treatment for the defoliation experiment.

| Date            | Sample Code | Fresh Leaf Weight (g)         |      |                  |      |      |      | Total per Treatment |
|-----------------|-------------|-------------------------------|------|------------------|------|------|------|---------------------|
|                 |             | 8 <sup>a</sup> Y <sup>b</sup> | 20Y  | 23R <sup>c</sup> | 24Y  | 18R  | 16Y  |                     |
| 20 June         | T           | 0.61                          | 0.61 | 0.64             | 0.55 | 0.52 | 0.56 | 3.49                |
| 22 June         | T1          | 0.61                          | 0.69 | 0.63             | 0.77 | 0.45 | 0.64 | 3.79                |
| 23 June         | T2          | 0.69                          | 0.63 | 0.65             | 0.61 | 0.46 | 0.62 | 3.66                |
| 24 June         | T3          | 0.90                          | 0.54 | 0.69             | 0.57 | 0.38 | 0.55 | 3.63                |
| 25 June         | T4          | 0.91                          | 0.76 | 0.60             | 0.68 | 0.38 | 0.53 | 3.86                |
| 26 June         | T5          | 1.01                          | 0.82 | 0.77             | 0.73 | 0.19 | 0.46 | 3.98                |
| 27 June         | T6          | 1.21                          | 0.76 | 0.78             | 0.69 | 0.53 | 0.52 | 4.49                |
| 30 June         | T7          | 0.76                          | 1.16 | 0.67             | 1.00 | -    | 0.13 | 3.72                |
| 20 July         | T8          | 0.66                          | 0.86 | 1.02             | 1.15 | 0.50 | 0.70 | 4.89                |
| 14 Sept.        | T9          | 0.52                          | 0.84 | 1.00             | 1.03 | -    | 0.83 | 4.22                |
| Total per Plant |             | 7.88                          | 7.67 | 7.45             | 7.78 | 3.41 | 5.54 |                     |

<sup>a</sup> plant identification number

<sup>b</sup> Y tagged to plants transplanted from 10-cubic-inch pots

<sup>c</sup> R tagged to plants transplanted from 60-cubic-inch pots

The leaves harvested on 14 September, 1997, had been present since 20 June, 1997, and showed no signs of senescence in contrast to the control plants which had started to shed their dried ephemeral leaves. The control plants had also developed fascicles of persistent small leaves. It was observed that ephemeral leaves on defoliated plants were green throughout the experiment while ephemeral leaves on control plants had undergone senescence at the beginning of the experiment.



### Chemical Extraction

The leaves were stripped from clipped branches, and 3.50 g ( $\pm$  0.05 g) of fresh leaves were extracted in a total of 15 ml of methylene chloride ( $\text{MeCl}_2$ ) (HPLC grade) for chemical analysis (Kelsey *et al.*, 1982; Gershenson and Croteau, 1991). The vials to be used were rinsed three times with 15 ml of  $\text{MeCl}_2$ , under the hood, one week before the beginning of the extraction procedure. The 20-ml vial used has a septum cap which prevents leakage of the volatile compounds extracted by  $\text{MeCl}_2$ . Two types of procedures were used for chemical extraction of the leaves. The first technique (Protocol 1) was used to detect terpenoid composition of the first samples, while the second technique (Protocol 2) was used for terpenoid quantification studies in clipping and defoliation experiments.

For the first extraction (Protocol 1), 10 ml of  $\text{MeCl}_2$  were used to cover the leaves for 10 days and the extract was poured into a vial. The leaves were thereafter rinsed one time in 5 ml of the same chemical. The two extracts were pooled and stored at 4°C. The extracts were analyzed, using Gas Chromatography/Mass Spectroscopy (GC/MS), in August of the same year to determine terpenoid composition of the leaves. This procedure was used for the samples extracted prior to the defoliation and clipping experiment.

A second extraction method (Protocol 2) was found to be an improvement because there was less detectable volatile from plants present in the air of the small weighing room of the Plant Growth Center when compared to the first extraction technique (Protocol 1) while performing the defoliation and clipping experiments. The experiment was

redesigned to do 5 ml of methylene chloride extraction for 5 min, and thereafter transfer the leaves into another 5 ml of the same solvent for ten days, and then rinse with an additional 5 ml of solvent (Mabry and Gill, 1979; Kelsey and Shafizadeh, 1980). The total quantity of extract was maintained at 15 ml throughout the experiments so that comparisons could be made. The three extracts were pooled for quantitative analysis using GC/MS.

### Chemical Analysis

Qualitative analyses were done to compare the terpenoid compounds from the leaves of greenhouse-grown plants to those produced by the leaves harvested from field-grown plants (Kelsey *et al.*, 1982; Buttkus *et al.*, 1997). Quantitative analysis is a fairly new technology that allows the quantification of volatiles produced by the leaves of induced versus non-induced plants.

Qualitative Analysis. The analyses were done with the addition of internal standards. Volatile compounds were separated using a column 30 m x 0.25 mm DB-5 with 0.25  $\mu$ m film thickness and a sample volume of 1  $\mu$ l was injected into the column. The temperature protocol was a starting temperature of 30 °C for 2 min, a temperature increase of 5 °C per min to 150 °C, followed by another temperature increase programmed at 25 °C per min to a final temperature held at 300 °C for 10 min, for a total run time of 44 min. Confirmation of NIST (National Institute of Standards and Technology software used with the analytical instrument) mass spectral library matches were made, when possible, by matching retention time and spectra with authentic samples. Confirmed

identifications were made by comparing the retention times and mass spectra of sample chromatograms to those of known purified compounds run under identical conditions.

The percentage composition of the terpenoid essence in the leaf extract was given as peak area percentage. The relative area (ratio of absolute area of each compound to the absolute area of the internal standard) was also used to compare the level of chemicals in some of the extracts ( $E_1$ ,  $E_2$ ,  $E_3$ , K,  $L_1$ ,  $L_2$ , SX, and TX).

Terpenoid compounds which may be important for further pesticide research should be confirmed by matching the retention time of terpenoid designated by library search with the pure compound prior to any experiment.

Quantitative Analysis. The quantitative analysis was done with a Hewlett Packard 5890 Series II Plus gas chromatograph interfaced to a mass spectrometer. The column was 30 m x 0.25 mm DB-5 with 0.25  $\mu$ m film thickness. The temperature protocol was a starting temperature of 30 °C for 2 min, the temperature ramp was 5 °C per min to 160 °C, a second temperature ramp at 25 °C per min to 290 °C, with a hold time of 5 min. Liquid leaf extracts of 1  $\mu$ l were injected with helium at an initial pressure of 40 kPa. The pressure was ramped with the temperature to maintain a constant helium flow throughout the duration of the analysis. The coupled mass spectrometer is a VG 70E-HF double focusing magnetic mass spectrometer (MS) (VG Instruments, Manchester, England). The MS was operated at a scan rate of 0.5 seconds per mass decade, ranging from 40 to 450 amu. The source temperature was 200 °C and the mass resolution was 1500.

### Interpretation of Chemical Analysis Data

Qualitative and quantitative data were obtained in the chemical analysis and the data appeared different because they were obtained from two types of instruments with different software for library identification.

Qualitative Analysis. The results of the plants before treatment were given in percentage area, considered as a rough percentage of each component in comparison to chromatographed compounds. The percentage area evaluates the proportional quantity of each component. The retention time represented the time when the component appears from the point of injection. The peak number represents the appearance rank of the component. The names of chemical components are printed on the sheet of data. The qualitative analysis was mostly applied to samples obtained before the clipping and defoliation experiments (samples coded A, E<sub>1</sub>, E<sub>2</sub>, E<sub>3</sub>, F<sub>1</sub>, F<sub>2</sub>, and F<sub>3</sub>; all analyses are in Appendix A).

Quantitative Analysis. The data sheet (Appendix B) was represented by bar graphs along with a numerical data sheet. The levels of terpenoid compounds were evaluated per leaf type by dividing the absolute area (found on the numerical data sheet) of each compound by the absolute area of the internal standard (also on the numerical data sheet) to obtain relative area. Bar graphs of the level of each terpenoid compound present in all three samples were drawn for comparison. This type of comparison was done on samples extracted before the clipping and defoliation experiments on samples coded A, E<sub>1</sub>, E<sub>2</sub>, E<sub>3</sub>,



K, L<sub>1</sub>, L<sub>2</sub> and F<sub>3</sub>. For the samples obtained from clipping and defoliation experiments the quantity of terpenoids extracted in the 15 ml of methylene chloride was calculated. Calibration curves were determined for terpenoid compounds encountered in these sagebrush extracts such as camphene, eucalyptol (cineole) and camphor by injecting 0 (blank), 10, 25, 50, 75 and 100 ng of each terpenoid compound and the instrument response was recorded. The linear regression equation  $y = mX + b$  was drawn from the graph with  $X = (y - b)/m$ . The value of X represents the quantity of each component in the extract and y represents the ratio of the absolute area of each component to the absolute area of the internal standard. The regression graphs obtained were  $y = 0.071272 X + 0.0012131$  for camphene,  $y = 0.078245 X - 0.03595$  for eucalyptol, and  $y = 0.0787785 X + 0.0375771$  for camphor. The mean number of the three calibration curves was used to evaluate the quantity of the remaining terpenoid compounds in the leaf extracts. This regression curve was  $y = 0.076098413 X + 0.000946791$  (Excel, 1997). The quantity of each terpenoid component in the leaf extracts obtained by the defoliation and clipping experiments was calculated and graphed to determine the quantity of terpenoid produced after branch defoliation and clipping *A. t. vaseyana* plants.

## Results

### Terpene Composition of Persistent and Ephemeral Leaves of *A. t. vaseyana*

Terpenoid compounds identified by library search which may be important for further research should be confirmed by matching the retention times with the pure compounds purchased from chemical companies.

Major chemical components are few and are the most abundant in the leaf extracts while minor components are 5-15% of the relative area of the major components.

Constitutive chemical components extracted from the persistent leaves harvested from the same plant were seasonally different (Tables 5-6) and different from the chemical profile of the ephemeral leaves of the same plant (Table 7). Persistent leaves from the same plant had as major compounds camphor (25.51%), eucarvone (16.74%), eucalyptol (11.31%), safranal (10.23%), and camphene (7.65%) (Table 6). The minor components were cyclobuta[1,2-b:4,3-b']bisthiopyran (3.46%),  $\alpha$ -cubebene (3.22%),  $\alpha$ -pinene (2.88%), p-mentha-6,8-dien-2-one (2.58%), isoborneol (2.23%), propanenitrile (1.99%), anisole,2,3,6-trimethyl (1.80%), 2,5-cyclohexadien-1-one (1.69%), and pleiocarpamine (1.33%) (Table 6).

The major components of the total extractable constituents from the ephemeral leaves of greenhouse-grown sagebrush plants were camphor (26.78%), eucarvone (18.48%), eucalyptol (15.60%), berbenone (8.19%), and camphene (7.06%) (Table 7). The minor components were 3,5-dimethyl-2-cyclohexen-1-one (3.46%), borneol (2.63%),  $\alpha$ -cubebene (2.90%), cis-geraniol (1.77%), and pugelone (1.35%) (Table 7). Ephemeral leaves did not possess  $\alpha$ -pinene or  $\beta$ -pinene (Table 7), while the persistent leaves from the same plant had  $\alpha$ -pinene and  $\beta$ -pinene (Table 5) or  $\alpha$ -pinene (Table 6).

Table 5: Chemical composition of the persistent leaves with no ephemeral leaves present from a 10-month-old plant (three-month-old leaves) of *Artemisia tridentata vaseyana*. Qualitative analysis performed on 19 December, 1996. (Sample Code A).

| Compound Name                                 | % Area <sup>a</sup> | Peak Number <sup>b</sup> | Retention Time (min) <sup>c</sup> |
|-----------------------------------------------|---------------------|--------------------------|-----------------------------------|
| Safranal (L <sup>d</sup> )                    | 28.38               | 9                        | 17.66                             |
| Camphor (C <sup>e</sup> )                     | 13.63               | 11                       | 18.19                             |
| 6-Nonynoic acid, methyl ester (L)             | 13.00               | 4                        | 12.89                             |
| Eucalyptol (Cineole) (C)                      | 9.50                | 5                        | 14.60                             |
| $\beta$ -Pinene (L)                           | 5.22                | 1                        | 10.67                             |
| Benzenemethanol (L)                           | 4.71                | 15                       | 23.19                             |
| $\alpha$ -Pinene (L)                          | 3.96                | 2                        | 11.45                             |
| 1H-Cyclopenta[1,3]cyclopropa[1,2] benzene (L) | 3.23                | 17                       | 27.53                             |
| 3,4-Pentadien-1-ol-2,2-dimethyl (L)           | 2.74                | 12                       | 19.73                             |
| Camphene (C)                                  | 2.62                | 3                        | 11.91                             |
| 3,4-Pentadienal,2,2-dimethyl (L)              | 2.48                | 12                       | 18.66                             |
| 5,5-Dimethyl-1,3-hexadiene (L)                | 2.39                | 10                       | 18.02                             |
| Cis-Butyric Acid (L)                          | 1.85                | 7                        | 15.82                             |
| 1-Methyl-2-methylene-cyclohexane (L)          | 1.76                | 6                        | 15.46                             |
| 7-Azabicyclo [4.1.0.]hept-3en-7-amid (L)      | 1.61                | 8                        | 17.42                             |
| 1-Hexyne (L)                                  | 1.47                | 13                       | 19.60                             |
| Caryophyllene (L)                             | 1.47                | 16                       | 25.83                             |

<sup>a</sup> rough % of each compound in comparison to chromatographed compounds

<sup>b</sup> rank of the compound when it appears from the point of injection

<sup>c</sup> time when the compound appears from the point of injection

<sup>d</sup> compound identified by library search (software of the instrument)

<sup>e</sup> compound confirmed by running authentic compound into the GC/MS

Table 6: Chemical composition of the persistent leaves from a 12-month-old plant (three-month-old leaves) of *Artemisia tridentata vaseyana*. Qualitative analysis performed on 19 December, 1996 (Same plant as in Table 5. Sample Code E<sub>1</sub>).

| Compound Name                           | % Area <sup>a</sup> | Peak Number <sup>b</sup> | Retention Time (min) <sup>c</sup> |
|-----------------------------------------|---------------------|--------------------------|-----------------------------------|
| Camphor (C <sup>d</sup> )               | 25.51               | 8                        | 17.90                             |
| Eucarvone (L <sup>e</sup> )             | 16.74               | 14                       | 24.87                             |
| Eucalyptol (cineole) (C)                | 11.31               | 4                        | 14.31                             |
| Safranal (L)                            | 10.23               | 7                        | 17.26                             |
| Camphene (C)                            | 7.64                | 2                        | 11.62                             |
| Cyclobuta[1,2-b:4,3-b']bisthiopyran (L) | 3.46                | 6                        | 16.57                             |
| $\alpha$ -Cubebene (L)                  | 3.22                | 16                       | 26.86                             |
| $\alpha$ -Pinene (L)                    | 2.88                | 1                        | 11.15                             |
| P-Mentha-6,8-dien-2-one (L)             | 2.58                | 12                       | 21.53                             |
| Isoborneol (L)                          | 2.23                | 10                       | 18.46                             |
| Propanenitrile (L)                      | 1.99                | 11                       | 20.62                             |
| Anisole,2,3,6-trimethyl (L)             | 1.80                | 15                       | 25.47                             |
| 2,5-Cyclohexadien-1-one (L)             | 1.69                | 9                        | 18.35                             |
| Pleiocarpamine (L)                      | 1.33                | 13                       | 23.72                             |
| 1-Pyrrolidinyloxy,3-amino-2,2,5,5 (L)   | 1.30                | 5                        | 15.44                             |

<sup>a</sup> rough % of each compound in comparison to chromatographed compounds

<sup>b</sup> rank of the compound when it appears from the point of injection

<sup>c</sup> time when the compound appears from the point of injection

<sup>d</sup> compound confirmed by running authentic chemical compound in the GC/MS

<sup>e</sup> compound identified by library search (software of the instrument)

Table 7: Chemical composition of the ephemeral leaves from a 12-month-old plant (three-month-old leaves) of *Artemisia tridentata vaseyana*. Qualitative analysis performed on 19 December, 1996 (Same plant as in Tables 5 and 6. Sample Code E<sub>2</sub>).

| Compound Name                       | % Area <sup>a</sup> | Peak Number <sup>b</sup> | Retention Time (min) <sup>c</sup> |
|-------------------------------------|---------------------|--------------------------|-----------------------------------|
| Camphor (C <sup>d</sup> )           | 26.78               | 5                        | 17.86                             |
| Eucarvone (L <sup>e</sup> )         | 18.48               | 10                       | 24.82                             |
| Eucalyptol (1,8-Cineole) (C)        | 15.60               | 2                        | 14.31                             |
| Berbenone (L)                       | 8.19                | 4                        | 17.23                             |
| 3,5-Dimethyl-2-cyclohexen-1-one (L) | 3.46                | 8                        | 21.50                             |
| $\alpha$ -Cubebene (L)              | 2.90                | 11                       | 26.86                             |
| Borneol (L)                         | 2.63                | 7                        | 18.45                             |
| Cis-Geraniol (L)                    | 1.77                | 9                        | 23.64                             |
| Pugelone (L)                        | 1.35                | 6                        | 18.34                             |

<sup>a</sup> rough % of each compound in comparison to chromatographed compounds

<sup>b</sup> rank of the compound when it appears from the point of injection

<sup>c</sup> time when the compound appears from the point of injection

<sup>d</sup> compound confirmed by running authentic chemical compound in the GC/MS

<sup>e</sup> compound identified by library search (software of the instrument)

Based upon the methodology used to extract the leaves, only lipophilic compounds such as monoterpenes and sesquiterpenes of interest are extracted. Current year leaves harvested from different branches of the same plant showed persistent leaves to have 16 compounds (Table 6), while ephemeral leaves possessed only 9 (Table 7). Persistent leaves had seven more minor compounds than the ephemeral leaves. Both types of leaves had almost the same major compounds, except the compound berbenone was in ephemeral leaves while safranal was in the persistent leaves of a 12-month-old plant.



The terpenoid profile of the ephemeral leaves was determined prior to the clipping and the defoliation experiment (Table 8). The major chemical components were berbenone (2-pinene-4-one), camphor, eucalyptol, camphene and 1,3-cyclohexadiene. The minor components were  $\beta$ -phellandrine, p-menth-1-en-4-ol, 2-norpinene, berbenone (2-pinene-4-one), 1-(2-pyrazine)butanone, cyclodiexanol (p-menth-8-en-1-ol), 2-cyclodiexene-1-one, 1,4-cyclohexadiene, androstan-17-one, norbornane, and borneol (Table 8).

The same sample was analyzed by two types of instruments made by different manufacturers and the software for each identified the compounds differently. However, components such as camphor and eucalyptol were major components in both analyses (Tables 7 and 8). The pretreatment terpenoid profile of the ephemeral leaves (Table 8) was compared to the terpenoid profiles produced in the clipping and defoliation experiments.

#### Comparison of the Terpenoids Produced by Different Ages of Ephemeral Leaves

Chemical composition of each type of leaf harvested from plants that possessed multiple branches which had leaves of variable phenological stages was performed. The level of terpenoid compounds per type of leaf was compared (Table 9). The quantity of terpenoid was higher in the younger ephemeral leaves (Sample K), than in the older ephemeral leaves harvested from branches which had fully grown persistent leaves (Sample L<sub>1</sub>) and in the persistent leaves harvested from the same branches bearing older ephemeral leaves (Sample L<sub>2</sub>). Ephemeral leaves harvested from branches that have

Table 8: Terpenoid profile of the ephemeral leaves from *Artemisia tridentata vaseyana* before the clipping and the defoliation experiment. Quantitative analysis performed on 2-3 May, 1997.

| Scan Number <sup>a</sup> | Compound Name                               | Relative Area <sup>b</sup>         |                                    |
|--------------------------|---------------------------------------------|------------------------------------|------------------------------------|
|                          |                                             | Sample E <sub>3</sub> <sup>c</sup> | Sample E <sub>2</sub> <sup>c</sup> |
| 886                      | Verbenone (2-pinen-4-one) (L <sup>d</sup> ) | 2.473                              | 1.913                              |
| 925                      | Verbenone (Hexene-1-methanol) (L)           | 2.243                              | 1.754                              |
| 682                      | Eucalyptol (C <sup>e</sup> )                | 2.018                              | 1.825                              |
| 496                      | Camphene (C)                                | 0.596                              | 0.480                              |
| 1404                     | Verbenone (L)                               | 0.532                              | 0.344                              |
| 555                      | $\beta$ -Phellandrene (L)                   | 0.236                              | 0.149                              |
| 465                      | 2-Norpinene (L)                             | 0.155                              | 0.113                              |
| 1446                     | Cinerone (L)                                | 0.138                              | 0.084                              |
| 839                      | Verbenone (L)                               | 0.123                              | 0.008                              |
| 1018                     | Unknown (L)                                 | 0.104                              | 0.064                              |
| 1177                     | 2-Cyclodiexen-1-one (L)                     | 0.088                              | 0.557                              |
| 827                      | Unknown (L)                                 | 0.072                              | 0.068                              |
| 961                      | Androstan-17-one (L)                        | 0.072                              | 0.606                              |
| 1204                     | Norbornane (L)                              | 0.048                              | 0.037                              |
| 967                      | Verbenone (L)                               | 0.019                              | 0.166                              |

<sup>a</sup> time when the compound appears from the point of injection

<sup>b</sup> the ratio of the absolute area of each compound to the absolute area of the internal standard

<sup>c</sup> each sample was diluted by a factor of 5 prior to analysis

<sup>d</sup> compound confirmed by running authentic chemical compound in the GC/MS

<sup>e</sup> compound identified by library search (software of the instrument)

already grown persistent leaves would not be suitable for evaluating the terpenoid levels produced by ephemeral leaves during the clipping or defoliation experiment because the level of terpenoids produced in the younger ephemeral leaves is greater (Table 9). The clipping experiments were therefore performed on the ephemeral leaves prior to the onset

Table 9: Level of terpenoid compounds per leaf type from a 16-month-old plant of *Artemisia tridentata vaseyana* grown in a 16 ft<sup>3</sup> container. Quantitative analysis was performed on 3 May, 1997.

| Scan Number <sup>a</sup> | Compound Name                               | Relative Area <sup>b</sup> |                             |                             |
|--------------------------|---------------------------------------------|----------------------------|-----------------------------|-----------------------------|
|                          |                                             | K <sup>c</sup>             | L <sub>1</sub> <sup>d</sup> | L <sub>2</sub> <sup>e</sup> |
| 889                      | Verbenone/2-pinene-4-one (L <sup>f</sup> )  | 0.881                      | 0.536                       | 0.677                       |
| 684                      | Eucalyptol (C <sup>g</sup> )                | 0.560                      | 0.286                       | 0.212                       |
| 465                      | 2-Norpinene (L)                             | 0.412                      | 0.031                       | 0.043                       |
| 1405                     | Verbenone (L)                               | 0.272                      | 0.158                       | 0.274                       |
| 555                      | β-Phellandrene (L)                          | 0.049                      | 0.027                       | -                           |
| 1018                     | Unknown (L)                                 | 0.047                      | 0.017                       | 0.014                       |
| 839                      | Unknown (L)                                 | 0.044                      | 0.028                       | 0.040                       |
| 1177                     | 2-Cyclohexen-1-one (L)                      | 0.044                      | 0.026                       | 0.048                       |
| 827                      | Unknown (L)                                 | 0.033                      | 0.013                       | -                           |
| 1446                     | Cinerone (L)                                | 0.031                      | 0.030                       | 0.042                       |
| 1115                     | Berbenone/Bicyclo[3.1.1]hept-3-en-2-one (L) | 0.023                      | 0.013                       | 0.025                       |
| 922                      | Verbenone (Hexene-1-methanol) (L)           | 0.022                      | -                           | -                           |
| 1321                     | Verbenone/2-pinene-4-one (L)                | 0.016                      | -                           | 0.022                       |
| 969                      | Unknown (L)                                 | 0.010                      | -                           | -                           |
| 1055                     | Berbenone/Bicyclo[3.1.1]hept-3-en-2-one (L) | 0.010                      | -                           | 0.011                       |
| 1869                     | Methyl jasmonate (L)                        | 0.010                      | -                           | -                           |
| 753                      | 1, 3 - Cyclohexadiene (L)                   | 0.009                      | -                           | 0.008                       |
| 1382                     | Methyl cinnamate (L)                        | 0.009                      | -                           | -                           |

<sup>a</sup> time when the compound appears from the point of injection

<sup>b</sup> ratio of compound absolute area to internal standard absolute area

<sup>c</sup> ephemeral leaves from branches with no persistent leaves

<sup>d</sup> ephemeral leaves from branches with mature persistent leaves

<sup>e</sup> persistent leaves from the same branches as the L<sub>1</sub> ephemeral leaves

<sup>f</sup> compound identified by library search (software of the instrument)

<sup>g</sup> compound confirmed by running authentic chemical compound in the GC/MS

Levels of terpenoid components per type of leaf harvested from different parts on the plant were evaluated prior to the clipping experiment (Table 10). Samples F<sub>1</sub>, F<sub>2</sub>, and F<sub>3</sub> were extracted from ephemeral leaves, persistent leaves, and the persistent leaves from the crown of each plant, respectively, of the ten plants sampled for this experiment. The leaves from the crown had all the compounds present in both ephemeral and persistent leaves (Table 10), which could have been misleading in the evaluation of terpenoid content in the clipping of branches in the ephemeral leaf experiment. The leaves from the tip of each branch of sagebrush resemble ephemeral leaves in that they are not organized into fascicles as are the persistent leaves, however their terpenoid profile classified them as persistent leaves (Table 10). Therefore, the leaves from the crown of each branch were not included in the extractions of the ephemeral leaves during the clipping experiments.

#### Terpenoids Produced by the Ephemeral Leaves Harvested from Clipped Branches

The plants reacted sharply 48 hours after the initial clipping (22 June, 1997) by increasing their overall production of terpenoid compounds; most of the biggest increases were registered 15 days after the beginning of the experiment (Figures 45-49). There was a decrease of terpenoid levels below the initial level observed on day 1 when the clipping was stopped on days 30 and 54 (Figures 45-49, Table 11).

The largest increase was recorded in the quantity of major terpenoid compounds produced by ephemeral leaves of the clipped *A. t. vaseyana* plant (Figure 45). The terpenoid eucarvone increased to twice that of the initial quantity 15 days after the first clipping, from 28.7 µg/g of dried leaves to 47.7 µg/g. The quantity of the terpenoid

Table 10: Comparison of terpenoid component levels of different types of leaf from *Artemisia tridentata vaseyana* plants.

| Compound Name                | % Area <sup>a</sup>                |                                    |                                    | Peak Number <sup>e</sup> | Retention Time (min) <sup>f</sup> |
|------------------------------|------------------------------------|------------------------------------|------------------------------------|--------------------------|-----------------------------------|
|                              | Sample F <sub>1</sub> <sup>b</sup> | Sample F <sub>2</sub> <sup>c</sup> | Sample F <sub>3</sub> <sup>d</sup> |                          |                                   |
| Camphor (C <sup>g</sup> )    | 24.25                              | 38.60                              | 25.62                              | 6                        | 17.91                             |
| Eucalyptol (1,8-cineole) (C) | 19.60                              | 14.54                              | 20.49                              | 3                        | 14.33                             |
| Eucarvone (L <sup>h</sup> )  | 17.57                              | -                                  | 21.01                              | 9                        | 24.85                             |
| Saphranine (L)               | 8.30                               | 10.92                              | 9.67                               | 5                        | 17.27                             |
| Camphene (C)                 | -                                  | 8.49                               | 5.80                               | 2                        | 11.63                             |
| $\alpha$ -Pinene (L)         | -                                  | 3.68                               | 4.29                               | 1                        | 11.16                             |
| Bornyl-acetate (L)           | 2.99                               | 3.64                               | 3.34                               | 8                        | 21.53                             |
| (2H)-Naphthalenone (L)       | 2.82                               | 2.95                               | 2.34                               | 10                       | 25.50                             |
| Bicyclo[2.2.1]hept-2-ene (L) | 2.63                               | 3.37                               | 2.63                               | 4                        | 16.58                             |
| 1-Decyne (L)                 | 2.56                               | 2.34                               | -                                  | 7                        | 18.36                             |

<sup>a</sup> rough % of each compound in comparison to chromatographed compounds

<sup>b</sup> F<sub>1</sub>: ephemeral leaves completely mature (3.59 g of fresh leaves)

<sup>c</sup> F<sub>2</sub>: persistent leaves fully developed (3.59 g of fresh leaves)

<sup>d</sup> F<sub>3</sub>: persistent leaves from the crown (3.55 g of fresh leaves)

<sup>e</sup> rank of the compound when it appears from the point of injection

<sup>f</sup> time when the compound appears from the point of injection

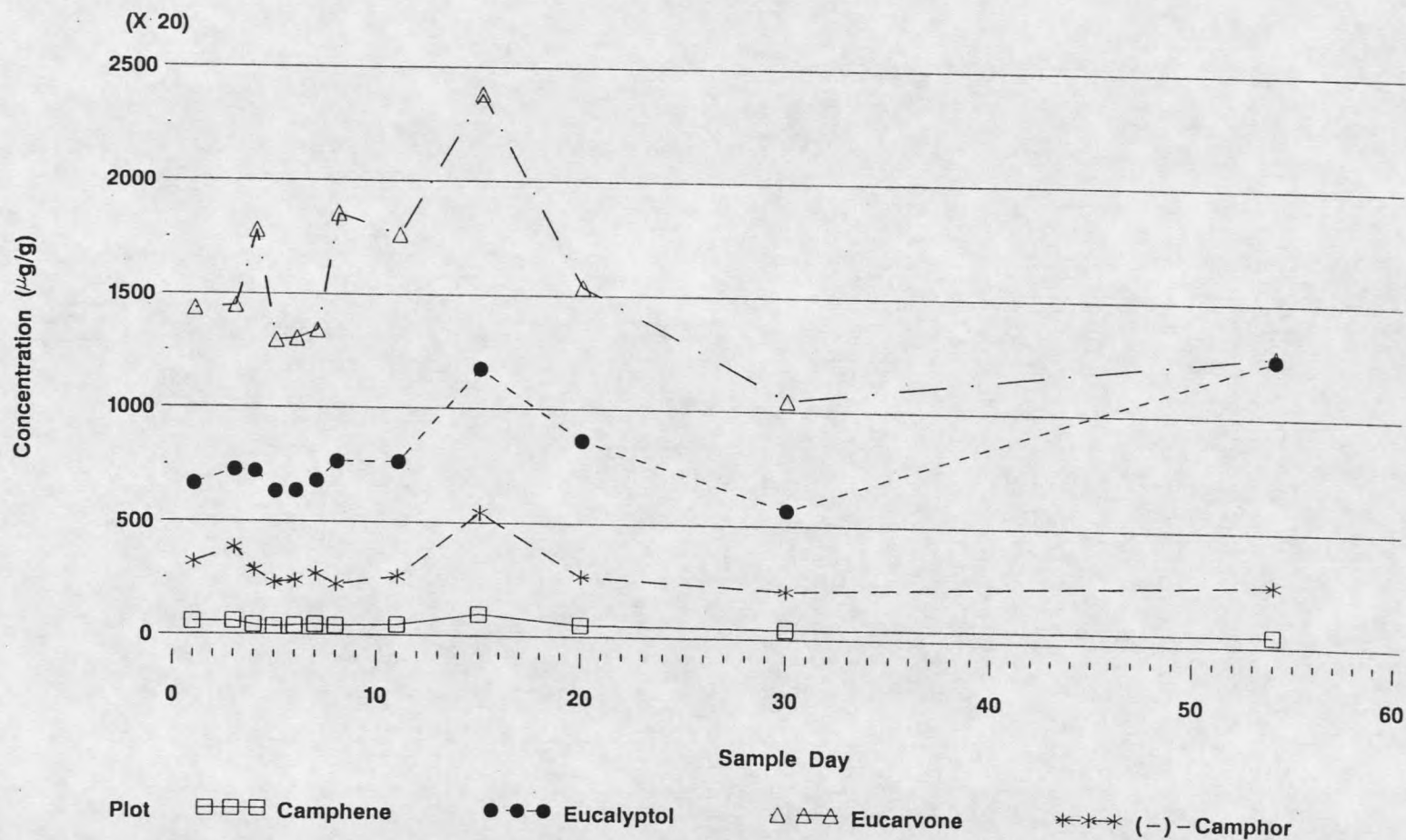
<sup>g</sup> compound confirmed by running authentic chemical compound in the GC/MS

<sup>h</sup> compound identified by library search (software of the instrument)



Figure 45

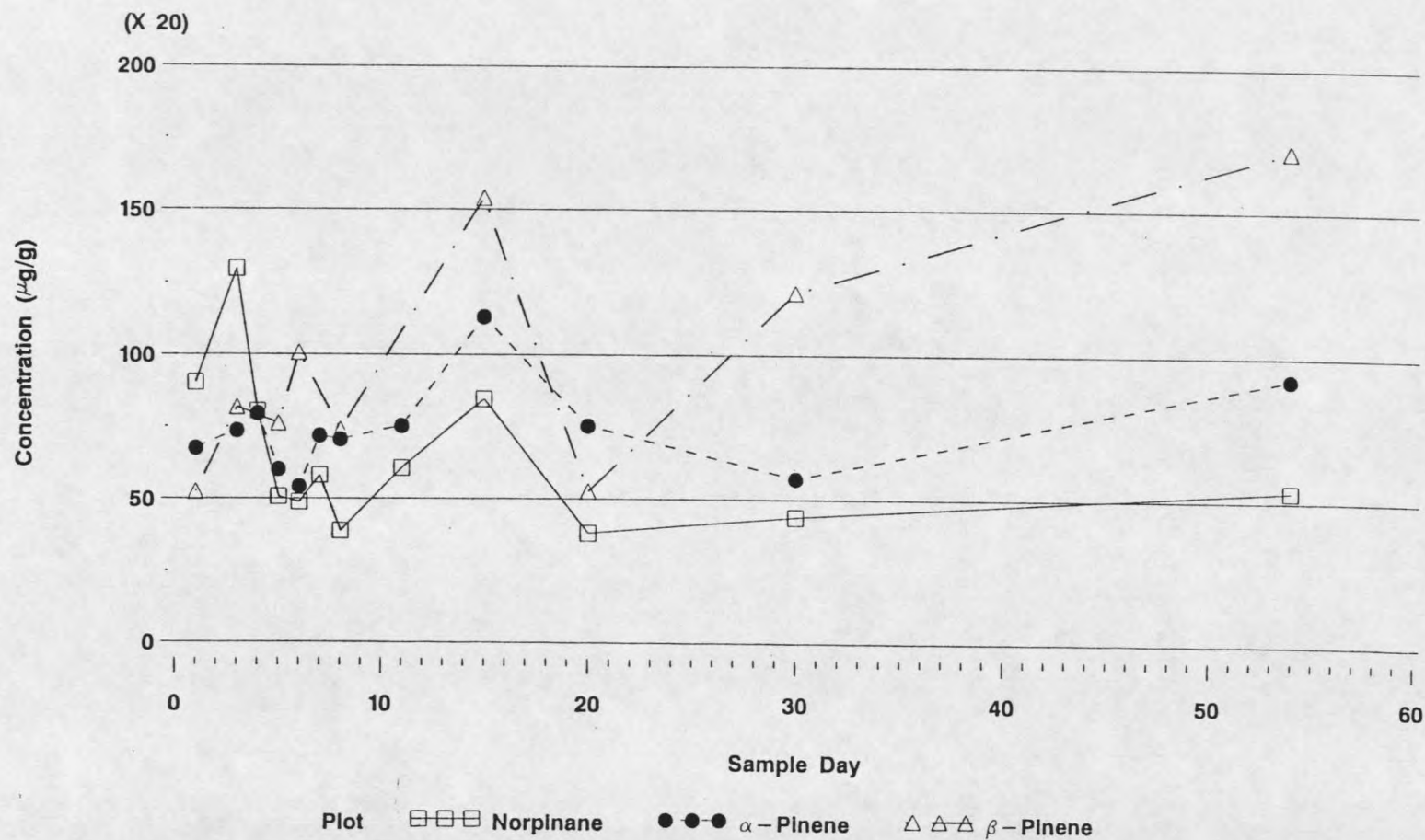
Terpenoids Produced by Ephemeral Leaves from Clipped  
Branches of *Artemisia tridentata* vaseyana



On Day 1, the first leaves were removed and analyzed; no clipping before the sampling. Leaves were then clipped and sampled on days 3, 4, 5, 6, 7, 8, 10, 30, and 54 after the original clipping. (X 20) = diluted by a factor of 20.

Figure 46

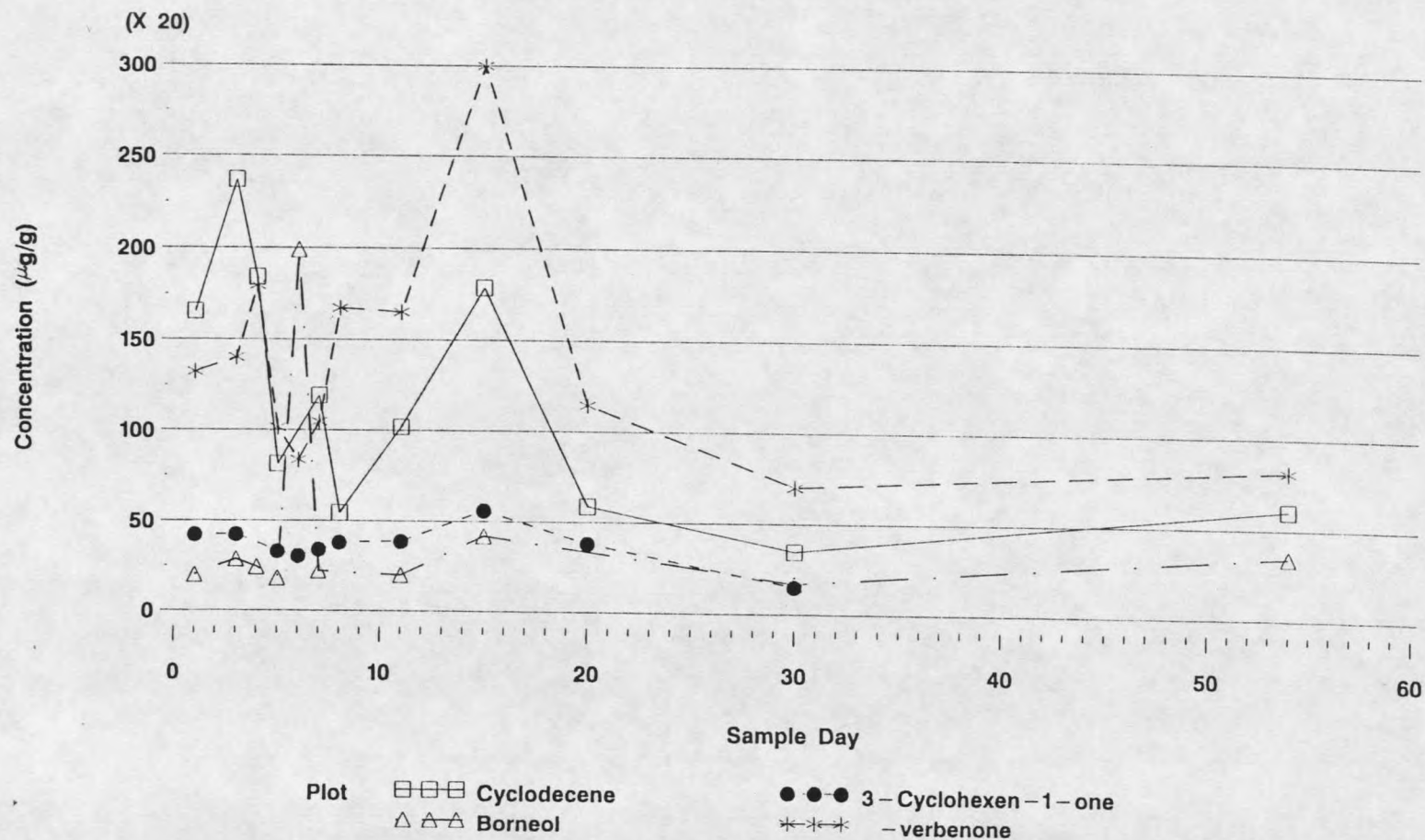
Terpenoids Produced by Ephemeral Leaves from Clipped  
Branches of *Artemisia tridentata vaseyana*



On Day 1, the first leaves were removed and analyzed; no clipping before the sampling. Leaves were then clipped and sampled on days 3, 4, 5, 6, 7, 8, 10, 30, and 54 after the original clipping. (X 20) = diluted by a factor of 20.

Figure 47

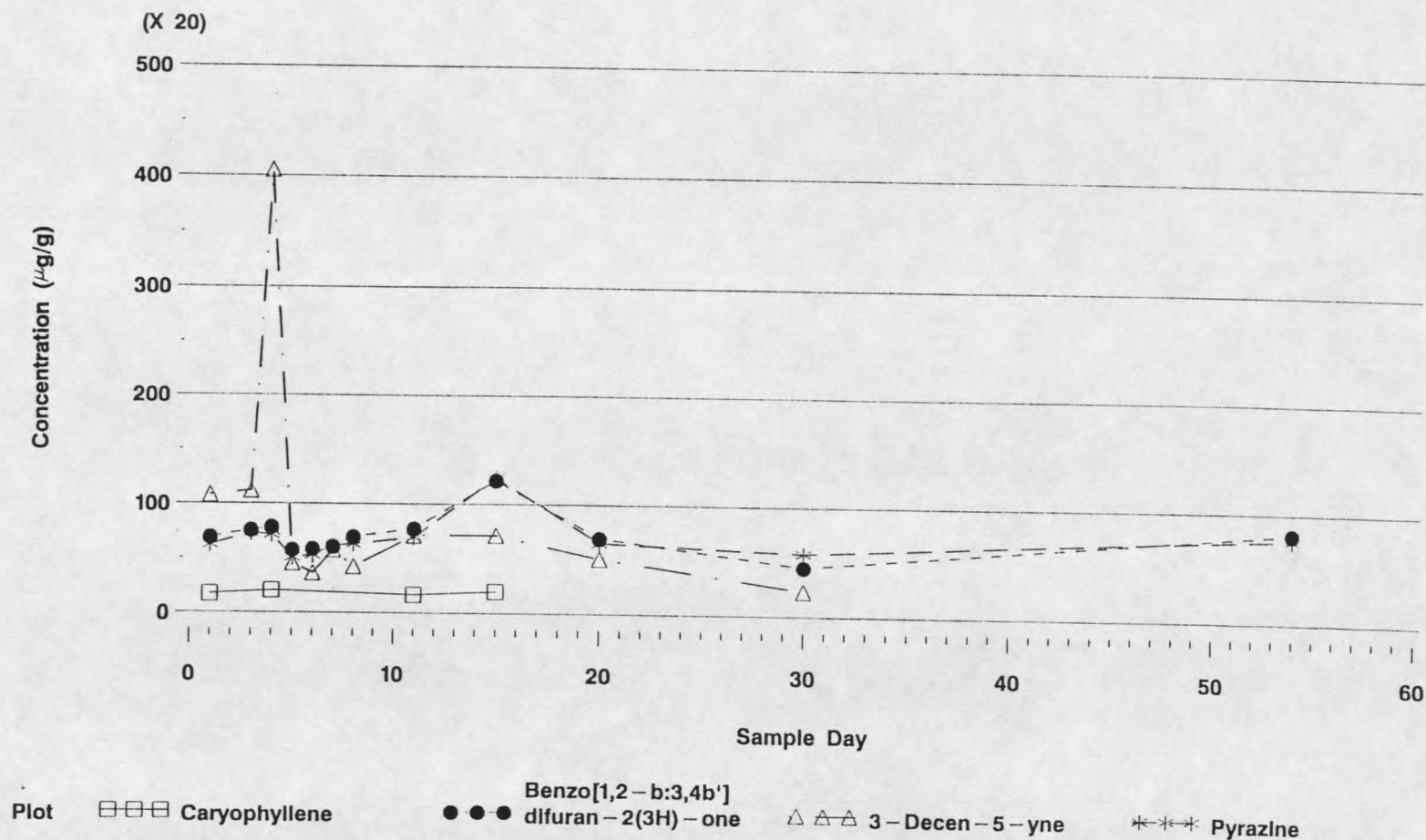
Terpenoids Produced by Ephemeral Leaves from Clipped  
Branches of *Artemisia tridentata vaseyana*



On Day 1, the first leaves were removed and analyzed; no clipping before the sampling. Leaves were then clipped and sampled on days 3, 4, 5, 6, 7, 8, 10, 30, and 54 after the original clipping. (X 20) = diluted by a factor of 20.

Figure 48

Terpenoids Produced by Ephemeral Leaves from Clipped  
Branches of *Artemisia tridentata vaseyana*

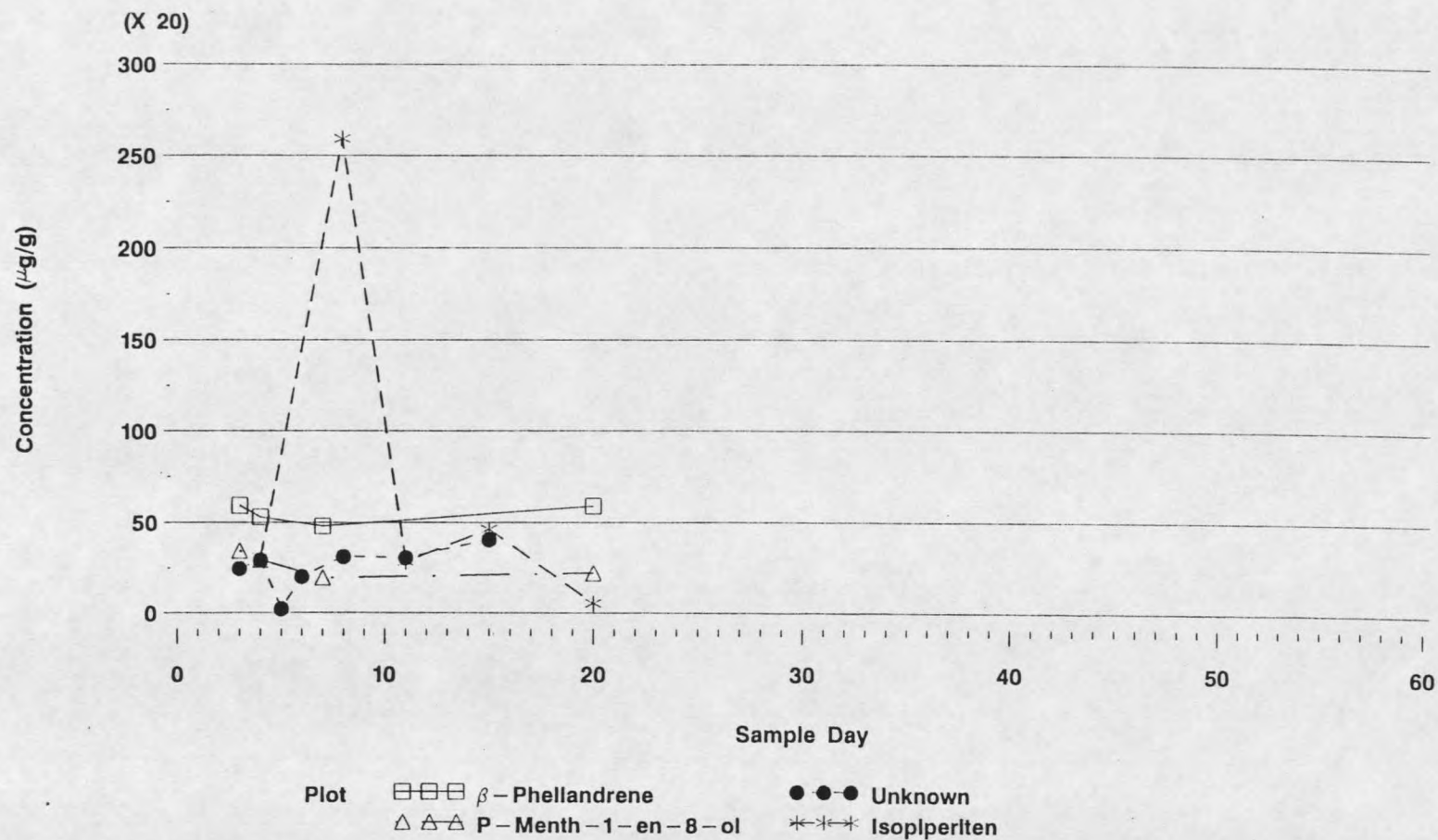


On Day 1, the first leaves were removed and analyzed; no clipping before the sampling. Leaves were then clipped and sampled on days 3, 4, 5, 6, 7, 8, 10, 30, and 54 after the original clipping. (X 20) = diluted by a factor of 20.



Figure 49

Terpenoids Produced by Ephemeral Leaves from Clipped  
Branches of *Artemisia tridentata vaseyana*



On Day 1, the first leaves were removed and analyzed; no clipping before the sampling. Leaves were then clipped and sampled on days 3, 4, 5, 6, 7, 8, 10, 30, and 54 after the original clipping. (X 20) = diluted by a factor of 20.



Table 11: Amount of terpenoid produced by the ephemeral leaves on day 1, 15, 30 and 54 during the clipping experiment. Quantitative analysis performed on the samples.

| Compound Name                                 | Concentration ( $\mu\text{g/g}$ dried leaves) (20 x) <sup>a</sup> |         |         |         |
|-----------------------------------------------|-------------------------------------------------------------------|---------|---------|---------|
|                                               | Day 1                                                             | Day 15  | Day 30  | Day 54  |
| Eucarvone (L <sup>b</sup> )                   | 1434.89                                                           | 2383.01 | 1047.40 | 1269.81 |
| Eucalyptol (C <sup>c</sup> )                  | 664.54                                                            | 1172.66 | 567.62  | 1251.87 |
| Camphor (C)                                   | 321.12                                                            | 545.17  | 211.65  | 266.85  |
| Cyclodecene (L)                               | 165.69                                                            | 178.78  | 34.60   | 60.33   |
| Verbenone (L)                                 | 132.31                                                            | 299.96  | 70.00   | 81.50   |
| 3-Decen-5-yne (L)                             | 108.27                                                            | 71.82   | 23.16   | -       |
| Norpinane (L)                                 | 90.35                                                             | 84.62   | 43.91   | 53.67   |
| Benzo[1,2-b:3,4-b'] difuran-2(3H)-one (L)     | 69.51                                                             | 122.08  | 45.19   | 81.56   |
| $\alpha$ -pinene (L)                          | 67.66                                                             | 113.24  | 57.32   | 92.50   |
| Pyrazine (L)                                  | 62.47                                                             | 124.57  | 57.88   | 76.40   |
| Camphene (C)                                  | 56.00                                                             | 94.64   | 42.87   | 48.68   |
| Bicyclo[4.1.0] heptane ( $\beta$ -pinene) (L) | 52.51                                                             | 154.09  | 121.67  | 171.21  |
| 3-Cyclohexen-1-one (L)                        | 42.34                                                             | 56.03   | 15.13   | -       |
| Borneol (L)                                   | 20.00                                                             | 41.89   | 16.87   | 34.32   |
| Caryophyllene (L)                             | 17.11                                                             | 19.92   | -       | -       |
| Isopiperiten (L)                              | -                                                                 | 46.25   | -       | -       |
| Unknown (L)                                   | -                                                                 | 40.57   | -       | -       |
| $\beta$ -Phyllandrene (L)                     | -                                                                 | -       | -       | -       |
| P-menth-1-en-8-ol (L)                         | -                                                                 | -       | -       | -       |

Day 1 - first clipping of the branches; leaves were extracted immediately. Day 15 - branches were clipped on days 1, 3, 4, 5, 6, 7, 8, 11 and 15; leaves harvested on day 15 were extracted immediately. Day 30 - branches were clipped on days 1, 3, 4, 5, 6, 7, 8, 11, 15, 21, and 30; leaves harvested on day 30 were extracted immediately. Day 54 - branches were clipped on days 1, 3, 4, 5, 6, 7, 8, 11, 15, 21, 30, and 54; leaves harvested on day 54 were extracted immediately. The analysis was performed on 17 October, 1997.

<sup>a</sup> diluted by a factor of 20

<sup>b</sup> compound identified by library search (software of the instrument)

<sup>c</sup> compound confirmed by running authentic chemical compound in the GC/MS

terpenoid eucarvone increased to twice that of the initial quantity 15 days after the first clipping, from 28.7  $\mu\text{g/g}$  of dried leaves to 47.7  $\mu\text{g/g}$ . The quantity of the terpenoid compound camphor increased from 6.5  $\mu\text{g/g}$  of dried leaves to 10.9  $\mu\text{g/g}$ , an 82% increase. The increases of the terpenoid compounds eucalyptol (1,8-cineole) and camphene were less dramatic and were 10% and 1%, respectively. The daily clipping of the plants for six consecutive days (days 3-8) did not show any dramatic change in the terpenoid quantities produced during those days. When a pause of 72 hours (from day 11 to day 15) was given to the plants, a sharp increase of terpenoid compounds was recorded on day 15 (Figure 45). The plants apparently needed time to replenish their terpenoid production resources.

The interruption of the clipping for five (day 20) and ten (day 30) days apparently caused a decrease in each terpenoid compound produced by the plants. The quantity of eucalyptol produced decreased below the level recorded for the first clipping (Figure 45), and was not due to loss of leaves because the leaves involved in the clipping experiment stayed green throughout the experiment.

A maximum increase of 50% was recorded 2 days post-clipping for the minor terpenoid compound norpinane and an increase of 200% for  $\beta$ -pinene and 100% for  $\alpha$ -pinene 15 days post-clipping (Figure 46).  $\beta$ -pinene levels increased to maximum even after the clipping was spaced for 10 and 24 days (Figure 46).

An increase of 50% was recorded from the second clipping (22 June) for the minor compound cyclodecene, an intermediate compound, and a 10% increase was found for the ketone 3-cyclohexen-1-one and borneol (Figure 47). An increase of 120% was observed

for verbenone 15 days post-clipping. Verbenone is an anti-aggregation pheromone for the pine bark beetle *Dendroctonus ponderosae* Hopkins (Figure 47) (Kostyk, 1992; Amman *et al.*, 1989).

The concentration of the minor compounds caryophyllene and (bicyclo[7.2.0]undec-4-ene) did not change during the course of the clipping experiment while benzo[1,2-b:3,4-b']difuran-2(3H)-one and pyrazine concentrations increased by 100% 15 days post-clipping (Figure 48). The minor compound 3-decen-5-yne increased 300% 4 days post-clipping. All four compounds decreased below their level at day one of the clipping experiment when the stimulus was spaced (Figure 48 and Table 11).

Components such as  $\beta$ -phellandrene, p-menth-1-en-8-ol, and isopiperiten were produced only after the branches were clipped the third time, and were no longer detectable after branch clipping was discontinued (Figure 49). Isopiperiten registered the largest increase among the newly produced compounds in the clipping experiment. A cyclical increase was observed on day 5 post-clipping (400%), decreased sharply on day 12 and increased slightly on day 15 (Figure 49).

#### Comparison of the Terpenoids Produced by the Ephemeral Leaves Before and After the Clipping Experiment.

Terpenoid levels before (Table 8) and after the clipping experiment (Table 12) show the highest level at day 1 of the clipping experiment. The extracts obtained from the clipping experiment were diluted by a factor of 20 prior to analysis while samples analyzed before the clipping experiment were diluted by a factor of 5, because they were less concentrated, to obtain the same profile of peak area chromatogram. This result was not a

Table 12: Amount of terpenoids produced by the ephemeral leaves on day 1, 15, 30 and 54 during the clipping experiment.

| Compound Name                                 | Relative Area (diluted 20 x) <sup>a</sup> |        |        |        |
|-----------------------------------------------|-------------------------------------------|--------|--------|--------|
|                                               | Day 1                                     | Day 15 | Day 30 | Day 54 |
| Eucarvone (L <sup>b</sup> )                   | 5.824                                     | 9.673  | 4.253  | 5.155  |
| Eucalyptol (C <sup>c</sup> )                  | 2.737                                     | 4.858  | 2.333  | 5.188  |
| Camphor (C)                                   | 1.387                                     | 2.328  | 0.927  | 1.159  |
| Cyclodecene (L)                               | 0.673                                     | 0.727  | 0.141  | 0.246  |
| Verbenone (L)                                 | 0.538                                     | 1.218  | 0.285  | 0.332  |
| 3-Decen-5-yne (L)                             | 0.440                                     | 0.292  | 0.095  | -      |
| Norpinane (L)                                 | 0.368                                     | 0.344  | 0.179  | 0.219  |
| Benzo[1,2-b:3,4-b']difuran-2(3H)-one (L)      | 0.283                                     | 0.496  | 0.184  | 0.332  |
| $\alpha$ -pinene (L)                          | 0.276                                     | 0.461  | 0.234  | 0.376  |
| Pyrazine (L)                                  | 0.254                                     | 0.507  | 0.236  | 0.311  |
| Camphene (C)                                  | 0.214                                     | 0.361  | 0.153  | 0.186  |
| Bicyclo[4.1.0] heptane ( $\beta$ -pinene) (L) | 0.214                                     | 0.626  | 0.495  | 0.696  |
| 3-Cyclohexen-1-one (L)                        | 0.173                                     | 0.228  | 0.062  | -      |
| Borneol (L)                                   | 0.082                                     | 0.178  | 0.069  | 0.140  |
| Caryophyllene (L)                             | 0.070                                     | 0.082  | -      | -      |
| Isopiperiten (L)                              | -                                         | 0.189  | -      | -      |
| Unknown (L)                                   | -                                         | 0.166  | -      | -      |
| $\beta$ -Phyllandrene (L)                     | -                                         | -      | -      | -      |
| P-menth-1-en-8-ol (L)                         | -                                         | -      | -      | -      |

Day 1 - first clipping of the branches; leaves were extracted immediately. Day 15 - branches were clipped on days 1, 3, 4, 5, 6, 7, 8, 11 and 15; leaves harvested on day 15 were extracted immediately. Day 30 - branches were clipped on days 1, 3, 4, 5, 6, 7, 8, 11, 15, 21, and 30; leaves harvested on day 30 were extracted immediately. Day 54 - branches were clipped on days 1, 3, 4, 5, 6, 7, 8, 11, 15, 21, 30, and 54; leaves harvested on day 54 were extracted immediately. The analysis was performed on 17 October, 1997.

<sup>a</sup> diluted by a factor of 20

<sup>a</sup> compound identified by library search (software of the instrument)

<sup>b</sup> compound confirmed by running authentic chemical compound in the GC/MS

surprise because the ephemeral leaves extracted before the clipping experiment were produced in winter (February to April) while the ephemeral leaves involved in the clipping experiment were produced in spring (April to June). Sagebrush terpenoids are plant defensive compounds (constitutive and induced) against herbivorous pests (Wiens *et al.*, 1991; Langenheim, 1994); the ephemeral leaves might produce more volatiles in spring, when herbivorous pests are likely to be present, than in winter.

#### Terpenoids Produced by the Ephemeral Leaves on Defoliated Branches

Branch defoliation affected the quantity and type of terpenoid compounds produced in the ephemeral leaves. All these compounds increased to day 7, and decreased when the defoliation experiment was spaced to day 31 (Table 13, Figures 50-53). There was a 55% increase in the quantity of the major compounds eucarvone, eucalyptol, camphor and camphene (Figure 50). The maximum increase occurred seven days after the beginning of the defoliation procedure.

The minor components increased by 100% except for the compound bicyclo[4.1.0] heptane (Figure 51). Bicyclo[4.1.0] heptane is an intermediate product for camphor (Erman, 1985) and decreased quantitatively on day 4, 5, and 6 during the defoliation period. It thereafter increased sharply and showed the same pattern from day 10 onward (Figure 51). The variations found with the compound bicyclo[4.1.0] heptane were defoliation independent. This response is similar to that of an intermediate compound with a turn-over period of 72 hours (Figure 51). Bicyclo[4.1.0] heptane is also an intermediate compound for the terpenoids eucarvone and 3-carene (Erman, 1985).



Table 13: Amount of terpenoid produced by the ephemeral leaves harvested from defoliated branches of *Artemisia tridentata vaseyana* on day 1, 7, 31 and 54.

| Compound Name                           | Concentration ( $\mu\text{g/g}$ of dried leaves) ( $\times 20$ ) <sup>a</sup> |         |         |         |
|-----------------------------------------|-------------------------------------------------------------------------------|---------|---------|---------|
|                                         | Day 1                                                                         | Day 7   | Day 31  | Day 54  |
| Eucarvone (L <sup>b</sup> )             | 564.330                                                                       | 934.883 | 589.205 | 705.002 |
| Eucalyptol (C <sup>c</sup> )            | 406.231                                                                       | 559.351 | 583.098 | 705.152 |
| Camphor (C)                             | 394.132                                                                       | 593.695 | 504.094 | 499.227 |
| Bicyclo[4.1.0.] heptane (L)             | 91.503                                                                        | 106.141 | 56.781  | 70.954  |
| Camphene (C)                            | 63.285                                                                        | 88.664  | 71.642  | 73.716  |
| Benzo[1,2-b:3,4-b']difuran-2(3H)one (L) | 39.503                                                                        | 62.184  | 30.738  | 33.063  |
| Pyrazine (L)                            | 34.829                                                                        | 57.916  | 27.043  | 47.588  |
| $\alpha$ -Pinene (L)                    | 27.179                                                                        | 50.270  | -       | 34.302  |
| Verbenone (L)                           | 20.059                                                                        | 69.799  | -       | 26.557  |
| 3-Cyclohexen-1-one (L)                  | 19.232                                                                        | 33.107  | 31.221  | -       |
| Norpinane (L)                           | -                                                                             | 53.605  | -       | -       |
| Cyclodecene (L)                         | -                                                                             | 46.384  | -       | -       |
| Unknown (L)                             | -                                                                             | 19.538  | 20.345  | -       |
| 3-Decen-5-yne (L)                       | -                                                                             | -       | -       | -       |

Day 1 - defoliated for the first time; leaves were extracted immediately. Day 7 - defoliated on days 1, 3, 4, 5, 6, and 7; leaves obtained on day 7 were extracted immediately. Day 31 - defoliated on days 1, 3, 4, 5, 6, 7, 8, 10, and 31; leaves obtained on day 31 were extracted immediately. Day 54 - defoliated on days 1, 3, 4, 5, 6, 7, 8, 9, 10, 31, and 54; the leaves obtained on day 54 were extracted immediately. The analysis was performed on 17 October, 1997.

<sup>a</sup> samples diluted by a factor of 20

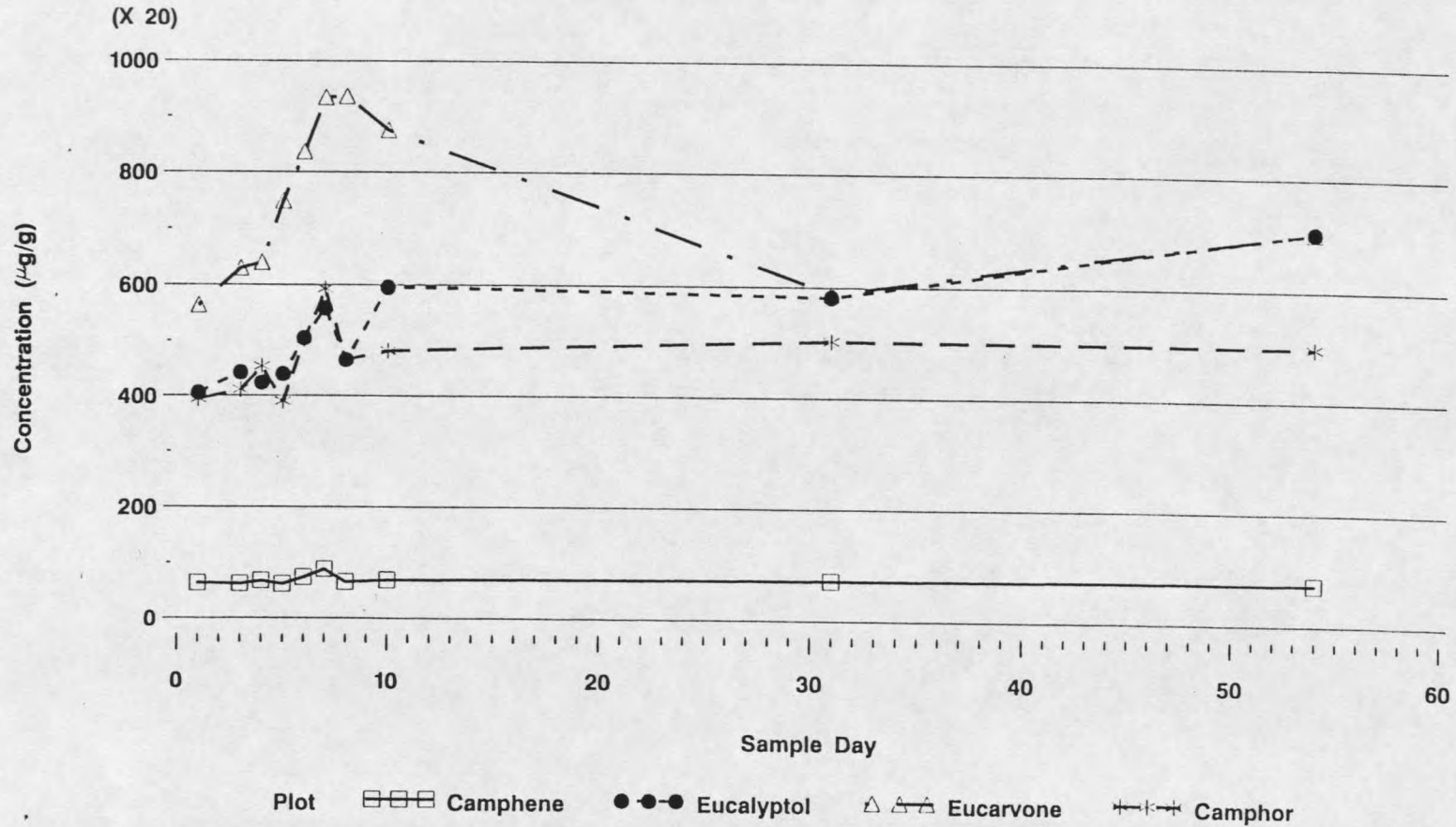
<sup>b</sup> compound identified by library search (software of the instrument)

<sup>c</sup> compound confirmed by running authentic chemical compound in the GC/MS

Terpenoid compounds such as verbenone, benzo[1,2-b:3,4-b']difuran-2(3h)-one and pyrazine were minor components that sharply decreased when the experiment was spaced for 31 days. Benzo[1,2-b:3,4-b']difuran-2(3h)-one and pyrazine decreased below

Figure 50

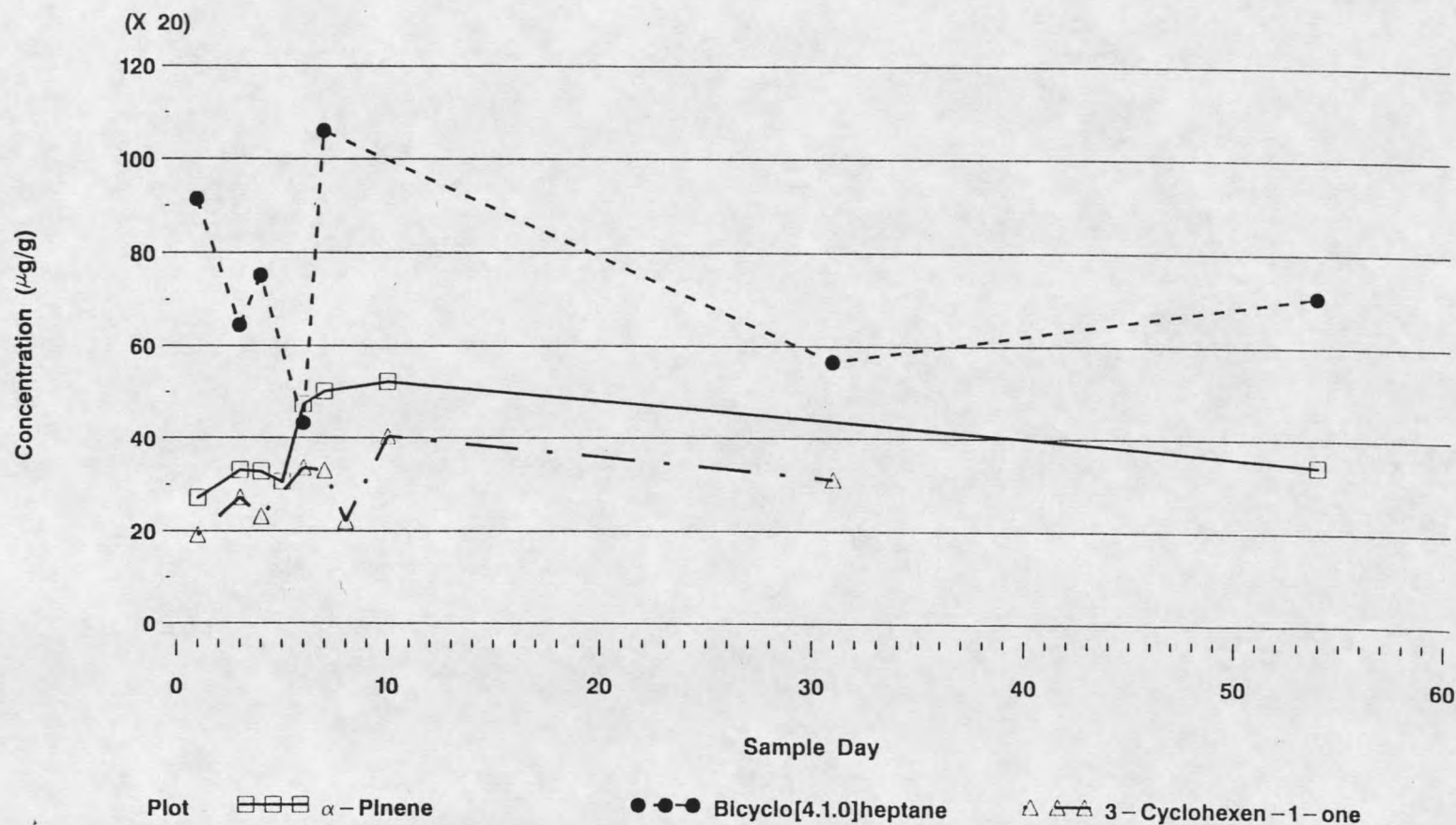
Terpenoids Produced by Ephemeral Leaves from Defoliated  
Branches of *Artemisia tridentata vaseyana*



On Day 1, the first leaves were removed and analyzed; no defoliation before this sampling. The branches were defoliated and the leaves were extracted on days 3, 4, 5, 6, 7, 8, 10, 31 and 54 after the original defoliation. (X 20) = diluted by a factor of 20.

Figure 51

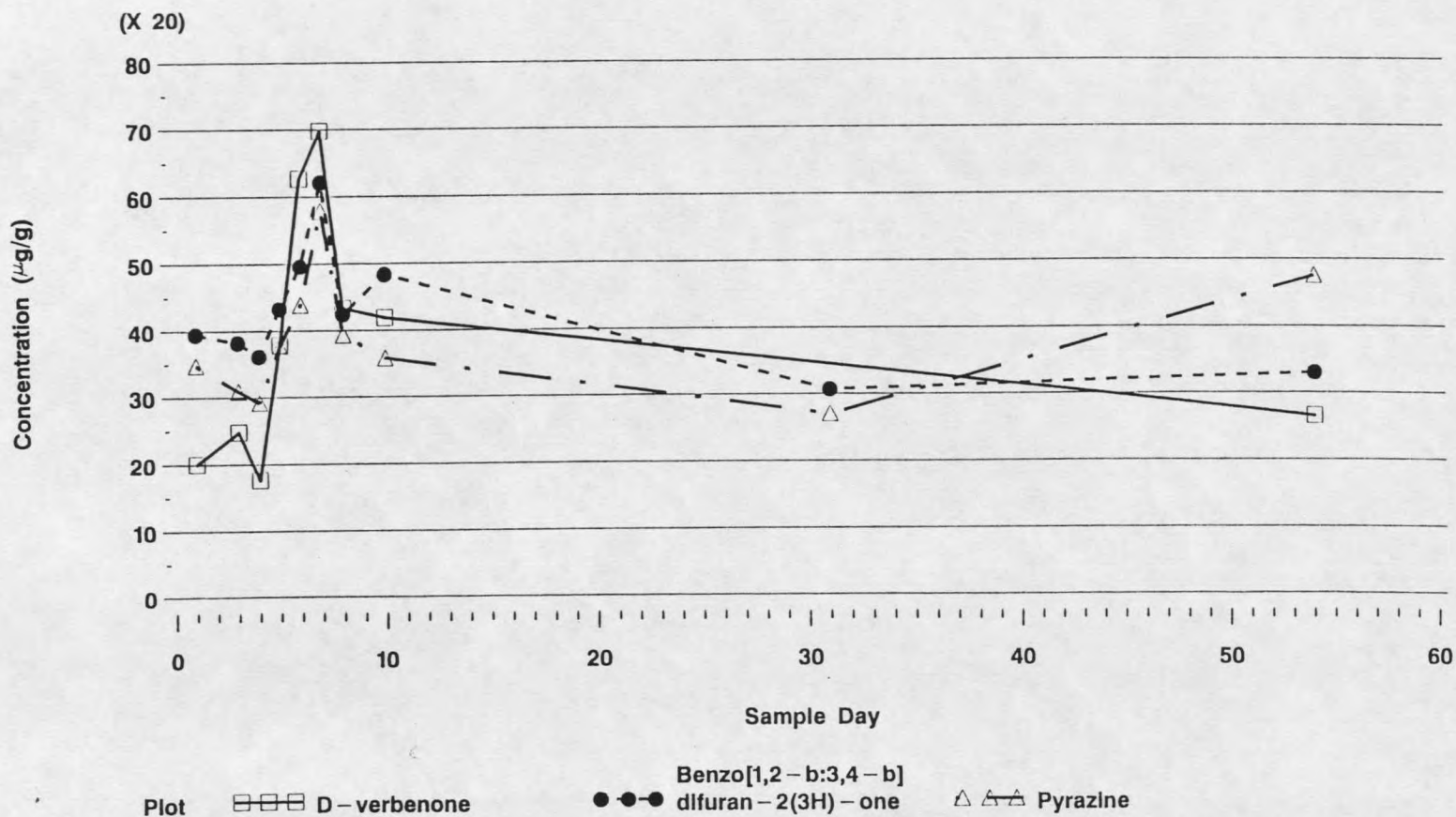
Terpenoids Produced by Ephemeral Leaves from Defoliated  
Branches of *Artemisia tridentata vaseyana*



On Day 1, the first leaves were removed and analyzed; no defoliation before this sampling. The branches were defoliated and the leaves were extracted on days 3, 4, 5, 6, 7, 8, 10, 31 and 54 after the original defoliation. (X 20) = diluted by a factor of 20.

Figure 52

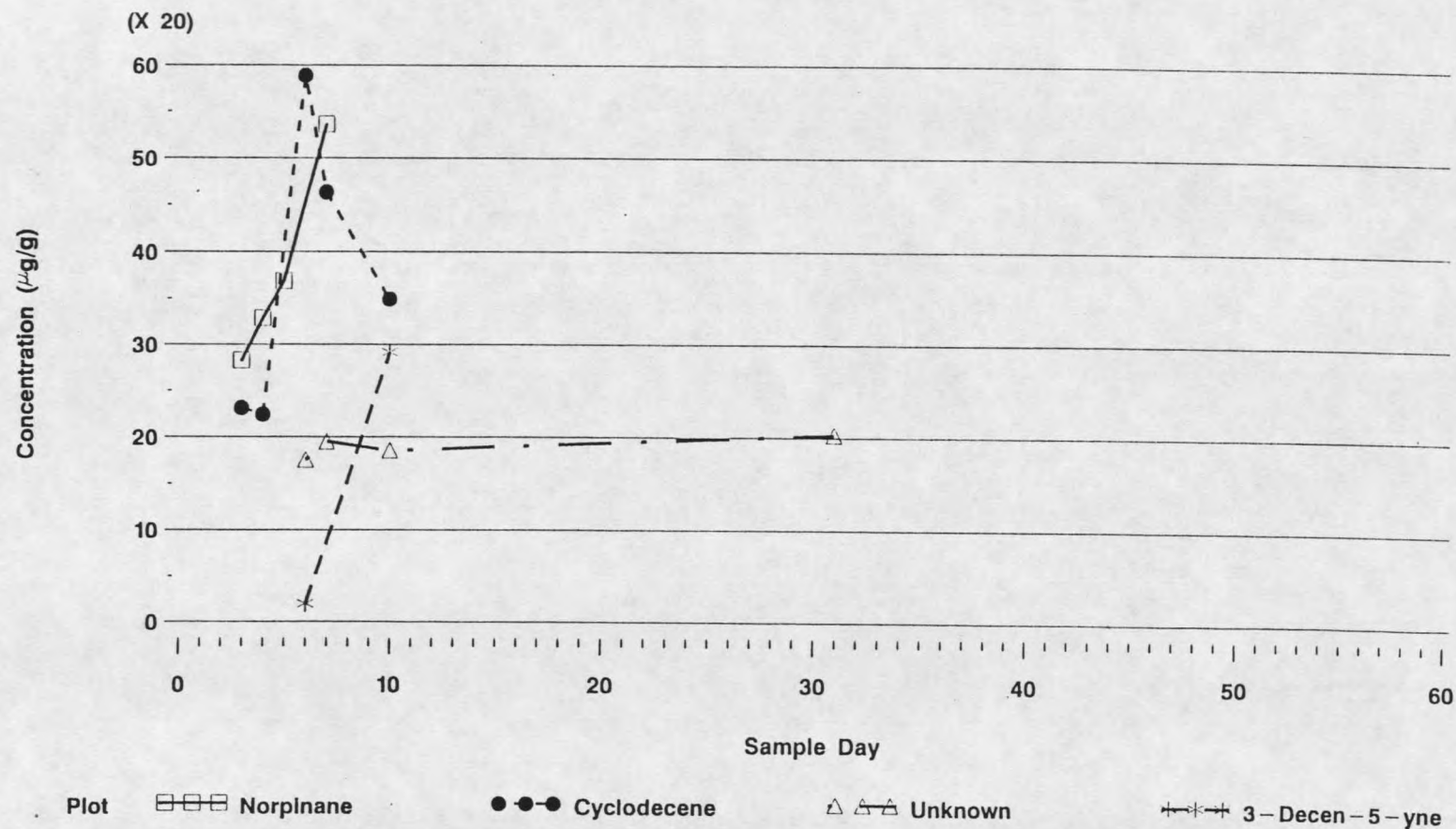
Terpenoids Produced by Ephemeral Leaves from Defoliated  
Branches of *Artemisia tridentata vaseyana*



On Day 1, the first leaves were removed and analyzed; no defoliation before this sampling. The branches were defoliated and the leaves were extracted on days 3, 4, 5, 6, 7, 8, 10, 31 and 54 after the original defoliation. (X 20) = diluted by a factor of 20.

Figure 53

Terpenoids Produced by Ephemeral Leaves from Defoliated  
Branches of *Artemisia tridentata* vaseyana



On Day 1, the first leaves were removed and analyzed; no defoliation before this sampling. The branches were defoliated and the leaves were extracted on days 3, 4, 5, 6, 7, 8, 10, 31 and 54 after the original defoliation. (X 20) = diluted by a factor of 20.



the level registered on day one of the experiment while verbenone did not decrease to the low level observed on day one of the experiment. Verbenone increased 250% while benzo[1,2-b:3,4-b]difuran-2(3h)-one and pyrazine registered a 50% increase.

Terpenoid compounds such as norpinane, 3-cyclodecene, 3-decen-5-yne, and an unknown compound (the library search could not identify this compound) were produced in the ephemeral leaves as a response to the defoliation of the branches. Norpinane is an intermediate compound for  $\alpha$ -pinene (Erman, 1985), and may have been the reason for the dramatic increase in  $\alpha$ -pinene on day 7 of the defoliation experiment (Figure 53).

#### Comparison of the Terpenoids Produced Before and After the Defoliation Experiment

Levels of terpenoid produced before (Table 8) and after the defoliation experiment (Table 14) were compared. Chemical components such as camphor and eucalyptol (major components) were produced both before and after the defoliation experiment. Camphene, a minor component in the post-treatment, is a major component pre-treatment. It would be very difficult to compare the levels of terpenoids for reasons expressed earlier, therefore, the pre-treatment experiment for controls is redesigned in Chapter 4.

#### Variations in Terpenoid Compounds Produced by Sibling Plants

The number of terpenoid compounds produced per leaf type varies for each plant. The six plants in the defoliation experiment had fewer compounds than the plants in the clipping experiment. Norpinane, cyclodecene, 3-decen-5-yne considered as novel compounds in the defoliation experiment (Figure 53) were already produced on day 1 by

Table 14: Amount of terpenoid produced by the ephemeral leaves harvested from defoliated branches of *Artemisia tridentata vaseyana* on day 1, 7, 31 and 54.

| Compound Name                           | Relative Area (diluted 20 x) <sup>a</sup> |       |        |        |
|-----------------------------------------|-------------------------------------------|-------|--------|--------|
|                                         | Day 1                                     | Day 7 | Day 31 | Day 54 |
| Eucarvone (L <sup>b</sup> )             | 2.291                                     | 3.795 | 2.392  | 2.862  |
| Eucalyptol (C <sup>c</sup> )            | 1.659                                     | 2.298 | 2.397  | 2.907  |
| Camphor (C)                             | 1.694                                     | 2.532 | 2.156  | 2.135  |
| Bicyclo[4.1.0.] heptane (L)             | 0.372                                     | 0.432 | 0.231  | 0.289  |
| Camphene (C)                            | 0.242                                     | 0.338 | 0.274  | 0.281  |
| Benzo[1,2-b:3,4-b']difuran-2(3H)one (L) | 0.161                                     | 0.253 | 0.126  | 0.135  |
| Pyrazine (L)                            | 0.142                                     | 0.236 | 0.111  | 0.194  |
| $\alpha$ -Pinene (L)                    | 0.111                                     | 0.205 | -      | 0.140  |
| Verbenone (L)                           | 0.082                                     | 0.284 | -      | 0.109  |
| 3-Cyclohexen-1-one (L)                  | 0.079                                     | 0.135 | 0.128  | -      |
| Norpinane (L)                           | -                                         | 0.219 | -      | -      |
| Cyclodecene (L)                         | -                                         | 0.189 | -      | -      |
| Unknown (L)                             | -                                         | 0.080 | 0.084  | -      |
| 3-Decen-5-yne (L)                       | -                                         | -     | -      | -      |

Day 1 - defoliated for the first time; leaves were extracted immediately. Day 7 - defoliated on days 1, 3, 4, 5, 6, and 7; leaves obtained on day 7 were extracted immediately. Day 31 - defoliated on days 1, 3, 4, 5, 6, 7, 8, 10, and 31; leaves obtained on day 30 were extracted immediately. Day 54 - defoliated on days 1, 3, 4, 5, 6, 7, 8, 9, 10, 31, and 54; the leaves obtained on day 54 were extracted immediately. The analysis was performed on 17 October, 1997.

<sup>a</sup> samples diluted by a factor of 20

<sup>b</sup> compound identified by library search (software of the instrument)

<sup>c</sup> compound confirmed by running authentic chemical compound in the GC/MS

clipped plants (Figures 45-48). Compounds such as borneol and  $\beta$ -pinene, present in the clipping experiment (Figures 46-48), were not observed in the extracts of the leaves

harvested during the defoliation experiment. The plants in the clipping experiment had 19

compounds (Table 11), while the ones in the defoliation experiment had 14 (Table 13). Of the five additional terpenoid components observed in the clipping experiment extracts, three ( $\beta$ -phellandrene, p-menth-1-en-1-one and isopiperiten) were new compounds for both sets of plants (used in defoliation and clipping experiments) and might have been induced by the clipping of the branches (Figure 49).

### Discussion

Terpenoids with 10 atoms of carbons ( $C_{10}$ ) are monoterpenoids,  $C_{15}$  compounds are sesquiterpenoids,  $C_{20}$  (di-),  $C_{30}$  (tri-),  $C_{40}$  (tetra-) and  $C > 40$  are poly-terpenoids. Terpenoids characteristically occur in plants as mixtures of compounds; the  $C_{10}$  and  $C_{15}$  compounds are referred to as "lower terpenoids" when they occur together and are commonly called "essential oils". On the other hand, all compounds containing 20 carbons and above are often referred to as "higher terpenoids" (Langenheim, 1994). Mono- and sesquiterpenoids are considered volatile, whereas others, such as di- and triterpenoids generally are non-volatile and the term "resin" refers to a combination of volatile and non-volatile terpenoids (Gershenzon and Croteau, 1991; Langenheim, 1994). Compounds of the ephemeral leaves were mostly volatile (monoterpenoids and sesquiterpenoids) because the chemical formula obtained through the library search showed them to be  $C_{10}$  or  $C_{15}$  compounds. No diterpenes or tetraterpenoids were present in the extracts of the ephemeral leaves used in the clipping experiment. The life span of the ephemeral leaves is three months. Such fast growing leaves cannot allocate resources to producing diterpenoids and tetraterpenoids, which require an enormous amount of

energy input (Gershenson *et al.*, 1989). The sagebrush plant can produce multiple types of terpenoid compounds, depending on the type of environmental stimulus encountered by the plant (Welch and McArthur, 1981). The summer ephemeral leaves produced one third fewer terpenoid compounds and different compounds before treatments in normal conditions than did the persistent winter leaves (Tables 5-8). Comparison of the number of terpenoid compounds produced by each type of leaf from the same plant clearly showed that persistent leaves always had more minor terpenoid components than ephemeral leaves. Such patterns were consistently found in all the extracts analyzed in these experiments, and are supported by many previously published documents on the analysis of the persistent leaves of *A. t. vaseyana* (Welch and McArthur, 1981; Wilt *et al.*, 1992; Weber *et al.*, 1994).

Both types of leaf had the same major terpenoid components which were camphor, eucalyptol, and eucarvone. The minor components, such as camphene, were common to both types of leaf (Tables 5, 6 and 7). Chemical dimorphism of the ephemeral-persistent leaves found in the big mountain sagebrush is not a new phenomenon. It is also found in the juvenile-adult leaves of some gymnosperm plants (Hayman *et al.*, 1986). Adams and Hagerman (1976) found significant differences in monoterpene and sesquiterpene levels between juvenile and adult foliage of *Juniperus scopulorum* L. The plants in the clipping experiments produced fewer persistent leaves than the plants in rows 2-4 (Table 1) which were not clipped and 95% of the plants involved in the clipping and defoliation experiments had died by the end of the experiment.

### Differences in the Terpenoids Produced by Sibling Plants

Inter- and intra-population variation of monoterpenoids reflect at least the following four major variables: (1) individual genetic divergence; (2) the broad environmental conditions of the population and the conditions prevailing at the time of the sampling; (3) the ontogenetic and phenological stages; and (4) the techniques of extraction and analysis (Burbott and Loomis, 1967; Loomis, 1969; Adams, 1970; Gershenson and Croteau, 1991). Different profiles of terpenoids could be produced by the ephemeral leaves of sibling plants under the same environmental conditions, depending upon the individual genetic variation of each plant. The plants used in the clipping experiment produced five terpenoid compounds more than the plants used in the defoliation experiment on day one of the experiment even though they were grown under the same environmental conditions. Of the compounds observed in the defoliation experiment, three out of four were constitutive terpenoids that were already present in the plants used in the clipping experiment (Figure 46). The compounds norpinane, 3-bromo-cyclodecene, and 3-decen-5-yne were considered new induced compounds because they were not found in the first extract obtained in the defoliation experiment, and seem to have been produced as a reaction to the defoliation of the branches. The later compounds were produced in a non-stressed state by the plants used in the clipping experiment. Volatiles produced by defoliated plants might have induced the production of these compounds in the set of plants used in the clipping experiment, because the defoliation experiment was carried out first while both sets of plants were kept in the weighing room during the experiment.



### Terpenoids Produced by the Ephemeral Leaves After Branch Clipping

The ephemeral leaves of the mountain big sagebrush, *A. t. vaseyana*, involved in the clipping and defoliation experiments produced basically the following four major monoterpenoids: camphene, camphor, eucalyptol, and eucarvone on day one of the experiment (Table 11). These comprised 45-92% of the essential oil produced in the glands. Ephemeral leaves extracted before the clipping or defoliation experiment possessed the same major components (Table 7). The others were minor components and could have been either transitional products or synergetic compounds produced to increase the activity of the major components (Langenheim, 1994).

There is considerable variety in the nature of terpenoid defenses induced by herbivory. In some cases, herbivore damage causes an increase in the concentration of terpenoids already present in the plant, whereas, in other cases, substances appear that are undetectable before herbivory (Akazawa *et al.*, 1960). Both cases were encountered in the clipping experiments carried out on the ephemeral leaves of *A. t. vaseyana* (Figures 45-53). The major terpenoid compounds eucarvone and camphor registered major increases during the clipping and defoliation experiments (Figures 45 and 50). The terpenoids camphor and eucarvone produced on 20 June, 1997, increased, over 15 days of clipping, from 6.4 mg/g to 10.9 mg/g for camphor, a 70% increase, and from 28.7 mg/g to 47.66 mg/g for eucarvone, a 66% increase. The induction time for herbivore-caused terpenoid increases ranges from less than an hour to more than a year with the most rapid response reported from the species of *Cucurbita* (Carroll and Hoffman, 1980; Cook and

Hain, 1987) and the slowest are trees such as the oak tree (Gershenson and Croteau, 1991). It was not surprising that the compound eucalyptol had increased only from 13.29 mg/g to 23.45 mg/g (76%) in the clipping experiment. Eucalyptol acts mostly as a feeding deterrent (Verma and Meloan, 1981) and as an herbicide (Duke *et al.*, 1988). The herbicide cinmethylin is a close analog of eucalyptol, and is a potent growth inhibitor (Duke *et al.*, 1988). Muller and Muller (1964) found that among the volatiles produced by *Salvia* species, camphor and cineole are the most toxic to root growth of seedlings. Eucalyptol acting as a repellent and herbicide is a defense chemical. It can defend the plant from browsing or mechanistic wounding. The increase in the quantity of camphor produced as a reaction to clipping is predictable because the response is the same as that caused by herbivory. Camphor or toxaphene, the chlorinated analogue, was used for a number of years as an insecticide, and, at one time, it topped all other insecticides in sales in the U.S. (Green *et al.*, 1987), before its use was limited by the Environmental Protection Agency. Several specific chlorinated camphors were found to be more highly insecticidal than others (Savage and Casida, 1979). Eucarvone is a naturally occurring monocyclic terpene. However, due to its formation from carenone, this terpenoid is also converted to 3-carene (Erman, 1985). The increase in monoterpene biosynthesis after wounding of the grand fir stem is the result of the inducible activities of 3-carene,  $\beta$ -phellandrene, and  $\alpha$ - and  $\beta$ -pinene synthase (Gijzen *et al.*, 1991).

There was also an increase of the minor compound bicyclo[4.1.0]heptane ( $\beta$ -pinene), a precursor of the terpenoids 3-carene and eucarvone (Erman, 1985). This compound increased after the first clipping with a turn over period of 15 days (Figure 45).

Clipping of the branches caused the production of new compounds such as  $\beta$ -phellandrene, p-menth1-en-8-ol, and isopiperiten (Figure 53). Isopiperiten is an intermediate product for 3-carene, which explains the increased production of 3-carene in the ephemeral leaves. The increased production of 3-carene, the new secretion of  $\beta$ -phellandrene in combination with the increase of  $\alpha$ -pinene and norpinane, an intermediate for  $\beta$ -pinene, showed that *A. t. vaseyana* was reacting in a manner similar to that of the pine tree trunk when it is attacked by bark beetles (Gijzen *et al.*, 1991).

The clipping of branches of *A. t. vaseyana* apparently provoked the ephemeral leaves to increase production of the major compounds 3-carene, 1,8-cineole and camphor, the minor compounds  $\alpha$ -pinene and norpinane, and the secretion of components such as  $\beta$ -phellandrene and isopiperiten. This reaction, which was also observed in pine trees (a gymnosperm) attacked by bark beetles (Lewinsohn *et al.*, 1993), might be a generalized pattern of defense for higher plants that contain terpenoids.

#### Terpenoids Produced by Ephemeral Leaves After Defoliation of the Branches

The defoliation of the branches of *A. t. vaseyana* generated a steady increase of terpenoid components which reached their maximum at day 7 post-defoliation. The increase was 60% for the major components eucarvone, eucalyptol, and camphor (Figure 52). The increase in minor components was 200% for verbenone, 60% for 3-cyclohexene-1-ol,  $\alpha$ -pinene, benzo[1,2-b:3,4-b']difuran-2(3H)-one, and pyrazine (Figures 45-46). Verbenone is an  $\alpha$ -pinene oxidation product (Hughes, 1974) and acts as anti-aggregation pheromone for some species of bark beetle. The compound bicyclo[4.1.0]heptane showed

a turn-over period of 72 hours (Figure 51), because it is the period of transformation of this compound into eucarvone. New compounds such as norpinane, a precursor of  $\beta$ -pinene, increased 100% and its production stopped at day 7 post-defoliation (Figure 53).

Plants involved in the clipping experiment produced quantitatively more terpenoid than the plants used in the defoliation experiment. Defoliation of the branches caused an increase in the same type of major terpenoids, eucarvone, camphor, eucalyptol,  $\alpha$ - and  $\beta$ - pinene, as did the clipping experiment. However, the increase was dependent on the type of stimulus applied. Defoliation of the branches caused a 60% increase of the major components and 100% of the minor components at 7 days post-defoliation, while the maximum increase in the clipping experiment was observed 15-days post-clipping. The delayed reaction observed in the clipping experiment may be that clipping of the branches is more traumatic than defoliation and the plant needs to manufacture extra protein and carbohydrate to repair the damage (Rumeau *et al.*, 1990; Zhou *et al.*, 1992; Wycoff *et al.*, 1995).

#### Implications for Pesticide Research

A survey of the ecological effectivity of terpenoids shows them universally to be concentration or dosage dependent; either as individual compounds, combined components or with respect to their total quantity (Harborne, 1991). If defenses are costly, it should be advantageous for plants to be less well defended when enemies are absent than when plants are subjected to attack. It was thus expected that plants would increase their commitment to defense when they were attacked by herbivores or other

enemies (Haukioja and Kabala, 1975). The quantity of terpenoids or alkaloids obtained is always below the level of the quantity produced by the plants under attack. Therefore, bioassays should be done using plant material harvested from stimulated plants. The ephemeral leaves of greenhouse-grown plants of *A. t. vaseyana* produced, from dried leaves 15 days of clipping, 10.9 mg/g of camphor, 23.45 mg/g of eucalyptol and 47.66 mg/g of eucarvone. Leaves of the big mountain sagebrush collected from the same field were previously used to control an infestation of beetles on stored products. The chemical components were not effective against the rice weevil, *Sitophilus oryzae* L., in wheat (Weaver *et al.*, 1995). The terpenoid components of these persistent leaves were numerous (24 components) with two major compounds camphor at 9.708 mg/g and 1,8-cineole (eucalyptol) 4.004 mg/g. The remaining 22 were minor components and had levels of terpenoids ranging from 0.844 to 0.047 mg/g. *Monarda fistulosa* L. was also tested in the same experiment (Weaver *et al.*, 1995) and appeared to be more efficient than *A. t. vaseyana* in controlling the pests under investigation. *M. fistulosa* leaves used in the experiment had only ten compounds: the three major components were carvacrol 26.285 mg/g, thymol 3.412 mg/g and para-cymene 2.079 mg/g. The seven minor components had concentrations ranging from 0.569-0.132 mg/g. *M. fistulosa* was probably more effective in controlling these pests because it possessed higher concentrations of major and minor components and probably more vapor pressure to reach the stored-grain pests than the compounds obtained from the sagebrush leaves. This result supports my recommendation to use sagebrush ephemeral leaves instead of persistent leaves in pesticide research. Sagebrush ephemeral leaves possess fewer



terpenoid components with better vapor concentration. This concept is not new and is referred to as a dilution factor (Langenheim, 1994).

Methyl bromide has a unique volatile profile required of a fumigant: it has a melting point (mp) of -94 °C and a boiling point (bp) of 4 °C, while the alternative compounds extracted from sagebrush seem to be less volatile: camphor (mp = 175-177 °C; bp = 209 °C); 1,8-cineole (mp = 1-2 °C, bp = 209 °C; and camphene (mp = 33-38 °C, bp = 159-160 °C). Most of camphor produced in the USA is synthesized from  $\alpha$ -pinene obtained from oil of turpentine. The last step in the preparation is the oxidation of a mixture of isoborneol and borneol to camphor that yields synthesized camphor as a white crystalline compound which explains its high boiling point. Similarly, camphor can be prepared from camphene, which is a structural isomer of  $\alpha$ -pinene (Williamson, 1998). Alpha-pinene is more volatile than both camphene and camphor ( $\alpha$ -pinene, mp = -61 °C, bp = 165-167 °C). The same pathway,  $\alpha$ -pinene to isoborneol to borneol to camphor, was used by *M. piperita* to produce volatile camphor instead of crystalline camphor (Croteau *et al.*, 1981). Terpenoid compounds from ephemeral leaves could be used to study the production of volatile instead of crystalline camphor.

Being too volatile seems to be a drawback and is actually the reason for methyl bromide now being discontinued. Methyl bromide used in agriculture is released into the atmosphere following soil fumigation. Eighty-seven percent of the applied methyl bromide was emitted within 7 days after commercial fumigation and removal of plastic film (Yagi *et al.*, 1993). Another study showed that 36% of methyl bromide was released within 24 hours and 63% was released 18 days later after commercial fumigation in an

inland valley of California, (Yates *et al.*, 1996). Management practices are being investigated and may reduce emissions of the fumigant into the atmosphere. However, some of these practices may reduce the biocidal properties of the fumigant to soilborne pathogens and pests (Munnecke *et al.*, 1979). Therefore, it is important to study volatile profiles of sagebrush ephemeral leaves because they already have the quality of being less volatile than methyl bromide and have been shown individually to repel or control agricultural pests.

## CHAPTER 4

INDUCED TERPENOID INCREASE IN THE LEAVES  
OF *ARTEMISIA TRIDENTATA VASEYANA* PLANT:  
THE NEW EXPERIMENTAL DESIGNIntroduction

Plants of *Artemisia tridentata vaseyana* grown in a greenhouse were defoliated for the first time when only ephemeral or persistent leaves were present. Each type of leaves were extracted for the first time and I considered the terpenoid profile to be constitutive because no stimuli were ever applied to the plant. Camphor, eucalyptol and eucarvone were major constituents of the leaves; the minor constituents are bicyclo[4.1.0]heptane, benzo[1,2-b:3,4-b']difuran-2(3H)one, pyrazine,  $\alpha$ -pinene, verbenone, 3-cyclohexen-1-ol and camphene. Because norpinane, cyclodecene and 3-decen-5-yne were not detected in the leaf extracted on day 1 of the defoliation experiment, I considered them to be induced but the experiment lacked controls. The project is redesigned to:

- 1) Analyze control plants for constitutive terpenoids throughout the experiment
- 2) Test for treatments (clipping or defoliation) that will either change constitutive or add induced terpenoids

Considering the importance of terpenoids in plant defense (Gershenson and Croteau, 1991), there are significantly fewer reports of induced defenses among terpenoids than among other classes of secondary metabolites, especially in leaf tissue (Gershenson and Croteau, 1991). This trend may be partially due to the existence of

physiological constraints on terpenoid biosynthesis in mature leaves. Terpenoid biosynthesis is restricted to specialized cells such as glandular trichomes that are usually only active early in leaf development (Gershenson and Croteau, 1991). No published study has evaluated the array of terpenoids produced by greenhouse-grown sagebrush plants or terpenoids produced after clipping of these plants. Such studies will permit the evaluation of terpenoid compounds secreted associated with plant herbivory.

The purpose of this part of the study is to assess the response of *A. t. vaseyana* plants to clipping or defoliation of its branches when ephemeral leaves or persistent leaves are present. Constitutive and newly produced compounds obtained after clipping or defoliation of the branches could be used as an effective tool to control invertebrate pests.

#### Results and Caveats Obtained from Previous Studies

Results from my previous studies (Chapter 3) suggested that terpenoids produced by mature ephemeral leaves can increase from 2 to 4 days after clipping the branches of two-year-old plants of *A. t. vaseyana* but the maximum was reached at about 15 days after the first treatment, and decreased thereafter (Table 11). Defoliation of the ephemeral leaves generated a maximum increase of terpenoids produced after seven days of experimentation (Table 13). However, I considered that the primary weakness of the original experiment was no temporal controls. It was not clear that changes in terpenoid contents were due only to treatments. There may have been internal daily or seasonal changes even though the plants were grown and kept in the same greenhouse throughout the experimental period to rule out the effect of seasonal changes. The following are the

suggested changes in procedures:

- 1) Addition of controls.
- 2) Shelf-life of the extracts will be studied prior to any clipping and defoliation experiments. Such a study will permit an adjustment for the loss of volatile in case extracts obtained during the clipping or defoliation experiments are not immediately analyzed.
- 3) Terpenoid constituents tentatively identified from previous experiments (Table 11) will be purchased and used to draw different calibration curves.
- 4) Within-plant variability of the terpenoid components will be assessed in the beginning of the experiment; this will permit an adjustment for within plant variability of terpenoid contents.
- 5) The quantity of wet leaves to be extracted should be reduced to  $\frac{1}{2}$  gram because previous extracts (Chapter 3) during the spring were too concentrated and had to be diluted twenty times prior to chemical analysis. The equivalent dry weight of  $\frac{1}{2}$  gram of wet weight should be measured in triplicate at the beginning of each experiment (Chapter 3). Terpenoid concentration in the extract will be based on the dry weight of the leaves.
- 6) Clipping should be performed for no more than 15 days because quantity of terpenoids produced by the leaves harvested from the branches clipped the first time was lower when compared to the terpenoids produced by the leaves harvested from the same branches clipped 15 days later. The clipping experiment will be conducted over a period of 15 days and the branches will be clipped (1, 3, 4, 5, 6,



7, 8, 9, 10, 11 and 15 days) (Table 11).

- 7) Defoliation experiment should be continued for seven days before maximum production of terpenoids might be reached. The defoliation experiment will be run for seven days (1, 3, 4, 5, 6, 7, and 8 days) (Table 13).
- 8) Clipping and defoliation experiments will be performed at the same time in two separate growth chambers by two different persons for two reasons: 1) two hours (from 1-3 pm) is not enough time for one person to carry out both experiments; and 2) suppression of the possibility for plants used in the clipping experiment to "talk" to the plants used in the defoliation experiment (Staswick and Lehman, 1999).
- 9) Plants used as controls in the experiment should be discarded to prevent them from "talking" to the remaining plants in the incubator (Staswick and Lehman, 1999).
- 10)  $^{13}\text{C}$  camphor will be used as a surrogate compound to the extraction to evaluate the quantity of volatile lost during the extraction procedures. An internal standard (benzofuran) should be added to all extracts prior to analyses.
- 11) Quantitative analyses should be performed.

### Hypotheses

I hypothesize that constitutive terpenoids from mature ephemeral and persistent leaves of *A. t. vaseyana* plants will increase and/or leaves will produce induced terpenoids after the clipping or defoliation of their branches. The following preliminary studies should be performed: 1) develop adequate extraction procedures; 2) determine the shelf-

life of the leaf extracts over a period of one year to adjust the quantity of volatile lost in case of temporary storage of the leaf extracts; and (3) perform defoliation and clipping experiments.

#### Time Table of the Procedures:

This study will be divided into two periods:

1) The first two years will be devoted to the following preliminary studies:

- Grow sagebrush plants from seed in a pest-free environment to minimize environmental factors such as insect infestation or mammals browsing on the plants which affect terpenoid levels.
- Develop an adequate method to extract volatile terpenoids from the leaves without terpenoid degradation and artifact formation often encountered when using steam distillation techniques.
- Determine the shelf-life of the best extracts chosen after multivariate analysis.
- Carry out an age-dependent study on both ephemeral and persistent leaves from two-year-old plants to assess the variations of terpenoids produced at the onset of persistent leaves and in the old dried leaves before treatments.

2) Second period of two years will be devoted to the clipping and defoliation experiment:

- Within-plant variability of the terpenoid components will be assessed in the beginning of the experiment; this will permit an adjustment for within plant variability of terpenoid contents
- Conduct the defoliation and clipping experiments when the leaves are at their

height of terpenoid production to assess the inducing potential of defoliation and clipping on the sagebrush branches.

### Materials and Methods

#### Materials

- 1) Seeds from a single plant of *A. t. vaseyana*, with a germination rate of 90-95%
- 2) Electronic balance
- 3) 2000 deep pots, 60 cubic inches (Stuewe & Sons, Inc., Corvallis, OR)
- 4) 2000 AZ 8" pots (AZALEA Pot, Delden Plastics, St. Paul, MN)
- 5) 10 gallons of 100% methylene chloride HPLC grade
- 6) 2200 20 ml vials with stopped caps and 1000 40 ml vials with stopped caps
- 7) 2 volumetric flasks of 5 ml each
- 8) 1 refrigerator
- 9) 10 pair of stainless steel scissors
- 10) 3 growth chambers set with the outdoor temperature at the beginning of each season
- 11) A cooler set at 4 °C to transport the vials for analysis.
- 12) 1,000 72-cell seed beds (No. 1206 insert for No.1020 flat 11" x 22", T. O. Plastics, made by Belden Plastics, St. Paul, MN)
- 13) Peter's 20-10-20 fertilizer
- 14) 4000 of 12 x 32 mm clear screw-cap vials with caps
- 15) 6 plastic vial holders for the 12 x 32 mm vials

- 16) 12 plastic vial holders for the vials with stopped caps
- 17) A ruler

### Methods

Plant Production in the Growth Chambers. Seeds will be vernalized at 4 °C for 10 days in a wetted Whatman Paper No. 3. Seeds will be sown in November, one per individual cell of a 72-cell seed bed, filled with pre-wetted Aqua 2000 potting media (one part of Bozeman silt loam, one part of peat moss, one part of washed concrete sand, and 1 lb per cubic yard of Aqua Gro 2000G, pasteurized at 80 °C). The ensemble will be covered with a clear plastic dome. The plants will be watered with tap water once every two days after seedling establishment. In addition, they will be watered twice per week with a dilute solution of Peter's 20-10-20 general purpose fertilizer (0.25 g/l). The seedlings will be transplanted, after two months growth into deep pots (60 cubic inches) where they will continue to grow for one year. The one-year-old plants will be transplanted into AZ 8" pots at the beginning of April for further growth.

The plants in AZ 8" pots will be randomly arranged in rows of six pots on the two benches of the growth chamber in the Plant Growth Center at Montana State University. The pots should be separated by 50 cm to prevent them from touching each other. Some of the small branches will grow and the large leaves will appear three weeks after transplantation and will be fully developed in three months. Plants involved in clipping and defoliation experiments should be fifteen months to two years old, the period required to develop secondary branching (Bilbrough and Richards, 1990).

Terpenoid biosynthesis is generally restricted to specialized types of cells that are usually active only in early leaf development (Fahn, 1979). Thus, evidence for the induced responses of terpenoids to herbivory should be sought not only in leaves present at the time of damage but also in leaves initiated immediately after damage has occurred (Gershenson and Croteau, 1991). The persistent leaves from the plants used in the ephemeral leaf defoliation experiment will also be included in the defoliation experiment of the persistent leaves. The clipping and defoliation experiments on the ephemeral leaves will be carried out in the first week of June when the leaves are three months old, and the experiment on the persistent leaves will be done at the beginning of November when the persistent leaves are three months old.

### First Period of the Study

#### Procedures for Terpenoid Extraction from the Leaves

Plants grown in the first growth chamber will be used for this experiment. Three methods of quantitative chemical extraction of terpenoids (described below) from the leaves will be tested and the best of the three methods will be selected by using multivariate analysis on different quantities of terpenoids obtained in each step of the extraction procedure. This method will be adopted as the extraction procedure for the remainder of the experiment. Multivariate analysis requires that the total number of plants used be at least equal to the number of terpenoid components in the extract and persistent leaves produce one and a half times more terpenoids than the ephemeral leaves (Table 5 vs. Table 7)). Therefore, the number of plants used for the persistent leaf experiment

should outnumber those selected for the experiment on the ephemeral leaves.

The vials to be used will be rinsed three times with 15 ml of  $\text{MeCl}_2$  under the hood one week prior to the beginning of the extraction procedure; the waste product will be retained as a blank in the analysis.

Thirty plants having the same height and the same number of branches will be numbered from one to thirty. Twenty of the plants will be randomly chosen for the extraction experiment using the random table, making five plants per treatment with four replicates. One branch of each plant will be clipped one time only and all twenty plants will be thereafter discarded preventing them from "talking" to the rest of the plants in the growth chamber. Forty out of fifty plants will be used for the persistent leaf experiment. The ephemeral or persistent leaves will be stripped from each cut branch and 0.5 g of fresh leaves will be extracted first with methylene chloride ( $\text{MeCl}_2$  HPLC grade); 5 ml of  $^{13}\text{C}$  camphor will be added as a surrogate to all the extracts to adjust for any loss encountered during extraction of the leaves. The procedures are the following: (1) Procedure for the First Extraction: 10 ml of  $\text{MeCl}_2$  will be used four times to cover the 0.5 g of fresh leaves for 5 minutes. The leaves will be consecutively dipped for 5 minutes in the four vials each containing 10 ml of methylene chloride. The leaves will then be ground using a mortar and pestle, and the ground leaves will be extracted with 10 ml of methylene chloride and filtered into the 40 ml vials. The mortar and pestle will be rinsed with 10 ml of methylene chloride and the extract filtered into the 40 ml vials. The ground leaves will be rinsed again with 10 ml of methylene chloride, filtered, and the three extracts of the ground leaves will be pooled. The five extracted products will be labeled as A to E per treatment



and the replicates will be numbered from 1 to 20. (2) Procedure for the Second

Extraction: The second procedure will be similar to the first one but will differ in extraction time. The extraction time in the second procedure will be 1 min. (3) Procedure for the Third Extraction: The leaves will be extracted in 10 ml the first time for 10 min, soaked for 10 days in another 10 ml of methylene chloride and the leaves will be rinsed twice more in 10 ml of methylene chloride. The leaves will be ground and extracted as in the first procedure. Three samples of 1 mm<sup>2</sup> of leaf from each treatment will be removed and weighed for the study of leaf anatomy, and the control 3 samples of 1 mm<sup>2</sup> will be from non-extracted leaves.

The length and weight of each cut branch will be measured. Leaf parameters such as total length, petiole length and width will be measured from ten leaves randomly chosen per branch. Each leaf will be weighed, and the leaf area measured using Zidas's thin pad as described in Chapter 2.

#### Plant Anatomy Study

In order to assess the effects of MeCl<sub>2</sub> on chloroplasts, microscopy studies will be conducted. Leaf samples from ephemeral and persistent leaves will be fixed overnight in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (Pease, 1964). The tissues will be rinsed for 45 min in the buffer, and stored overnight in sucrose buffer. The tissues will be postfixed for one hour in 1% OsO<sub>4</sub> in 0.5 M cacodylate buffer. The tissues will be then rinsed in distilled water, dehydrated in an alcohol series, and embedded in Spurr's resin. Thick-section samples obtained with glass knives on an ultramicrotome (Reichert-Jung

Ultracut E), will be stained with iodine and photographed using a phase contrast microscope. Thin-sections will also be stained and photographed with TEM. Prints from both phase contrast and electron transmission microscopes will be enlarged to assess the effects of methylene chloride on the chloroplasts of the cells of the leaves.

#### Shelf-Life Study on the Extracts Obtained in Leaf Extraction Procedure

Such a study will permit the adjustment for the loss of volatile in case the extracts obtained during the clipping or defoliation experiments were not immediately analyzed. The best extracts chosen from the preceding extraction procedures will be kept for one year to study the decay of the terpenoid components in each extract. Each of the samples will thereafter be analyzed every week, after the first analysis is done, for two months. The extracts will then be analyzed every month for seven months and every six months thereafter.

#### Age-Dependent Terpenoid Production by the Leaves.

This study will be done on both ephemeral and persistent leaves. The study of the ephemeral leaves will begin at the end of May and will last until the end of July. The study of the dried ephemeral leaves will continue until the end of October of the same year. The persistent leaves will be studied from August to the end of November of the same year. The study on dried persistent leaves will be done from July to August of the following year which is the senescent period of these leaves.

The leaves will be sampled from 20 plants randomly selected as described in the extraction procedures. The plants will be sampled on Monday of each week and the

experimental procedure will be carried out from 1 to 3 pm (maximum production of terpenoid by plants is at the beginning of the afternoon) (Nicholas, 1973) until 20 June and every two days thereafter to capture the process of terpenoid catabolism that occurs when persistent leaves begin their development and when the ephemeral leaves begin their senescence. One branch from each plant will be cut and the ephemeral or persistent leaves will be stripped from the branch. One-half gram of leaves will be extracted per plant using the extraction procedure selected from the first experiment. Leaf parameters such as weight, length, width and area will be measured on ten leaves per plant using the procedure cited earlier.

### Second Period of the Studies

#### Controls

Controls for the defoliation and clipping experiments will use the plants grown and kept in the third growth chamber. Only current year mature ephemeral and persistent leaves present at the beginning of the experiment should be extracted as controls. Controls could use all the plants grown in the growth chamber that reach the standardized level of growth. Twenty plants will be used daily as controls for defoliation or clipping experiments without regard to the type of leaf. Plants involved in the daily experiment will be discarded at the end of the experiment and will not be returned in the growth chamber, thus preventing them from "talking" to the remaining plants in the third growth chamber.

Only the leaves from below the crown will be extracted with methylene chloride. The leaves from the crown will be excluded because they are neither ephemeral nor

persistent leaves. The total weight and length of clipped branches and leaves harvested during the experiment will also be recorded per plant.

### Ephemeral Leaves

Study of Within-Plant Variability of Terpenoids Produced prior to the Clipping and Defoliation Experiment. This study will be to assess plant variability of terpenoid components prior to the clipping and defoliation experiments. Multivariate analysis (Tabachnick and Fidell, 1989) will be used to detect between plant variability of terpenoid content. Leaves harvested from twenty plants bearing only ephemeral leaves will be separately extracted with methylene chloride (HPLC grade) using the procedure chosen previously.

### Clipping or Defoliation-Induced Increase of Terpenoids Produced by the Leaves.

The first clipping and defoliation experiments should begin in June, before the onset of the small persistent leaves. The exact date should be chosen to match the date of highest terpenoid production, determined during the age-dependent study, by the leaves of growth chamber-grown plants. All the leaves should be fully mature before the beginning of the experiment. Only current year mature ephemeral leaves present at the beginning of the experiment should be extracted in the clipping and defoliation experiments. Both clipping and defoliation experiments on ephemeral leaves will use one row of pots separated by two intact rows. Each row will have five plants. We will, therefore, use four rows of plants each separated by two intact rows of plants that are not to be touched. The

clipping and defoliation experiments will use the set of plants in the first growth chamber. These experiments should be performed outside the growth chambers, in a place reserved for experimental procedures.

Clipping and Defoliation Experiments. Five plants will be used per treatment, with four replicates making 20 plants for the ephemeral leaf experiment. The experiment will be conducted every day from 1 to 3 pm in the growth chamber reserved for the experiments. The plants used in both experiments (clipping and defoliation) will be returned to their respective growth chamber and will be monitored until December of the following year. Mortality rate of the clipped or defoliated plants will be calculated in comparison to the mortality rate shown among the intact rows of plants that separate them. The branches at the same level will be clipped to make a flat-topped plant (Chapter 3). The size and weight of the branches clipped per plant will be measured and recorded. Ten leaves will also be selected and leaf parameters will be measured with the procedure described earlier (Chapter 3).

(1) Clipping experiment. The first clipping will begin in June. The leader branches will be clipped from the top toward the bottom by a pair of scissors. The scissors will be cleaned using balls of cotton wetted with absolute ethanol (HP Grade), and wiped dry with cotton balls before cutting the next plant. One-half gram of leaves will be extracted per plant immediately, with four plants per treatment with four replicates, making a total of 20 plants. The clipping will be done on days 1, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 15. Only the leaves from below the crown will be extracted with methylene chloride. The leaves

from the crown will be excluded because they are neither ephemeral nor persistent leaves. The total weight and length of cut branches and leaves harvested during the clipping experiments will also be recorded per plant.

(2) Defoliation Experiment. The defoliation experiment will begin in June. The ephemeral leaves will be removed from below the crown of the short and long shoots, in a top to bottom direction. The terpenoids from 0.5 g (2-3 leaves) of freshly harvested leaves will be extracted with methylene chloride using the procedure selected earlier. The leaves will be hand-picked, with the petioles removed from the shoots one at a time. The leaves will be harvested from twenty plants on six consecutive days, 48 hours after the first experiment, specifically on days 1, 3, 4, 5, 6, 7, and 8.

### Persistent Leaves

Study of Within-Plant Variability of Terpenoids Produced by Persistent Leaves prior to the Clipping and Defoliation Experiment. This study will assess plant variability of terpenoid components prior to the clipping and defoliation experiments. Multivariate analysis (Tabachnick and Fidell, 1989) will be used to detect within plant variability of terpenoid content. Leaves from forty plants bearing only persistent leaves will also be extracted. One branch of the plant will be clipped one time and all the plants involved in the experiment will be discarded.

Procedures for Defoliation and Clipping Experiments. The same model of experiment as in the ephemeral leaf defoliation and clipping will be applied to the



persistent leaves. The experiments will begin in October and will be done according to the procedure previously described for the ephemeral leaves. The clipping will be carried out on leader branches. Short shoots will be removed from the leader shoots in the defoliation experiment. The plants grown in the second incubator will be used in this experiment. Clipping or defoliation of the subsequent ephemeral leaves from plants involved in both defoliation and clipping experiments of persistent leaves will also be included.

Clipping and Defoliation Experiments. The plants used in both experiments will be monitored until December of the following year, and the mortality rate of the clipped or defoliated plants will be calculated in comparison to the mortality rate shown among plants in the intact rows.

Eight plants will be used per treatment, with five replicates making 40 plants for the persistent leaf experiment. The experiment will be conducted every day from 1 to 3 p m in the growth chamber reserved for the experiments. The branches at the same level will be clipped 2 cm long to make a flat-topped plant (Chapter 3). The size and weight of the branches clipped per plant will be measured and recorded. Ten leaves will also be selected per plant and leaf parameters will be measured in the procedure described earlier (Chapter 3).

(1) Clipping experiment. The first clipping will begin in October. The leader branches will be clipped (2 cm) from the top toward the bottom by a pair of scissors. The scissors will be cleaned using balls of cotton wetted with absolute ethanol (HP Grade), and wiped dry with cotton balls before cutting the next plant. One-half gram of leaves will

be extracted per plant immediately, with eight plants per treatment with five replicates, making a total of 40 plants. The plants clipped in October will be kept in the second incubator and will not be discarded. The clipping will be done on days 1, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 15. Only the leaves from below the crown will be extracted with methylene chloride. The leaves from the crown will be excluded because they are neither ephemeral nor persistent leaves. The total weight and length of cut branches and leaves harvested during the clipping experiments will also be recorded per plant.

(2) Defoliation Experiment. The defoliation experiment will begin in October. The persistent leaves will be removed from below the crown of the leader branches (long shoot) and short shoots, in a top to bottom direction. The terpenoids from 0.5 g of freshly harvested leaves will be extracted with methylene chloride using the procedure selected earlier. The leaves will be hand-picked, with the petioles removed from the branch one at a time. The leaves will be harvested from forty plants on six consecutive days 48 hours after the first experiment.

#### Quantitative Chemical Analysis

A microliter of benzofuran should be added to each sample as an internal standard prior to any analysis. Samples of all of the solvents used to make stock solutions will be drawn to analyze for purity. Terpenoid compounds tentatively identified in the leaf extracts (Chapter 3) will be purchased from chemical companies and used to make stock solutions. Calibration curves will be determined for terpenoid compounds encountered in sagebrush extracts by injecting a blank (0), 10, 25, 50, 75 and 100 ng of each terpenoid

compound and the instrument response will be recorded. The linear regression equation  $y = mX + b$  will be drawn from the graph with  $X = (y - b)/m$ . The value of  $X$  will represent the quantity of each component in the extract and  $y$  will represent the ratio of the absolute area of each component to the absolute area of the internal standard.

The quantitative analysis will be done with a Hewlett Packard 5890 Series II Plus gas chromatograph interfaced to a mass spectrometer. The column will be 30 m x 0.25 mm DB-5 with 0.25  $\mu$ m film thickness. The temperature protocol will be a starting temperature of 30 °C for 2 min, a temperature ramp of 5 °C per min to 160 °C, a holding time of 2 min, a second temperature ramp of 5 °C per min to 290 °C, with a holding time of 5 min. Volatile chemical samples of 1  $\mu$ l will be injected with helium at an initial pressure of 40 kPa. The pressure will be ramped with the temperature to maintain a constant helium flow throughout the duration of the analysis. The coupled mass spectrometer is a VG 70E-HF double focusing magnetic mass spectrometer (MS) (VG Instruments, Manchester, England). The MS will be operated at a scan rate of 0.5 seconds per mass decade, ranging from 40 to 450 amu. The source temperature will be 200 °C and the mass resolution will be 1500.

Table 15: Total number of plants required for the whole experiment.

| Type of Study                            | Total Number of Plants Needed       |                               |
|------------------------------------------|-------------------------------------|-------------------------------|
|                                          | Ephemeral Leaves                    | Persistent Leaves             |
| First Period                             |                                     |                               |
| Extraction                               | 20 plants/procedure x 3 = 60 plants | 40 plants/procedure x 3 = 120 |
| Shelf-life                               | Same set of plants                  | Same set of plants            |
| Age-dependent                            | May: 20 plants/week x 4 = 80 plants | Aug-Nov: 320 plants           |
|                                          | June: 20 plants/2days = 300 plants  | July-Aug: 160 plants          |
|                                          | July-Oct: 320 plants                |                               |
| Second Period                            |                                     |                               |
| Variability of Terpenoids Between Plants | 20 plants                           | 40 plants                     |
| Clipping experiment                      | 240 plants                          | 260 plants                    |
| Defoliation                              | 160 plants                          | 180 plants                    |
| <b>Total</b>                             | 1160 plants                         | 1080 plants                   |

## CHAPTER 5

ATTEMPT TO ISOLATE A TERPENOID CYCLASE GENE FRAGMENT  
FROM THE LEAVES OF *A.T. VASEYANA*IntroductionTerpenoid Metabolism

Terpenoids are the largest and most diverse class of organic compounds found in plants. They exhibit enormous chemical variety and complexity but are united by a common biosynthetic origin, combinations of five-carbon units named the isoprene unit. Terpenoids can decompose at high temperatures to give isoprene and are referred to as isoprenoids. Terpenoids are classified by the number of five-carbon units in their structures. Ten-carbon terpenoids, which contain two  $C_5$  units, are called monoterpenoids, 15-carbon terpenoids (three  $C_5$  units) are called sesquiterpenoids, and 20-carbon terpenoids (four  $C_5$  units) are called diterpenoids. Larger terpenoids include triterpenoids (six  $C_5$ ), tetraterpenoids (eight  $C_5$ ) and polyterpenoids (multiple  $C_5$  units). Mono- and sesquiterpenoids are semi-volatile compounds while the remaining types are not (Gershenson and Croteau, 1991).

The five-carbon building blocks of terpenoids are synthesized by the mevalonic acid pathway in the following two stages.

Stage One. Three molecules of acetyl-CoA are joined together stepwise to form the six-carbon compound 3-hydroxy-3-methylglutaryl-CoA, which is then reduced to

mevalonic acid. Mevalonic acid is pyrophosphorylated and then undergoes decarboxylative elimination to yield isopentenyl diphosphate (IDP). IDP and its isomer, dimethylallyl pyrophosphate (DMAPP), are the five-carbon building blocks of terpenoids that combine to form larger molecules. Prenyltransferase catalyzes the condensation of IDP and DMAPP to geranyl pyrophosphate (GDP), and the subsequent 1'-4 additions of isopentenyl diphosphate to generate farnesyl (FDP) and geranylgeranyl diphosphate (GGDP).

Stage Two. Geranyl diphosphate (GDP) undergoes a range of cyclizations to produce the precursors of each class of terpenoids. GDP ( $C_{10}$ ) gives rise to monoterpenes, FDP ( $C_{15}$ ) to sesquiterpenes, and GGDP ( $C_{20}$ ) to diterpenes. These reactions, catalyzed by the terpenoid synthases (cyclases), produce the many thousands of different terpenoids known today. The synthases responsible for generating the parent compounds of the various types of terpenoids are quite similar in their properties. They all operate by electrophilic reaction mechanisms, as do the prenyltransferases (Poulter and Rilling, 1981).

A number of monoterpene synthases from angiosperms, gymnosperms, and bryophytes have been partially purified and characterized. All have similar properties (Alonso and Croteau, 1993; Savage *et al.*, 1994; Adam *et al.*, 1996). For monoterpenes however, plastids are the exclusive site of synthesis (Kleinig, 1989; Feron *et al.*, 1990; Perez *et al.*, 1990; Solaer *et al.*, 1992). A study of 45 species of higher plants revealed a correlation between the levels of monoterpene biosynthesis and the presence of leucoplasts (Cheniclet and Carde, 1985). Immunogold labeling has indicated that the monoterpene



cyclase, limonene synthase, is located specifically in the leucoplasts of mint gland secretory cells (McGarvey and Croteau, 1995). Diterpenes are also biosynthesized in plastids (Dudley *et al.*, 1986; Laferriere and Beyer, 1991). GGDP synthase has been directly localized to the plastid stroma by immunocytochemical methods (Simcox *et al.*, 1975; Moore and Coolbaugh, 1976; Railton *et al.*, 1984; Chemiclet *et al.*, 1992; Kuntz *et al.*, 1992). Recently, the Arabidopsis gene for the A activity of ent-kaurene synthase (a diterpene) has been cloned, and the *in vitro*-translated protein was shown to be efficiently processed and imported into isolated chloroplasts (Sun and Kamiya, 1994). Synthesis of FDP and its derived sesquiterpenes takes place in the cytosol and the cytosol/ER (endoplasmic reticulum) boundary, as does the synthesis of triterpenes (Gleizes *et al.*, 1980; Belingheri *et al.*, 1988; Feron *et al.*, 1990; Hugueney and Camara, 1990).

#### Molecular Cloning of Terpenoid Synthase Genes

More than 30 plant terpenoid synthases have now been cloned as cDNAs. Purification of plant terpenoid synthases is difficult. A general similarity-based cloning technique was developed (Steele *et al.*, 1995) that was based on the use of consensus sequence elements which flank a highly conserved aspartate-rich region. Degenerate primers, designed to amplify sequences of this region using the Polymerase Chain Reaction (PCR), have been employed to isolate specific probes of terpenoid synthases (cyclases) from angiosperms and gymnosperms.

Such probes were used to obtain cDNAs encoding taxadiene synthase from the Pacific yew (Wildung and Croteau, 1996), monoterpene and sesquiterpene synthases from

grand fir (Colby *et al.*, 1998; Steele *et al.*, 1998), three different monoterpene synthases from common sage, and germacrene C (a diterpene) synthase from tomato (Colby *et al.*, 1998). The first crystal structure of a plant terpene synthase was recently solved for tobacco epi-aristolochene synthase (Staks *et al.*, 1997), and it was revealed that the protein is composed entirely of  $\alpha$ -helices with short, connecting loops and turns, forming a two-layer  $\alpha$ -barrel active site. This structure resembles that of microbial synthase (Back and Chapell, 1996; Wendt *et al.*, 1997), and modeling studies suggest that all terpenoid synthases have the same overall fold structure. This common three-dimensional framework, when coupled to direct mutagenesis, provides the means for evaluating the functional relevance of conserved aspartate amino acid residues. The hydrophobic, aromatic-rich active-site pocket of epi-aristolochene synthase is formed by  $\alpha$ -helices of the C-terminal domain to accommodate the olefin chain of the substrate, whereas the diphosphate moiety is complexed by two  $Mg^{2+}$  ions at the entrance of the active site. One  $Mg^{2+}$  is coordinated by conserved residues corresponding with Asp<sup>566</sup>, Asp<sup>570</sup>, and Glu<sup>644</sup>. Both aspartate residues are part of an absolutely conserved aspartate-rich configuration, DD(xx)D. The second  $Mg^{2+}$  is coordinated by a third aspartate residue Asp<sup>714</sup>, a non-conserved Thr-residue, a well conserved Glu /Asp-residue, and an absolutely conserved Arg<sup>710</sup> residue. These residues stabilize the negative charge of diphosphate after ionization and prevent its reaction with the carbocationic intermediates of the catalytic cycle.

Although the evolutionary relationship of plant and microbial terpene synthases and prenyltransferases is unclear, all of these enzymes contain the aspartate-rich region DD(xx)D. Replacement of any of the three aspartates (D) of the DD(xx)D sequence by

either Alanine (A) or Glutamine (Q) in the sequence of limonene synthase, reduces the catalytic activity of this synthase by 1,000-fold (Bohlmann *et al.*, 1998). The amino acid relatedness of the cloned plant terpenoid synthases allows subdivision of the family into six subfamilies, each distinguished by sharing a minimum of 40% identity among its members (Bohlmann *et al.*, 1998).

Genes for all classes of resin terpene synthases from the grand fir have been identified (Vogel *et al.*, 1996; Bohlman *et al.*, 1997; Steele *et al.*, 1998), and are transcriptionally activated by wounding (Bohlmann *et al.*, 1998; Steele *et al.*, 1998). Monoterpene synthase mRNA accumulation occurs within two hours post-wounding, and reaches its maximum quantity in two to four days (Steele *et al.*, 1998). The diterpene synthase gene expression is similarly induced, however, the time course is slightly delayed. These differences in the timing of induced gene expression are consistent with the different roles of terpenoid defense compounds. In the case of the bark beetle attack on pine trees, toxic monoterpenes are required immediately and diterpene resin acids are mobilized later. Sesquiterpene juvenile hormone analogues are produced later, presumably to interfere with insect reproduction, should the attack succeed (Bohlmann *et al.*, 1998; Steele *et al.*, 1998).

The objective of this study was to clone a portion of a cyclase gene coding region from *Artemisia* DNA, and to use this fragment as a probe to study the relationship between wounding and the differential expression of a cyclase gene of sagebrush.

## Materials and Methods

### Genomic DNA Extraction

DNA was extracted using a method adopted from that of Dellaporta *et al.*, (1983). One gram of ephemeral leaves from a greenhouse-grown *A. t. vaseyana* plant was ground to powder in liquid nitrogen using a mortar and pestle. Following this, 10 ml of extraction buffer (15 ml of 1M Tris, 0.5M EDTA, 3.75 ml 4M NaCl, 10% SDS, 14.4M mercaptoethanol, complete to 150 ml with sterile water) was added. The homogenate was transferred to a 30 ml Oakridge tube. The remaining 5 ml of extraction buffer was used to rinse the mortar and was added to the Oakridge tube. The tubes were incubated at 65 °C in a waterbath for 10 min. Five ml of 5M potassium acetate was added to the tubes, before incubating them on ice for 20 min. They were then centrifuged at 25,000 x g for 29 min. The supernatant was poured through a Miracloth filter into a clean 30 ml tube containing 10 ml of ice-cold isopropanol and 1 ml of 5M ammonium acetate. The solution was mixed and the tubes were incubated at -20 °C for 20 min to precipitate the DNA. A pellet of DNA was obtained by centrifugation at 20,000 x g for 15 min. The supernatant was gently poured off and the pellet was dried by inverting the tubes onto a paper towel for 1 hr. The pellet of DNA was dissolved in 0.7 ml TE, and the solution was transferred into 1.5 ml microfuge tubes. Cleaner DNA was obtained when it was precipitated a second time by adding 75 µl 3 M of sodium acetate, pH 7.0, and 500 µl of cold isopropanol. The DNA pellet was collected by centrifugation at 8000 rpm for 30 sec in a

microfuge. The DNA pellet was finally dissolved in 100-200  $\mu$ l TE, and stored at -20 °C until use.

### Polymerase Chain Reaction (PCR)

An attempt was made to amplify a fragment of DNA coding for a portion of the terpenoid synthase (cyclase) gene using the PCR. The degenerate primers were obtained from consensus sequences derived from three previously cloned cyclases Steele *et al.* (1995). These cyclases are limonene synthase (a monoterpene cyclase from *M. spicata*), epiaristolochene synthase (a sesquiterpene cyclase from *N. tabacum*), and casbene synthase (diterpene cyclase from *Ricinus communis*). The forward primer is 5' **ATGYTGCAGYTGTAYGAGGC** 3' and the reverse primer is 5' **YTKCATRTARTCVGGTARBYKRTC** 3' (Y = C/T, K = G/T, R = A/G, V = G/A/C, B = G/T/C).

A 50  $\mu$ l reaction mix was made of 2  $\mu$ l of 100 ng *A. t. vaseyana* DNA, 5  $\mu$ l of 10X buffer, 8  $\mu$ l of 1.25 mM dNTP mix, 2  $\mu$ l of 25 pmol of each of the primers (forward and reverse), 4.50  $\mu$ l of 25 mmol  $MgCl_2$ , and 0.15  $\mu$ l of 5 u/ $\mu$ l of Taq polymerase (Promega). The tubes were layered with mineral oil and deposited in a thermocycler heated to 94 °C. DNA fragments were amplified using the following cycle parameters: 94 °C for 4 min, then 30 times the cycle at 94 °C for 1 min, 42 °C for 2 min, and an extension time of 72 °C for 1.2 min. A final extension which lasted 7 min at 72 °C was scheduled at the end of the procedure. The tubes were cooled to 4 °C before being stored in the freezer. Fragments ranging from 300 to 680 bp were amplified and visualized on polyacrylamide gels. The

amplified 680 bp DNA fragment was close to the expected size of the cyclase gene fragment. The 680 bp PCR product was subcloned into the EcoRI restriction site of plasmid pBluescript (Stratagene) prior to sequencing. The dideoxy DNA sequencing method was first used to ensure the presence of the primers. Following this, the clone was sent to the DNA Sequencing Facility, Iowa State University, Ames, Iowa, for sequencing. Automated fluorescence-based sequencing was performed to determine the complete sequence of the 680 bp of the genomic DNA fragment. The deduced amino acid sequence of the isolated DNA fragment was obtained using GENEPRO Sequence Analysis Software (June 1993). The same software package was used to compare the sequence of the amplified DNA with the cDNA sequence of the sesquiterpene 5-epi-aristolochene synthase of *N. tabacum* (Genbank accession number L04680).

## Results

### Attempt to Isolate a Cyclase Gene Fragment from *A. t. vaseyana*

PCR amplification of *Artemisia* DNA with the cyclase gene degenerate primers produced a predicted 680 bp fragment. The fragment was translated in all 3 amino acid reading frames (Figures 54 and 55). Though this fragment is of a size consistent with that expected for the product from a cyclase gene (Kyoungwhan and Chapell, 1995; Bolhman *et al.*, 1998), no amino acid sequence with significant similarity to these cyclases is encoded. The PCR primers were designed to flank the consensus DD(xx)D sequence found in all cyclases (Bohlmann *et al.*, 1998) and no such conserved sequence is found in



any of the reading frames. The attempt to amplify and clone the probe fragment was unsuccessful and no studies of cyclase gene expression were performed.

### Discussion

The degenerate primers used in this study are the most conserved sequences from three diverse types of cyclases which are either membrane-bound or cytosolic (Steele *et al.*, 1995). These primers have been employed to amplify specific probes for terpenoid synthases from angiosperm and gymnosperm genomic DNA. These primers were so efficient that they were used for the cloning of 11 defense-related terpene synthases from grand fir, representing the largest group of synthases available from any plant source (Bohlman *et al.*, 1998; Steele *et al.*, 1998). All of the terpenoid synthases that have been cloned were from cDNA libraries enriched for terpenoid biosynthetic genes constructed from mRNA isolated from induced cells (Facchini and Chappell, 1992; Mau and West, 1994; Bohlmann *et al.*, 1998; Steele *et al.*, 1998). Because of the success of these primers in amplifying fragments of genomic DNA, they are a starting point for terpenoid synthase studies in all laboratories involved in such research. Other sets of primers were completely unsuccessful in amplifying any genomic DNA fragment from *Artemisia*, and prompted the adoption of the set developed by Steele *et al.* (1995) for this study.

Back and Chappell (1995) had made a schematic representation of amino acid sequence alignment for five terpene synthases, three of which were used to create the degenerate primers used in this study. They found that the intron-exon organization of the genes is conserved which implies that the exonic sequences might in fact represent

domains which serve similar functions in each enzyme. This set of degenerate primers cover a region of the gene which includes 3 exons and 2 introns. The amplified genomic DNA fragment from *Artemisia* might contain an intron sequence, and the conserved sequence DD(xx)D may be further away than the obtained 680 bp fragment. As this primer set has amplified the *Artemisia* genomic DNA fragments, further research should use the same set of primers along with the ones enclosed in brackets (Figure 56) to amplify cDNA obtained from glandular trichomes of ephemeral leaves of *A. t. vaseyana*. The wounding experiment, described in Chapter 3, showed that the ephemeral leaves could be stimulated when the branches were cut or defoliated. Leaves harvested from stimulated branches could produce mRNA rich in terpenoid synthase, which would be necessary to make a good synthase cDNA library.

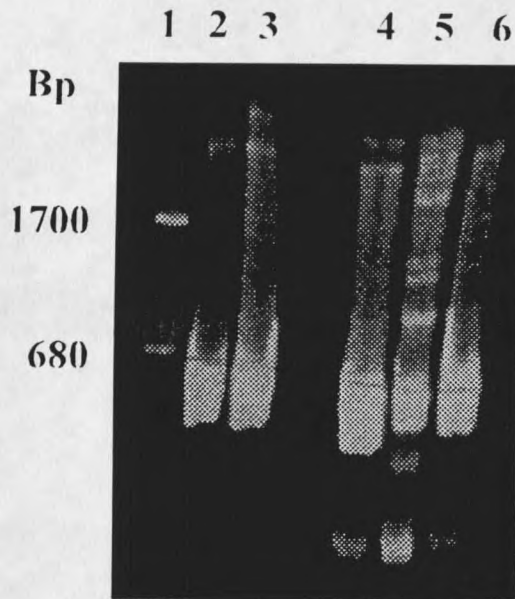


Figure 54: Polymerase Chain Reaction of the Genomic DNA of *A. t. vaseyana* Ephemeral Leaves. Lane 1, molecular mass standards Puc/RSA; Lanes 2-6, PCR products. Amplified DNA fragments of lanes 2 and 3 were cloned.

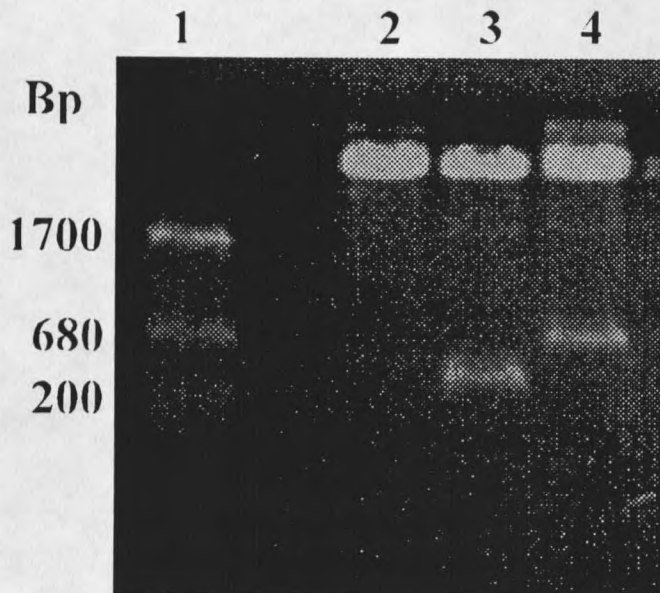


Figure 55: Clones of the Amplified DNA fragments of *A. t. vaseyana* Ephemeral Leaves. Lane 1, molecular mass standard Puc/RSA; Lanes 2-4, clones digested with *EcoRI*. The fragment sizes of 660 bp and 680 bp were sequenced.

|             |             |            |             |     |
|-------------|-------------|------------|-------------|-----|
| [ATGCTGCAGT | TGTATGAGGC] | TTTGGACACG | CATTTGTCAT  | 40  |
| GTGACCATTC  | CCACCACAGT  | TGTAGCATGT | GATTGAAATA  | 80  |
| TTTGCAATGC  | CTTCTCTGTG  | CATTCCCAGC | CATCTGATTT  | 120 |
| CTTCTCTGAG  | TAGTTCCCAT  | ACTGTTGCTG | TTACCCCCTG  | 160 |
| AAGAATTAAA  | TATGTTCCCT  | CCTGACTGAA | ACCTCTGAGA  | 200 |
| CCCATAGTTT  | CCACTGTTTC  | CTGAACCATA | TCTGCCCCGAA | 240 |
| GACCGATTCT  | GAATTTCCAC  | TCTACCATCG | TTAATAACAG  | 280 |
| CTTGATTCCCT | GGTATTTGAT  | GATGAATGAA | GACGATTGTT  | 320 |
| GGATTTGGTG  | AACTTGCCAA  | ATGCCTTAGC | AATTAATCCA  | 360 |
| AATGCTTCAT  | TAAGAGAGTT  | GAATGCATCC | TCTTCTTCAT  | 400 |
| CATAATCAGC  | GTTTGAGTAC  | AACATGGAAT | CTGAGTTGTT  | 440 |
| ATATGGAACC  | ATTTGATTGT  | CAAAGTATTG | TGATGGTGCT  | 480 |
| TCAAATGGGA  | TGATTTGGTT  | AGAAGGTTGT | GGTGAAAAAC  | 520 |
| TTGGTTGAAA  | AGATGATTGA  | CCAAATTCCT | GGTTTGGTAG  | 560 |
| TGGAAAGTTG  | GATTGAGCAA  | CGAGAGCTAG | TGAGTTAGGT  | 600 |
| GTTGGTGCTG  | G[GATGAAACC | GCCTCGTACA | A]          | 631 |

Figure 56: Genomic DNA Obtained from *A. t. vaseyana* Ephemeral Leaves. This fragment of 680 bp was named Toki. The primers are enclosed in brackets.

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APPENDICES

APPENDIX A

QUALITATIVE ANALYSES

Information from Data File:

File : C:\HPCHEM\1\DATA\BSB\HCA19DE6.D  
 Operator : [BSB1]HOWARD  
 Acquired : 19 Dec 96 11:29 am using AcqMethod HOWARD2  
 Sample Name: FRANCOISE GRNHS SAGE: A; REP 2  
 Misc Info : MECL EXT: 3.53G/20ML  
 Vial Number: 1

Search Libraries: C:\DATABASE\NBS75K.L Minimum Quality: 0

Unknown Spectrum: Peak average  
 Integration Params: current RTEINT parameters

| Pk# | RT    | Area% | Library/ID                         | Ref#  | CAS#        | Qual |
|-----|-------|-------|------------------------------------|-------|-------------|------|
| 1   | 6.40  | 1.11  | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | Methylene Chloride                 | 62705 | 000075-09-2 | 83   |
|     |       |       | Methylene Chloride                 | 62704 | 000075-09-2 | 80   |
|     |       |       | Methylene Chloride                 | 62703 | 000075-09-2 | 80   |
| 2   | 10.61 | 5.42  | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | 1,3,6-Octatriene, 3,7-dimethyl-, ( | 65760 | 003779-61-1 | 78   |
|     |       |       | .beta.-Pinene                      | 65804 | 000127-91-3 | 72   |
|     |       |       | Cyclohexene, 1,5,5-trimethyl-1-met | 6603  | 016609-28-2 | 53   |
| 3   | 10.74 | 2.10  | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | 1,1'-Bicyclopropyl                 | 62690 | 005685-46-1 | 39   |
|     |       |       | 1,1-Difluoro-1-silacyclo-3-pentene | 3674  | 034736-33-9 | 39   |
|     |       |       | 1,2,3,6-Tetrahydropyridine         | 62699 | 000694-05-3 | 39   |
| 4   | 11.37 | 4.05  | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | .alpha.-Pinene                     | 65808 | 000080-56-8 | 96   |
|     |       |       | Bicyclo[3.1.1]hept-2-ene, 3,6,6-tr | 6692  | 004889-83-2 | 94   |
|     |       |       | 1,4-Cyclohexadiene, 1-methyl-4-(1- | 65746 | 000099-85-4 | 87   |
| 5   | 11.83 | 2.71  | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | Camphene                           | 65768 | 000079-92-5 | 94   |
|     |       |       | Camphene                           | 65766 | 000079-92-5 | 94   |
|     |       |       | Camphene                           | 65764 | 000079-92-5 | 94   |
| 6   | 12.83 | 12.37 | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | Cycloheptene, 1-chloro-            | 65202 | 013294-30-9 | 68   |
|     |       |       | 9-Azabicyclo[6.1.0]non-4-ene, 9,9' | 38126 | 066387-82-4 | 42   |
|     |       |       | 7-Oxabicyclo[4.1.0]heptane, 3-oxir | 7391  | 000106-87-6 | 37   |
| 7   | 13.55 | 0.96  | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | Cyclohexene, 3-chloro-             | 64266 | 002441-97-6 | 58   |
|     |       |       | Cyclopropane, 1-chloro-2-ethenyl-1 | 3231  | 062337-93-3 | 53   |
|     |       |       | (tert-C4H9)C\$.CCH3                | 1122  | 000999-78-0 | 53   |
| 8   | 14.29 | 0.64  | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | Benzene, 1-methyl-4-(1-methylethyl | 65539 | 000099-87-6 | 94   |
|     |       |       | Benzene, 1-methyl-3-(1-methylethyl | 65580 | 000535-77-3 | 93   |
|     |       |       | Benzene, 1-methyl-3-(1-methylethyl | 65579 | 000535-77-3 | 90   |

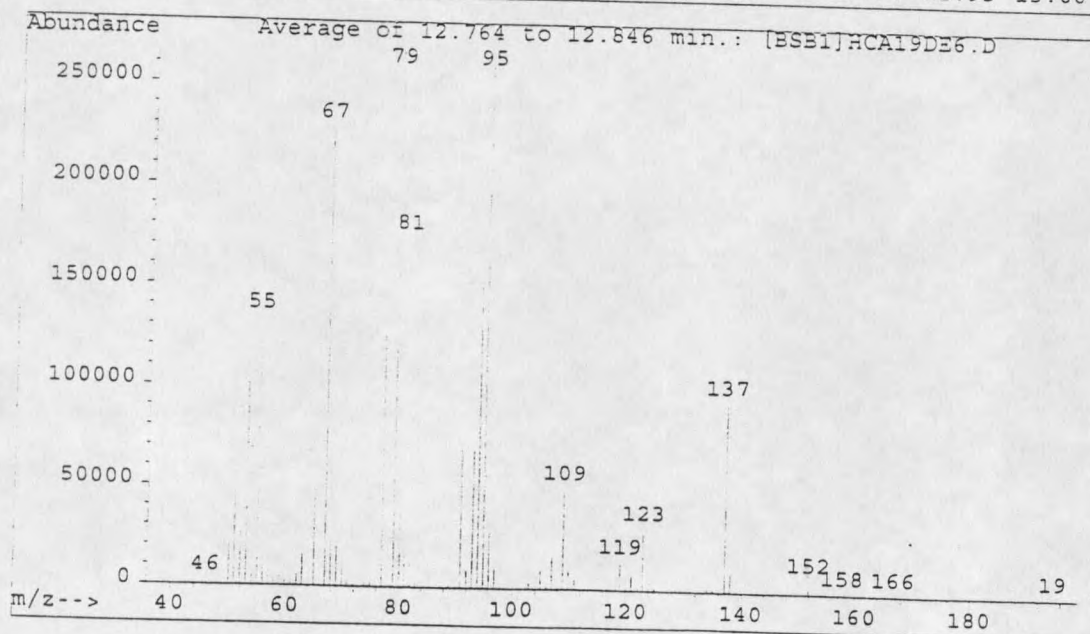
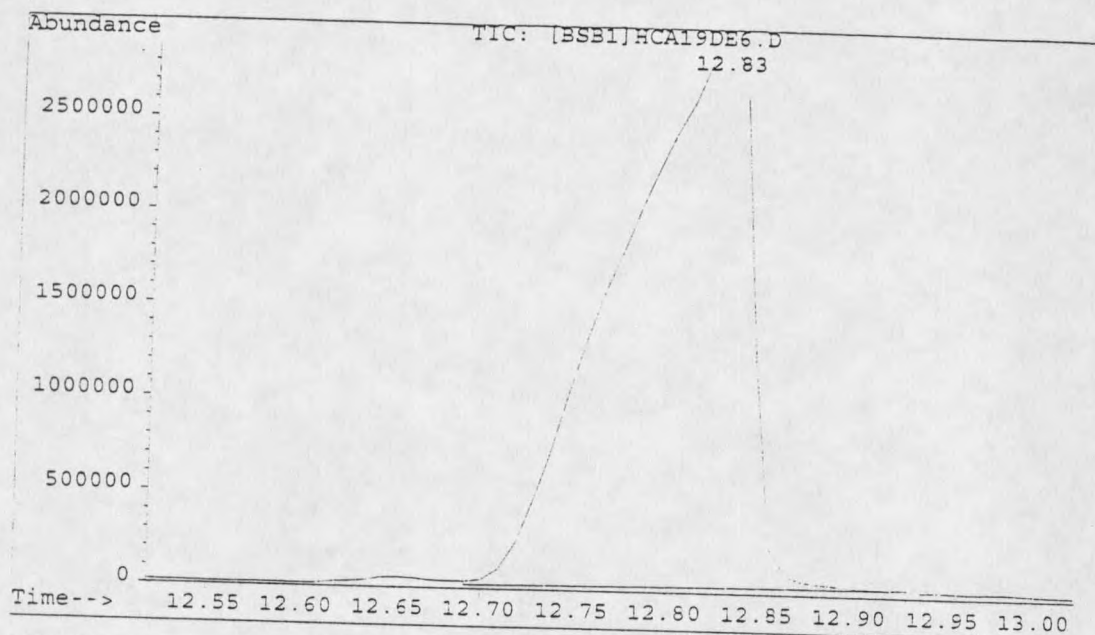
| Pk# | RT    | Area% | Library/ID                                                                                                                      | Ref#                    | CAS#                                      | Qual           |
|-----|-------|-------|---------------------------------------------------------------------------------------------------------------------------------|-------------------------|-------------------------------------------|----------------|
| 9   | 14.54 | 8.69  | C:\DATABASE\NBS75K.L<br>Eucalyptol<br>Eucalyptol<br>Eucalyptol                                                                  | 67088<br>67091<br>67087 | 000470-82-6<br>000470-82-6<br>000470-82-6 | 70<br>64<br>60 |
| 10  | 14.60 | 0.61  | C:\DATABASE\NBS75K.L<br>Eucalyptol<br>Eucalyptol<br>Ketone, 2,2-dimethylcyclohexyl met                                          | 67088<br>67087<br>67083 | 000470-82-6<br>000470-82-6<br>017983-26-5 | 59<br>55<br>45 |
| 11  | 15.39 | 1.11  | C:\DATABASE\NBS75K.L<br>3-Octyne, 5-methyl-<br>2-Undecyne<br>trans-Syn-cis-tricyclo[7.3.0.0(2,6                                 | 4258<br>67004<br>17334  | 062108-33-2<br>060212-29-5<br>073306-77-1 | 59<br>43<br>43 |
| 12  | 15.53 | 0.61  | C:\DATABASE\NBS75K.L<br>1,3-Cyclohexadiene, 1,3,5,5-tetram<br>1,3-Cyclohexadiene, 1,2,6,6-tetram<br>Phenol, 2-(1-methylpropyl)- | 6665<br>65788<br>66797  | 004724-89-4<br>000514-96-5<br>000089-72-5 | 56<br>53<br>46 |
| 13  | 16.29 | 0.72  | C:\DATABASE\NBS75K.L<br>(tert-C4H9)C.S.CCH3<br>Cyclopropane, 1-chloro-2-ethenyl-1<br>Cyclopropane, 1-chloro-2-(1-propen         | 1122<br>3231<br>3233    | 000999-78-0<br>062337-93-3<br>074752-94-6 | 59<br>53<br>45 |
| 14  | 16.36 | 0.84  | C:\DATABASE\NBS75K.L<br>Cyclohexene, 3-bromo-<br>Cyclopropane, 1-chloro-2-ethenyl-1<br>1,4-Hexadiene, 4-methyl-                 | 12265<br>3231<br>63147  | 001521-51-3<br>062337-93-3<br>001116-90-1 | 53<br>50<br>50 |
| 15  | 16.79 | 1.74  | C:\DATABASE\NBS75K.L<br>6,8-Nonadien-2-one<br>1,3,5-Hexatriene, (E)-<br>2-Hexen-4-yne, (E)-                                     | 7003<br>425<br>428      | 077828-54-7<br>000821-07-8<br>033625-96-6 | 59<br>59<br>59 |
| 16  | 17.25 | 1.91  | C:\DATABASE\NBS75K.L<br>3-Decen-1-yne, (Z)-<br>2-Nonen-4-yne, (Z)-<br>2H-Pyran-2-one, 4,6-dimethyl-                             | 6627<br>3966<br>4122    | 061827-98-1<br>056392-46-2<br>000675-09-2 | 30<br>30<br>30 |
| 17  | 17.49 | 8.44  | C:\DATABASE\NBS75K.L<br>2,4-Cycloheptadien-1-one, 2,6,6-tr<br>1,3-Cyclohexadiene-1-carboxaldehyd<br>Phenol, 4-butyl-            | 66800<br>9790<br>9808   | 000503-93-5<br>000116-26-7<br>001638-22-8 | 64<br>64<br>55 |
| 18  | 17.87 | 1.21  | C:\DATABASE\NBS75K.L<br>1,3-Hexadiene, 2,5-dimethyl-<br>exo-2-Bromonorbornane<br>5,5-Dimethyl-1,3-hexadiene                     | 2320<br>68413<br>2323   | 029253-64-3<br>002534-77-2<br>001515-79-3 | 59<br>53<br>50 |
| 19  | 17.93 | 1.93  | C:\DATABASE\NBS75K.L<br>2-Nonyne<br>3,5-Dimethylcyclohexene<br>1,4-Cyclohexanedimethanol                                        | 64823<br>2315<br>8402   | 019447-29-1<br>000000-00-0<br>000105-08-8 | 64<br>53<br>53 |



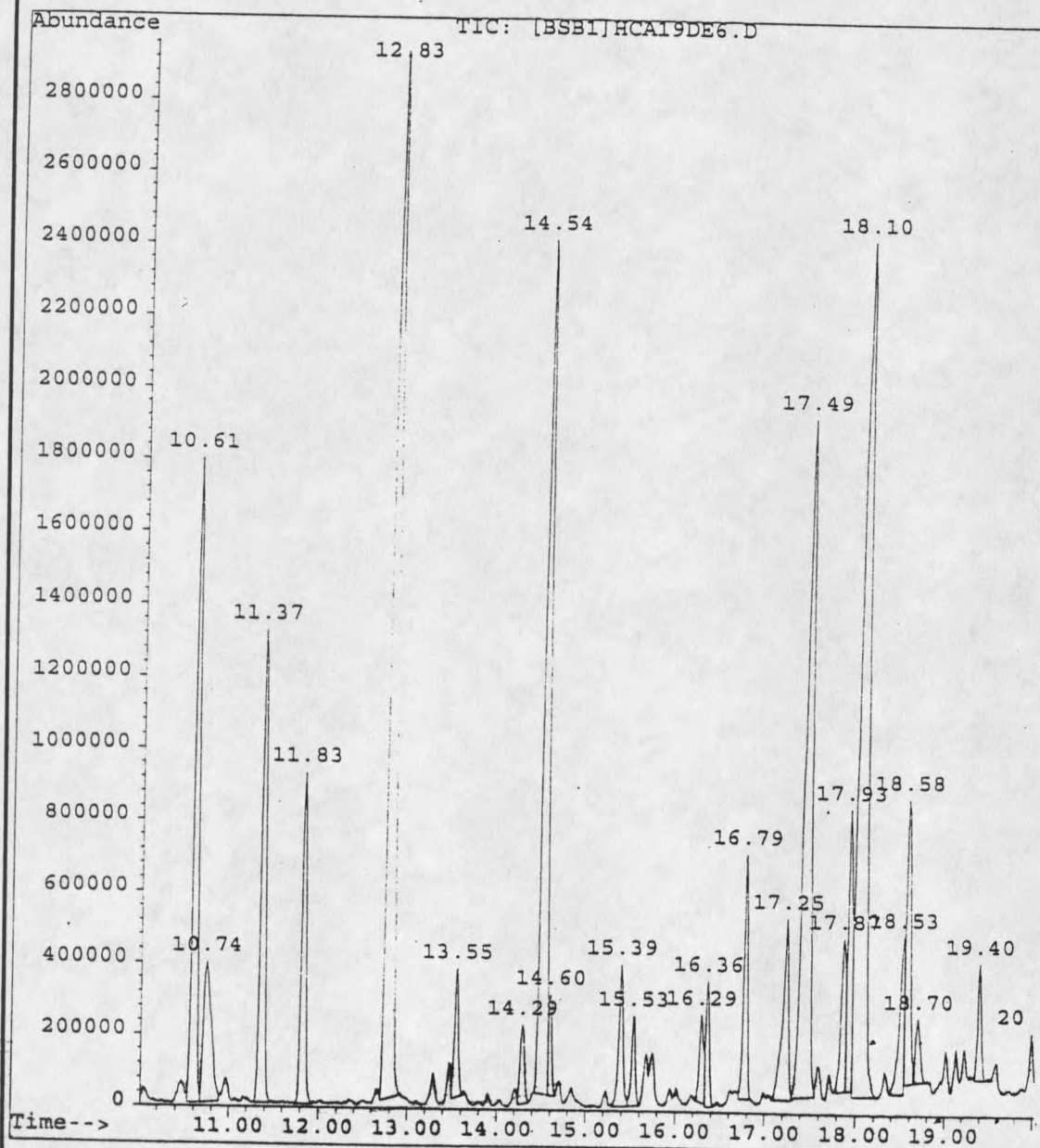
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|-----|-------|-------|------------------------------------|-------|-------------|------|
| 20  | 18.10 | 12.29 | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | Bicyclo[2.2.1]heptan-2-one, 1,7,7- | 10338 | 000464-48-2 | 97   |
|     |       |       | Bicyclo[2.2.1]heptan-2-one, 1,7,7- | 10341 | 021368-68-3 | 96   |
|     |       |       | Bicyclo[2.2.1]heptan-2-one, 1,7,7- | 66961 | 000464-48-2 | 96   |
| 21  | 18.53 | 1.32  | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | Bicyclo[3.3.1]heptan-3-one, 6,6-di | 9819  | 016812-40-1 | 42   |
|     |       |       | 1,3-Cyclooctadiene, (Z,Z)-         | 2155  | 003806-59-5 | 25   |
|     |       |       | 2-Octyne                           | 63876 | 002809-67-8 | 12   |
| 22  | 18.58 | 2.20  | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | 3,4-Pentadienal, 2,2-dimethyl-     | 2303  | 004058-51-9 | 50   |
|     |       |       | Ethyl propiolate                   | 1209  | 000623-47-2 | 43   |
|     |       |       | Furan, 2-pentyl-                   | 65913 | 003777-69-3 | 25   |
| 23  | 18.70 | 0.55  | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | Borneol                            | 67100 | 000507-70-0 | 53   |
|     |       |       | Borneol                            | 67097 | 000507-70-0 | 53   |
|     |       |       | Borneol                            | 67098 | 000507-70-0 | 53   |
| 24  | 19.40 | 0.78  | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | 2-Cyclohexen-1-one, 2-methyl-5-(1- | 66789 | 002244-16-8 | 38   |
|     |       |       | 2-Cyclohexen-1-one, 2-methyl-5-(1- | 66807 | 000099-49-0 | 38   |
|     |       |       | 2-Cyclohexen-1-one, 2-methyl-5-(1- | 66787 | 002244-16-8 | 35   |
| 25  | 20.00 | 0.60  | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | D-Verbenone                        | 9853  | 018309-32-5 | 70   |
|     |       |       | Bicyclo[3.1.1]hept-3-en-2-one, 4,6 | 66810 | 000080-57-9 | 58   |
|     |       |       | 1,3-Cyclooctadiene                 | 63792 | 001700-10-3 | 46   |
| 26  | 20.80 | 2.86  | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | 3-Hexyne, 2-methyl-                | 1106  | 036566-80-0 | 52   |
|     |       |       | 3,4-Heptadiene                     | 1112  | 002454-31-1 | 50   |
|     |       |       | 2,3-Hexadiene, 2-methyl-           | 1147  | 029212-09-7 | 50   |
| 27  | 21.35 | 0.55  | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | Cyclopropanecarboxylic acid, 3-eth | 7393  | 067528-58-9 | 53   |
|     |       |       | 1,3-Hexadiene, 2,5-dimethyl-       | 2320  | 029253-64-3 | 53   |
|     |       |       | Isobornyl acetate                  | 69497 | 000125-12-2 | 50   |
| 28  | 21.76 | 2.29  | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | 2-Cyclohexen-1-one, 2-methyl-5-(1- | 66787 | 002244-16-8 | 59   |
|     |       |       | 2-Cyclohexen-1-one, 2-methyl-5-(1- | 66808 | 000099-49-0 | 59   |
|     |       |       | 1H-Imidazole, 2-methyl-            | 62663 | 000693-98-1 | 52   |
| 29  | 23.09 | 1.79  | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | Pyridine, 2,5-dimethyl-            | 63719 | 000589-93-5 | 68   |
|     |       |       | Pyridine, 3,4-dimethyl-            | 63728 | 000583-58-4 | 64   |
|     |       |       | Phosphonic acid, (3,4-dimethyl-2-p | 32499 | 026384-82-7 | 64   |
| 30  | 23.82 | 0.61  | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | 9,12,15-Octadecatrienoic acid, met | 41563 | 000301-00-8 | 27   |
|     |       |       | Spiro[4.5]decane, 6-methylene-     | 9888  | 019144-01-5 | 25   |
|     |       |       | 1,5-Heptadiene, 2,3,6-trimethyl-   | 7094  | 033501-88-1 | 22   |

| Pk# | RT    | Area% | Library/ID                                                                                                                             | Ref#                    | CAS#                                      | Qual           |
|-----|-------|-------|----------------------------------------------------------------------------------------------------------------------------------------|-------------------------|-------------------------------------------|----------------|
| 31  | 23.91 | 0.66  | C:\DATABASE\NBS75K.L<br>1,7-Octadien-3-one, 2-methyl-6-met<br>1-Pentene, 5-bromo-<br>2,6-Nonadienal, (E,Z)-                            | 9854<br>9142<br>6994    | 041702-60-7<br>001119-51-3<br>000557-48-2 | 58<br>53<br>47 |
| 32  | 25.71 | 1.48  | C:\DATABASE\NBS75K.L<br>Caryophyllene<br>6-Propyl-cis-bicyclo(3.2.0)hept-6-<br>Caryophyllene                                           | 69933<br>9800<br>69934  | 000087-44-5<br>054396-48-4<br>000087-44-5 | 64<br>46<br>46 |
| 33  | 27.01 | 1.75  | C:\DATABASE\NBS75K.L<br>.alpha.-Cubebene<br>1H-Cyclopenta[1,3]cyclopropa[1,2]b<br>Naphthalene, 1,2,3,4,4a,5,6,8a-oct                   | 69900<br>23898<br>23957 | 017699-14-8<br>013744-15-5<br>039029-41-9 | 83<br>70<br>60 |
| 34  | 31.90 | 1.40  | C:\DATABASE\NBS75K.L<br>1,4-Naphthalenediol, 6-ethenyl-5,6<br>2H-Benzimidazol-2-one, 6-chloro-1,<br>Benzaldehyde, 2,4-difluoro-5-metho | 32780<br>32667<br>32632 | 077880-93-4<br>054986-47-9<br>000000-00-0 | 38<br>35<br>35 |
| 35  | 31.95 | 1.10  | C:\DATABASE\NBS75K.L<br>2H-Benzimidazol-2-one, 6-chloro-1,<br>Ferrocene, (3-hydroxypropyl)-<br>1-Chloro-2-methyl-1-morpholino-1,3      | 32667<br>32674<br>19620 | 054986-47-9<br>012093-88-8<br>000000-00-0 | 38<br>35<br>32 |
| 36  | 32.03 | 0.92  | C:\DATABASE\NBS75K.L<br>1H-Benzimidazol-4-ol, 1-(4-chlorop<br>2H-Benzimidazol-2-one, 6-chloro-1,<br>1,2,3,4-Phenazinetetrol            | 32666<br>32667<br>32646 | 054986-46-8<br>054986-47-9<br>025187-21-7 | 46<br>43<br>37 |
| 37  | 32.09 | 5.53  | C:\DATABASE\NBS75K.L<br>[1,1'-Biphenyl]-4,4'-diamine, 3,3'<br>1,1'-Biphenyl, 2,2',5-trimethoxy-<br>2-Propenoic acid, 2-cyano-3-(4-dim  | 71294<br>32755<br>71293 | 000119-90-4<br>019718-53-7<br>001886-52-8 | 64<br>59<br>58 |
| 38  | 32.90 | 0.64  | C:\DATABASE\NBS75K.L<br>Formaldehyde, methyl(2-propynyl)hy<br>Furan, 3-(4,8-dimethyl-3,7-nonadie<br>Nonahexacontanoic acid             | 1070<br>27259<br>62178  | 000000-00-0<br>023262-34-2<br>040710-32-5 | 47<br>43<br>25 |
| 39  | 33.79 | 2.30  | C:\DATABASE\NBS75K.L<br>Octadecane, 3-methyl-<br>Hexacosane<br>Pentadecane, 8,8-diheptyl-                                              | 37467<br>51010<br>54527 | 006561-44-0<br>000630-01-3<br>055268-72-9 | 91<br>90<br>87 |
| 40  | 34.91 | 1.23  | C:\DATABASE\NBS75K.L<br>Octadecane, 3-methyl-<br>Heptadecane, 8,8-dipentyl-<br>Tetratriacontane                                        | 37467<br>52250<br>58265 | 006561-44-0<br>055282-28-5<br>014167-59-0 | 91<br>90<br>87 |

File : C:\HPCHEM\1\DATA\BSB\HCA19DE6.D  
Operator : [BSB1]HOWARD  
Acquired : 19 Dec 96 11:29 am using AcqMethod HOWARD2  
Instrument : 5970 - In  
Sample Name: FRANCOISE GRNHS SAGE: A; REP 2  
Misc Info : MECL EXT: 3.53G/20ML  
Vial Number: 1

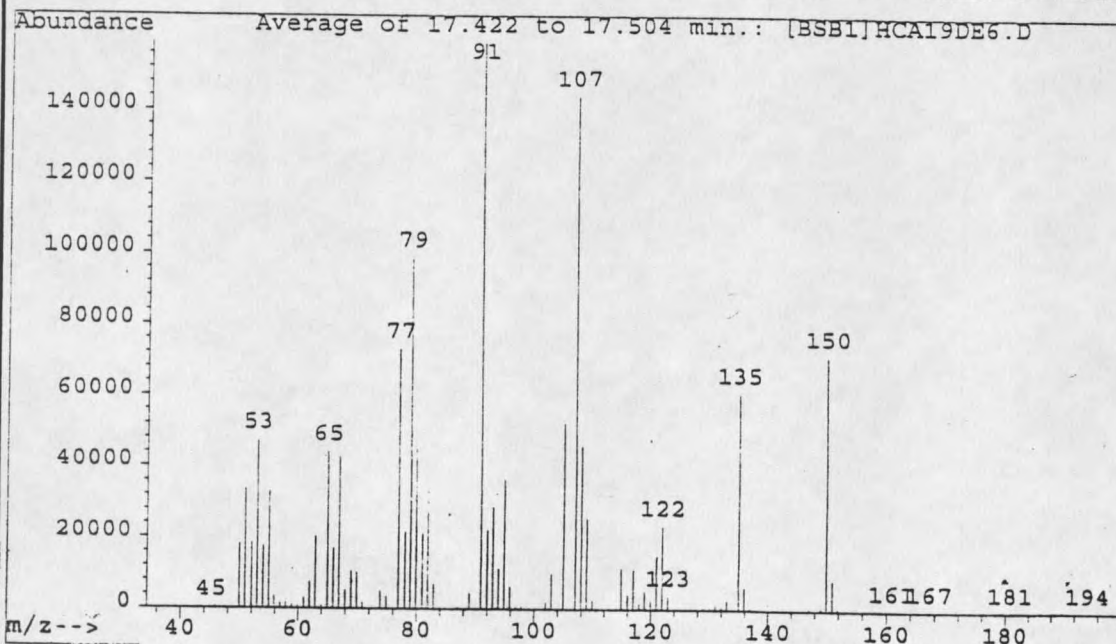
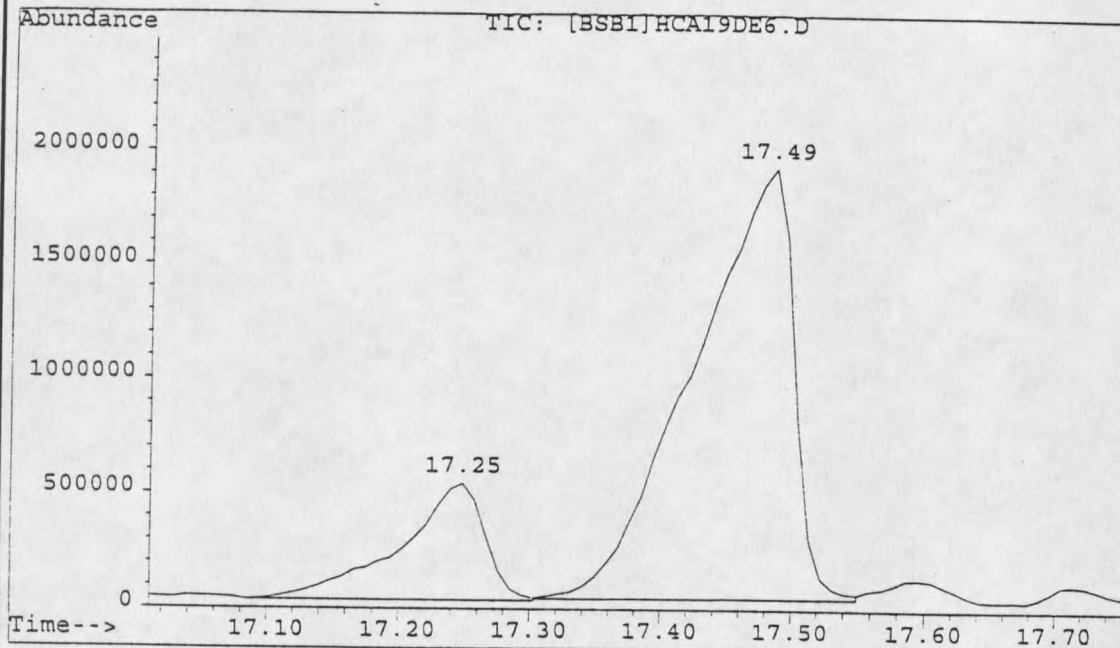


File : C:\HPCHEM\1\DATA\BSB\HCA19DE6.D  
Operator : [BSB1]HOWARD  
Acquired : 19 Dec 96 11:29 am using AcqMethod HOWARD2  
Instrument : 5970 - In  
Sample Name: FRANCOISE GRNHS SAGE: A; REP 2  
Misc Info : MECL EXT: 3.53G/20ML  
Vial Number: 1





File : C:\HPCHEM\1\DATA\BSB\HCA19DE6.D  
Operator : [BSB1]HOWARD  
Acquired : 19 Dec 96 11:29 am using AcqMethod HOWARD2  
Instrument : 5970 - In  
Sample Name: FRANCOISE GRNHS SAGE: A; REP 2  
Misc Info : MECL EXT: 3.53G/20ML  
Vial Number: 1

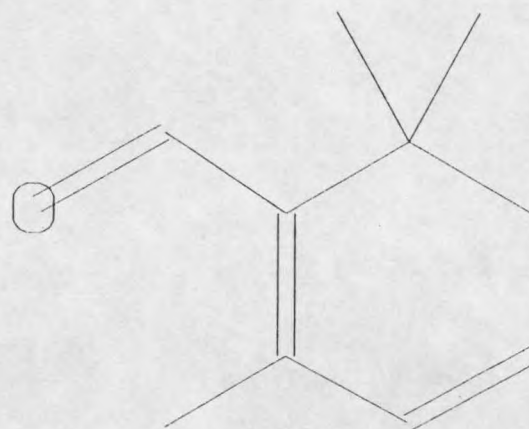
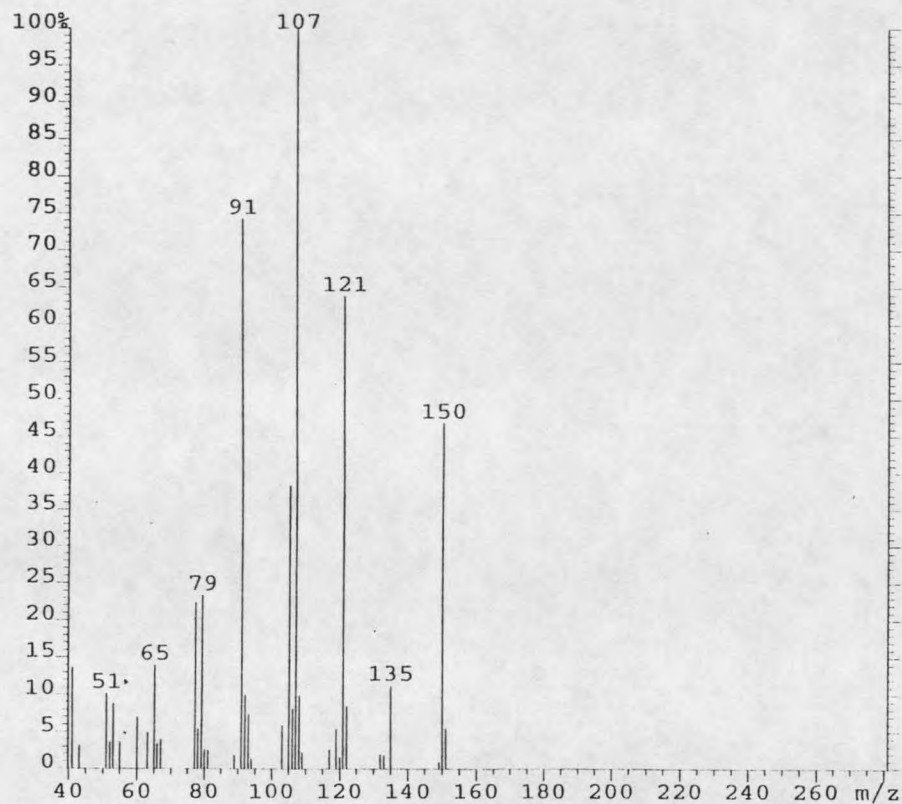


Libraries 1: NIST (62235) 2: NISTREP (12593)

Found 8 unverified entries for CAS number 116-26-7

NIST #9790 MF:C10H14O MW:150 CAS:116-26-7 NIST:4622

Name: 1,3-Cyclohexadiene-1-carboxaldehyde, 2,6,6-trimethyl- | Safranal SMILES: CC1=C(C=O)C(C)(C)C=C1

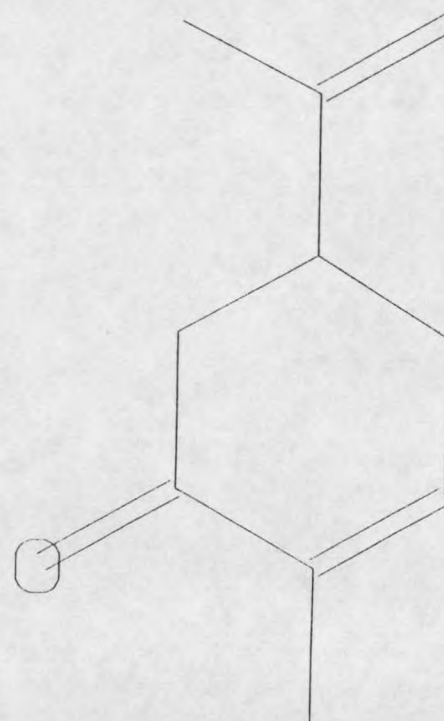
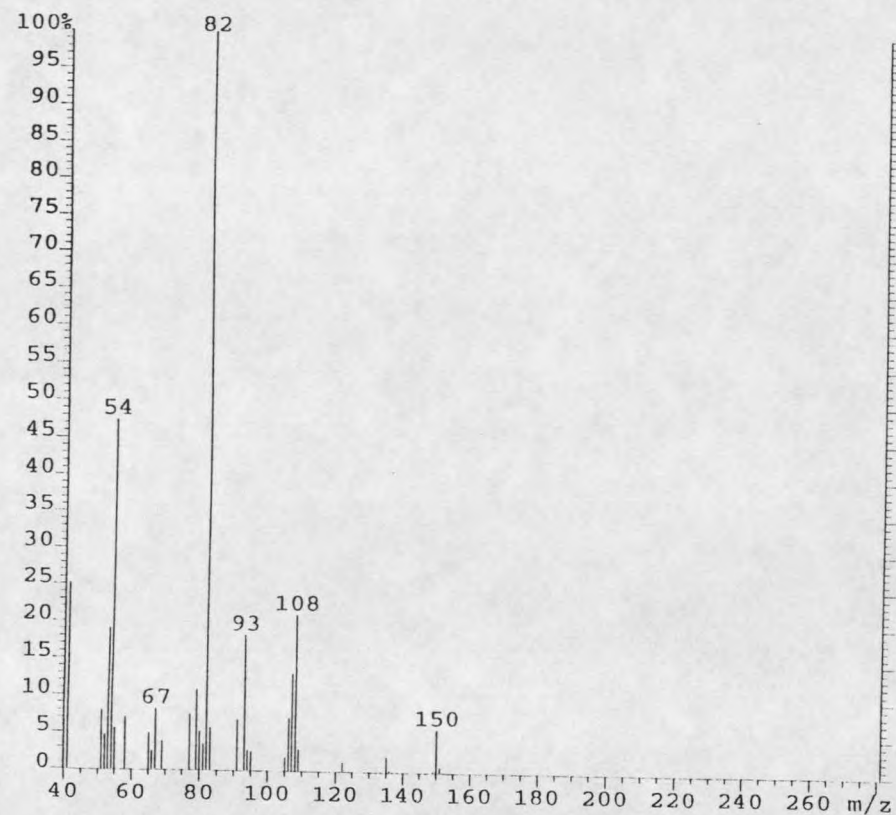


Libraries 1: NIST (62235) 2: NISTREP (12593)

Found 4 unverified entries for CAS number 6485-40-1

NIST #9836 MF:C10H14O MW:150 CAS:6485-40-1 NIST:28079

Name: 2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-, (R)- | p-Mentha-6,8-dien-2-one, (R)-(-)>



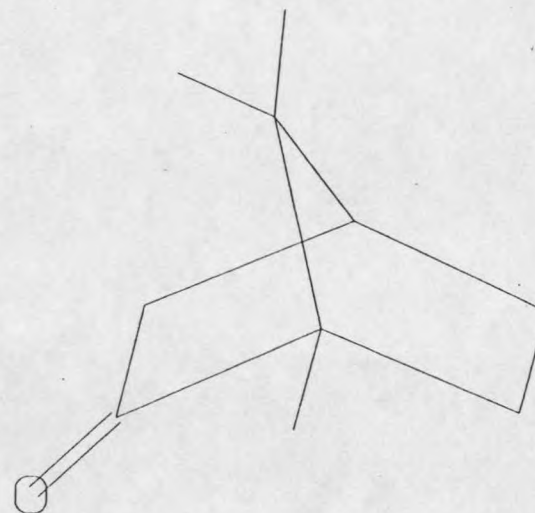
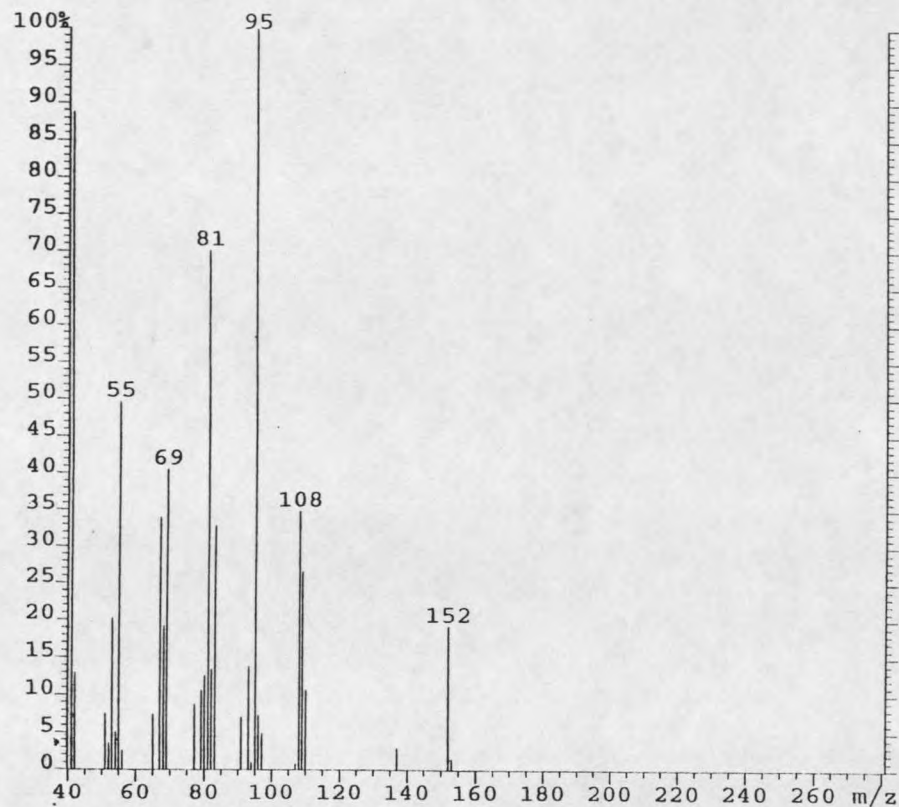


Libraries 1: NIST (62235) 2: NISTREP (12593)

Found 1 unverified entry for CAS number 21368-68-3

NIST #10341 MF:C10H16O MW:152 CAS:21368-68-3 NIST:73611

Name: Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (./-.)- | Camphor, (./-.)- | (./-.)-Camph>

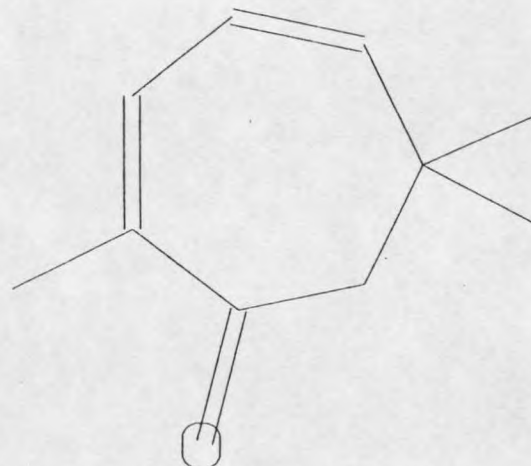
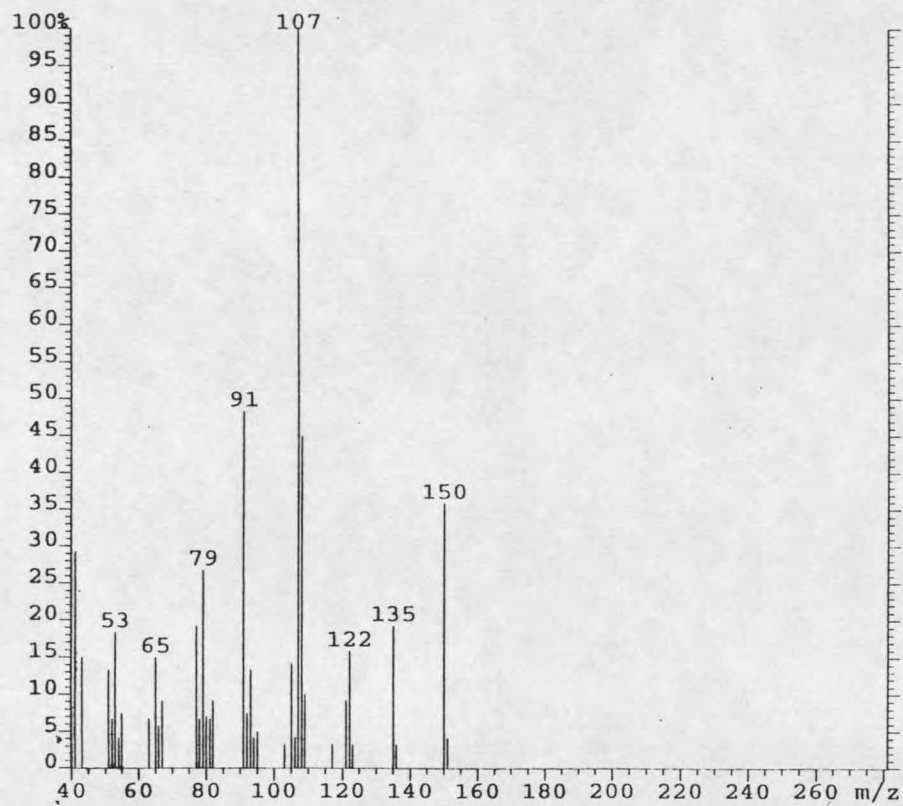


Libraries 1: NIST (62235) 2: NISTREP (12593)

Found 2 unverified entries for CAS number 503-93-5

NIST #9827 MF:C10H14O MW:150 CAS:503-93-5 NIST:4629

Name: 2,4-Cycloheptadien-1-one, 2,6,6-trimethyl- | Eucarvone SMILES: CC1=CC=CC(C)(C)CC1=O



## Information from Data File:

File : C:\HPCHEM\1\DATA\HC11APRA.D  
 Operator : howard c  
 Acquired : 11 Apr 97 12:01 pm using AcqMethod HOWARD2  
 Sample Name: Francoise's greenhouse plant extract: E1  
 Misc Info :  
 Vial Number: 1

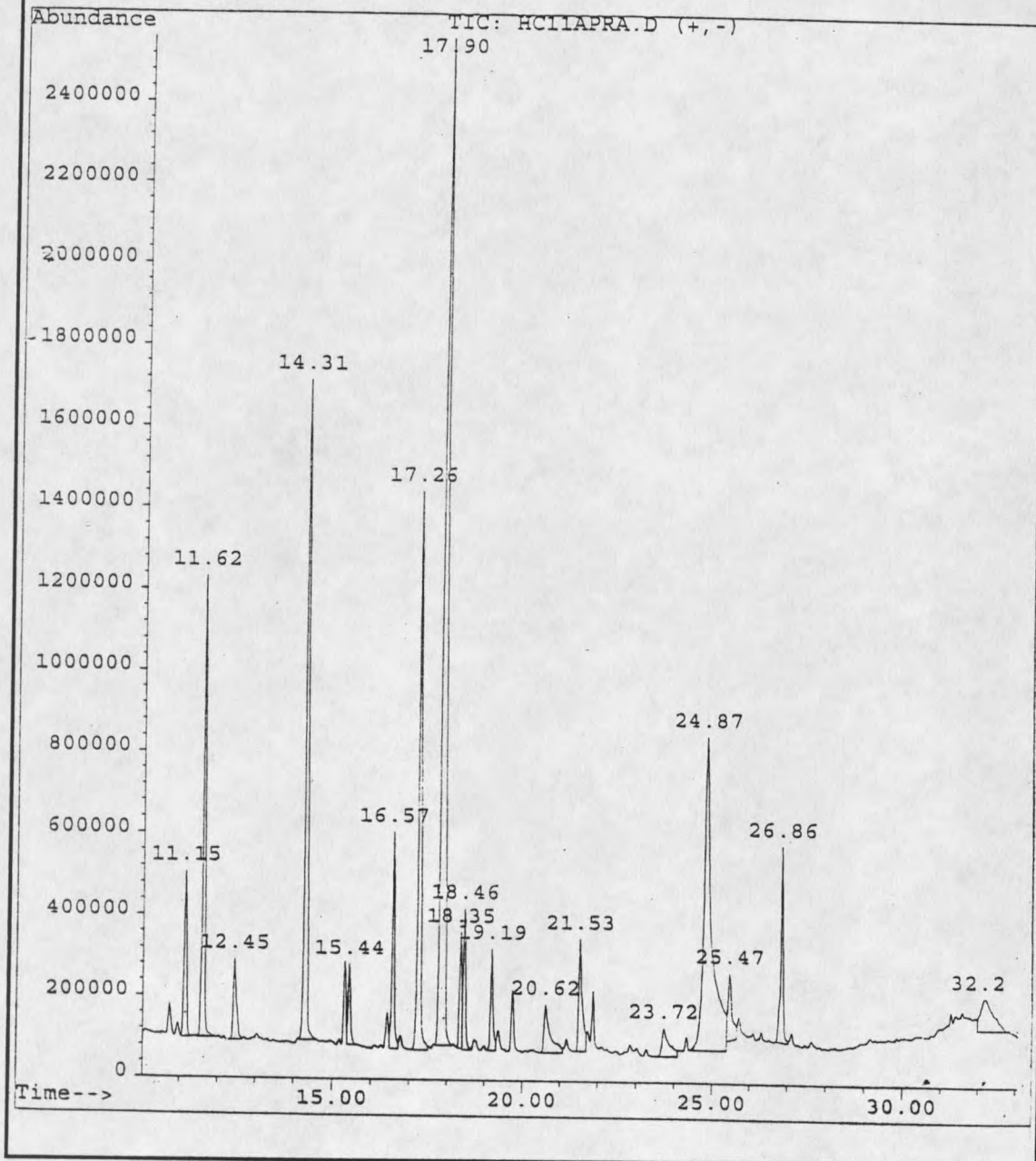
Search Libraries: C:\DATABASE\NBS75K.L Minimum Quality: 0

Unknown Spectrum: Peak average  
 Integration Params: current RTEINT parameters

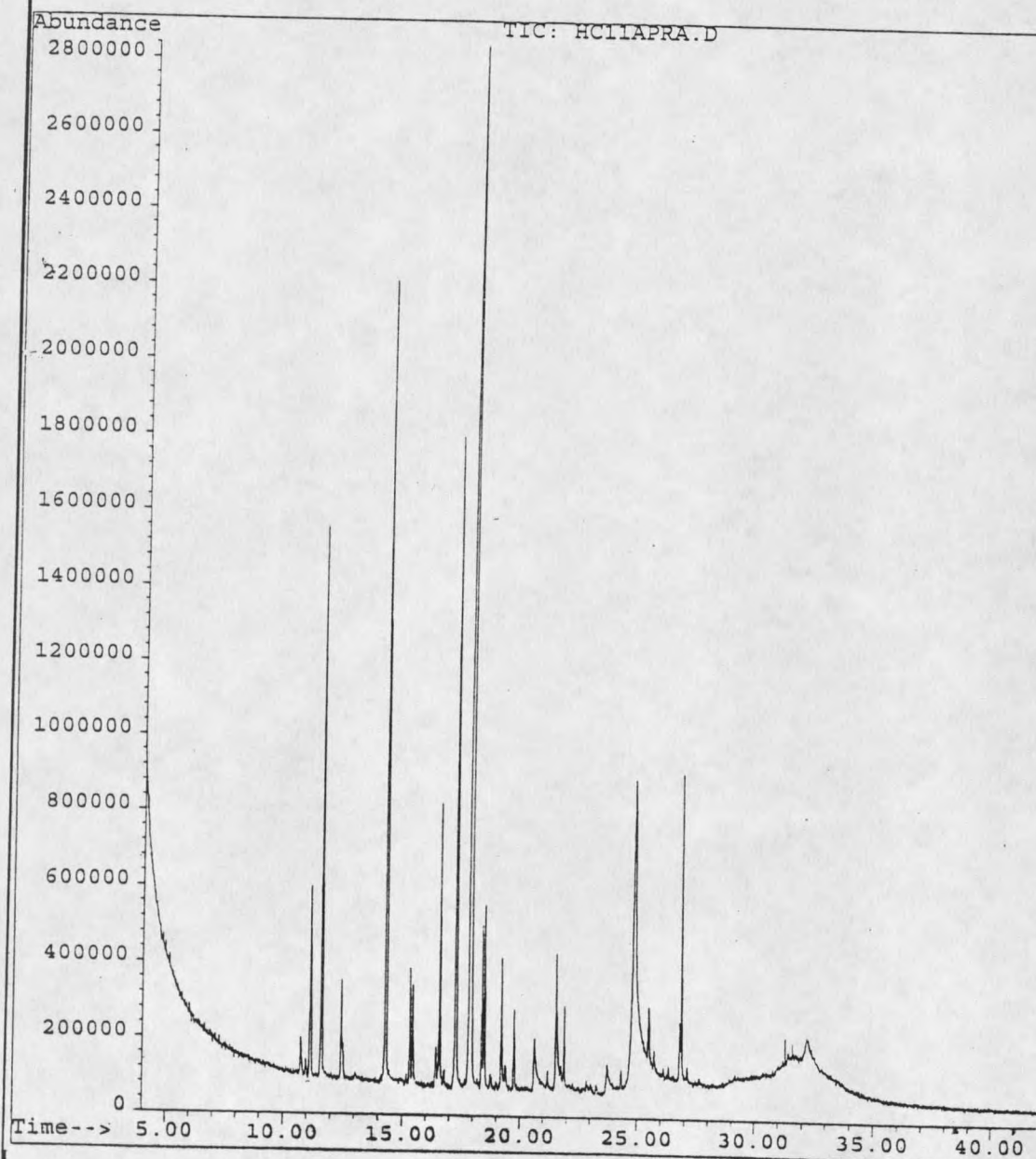
| PK# | RT    | Area% | Library/ID                          | Ref#  | CAS#        | Qual |
|-----|-------|-------|-------------------------------------|-------|-------------|------|
| 1   | 11.15 | 2.88  | C:\DATABASE\NBS75K.L                |       |             |      |
|     |       |       | .alpha.-Pinene                      | 65808 | 000080-56-8 | 59   |
|     |       |       | Bicyclo[3.1.1]hept-2-ene, 3,6,6-tr  | 6692  | 004889-83-2 | 55   |
|     |       |       | Bicyclo[3.1.1]heptane, 6,6-dimethy  | 6602  | 018172-67-3 | 53   |
| 2   | 11.62 | 7.64  | C:\DATABASE\NBS75K.L                |       |             |      |
|     |       |       | Camphene                            | 65764 | 000079-92-5 | 81   |
|     |       |       | Camphene                            | 65765 | 000079-92-5 | 81   |
|     |       |       | Camphene                            | 65766 | 000079-92-5 | 81   |
| 3   | 12.45 | 1.70  | C:\DATABASE\NBS75K.L                |       |             |      |
|     |       |       | 2-Methyl-4-propyl-1,3-oxathiane 3-  | 16604 | 000000-00-0 | 40   |
|     |       |       | Pyrrolidine, 1-methyl-2-octyl-      | 22123 | 003447-06-1 | 14   |
|     |       |       | 2,2'-Dipiperidine                   | 14633 | 000531-67-9 | 9    |
| 4   | 14.31 | 11.31 | C:\DATABASE\NBS75K.L                |       |             |      |
|     |       |       | Eucalyptol                          | 67088 | 000470-82-6 | 64   |
|     |       |       | Eucalyptol                          | 10893 | 000470-82-6 | 64   |
|     |       |       | Eucalyptol                          | 67089 | 000470-82-6 | 50   |
| 5   | 15.44 | 1.30  | C:\DATABASE\NBS75K.L                |       |             |      |
|     |       |       | 1-Pyrrolidinylloxy, 3-amino-2,2,5,5 | 11714 | 034272-83-8 | 49   |
|     |       |       | 2-Methyl-4-propyl-1,3-oxathiane 3-  | 16604 | 000000-00-0 | 40   |
|     |       |       | Ethyl-6-isopropyl-3-methyl-9-oxo-2  | 36985 | 000000-00-0 | 38   |
| 6   | 16.57 | 3.46  | C:\DATABASE\NBS75K.L                |       |             |      |
|     |       |       | Bicyclo[2.2.2]oct-2-ene             | 2141  | 000931-64-6 | 53   |
|     |       |       | Bicyclo(3.3.1)non-2-ene             | 3973  | 006671-66-5 | 47   |
|     |       |       | Cyclobuta[1,2-b:4,3-b']bisthiopyra  | 36484 | 074630-21-0 | 38   |
| 7   | 17.26 | 10.23 | C:\DATABASE\NBS75K.L                |       |             |      |
|     |       |       | 1,3-Cyclohexadiene-1-carboxaldehyd  | 9790  | 000116-26-7 | 38   |
|     |       |       | 1,3-Cyclohexadiene-1-carboxaldehyd  | 66783 | 000116-26-7 | 38   |
|     |       |       | Bicyclo[4.1.0]heptan-3-one, 7,7-di  | 9823  | 106974-32-7 | 30   |
| 8   | 17.90 | 25.51 | C:\DATABASE\NBS75K.L                |       |             |      |
|     |       |       | Bicyclo[2.2.1]heptan-2-one, 1,7,7-  | 10338 | 000464-48-2 | 98   |
|     |       |       | Camphor                             | 66978 | 000076-22-2 | 95   |
|     |       |       | Camphor                             | 66979 | 000076-22-2 | 95   |

| Pk# | RT    | Area% | Library/ID                                                                                                                            | Ref#                    | CAS#                                      | Qual           |
|-----|-------|-------|---------------------------------------------------------------------------------------------------------------------------------------|-------------------------|-------------------------------------------|----------------|
| 9   | 18.35 | 1.69  | C:\DATABASE\NBS75K.L<br>Cyclohexane, ethylidene-<br>2,5-Cyclohexadien-1-one, 4-ethyl-3<br>2,6-Dimethyl-1,3,6-heptatriene              | 63880<br>9817<br>3988   | 001003-64-1<br>017429-35-5<br>000928-67-6 | 90<br>90<br>90 |
| 10  | 18.46 | 2.23  | C:\DATABASE\NBS75K.L<br>trans,trans-1,9-Dimethylspiro[4.5]<br>trans,cis-1,9-Dimethylspiro[4.5]de<br>Isoborneol                        | 14141<br>14142<br>67125 | 000000-00-0<br>000000-00-0<br>000124-76-5 | 40<br>40<br>38 |
| 11  | 19.19 | 1.68  | C:\DATABASE\NBS75K.L<br>2-Methyl-4-propyl-1,3-oxathiane 3-<br>Methane, bromochloro-<br>Methane, bromochloro-                          | 16604<br>64995<br>64996 | 000000-00-0<br>000074-97-5<br>000074-97-5 | 42<br>40<br>40 |
| 12  | 20.62 | 1.99  | C:\DATABASE\NBS75K.L<br>Propanenitrile, 2,2,3-trichloro-<br>Ethanol, 2,2,2-trichloro-, dihydro<br>Methane, bromochloro-               | 11636<br>29394<br>64995 | 000813-74-1<br>000306-52-5<br>000074-97-5 | 42<br>36<br>33 |
| 13  | 21.53 | 2.58  | C:\DATABASE\NBS75K.L<br>2-Cyclohexen-1-one, 3,5-dimethyl-<br>2-Cyclohexen-1-one, 2-methyl-5-(1-<br>2-Cyclohexen-1-one, 2-methyl-5-(1- | 64787<br>66788<br>9836  | 001123-09-7<br>002244-16-8<br>006485-40-1 | 27<br>25<br>25 |
| 14  | 23.72 | 1.33  | C:\DATABASE\NBS75K.L<br>Ethanol, 2,2,2-trichloro-, dihydro<br>Propanenitrile, 2,2,3-trichloro-<br>1,16-Cyclocorynan-17-oic acid, 19,  | 29394<br>11636<br>45879 | 000306-52-5<br>000813-74-1<br>006393-66-4 | 53<br>42<br>37 |
| 15  | 24.87 | 16.74 | C:\DATABASE\NBS75K.L<br>Spiro[5.5]undec-1-ene<br>1,3-Cyclohexadiene-1-carboxaldehyd<br>2,4-Cycloheptadien-1-one, 2,6,6-tr             | 9897<br>9790<br>66800   | 000699-56-9<br>000116-26-7<br>000503-93-5 | 60<br>52<br>50 |
| 16  | 25.47 | 1.80  | C:\DATABASE\NBS75K.L<br>Benzene, 2-methoxy-1,3,4-trimethyl<br>Spiro[5.5]undec-1-ene<br>Pyrazine, 2,3-diethyl-5-methyl-                | 9866<br>9897<br>9761    | 021573-36-4<br>000699-56-9<br>018138-04-0 | 40<br>30<br>25 |
| 17  | 26.86 | 3.22  | C:\DATABASE\NBS75K.L<br>.alpha.-Cubebene<br>Copaene<br>Ylangene                                                                       | 69900<br>69922<br>23969 | 017699-14-8<br>003856-25-5<br>014912-44-8 | 92<br>60<br>55 |
| 18  | 32.20 | 2.71  | C:\DATABASE\NBS75K.L<br>Methane, bromochloro-<br>Methane, bromochloro-<br>Methane, bromochloro-                                       | 64996<br>64997<br>4820  | 000074-97-5<br>000074-97-5<br>000074-97-5 | 50<br>50<br>49 |

File : C:\HPCHEM\1\DATA\HC11APRA.D  
Operator : howard c  
Acquired : 11 Apr 97 12:01 pm using AcqMethod HOWARD2  
Instrument : 5970 - In  
Sample Name: Francoise's greenhouse plant extract: E1  
Misc Info :  
Vial Number: 1



File : C:\HPCHEM\1\DATA\HC11APRA.D  
Operator : howard c  
Acquired : 11 Apr 97 12:01 pm using AcqMethod HOWARD2  
Instrument : 5970 - In  
Sample Name: Francoise's greenhouse plant extract: E1  
Misc Info :  
Vial Number: 1



TIC: HC11APRA.D

Francoise's greenhouse plant extract: E1

Modified:added subtracted

| Peak# | Ret Time | Type | Width | Area     | Start Time | End Time |
|-------|----------|------|-------|----------|------------|----------|
| 1     | 11.149   | rVB  | 0.256 | 2000747  | 11.048     | 11.304   |
| 2     | 11.623   | rBV  | 0.374 | 5310837  | 11.505     | 11.879   |
| 3     | 12.455   | rBV  | 0.329 | 1182162  | 12.327     | 12.655   |
| 4     | 14.308   | rVB  | 0.448 | 7867198  | 14.144     | 14.592   |
| 5     | 15.441   | rVB  | 0.219 | 901760   | 15.377     | 15.596   |
| 6     | 16.573   | rVV  | 0.228 | 2408946  | 16.482     | 16.710   |
| 7     | 17.258   | rBV  | 0.411 | 7115171  | 17.085     | 17.496   |
| 8     | 17.898   | rBV  | 0.448 | 17743405 | 17.688     | 18.135   |
| 9     | 18.354   | rBV  | 0.128 | 1178801  | 18.281     | 18.409   |
| 10    | 18.464   | rVB  | 0.247 | 1549957  | 18.409     | 18.656   |
| 11    | 19.194   | rBV  | 0.237 | 1166376  | 19.067     | 19.304   |
| 12    | 20.619   | rBV  | 0.603 | 1383425  | 20.473     | 21.076   |
| 13    | 21.532   | rBV  | 0.265 | 1796055  | 21.432     | 21.697   |
| 14    | 23.724   | rBV  | 0.575 | 924586   | 23.505     | 24.080   |
| 15    | 24.866   | rBV  | 0.995 | 11644940 | 24.391     | 25.386   |
| 16    | 25.469   | rVV  | 0.265 | 1251862  | 25.386     | 25.651   |
| 17    | 26.857   | rBV  | 0.320 | 2239634  | 26.711     | 27.030   |
| 18    | 32.199   | rVB  | 0.785 | 1885951  | 31.989     | 32.775   |



## Information from Data File:

File : C:\HPCHEM\1\DATA\HC2MARA.D  
 Operator : Howard C.  
 Acquired : 2 Mar 97 7:30 pm using AcqMethod HOWARD2  
 Sample Name: Francoise; E2 (1/20/97)  
 Misc Info :  
 Vial Number: 1

Search Libraries: C:\DATABASE\NBS75K.L

Minimum Quality: 0

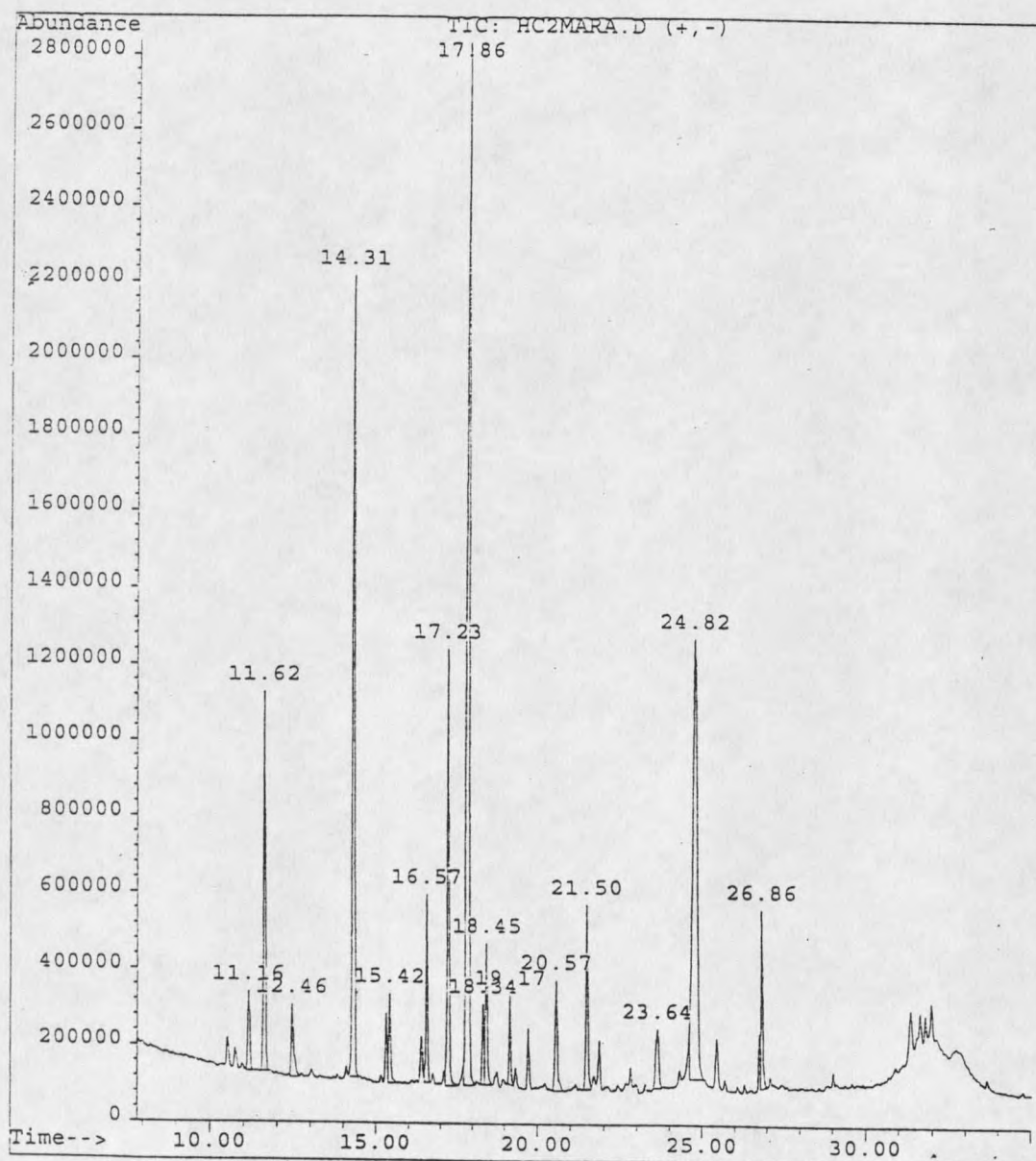
Unknown Spectrum: Peak average

Integration Params: current RTEINT parameters

| Pk# | RT    | Area% | Library/ID                         | Ref#  | CAS#        | Qual |
|-----|-------|-------|------------------------------------|-------|-------------|------|
| 1   | 11.16 | 1.56  | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | Methylene Chloride                 | 62705 | 000075-09-2 | 83   |
|     |       |       | Methylene Chloride                 | 62703 | 000075-09-2 | 83   |
|     |       |       | Methylene Chloride                 | 62704 | 000075-09-2 | 64   |
| 2   | 11.62 | 7.06  | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | Camphene                           | 65766 | 000079-92-5 | 84   |
|     |       |       | Camphene                           | 65765 | 000079-92-5 | 84   |
|     |       |       | Camphene                           | 65764 | 000079-92-5 | 83   |
| 3   | 12.46 | 1.64  | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | Methylene Chloride                 | 62705 | 000075-09-2 | 83   |
|     |       |       | Methylene Chloride                 | 62703 | 000075-09-2 | 83   |
|     |       |       | Methylene Chloride                 | 524   | 000075-09-2 | 72   |
| 4   | 14.31 | 15.60 | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | Eucalyptol                         | 67090 | 000470-82-6 | 83   |
|     |       |       | Eucalyptol                         | 67088 | 000470-82-6 | 64   |
|     |       |       | Eucalyptol                         | 10893 | 000470-82-6 | 62   |
| 5   | 15.42 | 1.62  | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | Methylene Chloride                 | 62705 | 000075-09-2 | 87   |
|     |       |       | Methylene Chloride                 | 62703 | 000075-09-2 | 86   |
|     |       |       | Methylene Chloride                 | 524   | 000075-09-2 | 72   |
| 6   | 16.57 | 3.08  | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | Phenol, 4-ethyl-                   | 3957  | 000123-07-9 | 90   |
|     |       |       | Isopropylpyrazine                  | 3932  | 009820-90-0 | 90   |
|     |       |       | 2-Isopropylpyrazine                | 3926  | 029460-90-0 | 90   |
| 7   | 17.23 | 8.19  | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | Methylene Chloride                 | 62705 | 000075-09-2 | 52   |
|     |       |       | Methylene Chloride                 | 62704 | 000075-09-2 | 49   |
|     |       |       | Methylene Chloride                 | 524   | 000075-09-2 | 49   |
| 8   | 17.86 | 26.78 | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | Bicyclo[2.2.1]heptan-2-one, 1,7,7- | 10338 | 000464-48-2 | 98   |
|     |       |       | Bicyclo[2.2.1]heptan-2-one, 1,7,7- | 66961 | 000464-48-2 | 97   |
|     |       |       | Bicyclo[2.2.1]heptan-2-one, 1,7,7- | 10341 | 021368-68-3 | 96   |

| PK# | RT    | Area% | Library/ID                         | Ref#  | CAS#        | Qual |
|-----|-------|-------|------------------------------------|-------|-------------|------|
| 9   | 18.34 | 1.35  | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | Benzyl Alcohol                     | 63784 | 000100-51-6 | 91   |
|     |       |       | 1,2,3-Butatriene, 1-chloro-        | 548   | 020658-21-3 | 90   |
|     |       |       | Benzyl Alcohol                     | 2119  | 000100-51-6 | 90   |
| 10  | 18.45 | 2.63  | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | Borneol                            | 67099 | 000507-70-0 | 70   |
|     |       |       | Methylene Chloride                 | 62704 | 000075-09-2 | 58   |
|     |       |       | Methylene Chloride                 | 62703 | 000075-09-2 | 58   |
| 11  | 19.17 | 1.46  | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | Methylene Chloride                 | 62705 | 000075-09-2 | 64   |
|     |       |       | Methylene Chloride                 | 62704 | 000075-09-2 | 64   |
|     |       |       | Methylene Chloride                 | 62703 | 000075-09-2 | 64   |
| 12  | 20.57 | 2.43  | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | Methylene Chloride                 | 62704 | 000075-09-2 | 58   |
|     |       |       | Methylene Chloride                 | 62703 | 000075-09-2 | 58   |
|     |       |       | Methylene Chloride                 | 524   | 000075-09-2 | 58   |
| 13  | 21.50 | 3.46  | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | 2-Cyclohexen-1-one, 3-methyl-      | 63863 | 001193-18-6 | 30   |
|     |       |       | 1H-Pyrazole, 1-methyl-             | 62662 | 000930-36-9 | 30   |
|     |       |       | 1H-Imidazole, 2-methyl-            | 62663 | 000693-98-1 | 27   |
| 14  | 23.64 | 1.77  | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | Methylene Chloride                 | 524   | 000075-09-2 | 53   |
|     |       |       | Methylene Chloride                 | 62703 | 000075-09-2 | 53   |
|     |       |       | Methylene Chloride                 | 62705 | 000075-09-2 | 49   |
| 15  | 24.82 | 18.48 | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | Phenol, 4-(2-methylpropyl)-        | 9799  | 004167-74-2 | 78   |
|     |       |       | Spiro[5.5]undec-1-ene              | 9897  | 000699-56-9 | 60   |
|     |       |       | 1,3-Cyclohexadiene-1-carboxaldehyd | 9790  | 000116-26-7 | 50   |
| 16  | 26.86 | 2.90  | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | .alpha.-Cubebene                   | 69900 | 017699-14-8 | 95   |
|     |       |       | .alpha.-Cubebene                   | 69901 | 017699-14-8 | 90   |
|     |       |       | .alpha.-Cubebene                   | 23919 | 017699-14-8 | 64   |

File : C:\HPCHEM\1\DATA\HC2MARA.D  
Operator : Howard C.  
Acquired : 2 Mar 97 7:30 pm using AcqMethod HOWARD2  
Instrument : 5970 - In  
Sample Name: Francoise; E2 (1/20/97)  
Misc Info :  
Vial Number: 1



## Information from Data File:

File : C:\HPCHEM\1\DATA\HC2MARB.D  
 Operator : Howard C.  
 Acquired : 2 Mar 97 8:32 pm using AcqMethod HOWARD2  
 Sample Name: Francoise; F1 (1/20/97)  
 Misc Info :  
 Vial Number: 1

Search Libraries: C:\DATABASE\NBS75K.L Minimum Quality: 0

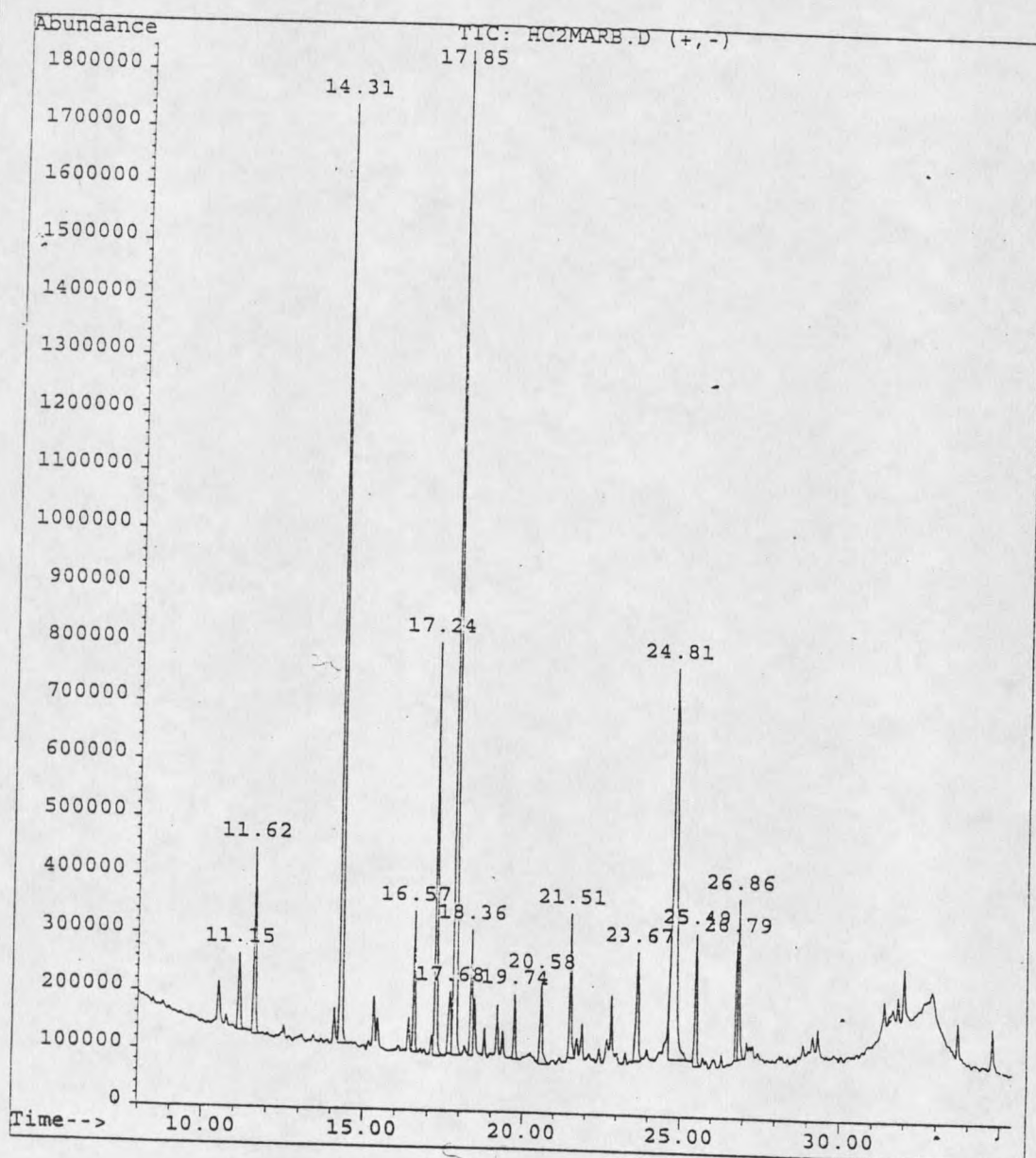
Unknown Spectrum: Peak average  
 Integration Params: current RTEINT parameters

| Pk# | RT    | Area% | Library/ID                         | Ref#  | CAS#        | Qual |
|-----|-------|-------|------------------------------------|-------|-------------|------|
| 1   | 11.15 | 1.82  | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | 2-Methyl-4-propyl-1,3-oxathiane 3- | 16604 | 000000-00-0 | 64   |
|     |       |       | Methane, bromochloro-              | 64996 | 000074-97-5 | 45   |
|     |       |       | Ethane, 2-chloro-1-(chloromethoxy) | 18140 | 000428-92-2 | 42   |
| 2   | 11.62 | 4.21  | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | 2-Methyl-4-propyl-1,3-oxathiane 3- | 16604 | 000000-00-0 | 40   |
|     |       |       | Methane, bromochloro-              | 64995 | 000074-97-5 | 36   |
|     |       |       | Propanenitrile, 3-chloro-          | 62968 | 000542-76-7 | 28   |
| 3   | 14.31 | 19.60 | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | Eucalyptol                         | 67090 | 000470-82-6 | 83   |
|     |       |       | Eucalyptol                         | 67087 | 000470-82-6 | 60   |
|     |       |       | Eucalyptol                         | 67088 | 000470-82-6 | 59   |
| 4   | 16.57 | 2.63  | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | Bicyclo[2.2.1]hept-2-ene, 1-methyl | 2173  | 000822-73-1 | 90   |
|     |       |       | Bicyclo[2.2.1]hept-2-ene, 2-methyl | 2158  | 000694-92-8 | 83   |
|     |       |       | 2H-Pyran, tetrahydro-              | 62831 | 000142-68-7 | 83   |
| 5   | 17.24 | 8.30  | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | 2-Methyl-4-propyl-1,3-oxathiane 3- | 16604 | 000000-00-0 | 33   |
|     |       |       | 1,3-Cyclohexadiene-1-carboxaldehyd | 9790  | 000116-26-7 | 25   |
|     |       |       | Methane, bromochloro-              | 4820  | 000074-97-5 | 23   |
| 6   | 17.68 | 1.67  | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | 2-Methyl-4-propyl-1,3-oxathiane 3- | 16604 | 000000-00-0 | 64   |
|     |       |       | Propanenitrile, 2,2,3-trichloro-   | 11636 | 000813-74-1 | 36   |
|     |       |       | Ethanol, 2,2,2-trichloro-          | 66531 | 000115-20-8 | 9    |
| 7   | 17.85 | 24.25 | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | Bicyclo[2.2.1]heptan-2-one, 1,7,7- | 10338 | 000464-48-2 | 98   |
|     |       |       | Bicyclo[2.2.1]heptan-2-one, 1,7,7- | 10341 | 021368-68-3 | 96   |
|     |       |       | Camphor                            | 66978 | 000076-22-2 | 93   |
| 8   | 18.36 | 2.56  | C:\DATABASE\NBS75K.L               |       |             |      |
|     |       |       | Bicyclo[2.2.1]heptan-2-one, 1,3,3- | 10301 | 001195-79-5 | 90   |
|     |       |       | 1-Decyne                           | 65923 | 000764-93-2 | 83   |
|     |       |       | 2,4-Heptadienal, (E,E)-            | 63861 | 004313-03-5 | 83   |

| Pk# | RT    | Area% | Library/ID                                                                                                                                                               | Ref#                    | CAS#                                      | Qual           |
|-----|-------|-------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------|-------------------------------------------|----------------|
| 9   | 19.74 | 1.25  | C:\DATABASE\NBS75K.L<br>2-Methyl-4-propyl-1,3-oxathiane 3-<br>Propanenitrile, 2,2,3-trichloro-<br>Methane, bromochloro-                                                  | 16604<br>11636<br>64995 | 000000-00-0<br>000813-74-1<br>000074-97-5 | 50<br>40<br>33 |
| 10  | 20.58 | 2.24  | C:\DATABASE\NBS75K.L<br>Methane, bromochloro-<br>Propanenitrile, 2,2,3-trichloro-<br>Methane, bromochloro-                                                               | 64995<br>11636<br>64997 | 000074-97-5<br>000813-74-1<br>000074-97-5 | 42<br>42<br>36 |
| 11  | 21.51 | 2.99  | C:\DATABASE\NBS75K.L<br><i>alpha-phenyl</i> 1H-Imidazole-1-ethanol, .alpha.-ph<br>2-Cyclohexen-1-one, 3-methyl-6-(1-<br>2-Cyclohexen-1-one, 3,5,5-trimethy               | 19869<br>9860<br>65922  | 024155-47-3<br>016750-82-6<br>000078-59-1 | 36<br>18<br>10 |
| 12  | 23.67 | 2.97  | C:\DATABASE\NBS75K.L<br>Cyclopentanecarboxylic acid, ethen<br>1-Pyrroline, 3-ethyl-<br>cis-2,6-Dimethyl-2,6-octadiene                                                    | 7375<br>1176<br>7050    | 016523-06-1<br>001192-29-6<br>002492-22-0 | 14<br>12<br>11 |
| 13  | 24.81 | 17.57 | C:\DATABASE\NBS75K.L<br>1,3-Cyclohexadiene-1-carboxaldehyd<br><i>berberone</i> Bicyclo[3.1.1]hept-3-en-2-one, 4,6<br><i>new-4 one</i> 3,7-Epoxy-2-methylene-6-methyloct- | 9790<br>66810<br>13995  | 000116-26-7<br>000080-57-9<br>000000-00-0 | 35<br>35<br>35 |
| 14  | 25.49 | 2.82  | C:\DATABASE\NBS75K.L<br>2-Methyl-4-propyl-1,3-oxathiane 3-<br>5-Hexen-2-one, 5-methyl-3-methylen<br>Propanenitrile, 3-chloro-                                            | 16604<br>4193<br>863    | 000000-00-0<br>051756-18-4<br>000542-76-7 | 39<br>28<br>28 |
| 15  | 26.79 | 2.07  | C:\DATABASE\NBS75K.L<br>1,1-Cyclopropanedicarbonitrile, 2,<br>4-Aminostyrene<br>[1,2,4]Triazolo[1,5-a]pyridine                                                           | 6145<br>3652<br>3638    | 013764-28-8<br>001520-21-4<br>000274-85-1 | 22<br>10<br>10 |
| 16  | 26.86 | 3.06  | C:\DATABASE\NBS75K.L<br>Acetonitrile, (3,5,5-trimethyl-2-c<br>Pyrido[2,3-d]pyrimidine, 4-methoxy<br>Acetonitrile, (3,5,5-trimethyl-2-c                                   | 12728<br>12642<br>12730 | 069697-21-8<br>028732-78-7<br>069697-22-9 | 11<br>11<br>11 |



File : C:\HPCHEM\1\DATA\HC2MARB.D  
Operator : Howard C.  
Acquired : 2 Mar 97 8:32 pm using AcqMethod HOWARD2  
Instrument : 5970 - In  
Sample Name: Francoise; F1 (1/20/97)  
Misc Info :  
Vial Number: 1



## Information from Data File:

File : C:\HPCHEM\1\DATA\HC1MARB.D  
 Operator : HOWARD C.  
 Acquired : 1 Mar 97 12:08 pm using AcqMethod HOWARD2  
 Sample Name: FRANCOISE: F2 (1/20/97)  
 Misc Info :  
 Vial Number: 1

Search Libraries: C:\DATABASE\NBS75K.L Minimum Quality: 0

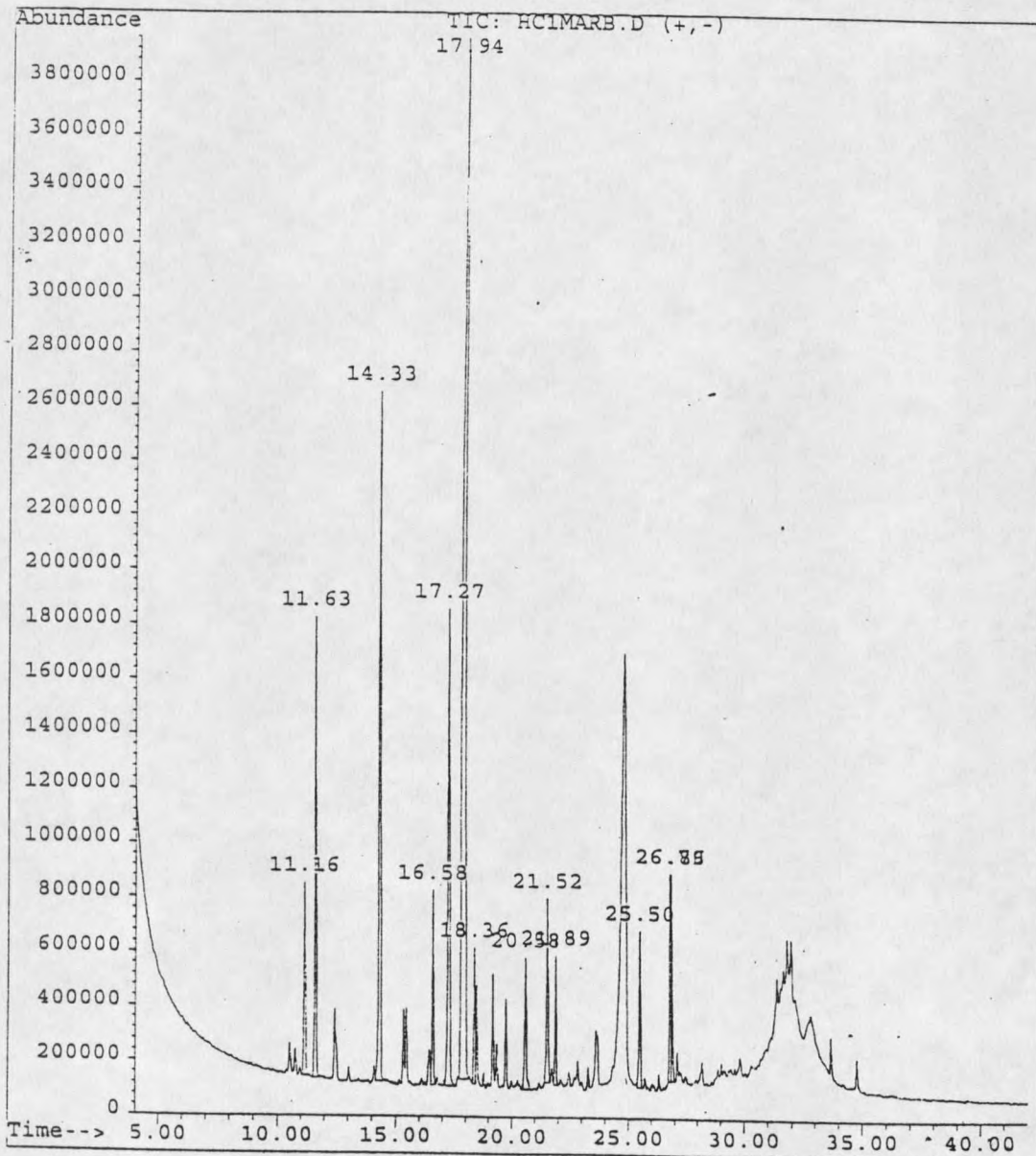
Unknown Spectrum: Peak average  
 Integration Params: current RTEINT parameters

| Pk# | RT    | Area% | Library/ID                                                                                                                                               | Ref#                    | CAS#                                      | Qual           |
|-----|-------|-------|----------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------|-------------------------------------------|----------------|
| 1   | 11.16 | 3.68  | C:\DATABASE\NBS75K.L<br>.alpha.-Pinene<br>3-Carene<br>Bicyclo[3.1.1]hept-2-ene, 3,6,6-tr                                                                 | 65808<br>65795<br>6692  | 000080-56-8<br>013466-78-9<br>004889-83-2 | 86<br>42<br>42 |
| 2   | 11.63 | 8.49  | C:\DATABASE\NBS75K.L<br>Camphene<br>Camphene<br>Camphene                                                                                                 | 65765<br>65764<br>65768 | 000079-92-5<br>000079-92-5<br>000079-92-5 | 90<br>90<br>78 |
| 3   | 14.33 | 14.54 | C:\DATABASE\NBS75K.L<br>Eucalyptol<br>Eucalyptol<br>Eucalyptol                                                                                           | 67091<br>67088<br>67090 | 000470-82-6<br>000470-82-6<br>000470-82-6 | 64<br>64<br>62 |
| 4   | 16.58 | 3.37  | C:\DATABASE\NBS75K.L<br>Bicyclo(3.3.1)non-2-ene<br>1-Penten-3-yne, 2-methyl-<br>6,8-Nonadien-2-one                                                       | 3973<br>426<br>7003     | 006671-66-5<br>000926-55-6<br>077828-54-7 | 47<br>43<br>43 |
| 5   | 17.27 | 10.92 | C:\DATABASE\NBS75K.L<br>1,3-Cyclohexadiene-1-carboxaldehyd<br>Spiro[5.5]undec-1-ene<br>Benzenemethanol, .alpha.-methyl-                                  | 9790<br>9897<br>64678   | 000116-26-7<br>000699-56-9<br>000098-85-1 | 46<br>41<br>38 |
| 6   | 17.94 | 38.60 | C:\DATABASE\NBS75K.L<br><i>camphor</i> -> Bicyclo[2.2.1]heptan-2-one, 1,7,7-<br>Bicyclo[2.2.1]heptan-2-one, 1,7,7-<br>Bicyclo[2.2.1]heptan-2-one, 1,7,7- | 10338<br>66961<br>10341 | 000464-48-2<br>000464-48-2<br>021368-68-3 | 98<br>97<br>96 |
| 7   | 18.36 | 2.34  | C:\DATABASE\NBS75K.L<br>Bicyclo[3.3.1]heptan-3-one, 6,6-di<br>3,4-Pentadienal, 2,2-dimethyl-<br>Butane, 1,3-dichloro-3-methyl-                           | 9819<br>2303<br>65983   | 016812-40-1<br>004058-51-9<br>000624-96-4 | 43<br>37<br>34 |
| 8   | 20.58 | 2.92  | C:\DATABASE\NBS75K.L<br>Methylene Chloride<br>Methylene Chloride<br>Methylene Chloride                                                                   | 62705<br>62703<br>62704 | 000075-09-2<br>000075-09-2<br>000075-09-2 | 58<br>53<br>52 |

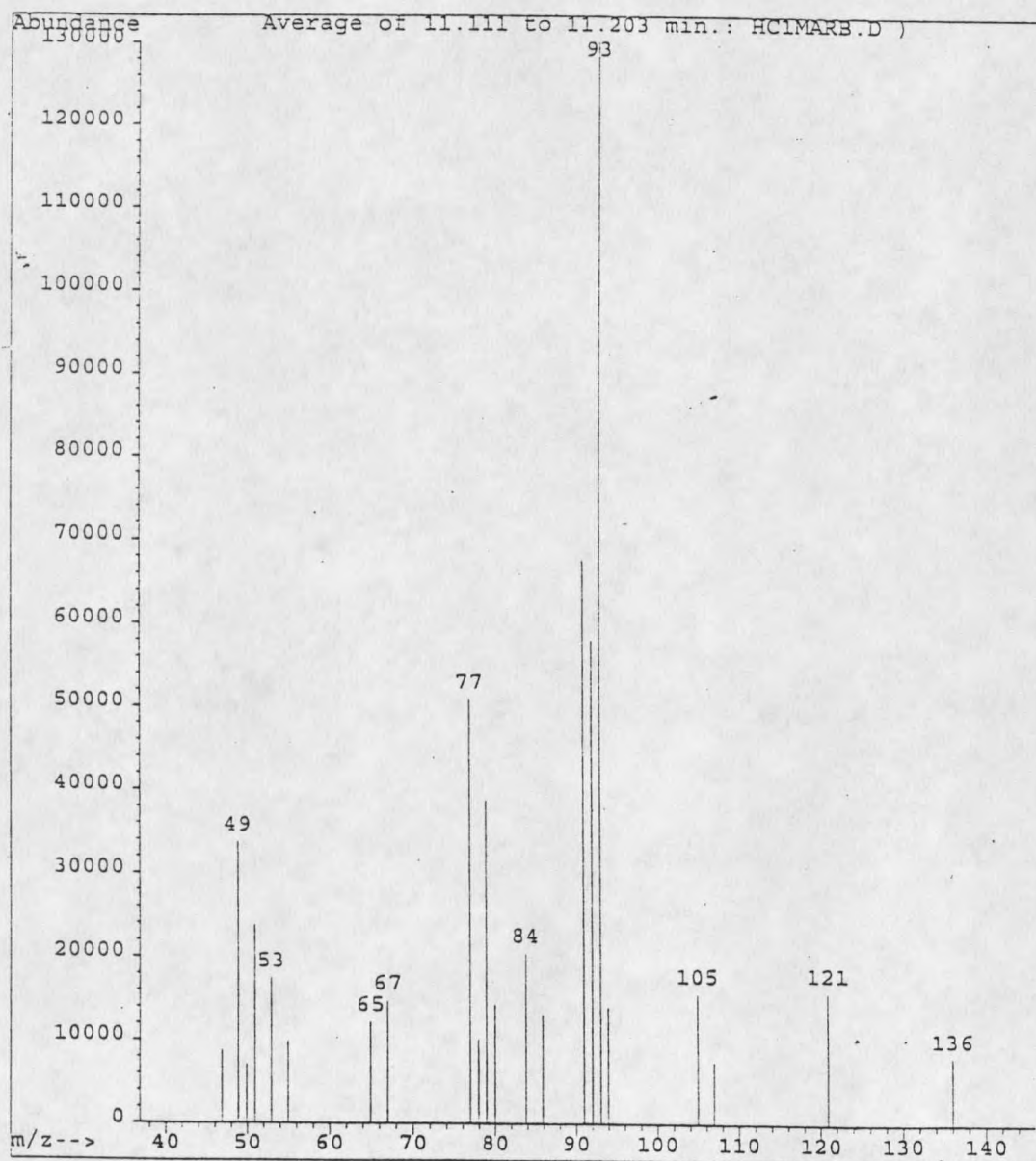


| Pk# | RT    | Area% | Library/ID                           | Ref#  | CAS#        | Qual |
|-----|-------|-------|--------------------------------------|-------|-------------|------|
| 9   | 21.52 | 3.64  | C:\DATABASE\NBS75K.L                 |       |             |      |
|     |       |       | 2-Cyclohexen-1-one, 3,5-dimethyl-    | 64787 | 001123-09-7 | 47   |
|     |       |       | 2-Cyclohexen-1-one, 3,5,5-trimethyl- | 65921 | 000078-59-1 | 38   |
|     |       |       | Furan, 3-pentyl-                     | 6968  | 006177-84-0 | 38   |
| 10  | 21.89 | 2.13  | C:\DATABASE\NBS75K.L                 |       |             |      |
|     |       |       | Bicyclo[2.2.1]heptan-2-ol, 1,3,3-t   | 21900 | 004057-31-2 | 94   |
|     |       |       | Bicyclo[2.2.1]heptan-2-ol, 1,7,7-t   | 21868 | 005655-61-8 | 89   |
|     |       |       | Bornyl acetate                       | 21896 | 000076-49-3 | 87   |
| 11  | 25.50 | 2.95  | C:\DATABASE\NBS75K.L                 |       |             |      |
|     |       |       | 1(2H)-Naphthalenone, 3,4,4a,5,8,8a   | 13537 | 021841-29-2 | 25   |
|     |       |       | 1,9-Decadiyne                        | 6205  | 001720-38-3 | 22   |
|     |       |       | 1,Z-5,E-7-Dodecatriene               | 13566 | 083085-83-0 | 14   |
| 12  | 26.79 | 3.46  | C:\DATABASE\NBS75K.L                 |       |             |      |
|     |       |       | 1,1-Cyclopropanedicarbonitrile, 2,   | 6145  | 013764-28-8 | 27   |
|     |       |       | Benzene, methyl(1-methylethyl)-      | 6208  | 025155-15-1 | 11   |
|     |       |       | Benzene, 1-methyl-4-(1-methylethyl   | 65535 | 000099-87-6 | 11   |
| 13  | 26.86 | 2.95  | C:\DATABASE\NBS75K.L                 |       |             |      |
|     |       |       | s-Triazolo[4,3-a]pyridine, 3,5,7-t   | 12684 | 004919-15-7 | 49   |
|     |       |       | Acetonitrile, (3,5,5-trimethyl-2-c   | 12728 | 069697-21-8 | 25   |
|     |       |       | Benzene, 1-methyl-2-(1-methylethyl   | 65581 | 000527-84-4 | 11   |

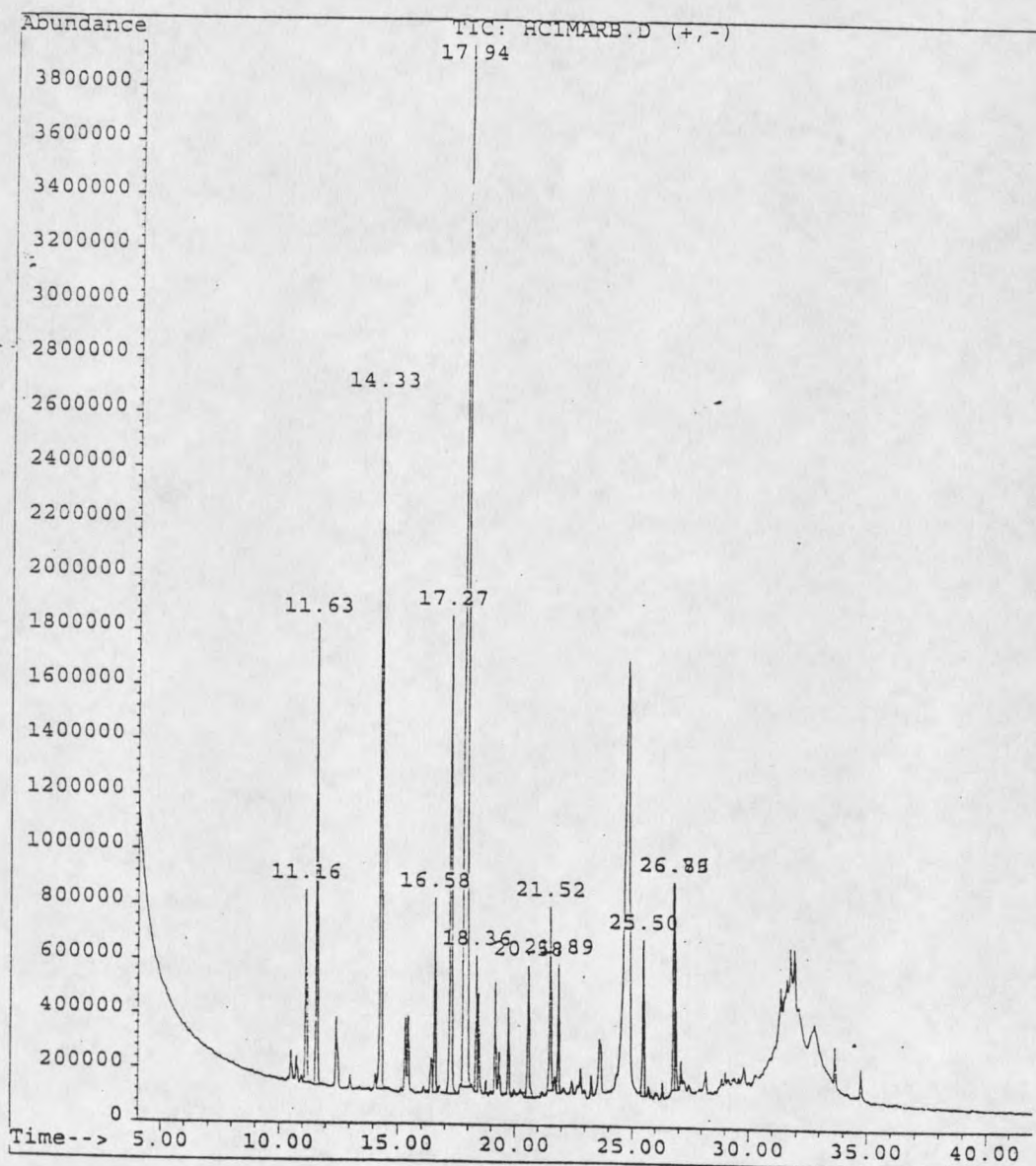
File : C:\HPCHEM\1\DATA\HC1MARB.D  
Operator : HOWARD C.  
Acquired : 1 Mar 97 12:08 pm using AcqMethod HOWARD2  
Instrument : 5970 - In  
Sample Name: FRANCOISE: F2 (1/20/97)  
Misc Info :  
Vial Number: 1



File : C:\HPCHEM\1\DATA\HC1MARB.D  
Operator : HOWARD C.  
Acquired : 1 Mar 97 12:08 pm using AcqMethod HOWARD2  
Instrument : 5970 - In  
Sample Name: FRANCOISE: F2 (1/20/97)  
Misc Info :  
Vial Number: 1



File : C:\HPCHEM\1\DATA\HC1MARB.D  
Operator : HOWARD C.  
Acquired : 1 Mar 97 - 12:08 pm using AcqMethod HOWARD2  
Instrument : 5970 - In  
Sample Name: FRANCOISE: F2 (1/20/97)  
Misc Info :  
Vial Number: 1





## Information from Data File:

File : C:\HPCHEM\1\DATA\HC1MARC.D  
 Operator : HOWARD C.  
 Acquired : 1 Mar 97 1:14 pm using AcqMethod HOWARD2  
 Sample Name: FRANCOISE: F3 (1/20/97)  
 Misc Info :  
 Vial Number: 1

Search Libraries: C:\DATABASE\NBS75K.L Minimum Quality: 0

Unknown Spectrum: Peak average  
 Integration Params: current RTEINT parameters

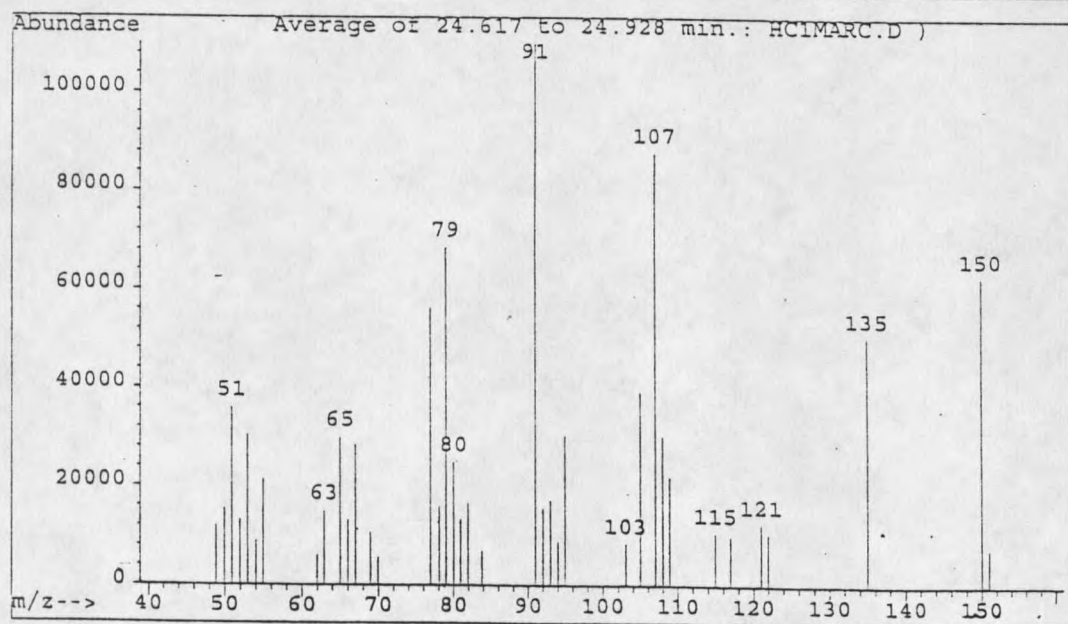
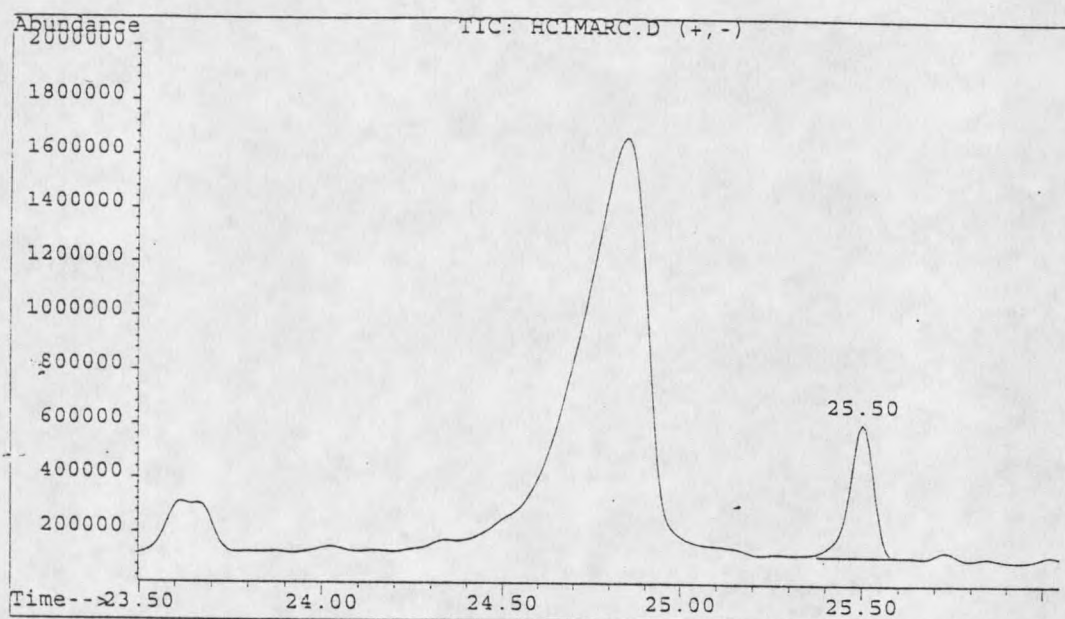
| Pk# | RT    | Area% | Library/ID                                                 | Ref#  | CAS#        | Qual |
|-----|-------|-------|------------------------------------------------------------|-------|-------------|------|
| 1   | 11.14 | 4.29  | C:\DATABASE\NBS75K.L<br>.alpha.-Pinene                     | 65808 | 000080-56-8 | 58   |
|     |       |       | Bicyclo[3.1.1]hept-2-ene, 3,6,6-tr                         | 6692  | 004889-83-2 | 55   |
|     |       |       | 2-Methyl-4-propyl-1,3-oxathiane 3-                         | 16604 | 000000-00-0 | 38   |
| 2   | 11.61 | 5.80  | C:\DATABASE\NBS75K.L<br>Camphene                           | 65765 | 000079-92-5 | 81   |
|     |       |       | Camphene                                                   | 65764 | 000079-92-5 | 80   |
|     |       |       | Camphene                                                   | 65766 | 000079-92-5 | 59   |
| 3   | 12.44 | 2.23  | C:\DATABASE\NBS75K.L<br>Cyclopenta[c]pyran-1,3-dione, 4,4a | 17738 | 066407-26-9 | 37   |
|     |       |       | Bicyclo[3.1.0]hexane, 4-methylene-                         | 6618  | 003387-41-5 | 25   |
|     |       |       | .alpha.-Pinene                                             | 65808 | 000080-56-8 | 25   |
| 4   | 14.33 | 20.49 | C:\DATABASE\NBS75K.L<br>Eucalyptol                         | 67091 | 000470-82-6 | 64   |
|     |       |       | Eucalyptol                                                 | 67088 | 000470-82-6 | 64   |
|     |       |       | Eucalyptol                                                 | 67090 | 000470-82-6 | 62   |
| 5   | 15.45 | 2.65  | C:\DATABASE\NBS75K.L<br>2-Methyl-4-propyl-1,3-oxathiane 3- | 16604 | 000000-00-0 | 42   |
|     |       |       | Propanenitrile, 2,2,3-trichloro-                           | 11636 | 000813-74-1 | 37   |
|     |       |       | Propanenitrile, 3-chloro-                                  | 863   | 000542-76-7 | 25   |
| 6   | 16.42 | 1.63  | C:\DATABASE\NBS75K.L<br>2-Methyl-4-propyl-1,3-oxathiane 3- | 16604 | 000000-00-0 | 45   |
|     |       |       | Propanenitrile, 2,2,3-trichloro-                           | 11636 | 000813-74-1 | 38   |
|     |       |       | Propanenitrile, 3-chloro-                                  | 863   | 000542-76-7 | 38   |
| 7   | 16.58 | 3.33  | C:\DATABASE\NBS75K.L<br>Bicyclo(3.3.1)non-2-ene            | 3973  | 006671-66-5 | 47   |
|     |       |       | Bicyclo[2.2.2]oct-2-ene                                    | 2141  | 000931-64-6 | 43   |
|     |       |       | 6,8-Nonadien-2-one                                         | 7003  | 077828-54-7 | 43   |
| 8   | 17.26 | 9.67  | C:\DATABASE\NBS75K.L<br>1,3-Cyclohexadiene-1-carboxaldehyd | 9790  | 000116-26-7 | 38   |
|     |       |       | 2-Cyclohexen-1-one, 3-methyl-6-(1-                         | 66794 | 000491-09-8 | 35   |
|     |       |       | Bicyclo[2.2.1]heptan-7-one, 2-(1-m                         | 9789  | 066929-96-2 | 25   |

## TMPLIBRP.TXT

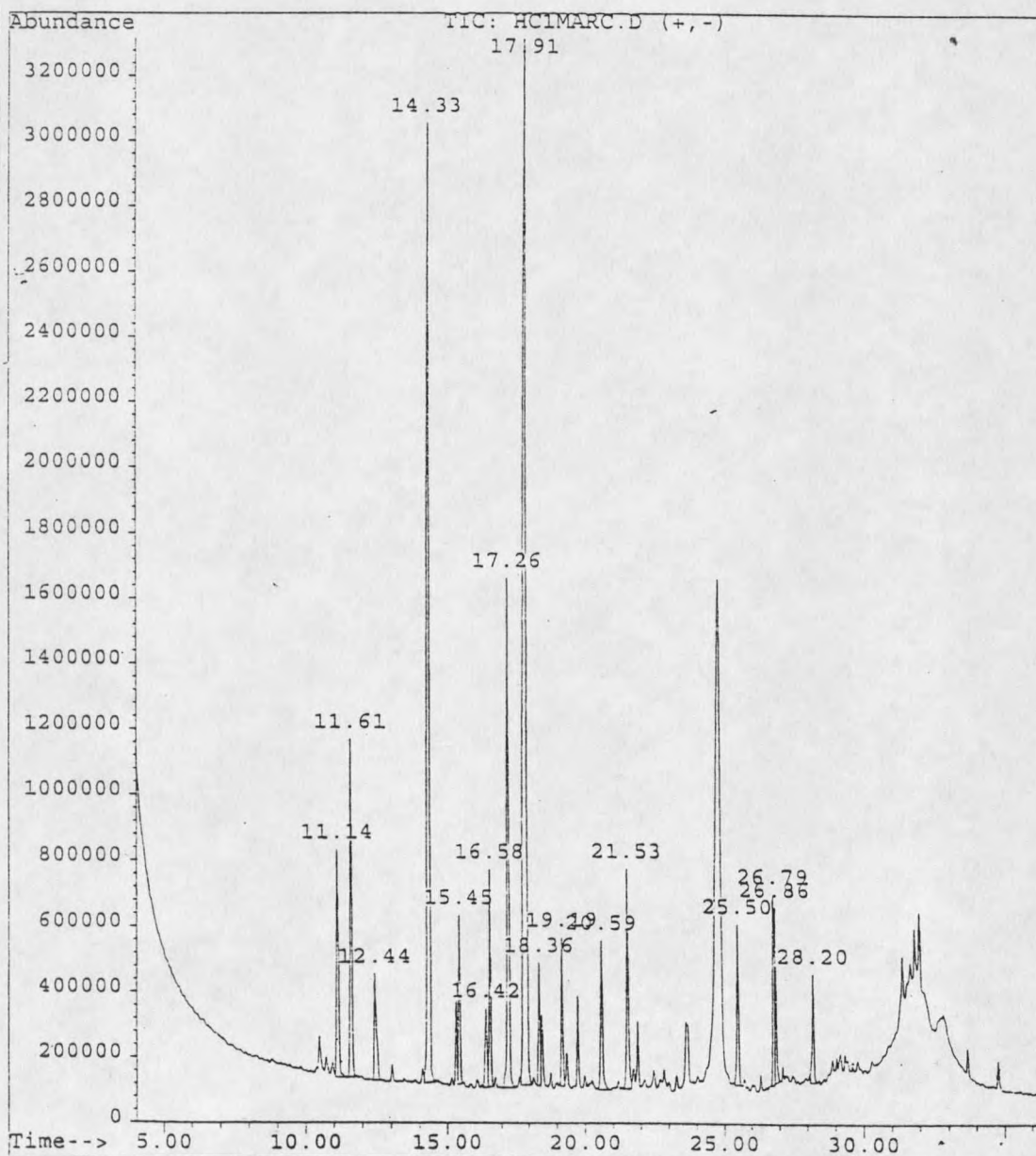
|       |       |                                       |       |             |    |
|-------|-------|---------------------------------------|-------|-------------|----|
|       |       | Bicyclo[2.2.2]oct-2-ene               | 2141  | 000931-64-6 | 46 |
|       |       | 1,3,5-Hexatriene, (E)-                | 425   | 000821-07-8 | 43 |
|       |       | 1-Penten-3-yne, 2-methyl-             | 62647 | 000926-55-6 | 43 |
| 17.26 | 8.53  | C:\DATABASE\NBS75K.L                  |       |             |    |
|       |       | Spiro[5.5]undec-1-ene                 | 9897  | 000699-56-9 | 41 |
|       |       | Methylene Chloride                    | 524   | 000075-09-2 | 41 |
|       |       | 1,3-Cyclohexadiene-1-carboxaldehyde,  | 9790  | 000116-26-7 | 38 |
| 17.91 | 25.62 | C:\DATABASE\NBS75K.L                  |       |             |    |
|       |       | Bicyclo[2.2.1]heptan-2-one, 1,7,7-tri | 10338 | 000464-48-2 | 98 |
|       |       | Bicyclo[2.2.1]heptan-2-one, 1,7,7-tri | 66961 | 000464-48-2 | 97 |
|       |       | Bicyclo[2.2.1]heptan-2-one, 1,7,7-tri | 10341 | 021368-68-3 | 96 |
| 19.19 | 1.86  | C:\DATABASE\NBS75K.L                  |       |             |    |
|       |       | Acetic acid, chloro-, methyl ester    | 2063  | 000096-34-4 | 47 |
|       |       | Methylene Chloride                    | 62705 | 000075-09-2 | 46 |
|       |       | Methylene Chloride                    | 62703 | 000075-09-2 | 46 |
| 20.59 | 2.49  | C:\DATABASE\NBS75K.L                  |       |             |    |
|       |       | Methylene Chloride                    | 62703 | 000075-09-2 | 62 |
|       |       | Methylene Chloride                    | 62705 | 000075-09-2 | 62 |
|       |       | Methylene Chloride                    | 62704 | 000075-09-2 | 58 |
| 21.53 | 3.34  | C:\DATABASE\NBS75K.L                  |       |             |    |
|       |       | 1H-Pyrazole, 3-methyl-                | 62660 | 001453-58-3 | 35 |
|       |       | 1H-Pyrazole, 1-methyl-                | 62662 | 000930-36-9 | 30 |
|       |       | 2-Cyclohexen-1-one, 3-methyl-         | 63863 | 001193-18-6 | 30 |
| 24.85 | 21.01 | C:\DATABASE\NBS75K.L                  |       |             |    |
|       |       | Spiro[5.5]undec-1-ene                 | 9897  | 000699-56-9 | 55 |
|       |       | 1,3-Cyclohexadiene-1-carboxaldehyde,  | 9790  | 000116-26-7 | 50 |
|       |       | Phenol, 2-butyl-                      | 9798  | 003180-09-4 | 46 |
| 25.50 | 2.34  | C:\DATABASE\NBS75K.L                  |       |             |    |
|       |       | Cyclohexane, 1,2-dimethyl-3,5-bis(1-m | 69324 | 062337-99-9 | 91 |
|       |       | Phenol, 4-(3-hydroxy-1-propenyl)-     | 9701  | 003690-05-9 | 90 |
|       |       | Benzene, 2,4-dimethyl-1-(1-methylethy | 9376  | 004706-89-2 | 90 |
| 28.20 | 1.59  | C:\DATABASE\NBS75K.L                  |       |             |    |
|       |       | 5-Hepten-3-one, 2-(5-ethenyltetrahydr | 31128 | 020482-11-5 | 55 |
|       |       | Methylene Chloride                    | 62705 | 000075-09-2 | 49 |



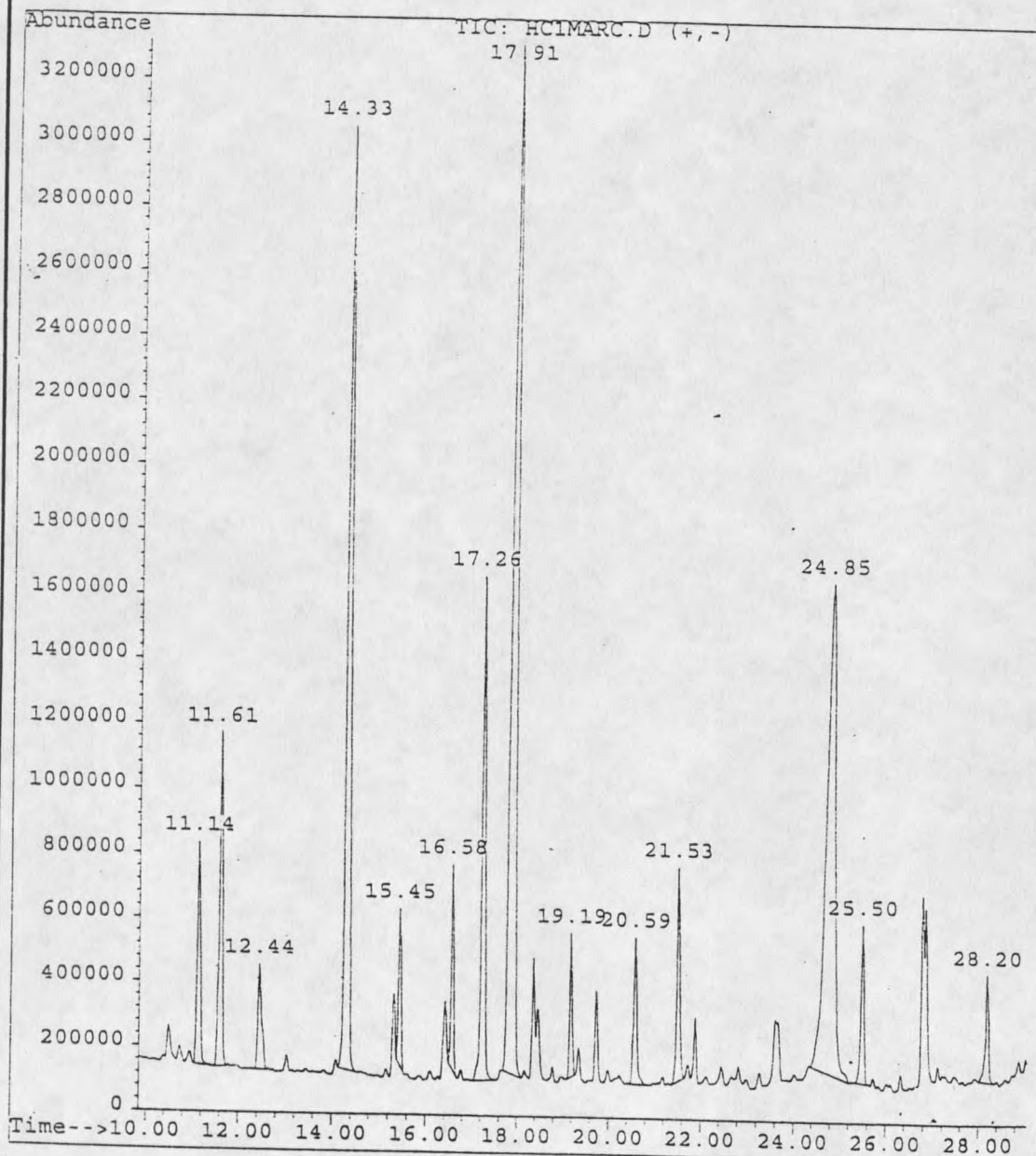
File : C:\HPCHEM\1\DATA\HC1MARC.D  
Operator : HOWARD C.  
Acquired : 1 Mar 97 1:14 pm using AcqMethod HOWARD2  
Instrument : 5970 - In  
Sample Name: FRANCOISE: F3 (1/20/97)  
Misc Info :  
Vial Number: 1



File : C:\HPCHEM\1\DATA\HC1MARC.D  
Operator : HOWARD C.  
Acquired : 1 Mar 97 1:14 pm using AcqMethod HOWARD2  
Instrument : 5970 - In  
Sample Name: FRANCOISE: F3 (1/20/97)  
Misc Info :  
Vial Number: 1



File : C:\HPCHEM\1\DATA\HC1MARC.D  
Operator : HOWARD C.  
Acquired : 1 Mar 97 1:14 pm using AcqMethod HOWARD2  
Instrument : 5970 - In  
Sample Name: FRANCOISE: F3 (1/20/97)  
Misc Info :  
Vial Number: 1



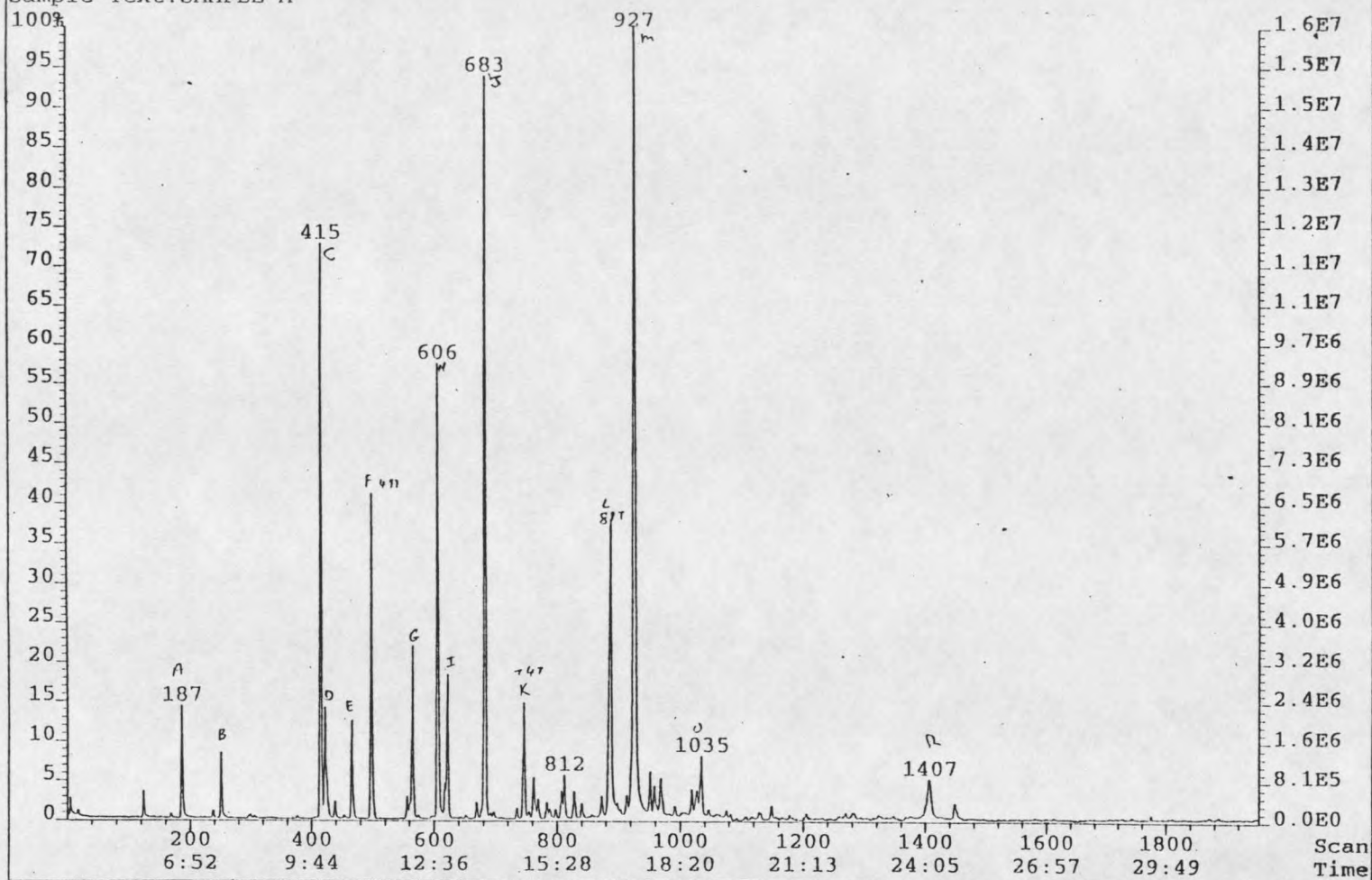
APPENDIX B

QUANTITATIVE ANALYSES

File: 2MAY97K #1-1953 Acq: 2-MAY-1997 16:24:08 GC EI+ Magnet 70S

TIC (+RP) Exp: GC-MSLR

Sample Text: SAMPLE A





MS\_USER:CHR\_DEFAULT.LIS 5-MAY-1997 07:56

Listing of peaks detected for -

data file 2MAY97K

data ident TIC (+RP) PKD(3,3,0.10%,0.0,0.00%,F,T)

Axis display range X\_SCAN (1.00, 1953.00)

Normalising intensity 1.61791E+07 at buffer index 927

Height threshold 2.00% of maximum value 1.60432E+07

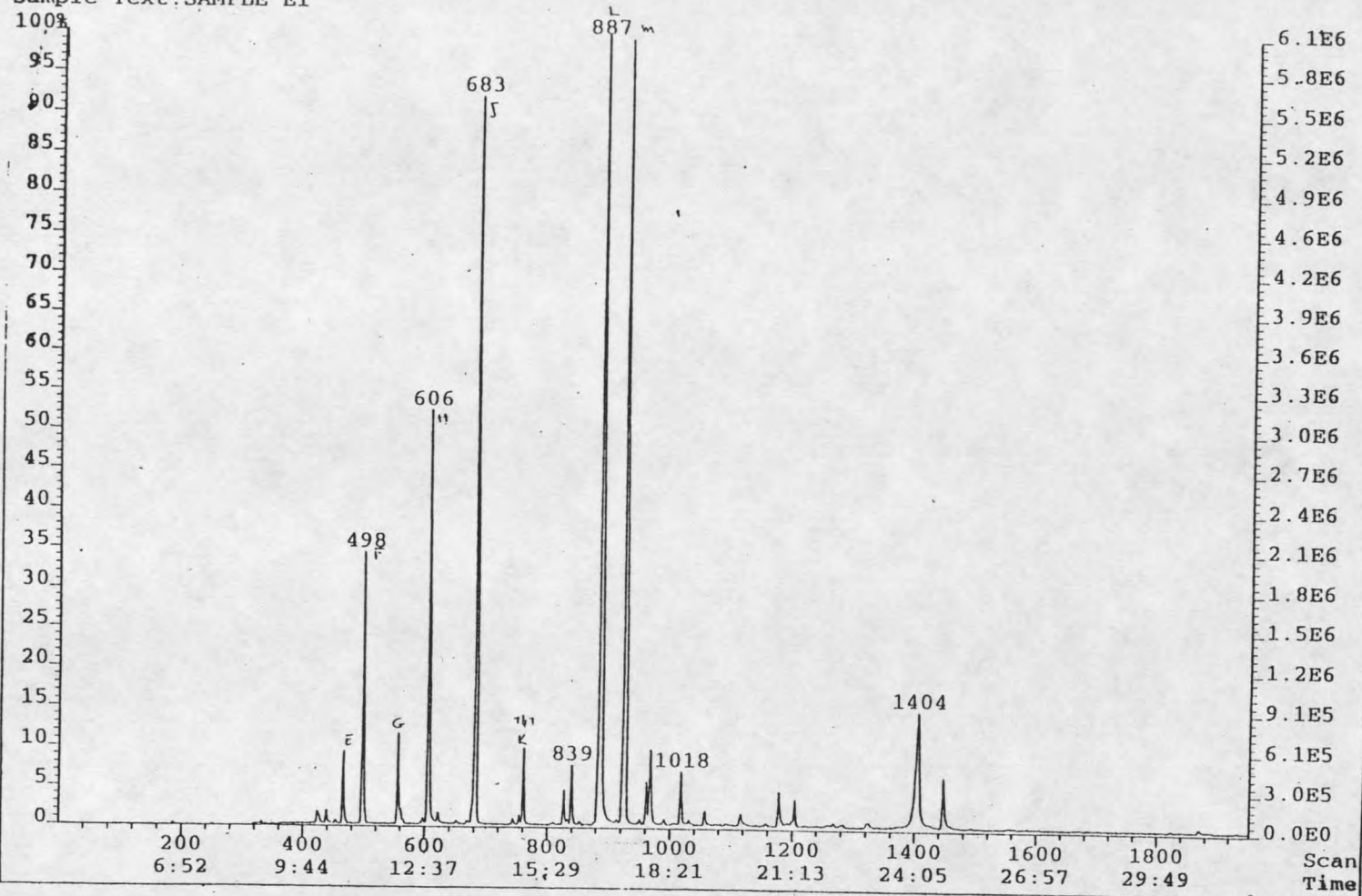
| SCAN AT | TIME AT | ABS         | REL       | ABS         | REL     | PEAK BASELINE START |             | PEAK BASELINE END |             |
|---------|---------|-------------|-----------|-------------|---------|---------------------|-------------|-------------------|-------------|
| MAX HT  | MAX HT  | HEIGHT      | HEIGHT(%) | AREA        | AREA(%) | X_SCAN,             | INTENSITY   | X_SCAN,           | INTENSITY   |
| 5       | 4:04    | 3.75231E+05 | 2.34      | 1.19208E+06 | 1.52    | 3.01,               | 7.83390E+04 | 15.00,            | 7.83390E+04 |
| 124     | 5:47    | 5.33472E+05 | 3.33      | 1.35449E+06 | 1.73    | 121.00,             | 6.17720E+04 | 132.66,           | 6.17720E+04 |
| A-187   | 6:41    | 2.28481E+06 | 14.24     | 6.75232E+06 | 8.63    | 183.00,             | 4.58510E+04 | 196.00,           | 4.58510E+04 |
| B-251   | 7:36    | 1.34561E+06 | 8.39      | 3.93846E+06 | 5.04    | 247.00,             | 3.81390E+04 | 260.97,           | 3.81390E+04 |
| C-415   | 9:57    | 1.17364E+07 | 73.15     | 3.39874E+07 | 43.45   | 408.00,             | 4.72170E+04 | 419.00,           | 4.72170E+04 |
| D-422   | 10:03   | 2.24801E+06 | 14.01     | 9.15994E+06 | 11.71   | 419.00,             | 4.72170E+04 | 434.00,           | 4.72170E+04 |
| E-438   | 10:17   | 3.42481E+05 | 2.13      | 1.10935E+06 | 1.42    | 436.00,             | 4.38930E+04 | 445.00,           | 4.38930E+04 |
| 466     | 10:41   | 1.98795E+06 | 12.39     | 5.90909E+06 | 7.55    | 462.06,             | 4.33560E+04 | 475.00,           | 4.33560E+04 |
| F-498   | 11:09   | 6.62322E+06 | 41.28     | 2.01933E+07 | 25.82   | 493.00,             | 3.67598E+04 | 508.82,           | 3.67598E+04 |
| G-565   | 12:06   | 3.51157E+06 | 21.89     | 1.18731E+07 | 15.18   | 558.00,             | 4.58040E+04 | 572.00,           | 4.58040E+04 |
| H-606   | 12:41   | 9.25452E+06 | 57.68     | 3.24080E+07 | 41.43   | 601.00,             | 4.84405E+04 | 614.00,           | 4.84405E+04 |
| I-618   | 12:52   | 7.17649E+05 | 4.47      | 2.08592E+06 | 2.67    | 614.00,             | 4.84405E+04 | 619.00,           | 4.84405E+04 |
| 622     | 12:55   | 2.92847E+06 | 18.25     | 9.12289E+06 | 11.66   | 619.00,             | 4.84405E+04 | 630.77,           | 4.84405E+04 |
| J-683   | 13:48   | 1.51304E+07 | 94.31     | 4.92743E+07 | 62.99   | 673.00,             | 5.41920E+04 | 688.00,           | 5.41920E+04 |
| K-746   | 14:42   | 2.34893E+06 | 14.64     | 7.66196E+06 | 9.80    | 741.00,             | 5.73940E+04 | 751.00,           | 5.73940E+04 |
| 762     | 14:56   | 8.19579E+05 | 5.11      | 3.22717E+06 | 4.13    | 757.00,             | 5.73940E+04 | 767.00,           | 5.73940E+04 |
| 769     | 15:02   | 3.86833E+05 | 2.41      | 1.28435E+06 | 1.64    | 767.00,             | 5.73940E+04 | 774.35,           | 5.73940E+04 |
| 783     | 15:14   | 3.20991E+05 | 2.00      | 1.03050E+06 | 1.32    | 780.00,             | 5.26730E+04 | 786.00,           | 5.26730E+04 |
| 807     | 15:34   | 5.62399E+05 | 3.51      | 1.59509E+06 | 2.04    | 802.00,             | 5.26730E+04 | 809.00,           | 5.26730E+04 |
| 812     | 15:39   | 8.75504E+05 | 5.46      | 2.88075E+06 | 3.68    | 809.00,             | 5.26730E+04 | 818.00,           | 5.26730E+04 |
| 828     | 15:52   | 5.51641E+05 | 3.44      | 2.19272E+06 | 2.80    | 821.00,             | 5.26730E+04 | 835.00,           | 5.26730E+04 |
| 873     | 16:31   | 4.37840E+05 | 2.73      | 1.80548E+06 | 2.31    | 866.00,             | 7.28020E+04 | 878.00,           | 7.28020E+04 |
| L-888   | 16:44   | 6.07649E+06 | 37.88     | 3.04874E+07 | 38.98   | 878.00,             | 7.28020E+04 | 896.00,           | 7.28020E+04 |
| 914     | 17:06   | 3.90850E+05 | 2.44      | 1.48045E+06 | 1.89    | 909.00,             | 1.35861E+05 | 917.00,           | 1.35861E+05 |
| M-927   | 17:18   | 1.60432E+07 | 100.00    | 7.82202E+07 | 100.00  | 917.00,             | 1.35861E+05 | 947.00,           | 1.35861E+05 |
| 952     | 17:39   | 8.66321E+05 | 5.40      | 2.86467E+06 | 3.66    | 948.00,             | 1.34298E+05 | 955.00,           | 1.34298E+05 |
| 959     | 17:45   | 5.74293E+05 | 3.58      | 2.55076E+06 | 3.26    | 955.00,             | 1.34298E+05 | 965.00,           | 1.34298E+05 |
| 969     | 17:54   | 7.41463E+05 | 4.62      | 3.26217E+06 | 4.17    | 965.00,             | 1.34298E+05 | 979.00,           | 1.34298E+05 |
| 1019    | 18:37   | 6.21427E+05 | 3.87      | 2.44123E+06 | 3.12    | 1015.00,            | 2.30800E+04 | 1022.00,          | 2.30800E+04 |
| 1027    | 18:44   | 5.95215E+05 | 3.71      | 3.21102E+06 | 4.11    | 1022.00,            | 2.30800E+04 | 1030.00,          | 2.30800E+04 |
| O-1035  | 18:51   | 1.30013E+06 | 8.10      | 6.21863E+06 | 7.95    | 1030.00,            | 2.30800E+04 | 1043.00,          | 2.30800E+04 |
| P-1407  | 24:11   | 7.51665E+05 | 4.69      | 6.39569E+06 | 8.18    | 1396.00,            | 9.87980E+04 | 1418.86,          | 9.87980E+04 |
| 1448    | 24:46   | 3.24964E+05 | 2.03      | 2.09443E+06 | 2.68    | 1444.09,            | 4.97140E+04 | 1462.90,          | 4.97140E+04 |



File: 2MAY97K #1-1953 Acq: 2-MAY-1997 18:45:09 GC EI+ Magnet 70S

TIC (+RP) S:3 Exp:GC-MSLR

Sample Text: SAMPLE E1



MS\_USER:CHR\_DEFAULT.LIS 5-MAY-1997 07:59

Listing of peaks detected for -

data file 2MAY97K

data ident TIC (+RP) S:3 PKD(3,3,3,0.10%,0.0,0.00%,F,T)

axis display range X\_SCAN (1.00, 1953.00)

Normalising intensity 6.06846E+06 at buffer index 887

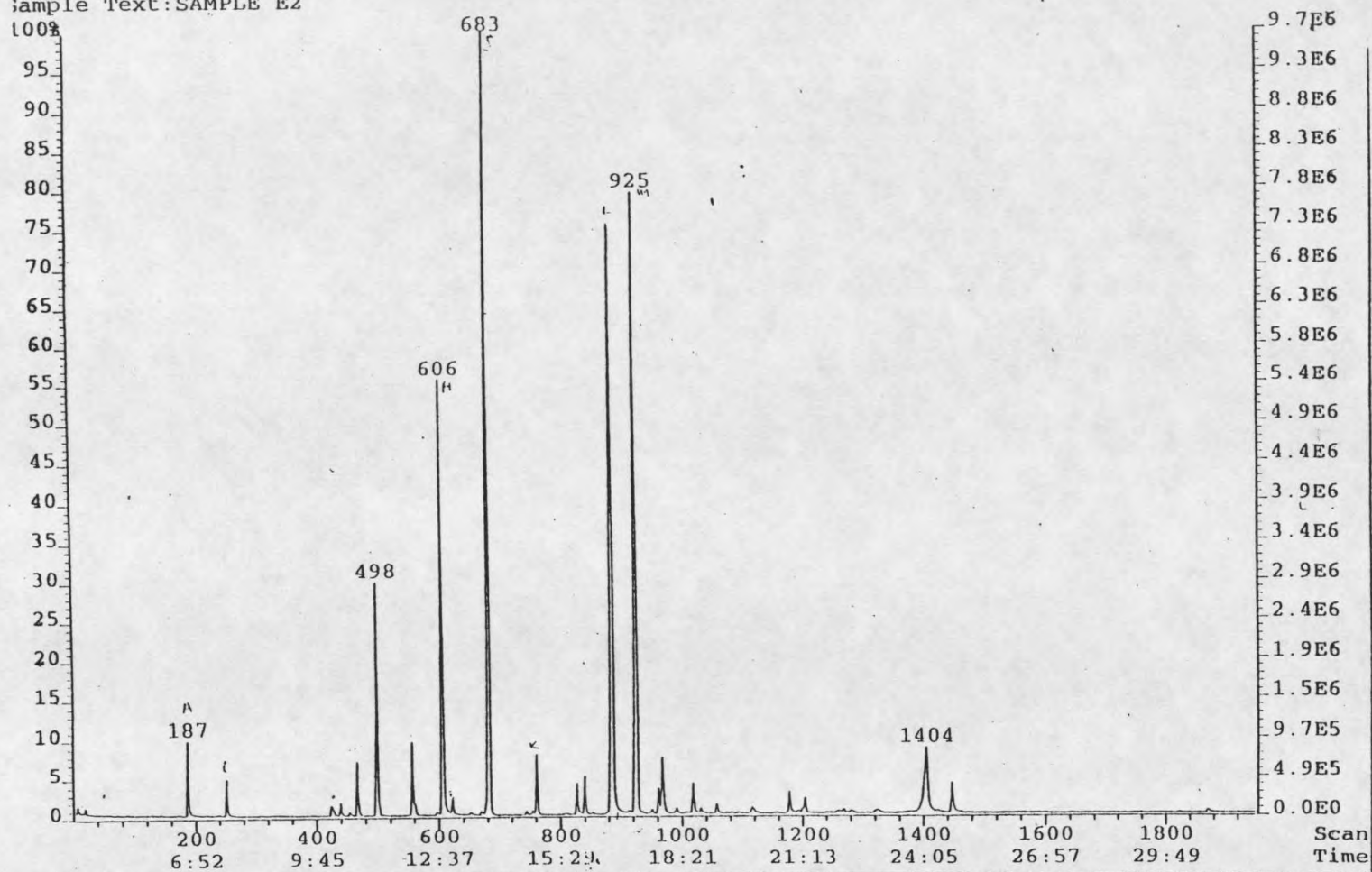
Height threshold 2.00% of maximum value 6.04309E+06

| SCAN AT<br>MAX HT | TIME AT<br>MAX HT | ABS<br>HEIGHT | REL<br>HEIGHT(%) | ABS<br>AREA | REL<br>AREA(%) | PEAK BASELINE START<br>X_SCAN, INTENSITY | PEAK BASELINE END<br>X_SCAN, INTENSITY |
|-------------------|-------------------|---------------|------------------|-------------|----------------|------------------------------------------|----------------------------------------|
| 466               | 10:41             | 5.65846E+05   | 9.36             | 1.74813E+06 | 6.04           | 462.00, 1.71510E+04                      | 475.00, 1.71510E+04                    |
| 498               | 11:09             | 2.07771E+06   | 34.38            | 6.49163E+06 | 22.45          | 494.00, 2.09908E+04                      | 506.79, 2.09908E+04                    |
| 556               | 11:59             | 6.94850E+05   | 11.50            | 2.05602E+06 | 7.11           | 552.00, 1.80320E+04                      | 559.00, 1.80320E+04                    |
| 606               | 12:42             | 3.16466E+06   | 52.37            | 1.09102E+07 | 37.73          | 601.00, 2.27320E+04                      | 615.00, 2.27320E+04                    |
| 683               | 13:48             | 5.57221E+06   | 92.21            | 2.00570E+07 | 69.36          | 673.00, 1.97110E+04                      | 690.00, 1.97110E+04                    |
| 761               | 14:55             | 5.90050E+05   | 9.76             | 1.93429E+06 | 6.69           | 757.00, 2.02220E+04                      | 771.00, 2.02220E+04                    |
| 827               | 15:52             | 2.74574E+05   | 4.54             | 8.90416E+05 | 3.08           | 823.08, 2.25280E+04                      | 831.00, 2.25280E+04                    |
| 839               | 16:02             | 4.64031E+05   | 7.68             | 1.58540E+06 | 5.48           | 836.00, 2.25280E+04                      | 847.94, 2.25280E+04                    |
| 887               | 16:43             | 6.04309E+06   | 100.00           | 2.89190E+07 | 100.00         | 878.00, 2.53680E+04                      | 909.00, 2.53680E+04                    |
| 926               | 17:17             | 6.00531E+06   | 99.37            | 2.60231E+07 | 89.99          | 917.00, 2.53680E+04                      | 937.69, 2.53680E+04                    |
| 961               | 17:47             | 3.38914E+05   | 5.61             | 1.08671E+06 | 3.76           | 956.00, 2.88310E+04                      | 964.00, 2.88310E+04                    |
| 967               | 17:52             | 5.81258E+05   | 9.62             | 2.45567E+06 | 8.49           | 964.00, 2.88310E+04                      | 977.90, 2.88310E+04                    |
| 1018              | 18:36             | 4.19528E+05   | 6.94             | 1.42084E+06 | 4.91           | 1013.00, 2.49350E+04                     | 1024.00, 2.49350E+04                   |
| 1178              | 20:54             | 2.68693E+05   | 4.45             | 1.13678E+06 | 3.93           | 1174.02, 3.05280E+04                     | 1187.00, 3.05280E+04                   |
| 1204              | 21:16             | 2.13564E+05   | 3.53             | 6.45274E+05 | 2.23           | 1201.00, 2.79824E+04                     | 1209.00, 2.79824E+04                   |
| 1404              | 24:08             | 8.65090E+05   | 14.32            | 7.00010E+06 | 24.21          | 1386.00, 4.74480E+04                     | 1419.00, 4.74480E+04                   |
| 1446              | 24:44             | 3.89831E+05   | 6.45             | 1.72644E+06 | 5.97           | 1442.00, 3.02701E+04                     | 1458.00, 3.02701E+04                   |

File: 2MAY97K 11-1953 Acq: 2-MAY-1997 19:55:10 GC EI+ Magnet 70S

LIC (+RP) S:4 Exp:GC-MSLR

Sample Text: SAMPLE E2



MS\_USER:CHR\_DEFAULT.LIS 5-MAY-1997 07:59

Listing of peaks detected for -

data file 2MAY97K

data ident TIC (+RP) S:4 PKD(3,3,3,0.10%,0.0,0.00%,F,T)

X-axis display range X\_SCAN (1.00, 1953.00)

Normalising intensity 9.73998E+06 at buffer index 683

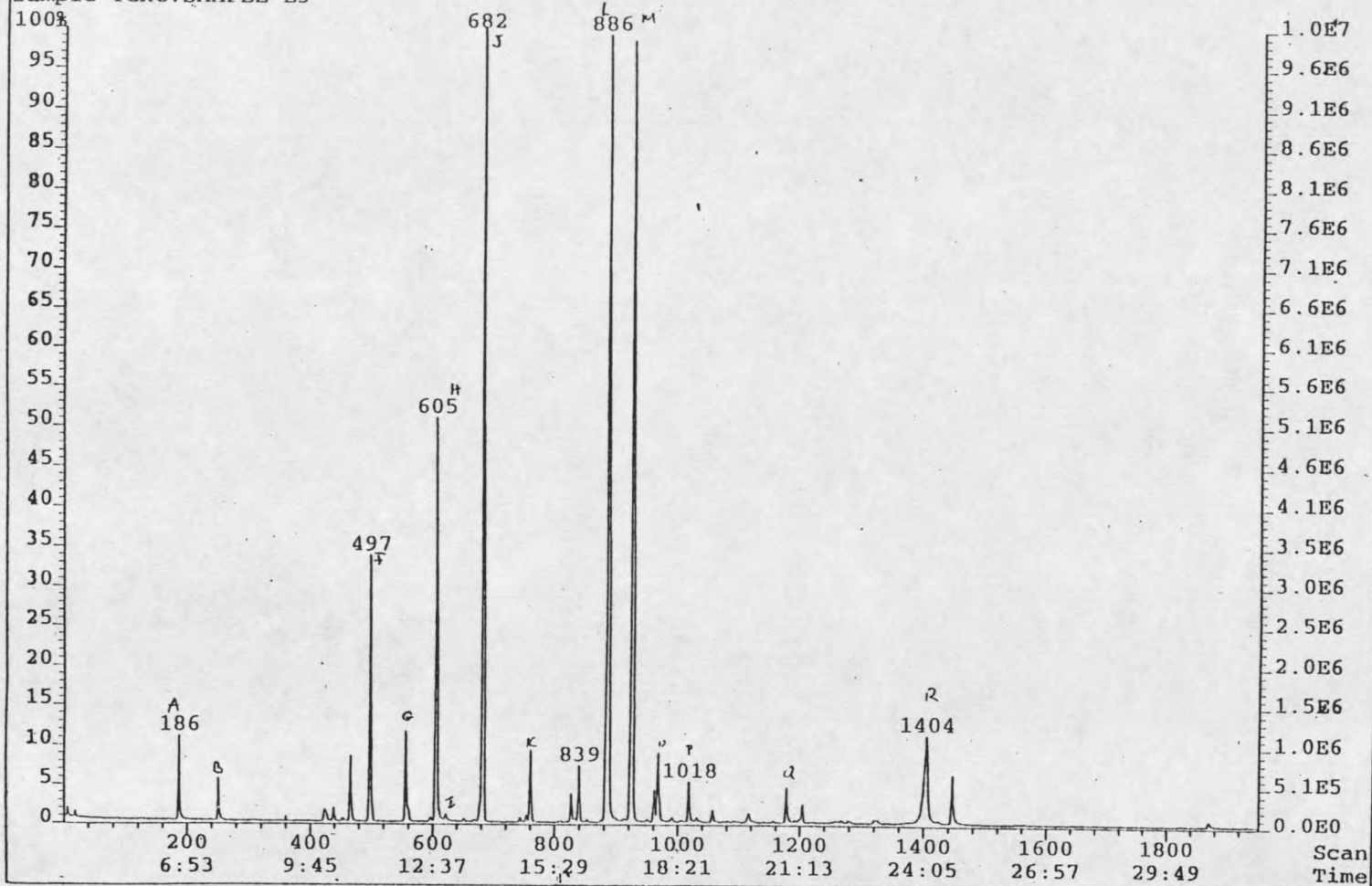
Height threshold 2.00% of maximum value 9.71435E+06

| SCAN AT | TIME AT | ABS         | REL       | ABS         | REL     | PEAK BASELINE START  | PEAK BASELINE END    |
|---------|---------|-------------|-----------|-------------|---------|----------------------|----------------------|
| MAX HT  | MAX HT  | HEIGHT      | HEIGHT(%) | AREA        | AREA(%) | X_SCAN, INTENSITY    | X_SCAN, INTENSITY    |
| 187     | 6:41    | 9.38614E+05 | 9.66      | 2.41971E+06 | 6.91    | 184.00, 4.24580E+04  | 197.00, 4.24580E+04  |
| 251     | 7:36    | 4.56721E+05 | 4.70      | 1.27067E+06 | 3.63    | 244.00, 3.92250E+04  | 259.00, 3.92250E+04  |
| 466     | 10:41   | 6.80976E+05 | 7.01      | 2.07617E+06 | 5.93    | 461.00, 2.94370E+04  | 473.00, 2.94370E+04  |
| 498     | 11:09   | 2.88776E+06 | 29.73     | 8.80432E+06 | 25.14   | 493.04, 3.28648E+04  | 509.68, 3.28648E+04  |
| 556     | 11:59   | 9.24573E+05 | 9.52      | 2.72561E+06 | 7.78    | 553.00, 2.75920E+04  | 560.00, 2.75920E+04  |
| 606     | 12:42   | 5.38344E+06 | 55.42     | 1.83110E+07 | 52.28   | 601.00, 3.79540E+04  | 618.00, 3.79540E+04  |
| 683     | 13:48   | 9.71435E+06 | 100.00    | 3.34253E+07 | 95.43   | 674.00, 2.56240E+04  | 697.00, 2.56240E+04  |
| 761     | 14:55   | 7.56116E+05 | 7.78      | 2.34645E+06 | 6.70    | 757.00, 3.23705E+04  | 766.89, 3.23705E+04  |
| 827     | 15:52   | 3.93012E+05 | 4.05      | 1.24789E+06 | 3.56    | 823.04, 2.90080E+04  | 832.00, 2.90080E+04  |
| 840     | 16:03   | 4.78338E+05 | 4.92      | 1.55140E+06 | 4.43    | 835.00, 2.90080E+04  | 849.00, 2.90080E+04  |
| 886     | 16:43   | 7.31969E+06 | 75.35     | 3.50259E+07 | 100.00  | 877.00, 3.65765E+04  | 907.00, 3.65765E+04  |
| 925     | 17:16   | 7.70929E+06 | 79.36     | 3.21198E+07 | 91.70   | 918.00, 3.65765E+04  | 940.93, 3.65765E+04  |
| 962     | 17:48   | 3.28020E+05 | 3.38      | 1.11001E+06 | 3.17    | 956.00, 3.03700E+04  | 964.00, 3.03700E+04  |
| 968     | 17:53   | 7.11487E+05 | 7.32      | 3.04298E+06 | 8.69    | 964.00, 3.03700E+04  | 978.86, 3.03700E+04  |
| 1018    | 18:36   | 3.66042E+05 | 3.77      | 1.17277E+06 | 3.35    | 1015.00, 4.67690E+04 | 1023.00, 4.67690E+04 |
| 1178    | 20:54   | 2.73724E+05 | 2.82      | 1.02048E+06 | 2.91    | 1175.00, 3.99240E+04 | 1188.00, 3.99240E+04 |
| 1204    | 21:16   | 2.00891E+05 | 2.07      | 6.78750E+05 | 1.94    | 1201.00, 3.32290E+04 | 1208.00, 3.32290E+04 |
| 1404    | 24:08   | 7.95121E+05 | 8.19      | 6.29886E+06 | 17.98   | 1390.16, 5.64450E+04 | 1418.99, 5.64450E+04 |
| 1447    | 24:45   | 3.77008E+05 | 3.88      | 1.55372E+06 | 4.44    | 1443.03, 4.01480E+04 | 1455.00, 4.01480E+04 |

File: 2MAY97K #1-1952 Acq: 2-MAY-1997 21:04:06 GC EI+ Magnet 70S

TIC (+RP) S:5 Exp: GC-MSLR

Sample Text: SAMPLE E3





MS\_USER:CHR\_DEFAULT.LIS 5-MAY-1997 07:59

Listing of peaks detected for -

data file 2MAY97K

data ident TIC (+RP) S:5 PKD(3,3,3,0.10%,0.0,0.00%,F,T)

X-axis display range X\_SCAN (1.00, 1952.00)

Normalising intensity 1.01327E+07 at buffer index 682

Height threshold 2.00% of maximum value 1.00969E+07

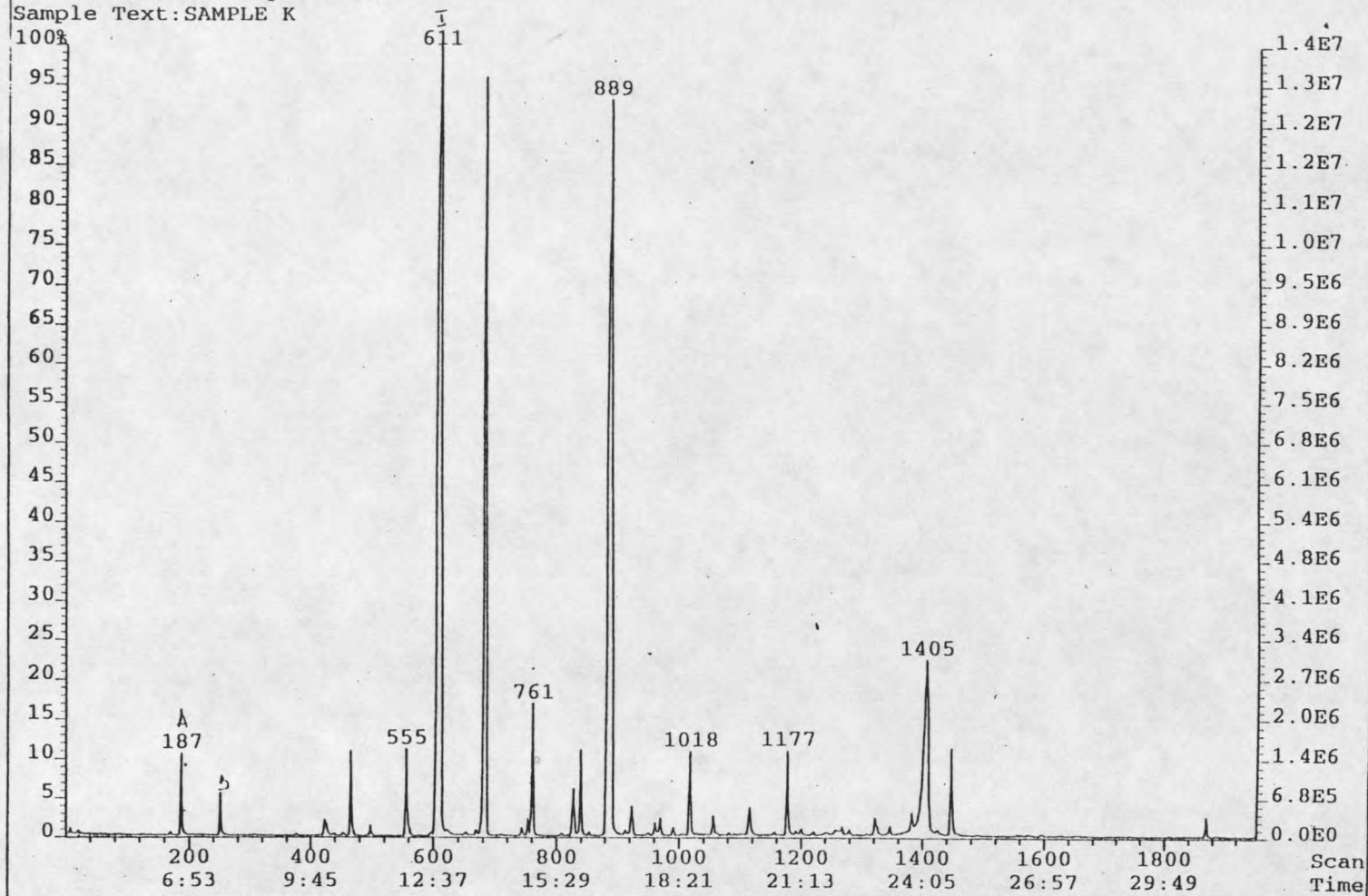
| SCAN AT | TIME AT | ABS         | REL       | ABS         | REL     | PEAK BASELINE START  | PEAK BASELINE END    |
|---------|---------|-------------|-----------|-------------|---------|----------------------|----------------------|
| MAX HT  | MAX HT  | HEIGHT      | HEIGHT(%) | AREA        | AREA(%) | X_SCAN, INTENSITY    | X_SCAN, INTENSITY    |
| 186     | 6:41    | 1.09000E+06 | 10.80     | 2.89480E+06 | 6.57    | 183.07, 4.36360E+04  | 198.00, 4.36360E+04  |
| 250     | 7:36    | 5.53958E+05 | 5.49      | 1.56178E+06 | 3.54    | 248.00, 3.64790E+04  | 263.00, 3.64790E+04  |
| 465     | 10:41   | 8.50206E+05 | 8.42      | 2.77491E+06 | 6.29    | 462.00, 3.33740E+04  | 474.00, 3.33740E+04  |
| 497     | 11:08   | 3.40381E+06 | 33.71     | 1.06223E+07 | 24.10   | 493.00, 3.18070E+04  | 508.00, 3.18070E+04  |
| 555     | 11:58   | 1.16970E+06 | 11.58     | 4.21796E+06 | 9.57    | 552.02, 3.16284E+04  | 570.88, 3.16284E+04  |
| 605     | 12:41   | 5.12798E+06 | 50.79     | 1.78206E+07 | 40.43   | 601.00, 4.00880E+04  | 617.00, 4.00880E+04  |
| 682     | 13:48   | 1.00969E+07 | 100.00    | 3.59750E+07 | 81.61   | 672.00, 3.58110E+04  | 689.95, 3.58110E+04  |
| 760     | 14:55   | 9.34043E+05 | 9.25      | 2.89007E+06 | 6.56    | 757.00, 3.12356E+04  | 768.00, 3.12356E+04  |
| 827     | 15:52   | 3.80577E+05 | 3.77      | 1.29019E+06 | 2.93    | 821.00, 2.88260E+04  | 830.00, 2.88260E+04  |
| 839     | 16:03   | 7.31705E+05 | 7.25      | 2.20561E+06 | 5.00    | 836.00, 2.88260E+04  | 848.00, 2.88260E+04  |
| 886     | 16:43   | 1.00061E+07 | 99.10     | 4.40814E+07 | 100.00  | 878.00, 4.10110E+04  | 898.00, 4.10110E+04  |
| 925     | 17:17   | 9.93652E+06 | 98.41     | 3.99846E+07 | 90.71   | 917.00, 3.75060E+04  | 935.00, 3.75060E+04  |
| 961     | 17:48   | 4.18771E+05 | 4.15      | 1.28766E+06 | 2.92    | 957.00, 4.13050E+04  | 964.00, 4.13050E+04  |
| 967     | 17:53   | 8.93635E+05 | 8.85      | 3.43338E+06 | 7.79    | 964.00, 4.13050E+04  | 980.96, 4.13050E+04  |
| 1018    | 18:37   | 5.23816E+05 | 5.19      | 1.85877E+06 | 4.22    | 1014.00, 3.53300E+04 | 1023.00, 3.53300E+04 |
| 1177    | 20:53   | 4.53219E+05 | 4.49      | 1.56909E+06 | 3.56    | 1174.00, 4.20880E+04 | 1186.00, 4.20880E+04 |
| 1204    | 21:17   | 2.43675E+05 | 2.41      | 8.55556E+05 | 1.94    | 1200.00, 3.56990E+04 | 1210.89, 3.56990E+04 |
| 1404    | 24:09   | 1.12549E+06 | 11.15     | 9.49566E+06 | 21.54   | 1386.00, 3.93230E+04 | 1417.00, 3.93230E+04 |
| 1446    | 24:45   | 6.24790E+05 | 6.19      | 2.46283E+06 | 5.59    | 1442.00, 3.93230E+04 | 1457.00, 3.93230E+04 |



File: 2MAY97K #1-1953 Acq: 3-MAY-1997 02:47:26 GC EI+ Magnet 70S

TIC (+RP) S:10 Exp:GC-MSLR

Sample Text: SAMPLE K



MS USER:CHR\_DEFAULT.LIS 5-MAY-1997 08:02

Listing of peaks detected for -

data file 2MAY97K

data ident TIC (+RP) S:10 PKD(3,3,3,0.10%,0.0,0.00%,F,T)

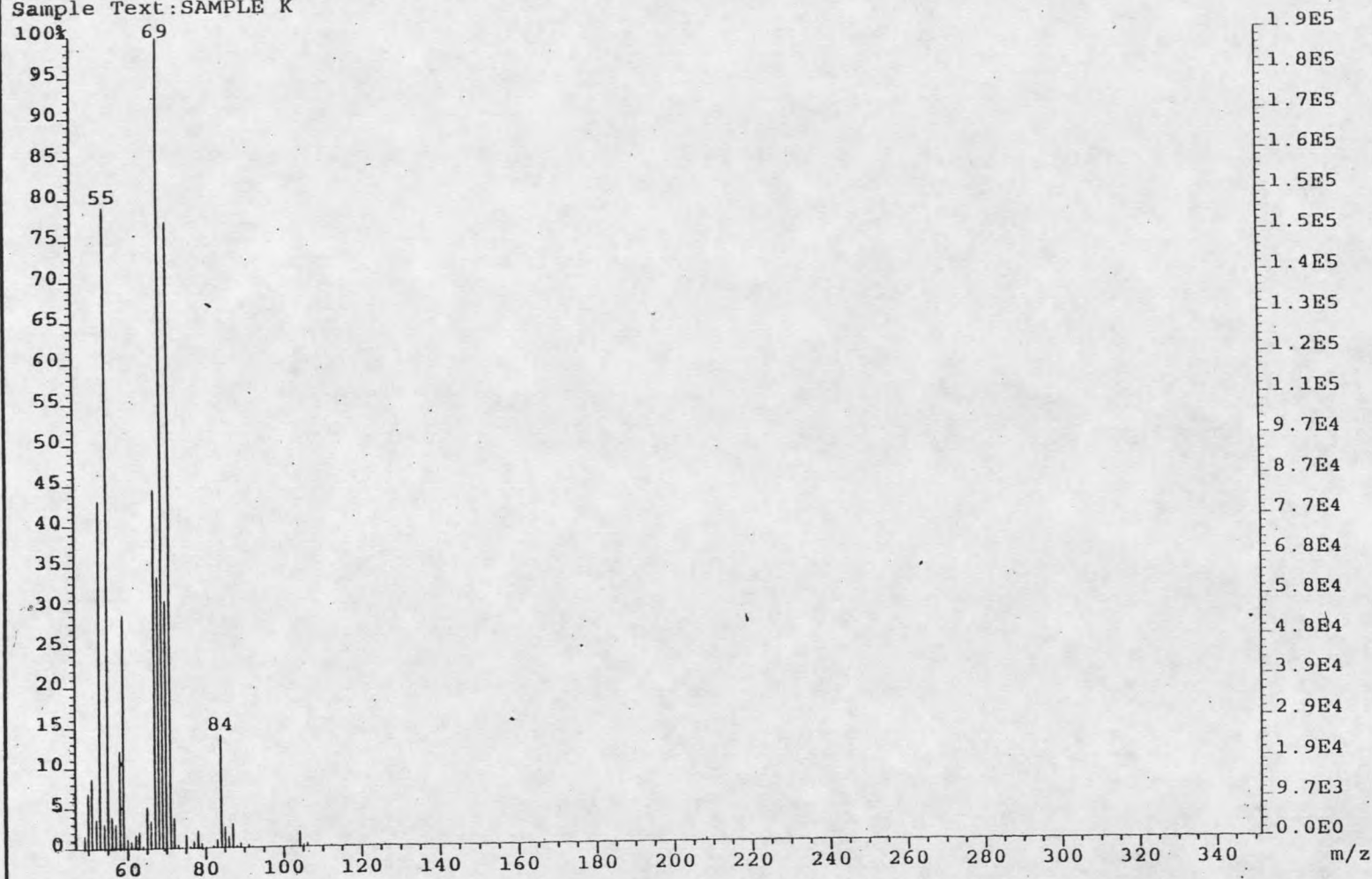
xis display range X\_SCAN (1.00, 1953.00)

Normalising intensity 1.36225E+07 at buffer index 611

Height threshold 2.00% of maximum value 1.35891E+07

| SCAN AT | TIME AT | ABS         | REL       | ABS         | REL     | PEAK BASELINE START  | PEAK BASELINE END    |
|---------|---------|-------------|-----------|-------------|---------|----------------------|----------------------|
| MAX HT  | MAX HT  | HEIGHT      | HEIGHT(%) | AREA        | AREA(%) | X_SCAN, INTENSITY    | X_SCAN, INTENSITY    |
| 187     | 6:41    | 1.43692E+06 | 10.57     | 4.03023E+06 | 3.75    | 182.00, 3.08540E+04  | 198.90, 3.08540E+04  |
| 251     | 7:37    | 7.51023E+05 | 5.53      | 2.26722E+06 | 2.11    | 247.00, 3.13240E+04  | 256.62, 3.13240E+04  |
| 422     | 10:04   | 2.87145E+05 | 2.11      | 1.63469E+06 | 1.52    | 419.00, 3.07990E+04  | 433.00, 3.07990E+04  |
| 465     | 10:41   | 1.48031E+06 | 10.89     | 4.43236E+06 | 4.12    | 462.00, 2.91980E+04  | 473.00, 2.91980E+04  |
| 555     | 11:58   | 1.51398E+06 | 11.14     | 5.33672E+06 | 4.96    | 552.00, 2.28530E+04  | 569.79, 2.28530E+04  |
| 611     | 12:46   | 1.35891E+07 | 100.00    | 1.07560E+08 | 100.00  | 599.00, 3.33780E+04  | 619.00, 3.33780E+04  |
| 684     | 13:49   | 1.30437E+07 | 95.99     | 6.02259E+07 | 55.99   | 673.00, 4.13495E+04  | 690.86, 4.13495E+04  |
| 753     | 14:48   | 3.18065E+05 | 2.34      | 9.45577E+05 | 0.88    | 749.00, 2.45110E+04  | 756.00, 2.45110E+04  |
| 761     | 14:55   | 2.29841E+06 | 16.91     | 8.02724E+06 | 7.46    | 756.00, 2.45110E+04  | 769.00, 2.45110E+04  |
| 827     | 15:52   | 8.27332E+05 | 6.09      | 3.54661E+06 | 3.30    | 822.00, 3.55150E+04  | 833.00, 3.55150E+04  |
| 839     | 16:02   | 1.49034E+06 | 10.97     | 4.77904E+06 | 4.44    | 833.00, 3.55150E+04  | 847.00, 3.55150E+04  |
| 889     | 16:45   | 1.26541E+07 | 93.12     | 9.48155E+07 | 88.15   | 875.00, 3.79425E+04  | 899.00, 3.79425E+04  |
| 922     | 17:14   | 5.09384E+05 | 3.75      | 2.38890E+06 | 2.22    | 915.00, 3.79425E+04  | 931.95, 3.79425E+04  |
| 969     | 17:54   | 3.14072E+05 | 2.31      | 1.11631E+06 | 1.04    | 966.00, 3.45560E+04  | 976.00, 3.45560E+04  |
| 1018    | 18:36   | 1.45390E+06 | 10.70     | 5.03440E+06 | 4.68    | 1012.00, 4.19640E+04 | 1024.88, 4.19640E+04 |
| 1055    | 19:08   | 3.39647E+05 | 2.50      | 1.10697E+06 | 1.03    | 1052.00, 4.43755E+04 | 1059.00, 4.43755E+04 |
| 1115    | 20:00   | 4.97018E+05 | 3.66      | 2.44982E+06 | 2.28    | 1109.00, 4.16210E+04 | 1126.00, 4.16210E+04 |
| 1177    | 20:53   | 1.45814E+06 | 10.73     | 4.75806E+06 | 4.42    | 1173.00, 5.51430E+04 | 1187.00, 5.51430E+04 |
| 1321    | 22:57   | 3.07947E+05 | 2.27      | 1.73257E+06 | 1.61    | 1314.00, 4.41060E+04 | 1331.00, 4.41060E+04 |
| 1382    | 23:50   | 3.72656E+05 | 2.74      | 1.61665E+06 | 1.50    | 1377.00, 8.26920E+04 | 1385.00, 8.26920E+04 |
| 1405    | 24:09   | 2.99123E+06 | 22.01     | 2.92251E+07 | 27.17   | 1385.00, 8.26920E+04 | 1420.00, 8.26920E+04 |
| 1446    | 24:45   | 1.50996E+06 | 11.11     | 5.36277E+06 | 4.99    | 1442.00, 5.25130E+04 | 1454.00, 5.25130E+04 |
| 1869    | 30:49   | 3.47648E+05 | 2.56      | 1.09406E+06 | 1.02    | 1865.00, 3.34730E+04 | 1876.64, 3.34730E+04 |

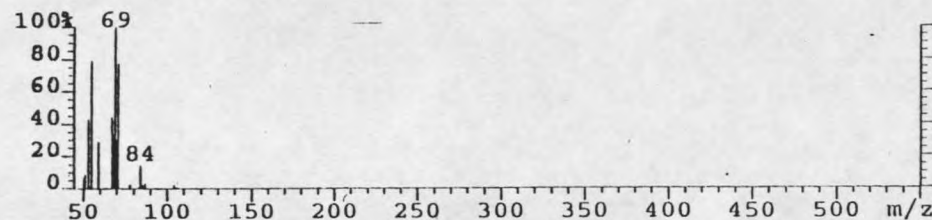
File: 2MAY97K Ident: 186\_188-198\_203 Mer Def 0.25 S:10 Acq: 3-MAY-1997 02:47:26 +6:41 Cal: 2MAY97K>  
70S EI+ Magnet BpM:69 BpI:193555 TIC:1039931 Flags: HALL  
Sample Text: SAMPLE K



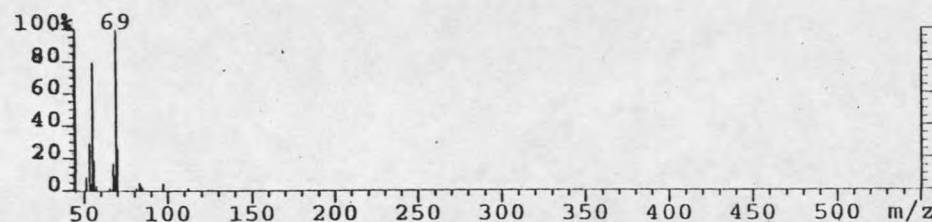
Libraries 1: NIST (62235) 2: NISTREP (12593)

|          |   |   |   |     |      |      |       |       |       |
|----------|---|---|---|-----|------|------|-------|-------|-------|
| Peaks:   | 8 | 7 | 6 | 5   | 4    | 3    | 2     | 1     | 0     |
| Matches: | 0 | 0 | 4 | 125 | 1066 | 4208 | 8036  | 14725 | 46664 |
| Total:   | 0 | 0 | 4 | 129 | 1195 | 5403 | 13439 | 28164 | 74828 |

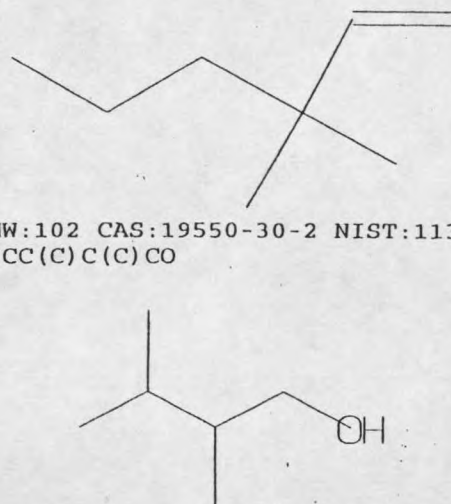
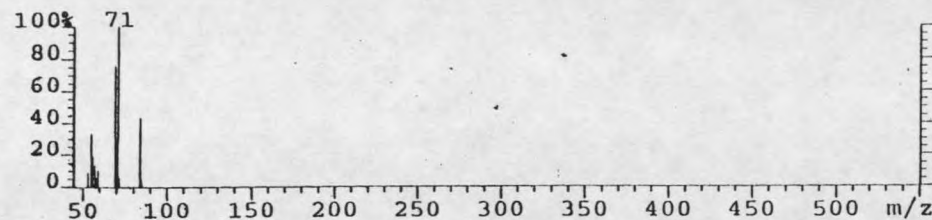
Normal Search: 26 of 129 Rank: Purity Mass Weighting: On Presearch Minimum: 20  
File: 2MAY97K Ident: 186\_188-198\_203 Mer Def 0.25 S:10 Acquired: 2-MAY-1997 16:47:26 +6:41  
70S EI+ Function: Magnet TIC: 1039931



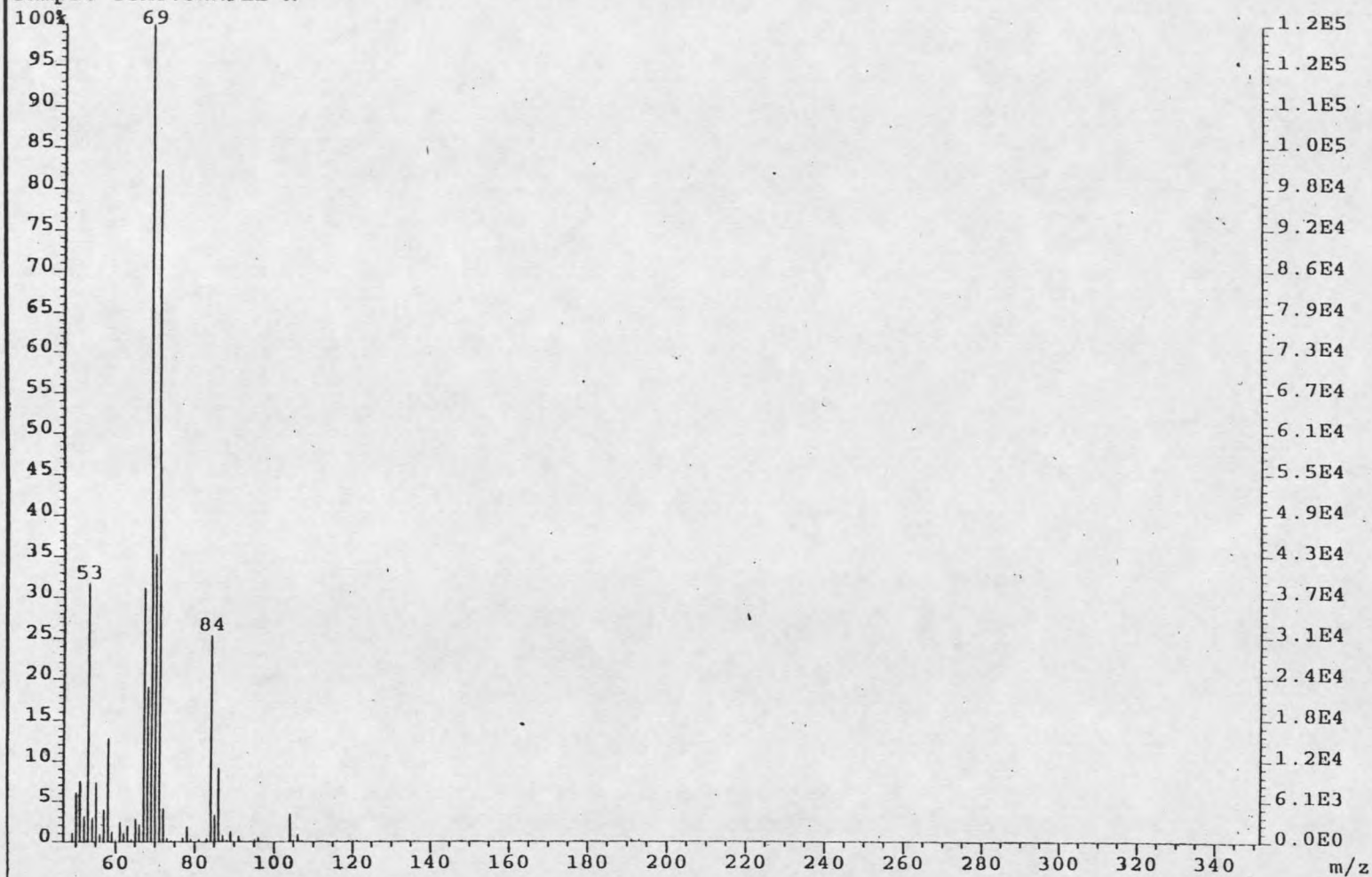
Rank: 1 NISTREP #1813 Purity: 678 Mixture: 906 Matched: 5 MF: C<sub>8</sub>H<sub>16</sub> MW: 112 CAS: 3404-77-1 NIST: 46310  
Name: 1-Hexene, 3,3-dimethyl- SMILES: CCCC(C)(C)C=C



Rank: 2 NIST #1776 Purity: 677 Mixture: 765 Matched: 5 MF: C<sub>6</sub>H<sub>14</sub>O MW: 102 CAS: 19550-30-2 NIST: 113688  
Name: 1-Butanol, 2,3-dimethyl- | 2,3-Dimethyl-1-butanol SMILES: CC(C)C(C)CO



File: 2MAY97K Ident: 249 252-240 246 Mer Def 0.25 S: 10 Acq: 3-MAY-1997 02:47:26 +7:36 Cal: 2MAY97K  
70S EI+ Magnet BpM: 69 BpI: 122186 TIC: 507853 Flags: HALL  
Sample Text: SAMPLE K

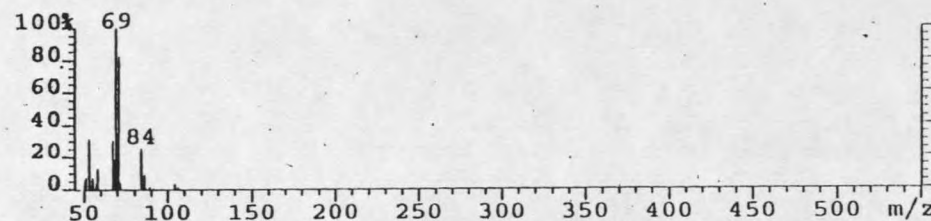




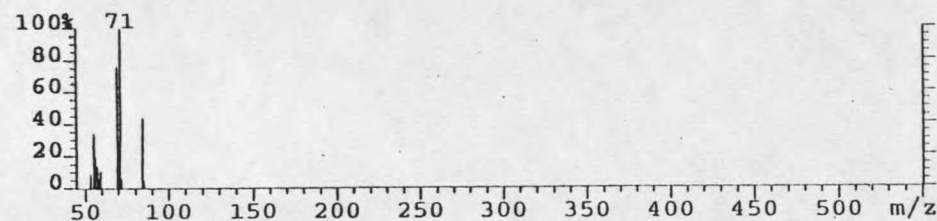
Libraries 1: NIST (62235) 2: NISTREP (12593)

|          |   |   |   |     |      |      |       |       |       |
|----------|---|---|---|-----|------|------|-------|-------|-------|
| Peaks:   | 8 | 7 | 6 | 5   | 4    | 3    | 2     | 1     | 0     |
| Matches: | 0 | 0 | 6 | 137 | 1007 | 4115 | 8116  | 16258 | 45189 |
| Total:   | 0 | 0 | 6 | 143 | 1150 | 5265 | 13381 | 29639 | 74828 |

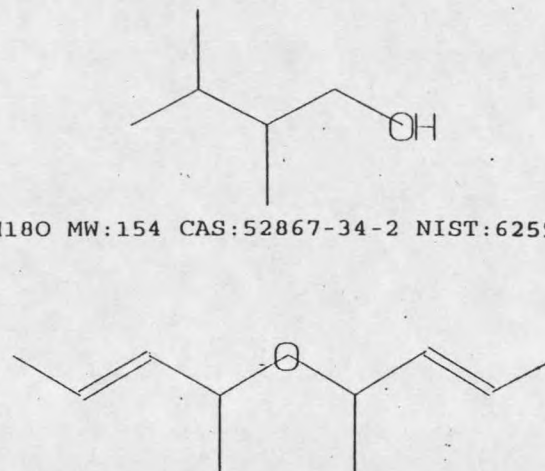
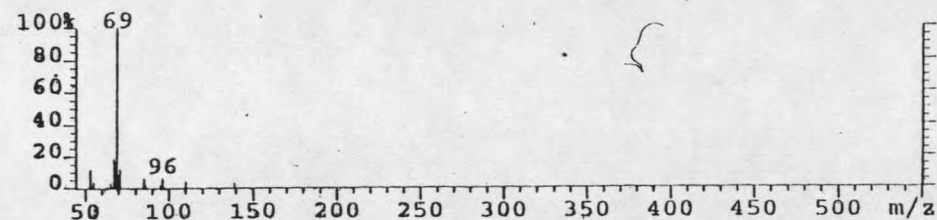
Normal Search: 37 of 143 Rank: Purity Mass Weighting: On Presearch Minimum: 20  
File: 2MAY97K Ident: 249 252-240 246 Mer Def 0.25 S: 10 Acquired: 2-MAY-1997 16:47:26 +7:36  
70S EI+ Function: Magnet TIC: 507853



Rank: 1 NIST #1776 Purity: 792 Mixture: 851 Matched: 5 MF: C<sub>6</sub>H<sub>14</sub>O MW: 102 CAS: 19550-30-2 NIST: 113688  
Name: 1-Butanol, 2,3-dimethyl- | 2,3-Dimethyl-1-butanol SMILES: CC(C)C(C)CO

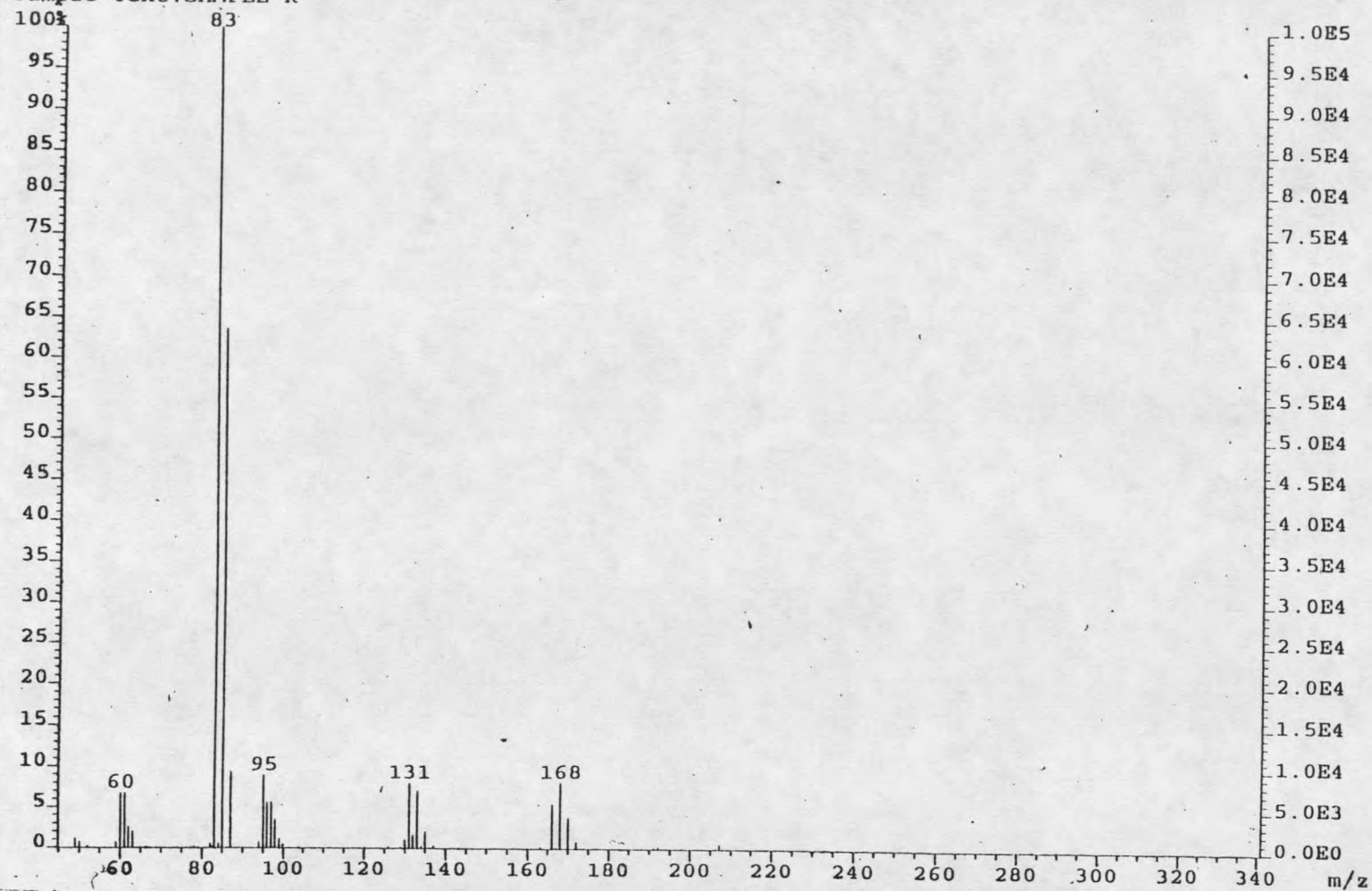


Rank: 2 NIST #10870 Purity: 664 Mixture: 672 Matched: 5 MF: C<sub>10</sub>H<sub>18</sub>O MW: 154 CAS: 52867-34-2 NIST: 62597  
Name: 2-Pentene, 4,4'-oxybis- SMILES: CC=CC(C)OC(C)C=CC





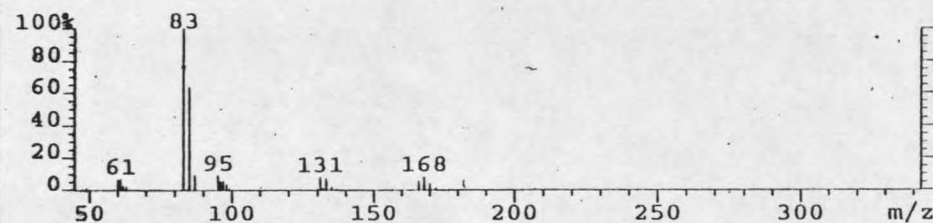
File: 2MAY97K Ident: 422\_425-402\_407 Mer Def 0.25 S:10 Acq: 3-MAY-1997 02:47:26 +10:05 Cal: 2MAY97>  
70S EI+ Magnet BpM: 83 BpI: 99924 TIC: 264706 Flags: HALL  
Sample Text: SAMPLE K



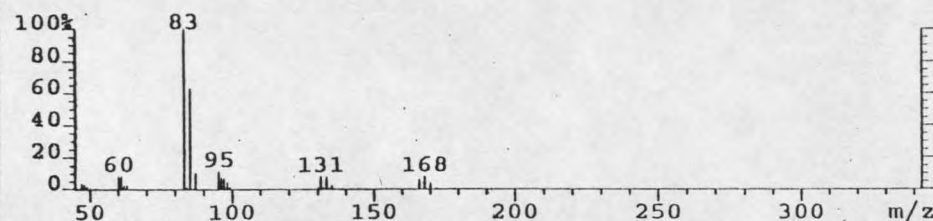
Libraries 1: NIST (62235) 2: NISTREP (12593)

|          |   |   |   |    |     |     |      |       |       |
|----------|---|---|---|----|-----|-----|------|-------|-------|
| Peaks:   | 8 | 7 | 6 | 5  | 4   | 3   | 2    | 1     | 0     |
| Matches: | 3 | 1 | 1 | 19 | 86  | 847 | 5098 | 20282 | 48491 |
| Total:   | 3 | 4 | 5 | 24 | 110 | 957 | 6055 | 26337 | 74828 |

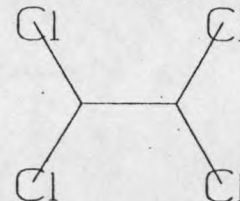
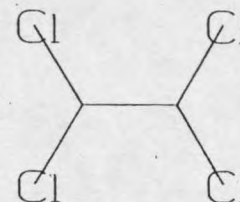
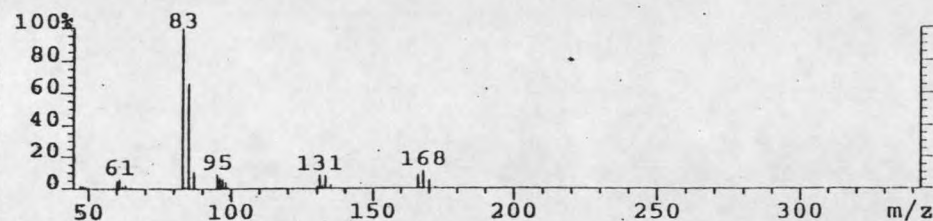
Normal Search: 10 of 24 Rank: Purity Mass Weighting: On Presearch Minimum: 20  
File: 2MAY97K Ident: 422 425-402 407 Mer Def 0.25 S: 10 Acquired: 2-MAY-1997 16:47:26 +10:05  
70S EI+ Function: Magnet TIC: 264706



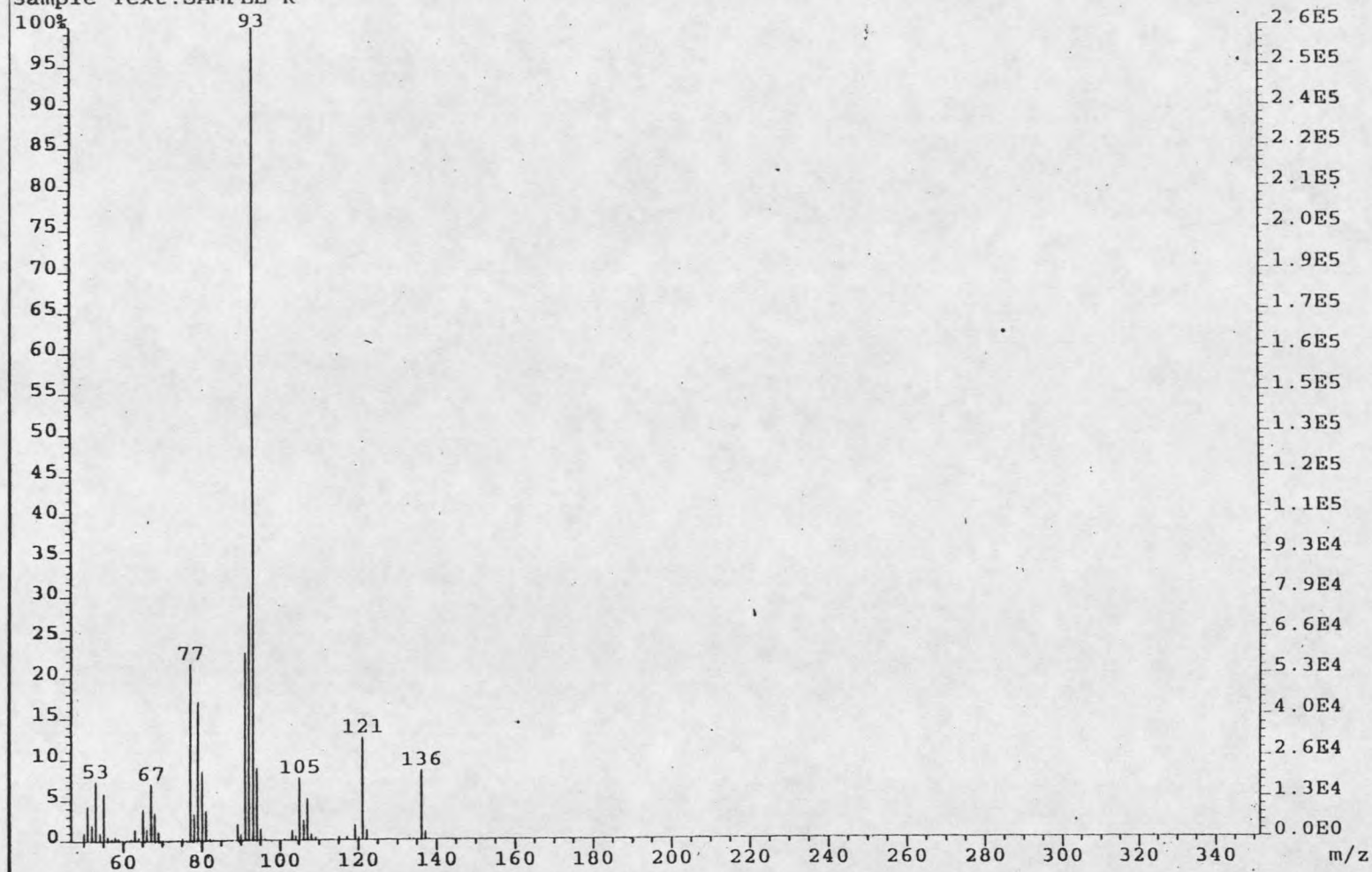
Rank: 1 NIST #13774 Purity: 997 Mixture: 998 Matched: 8 MF: C<sub>2</sub>H<sub>2</sub>Cl<sub>4</sub> MW: 166 CAS: 79-34-5 NIST: 34547  
Name: Ethane, 1,1,2,2-tetrachloro- | S-Tetrachloroethane | Acetylene tetrachloride SMILES: ClC(Cl)>



Rank: 2 NISTREP #5683 Purity: 996 Mixture: 996 Matched: 8 MF: C<sub>2</sub>H<sub>2</sub>Cl<sub>4</sub> MW: 166 CAS: 79-34-5 NIST: 34374  
Name: Ethane, 1,1,2,2-tetrachloro- SMILES: ClC(Cl)C(Cl)Cl



File: 2MAY97K Ident: 464 468-475 481 Mer Def 0.25 S: 10 Acq: 3-MAY-1997 02:47:26 +10:41 Cal: 2MAY97>  
70S EI+ Magnet BpM: 93 BpI: 264299 TIC: 824326 Flags: HALL  
Sample Text: SAMPLE K

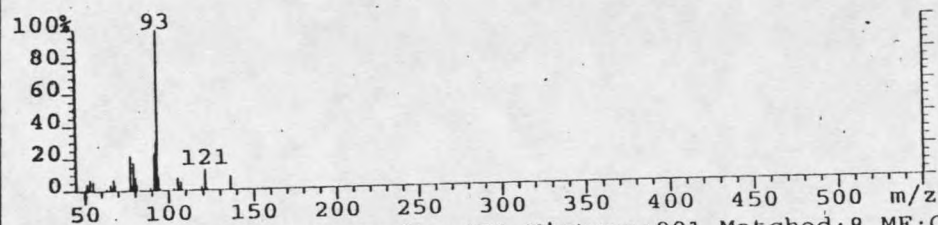


Libraries 1: NIST (62235) 2: NISTREP (12593)

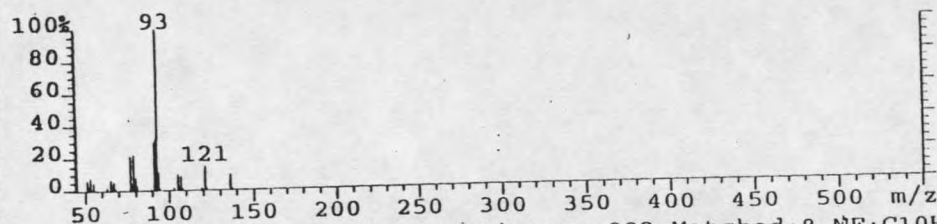
| Peaks:   | 8  | 7   | 6   | 5    | 4    | 3    | 2     | 1     | 0     |
|----------|----|-----|-----|------|------|------|-------|-------|-------|
| Matches: | 50 | 126 | 281 | 753  | 1850 | 3946 | 7907  | 14224 | 45691 |
| Total:   | 50 | 176 | 457 | 1210 | 3060 | 7006 | 14913 | 29137 | 74828 |

Normal Search: 41 of 50 Rank: Purity Mass Weighting: On Presearch Minimum: 20

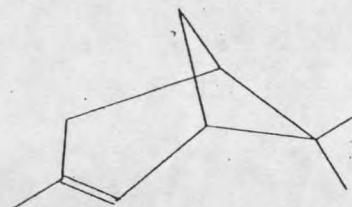
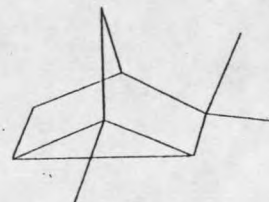
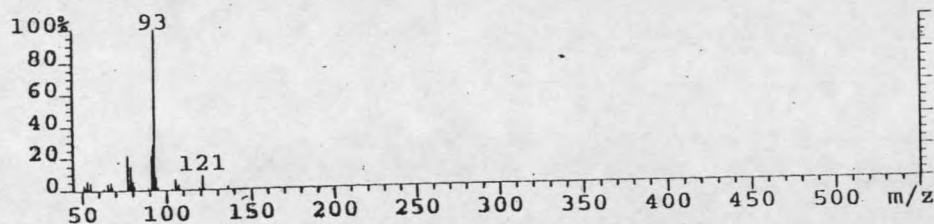
File: 2MAY97K Ident: 464\_468-475\_481 Mer Def 0.25 S: 10 Acquired: 2-MAY-1997 16:47:26 +10:41  
70S EI+ Function: Magnet TIC: 824326



Rank: 1 NISTREP #3509 Purity: 991 Mixture: 991 Matched: 8 MF: C<sub>10</sub>H<sub>16</sub> MW: 136 CAS: 488-97-1 NIST: 118832>  
Name: Tricyclo[2.2.1.0<sup>2,6</sup>]heptane, 1,3,3-trimethyl- SMILES: CC1(C)C3CC2C1C2(C)C3

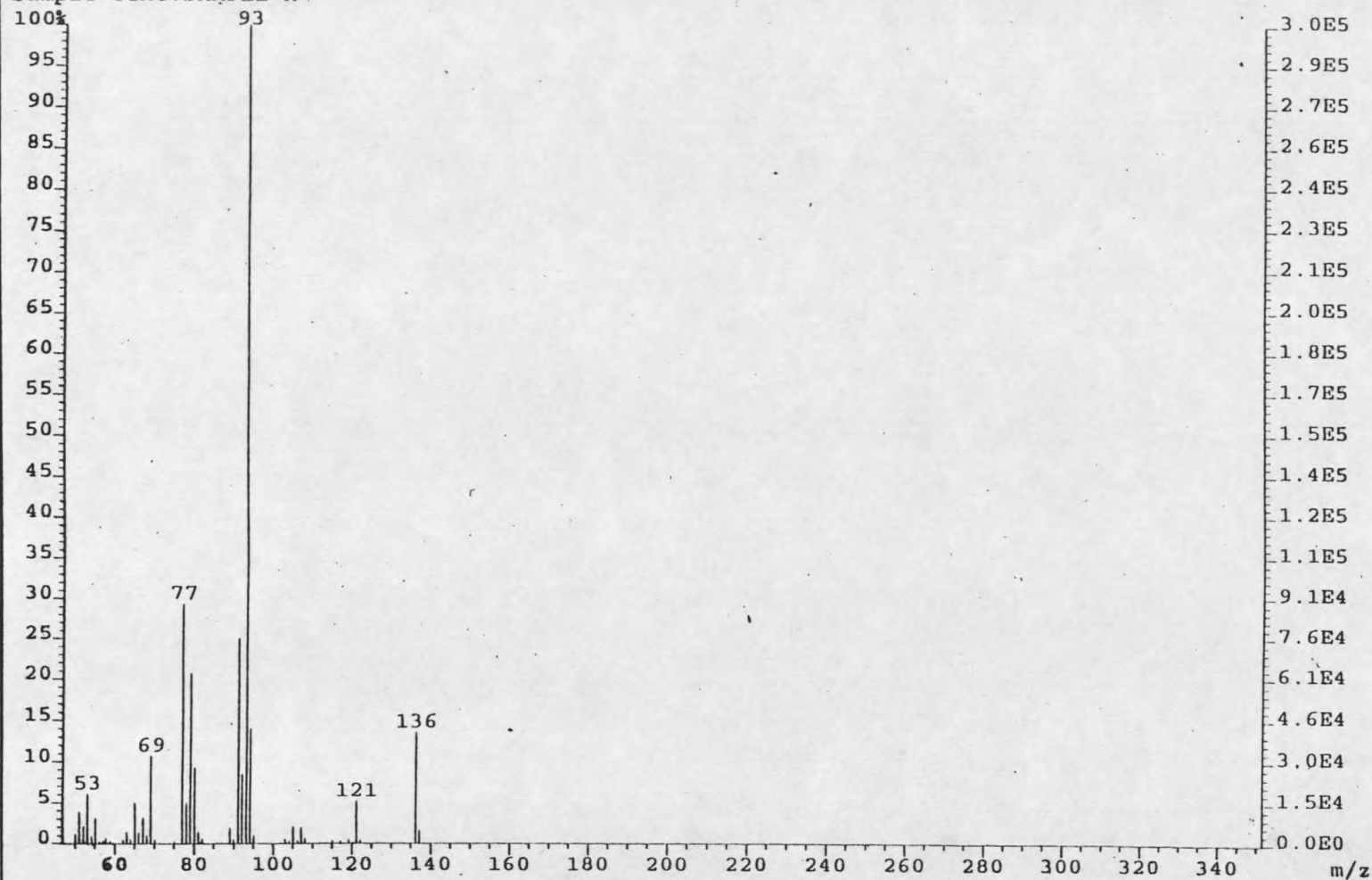


Rank: 2 NIST #6692 Purity: 991 Mixture: 992 Matched: 8 MF: C<sub>10</sub>H<sub>16</sub> MW: 136 CAS: 4889-83-2 NIST: 3320  
Name: Bicyclo[3.1.1]hept-2-ene, 3,6,6-trimethyl- | 2-Norpinene, 3,6,6-trimethyl- | 3-Methyl-apop>





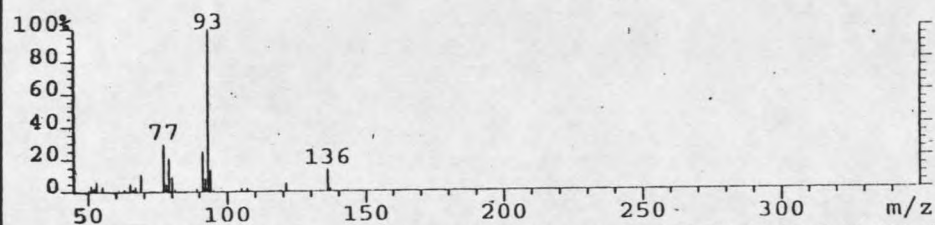
File: 2MAY97K Ident: 553-557-539\_545 Mer Def 0.25 S:10 Acq: 3-MAY-1997 02:47:26 +11:58 Cal: 2MAY97>  
70S EI+ Magnet BpM:93 BpI:304219 TIC:885727 Flags:HALL  
Sample Text: SAMPLE K



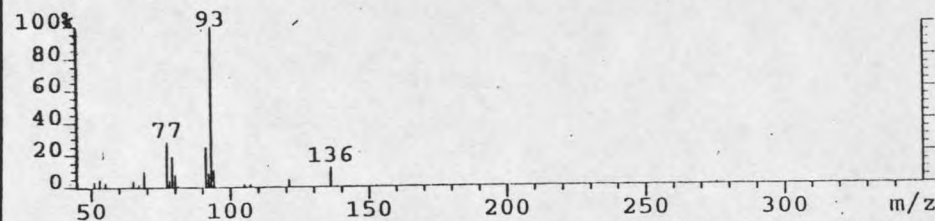
Libraries 1: NIST (62235) 2: NISTREP (12593)

| Peaks:   | 8  | 7  | 6   | 5   | 4    | 3    | 2     | 1     | 0     |
|----------|----|----|-----|-----|------|------|-------|-------|-------|
| Matches: | 16 | 72 | 189 | 633 | 1671 | 3778 | 6774  | 18448 | 43247 |
| Total:   | 16 | 88 | 277 | 910 | 2581 | 6359 | 13133 | 31581 | 74828 |

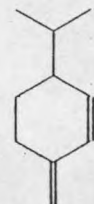
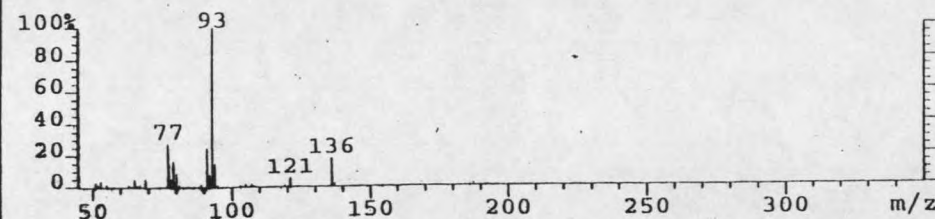
Normal Search: 72 of 88 Rank: Purity Mass Weighting: On Presearch Minimum: 20  
File: 2MAY97K Ident: 553\_557-539\_545 Mer Def 0.25 S:10 Acquired: 2-MAY-1997 16:47:26 +11:58  
70S EI+ Function: Magnet TIC: 885727



Rank: 1 NISTREP #3516 Purity: 997 Mixture: 998 Matched: 7 MF: C<sub>10</sub>H<sub>16</sub> MW: 136 CAS: 3387-41-5 NIST: 3315  
Name: Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)- SMILES: CC(C)C12CCC(=C)C1C2

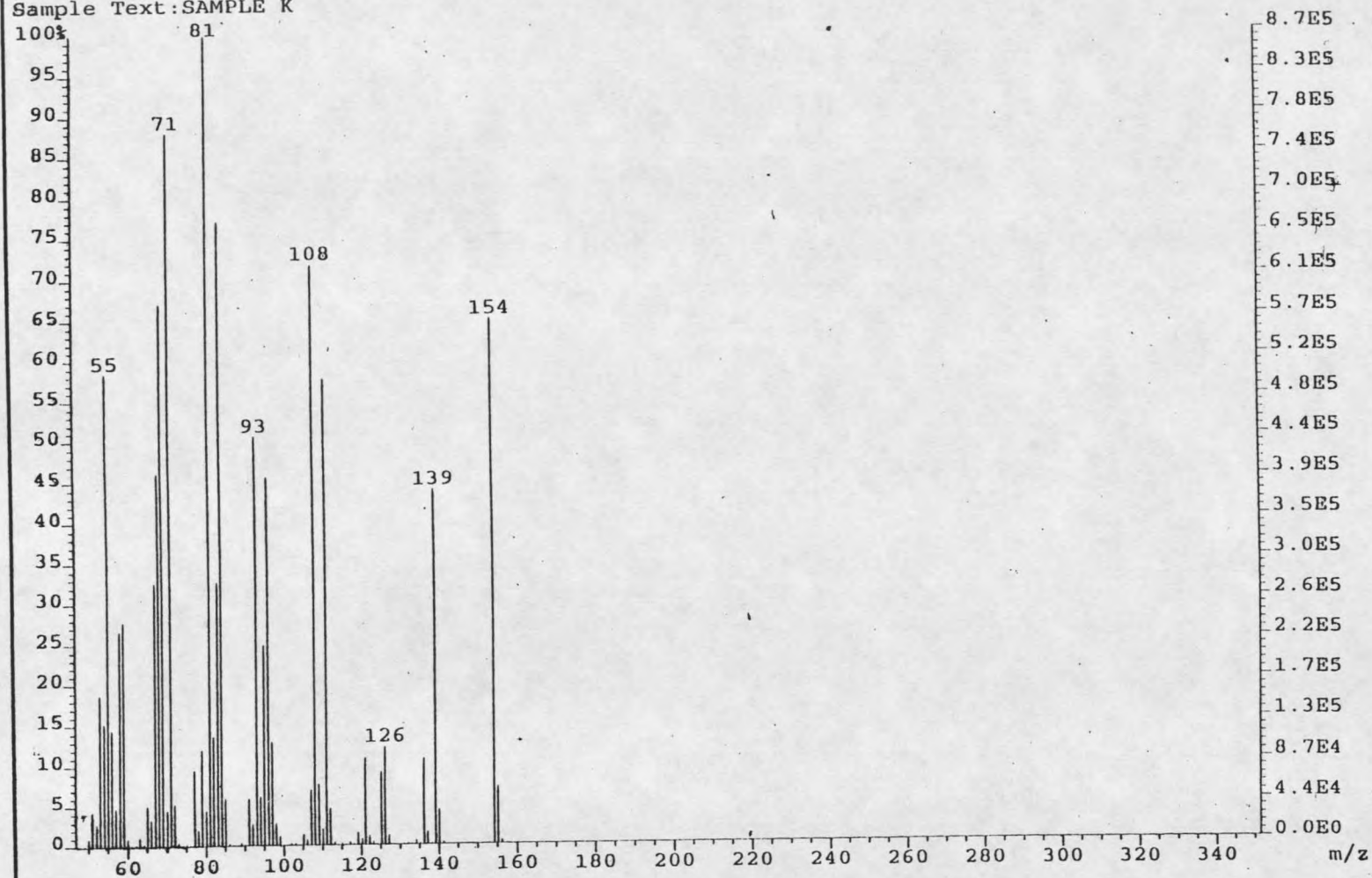


Rank: 2 NIST #6641 Purity: 994 Mixture: 994 Matched: 8 MF: C<sub>10</sub>H<sub>16</sub> MW: 136 CAS: 555-10-2 NIST: 3307  
Name: .beta.-Phellandrene | Cyclohexene, 3-methylene-6-(1-methylethyl)- | p-Mentha-1(7),2-diene^>





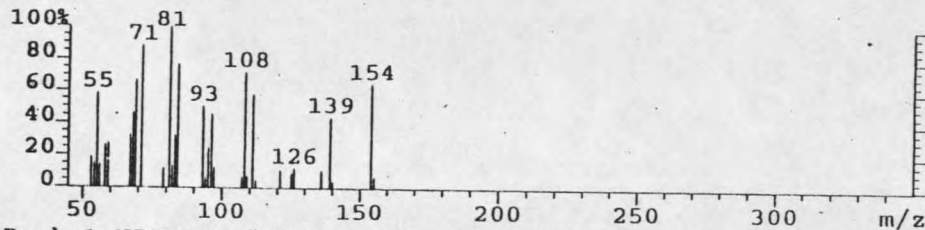
File: 2MAY97K Ident: 681\_685-694\_701 Mer Def 0.25 S:10 Acq: 3-MAY-1997 02:47:26 +13:48 Cal: 2MAY97>  
70S EI+ Magnet BpM: 81 BpI: 870394 TIC: 10176944 Flags: HALL  
Sample Text: SAMPLE K



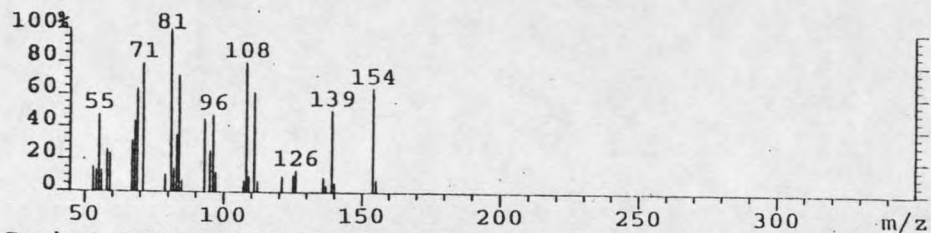
Libraries 1: NIST (62235) 2: NISTREP (12593)

|          |   |   |    |     |      |      |       |       |       |
|----------|---|---|----|-----|------|------|-------|-------|-------|
| Peaks:   | 8 | 7 | 6  | 5   | 4    | 3    | 2     | 1     | 0     |
| Matches: | 0 | 9 | 12 | 140 | 878  | 3466 | 8870  | 17276 | 44177 |
| Total:   | 0 | 9 | 21 | 161 | 1039 | 4505 | 13375 | 30651 | 74828 |

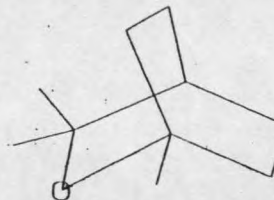
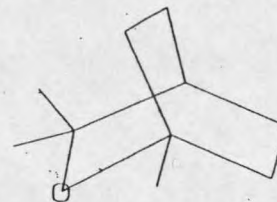
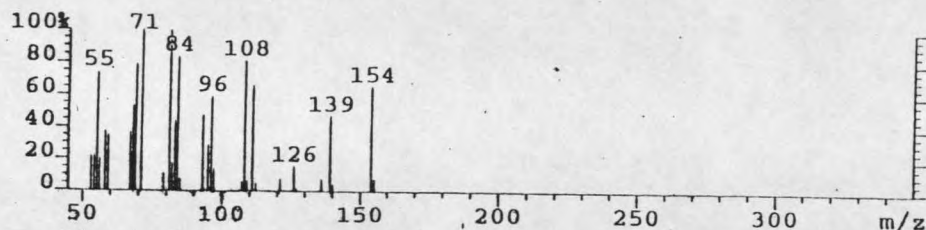
Normal Search: 13 of 21 Rank: Purity Mass Weighting: On Presearch Minimum: 20  
File: 2MAY97K Ident: 681\_685-694\_701 Mer Def 0.25 S: 10 Acquired: 2-MAY-1997 16:47:26 +13:48  
70S EI+ Function: Magnet TIC: 10176944



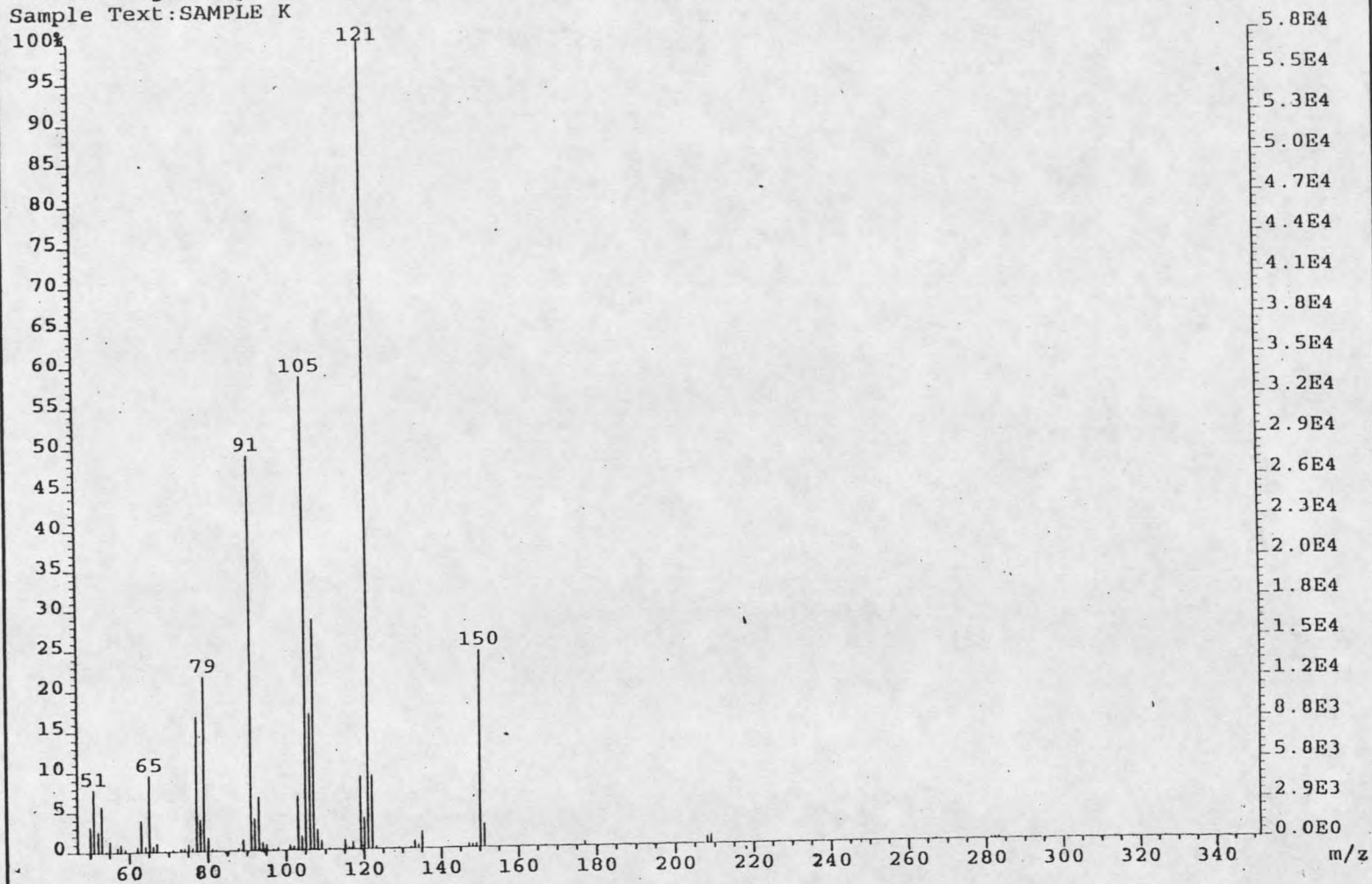
Rank: 1 NISTREP #4854 Purity: 992 Mixture: 992 Matched: 7 MF: C<sub>10</sub>H<sub>18</sub>O MW: 154 CAS: 470-82-6 NIST: 43952  
Name: Eucalyptol SMILES: CC12CCC(CC1)C(C)(C)O2



Rank: 2 NIST #10893 Purity: 987 Mixture: 990 Matched: 7 MF: C<sub>10</sub>H<sub>18</sub>O MW: 154 CAS: 470-82-6 NIST: 5106  
Name: Eucalyptol | Cineole | 2-Oxabicyclo[2.2.2]octane, 1,3,3-trimethyl- SMILES: CC12CCC(CC1)C(C)2



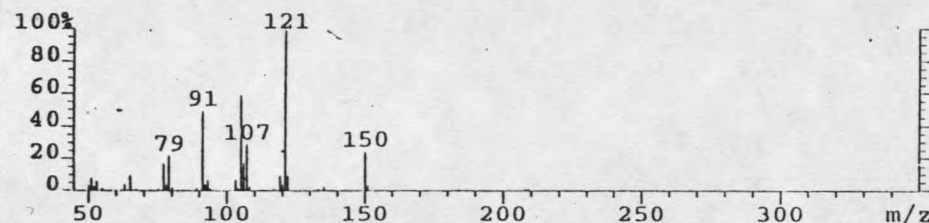
File: 2MAY97K Ident: 752\_754-730\_734 Mer Def 0.25 S:10 Acq: 3-MAY-1997 02:47:26 +14:48 Cal: 2MAY97>  
70S EI+ Magnet BpM:121 BpI:58370 TIC:252887 Flags:HALL  
Sample Text: SAMPLE K



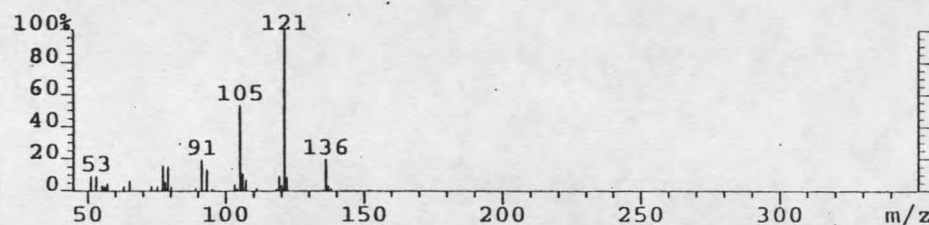
Libraries 1: NIST (62235) 2: NISTREP (12593)

| Peaks:   | 8 | 7   | 6   | 5    | 4    | 3    | 2     | 1     | 0     |
|----------|---|-----|-----|------|------|------|-------|-------|-------|
| Matches: | 8 | 92  | 338 | 850  | 2120 | 4043 | 7310  | 13581 | 46486 |
| Total:   | 8 | 100 | 438 | 1288 | 3408 | 7451 | 14761 | 28342 | 74828 |

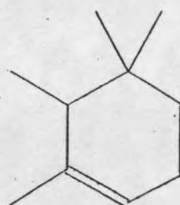
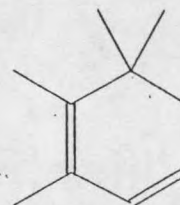
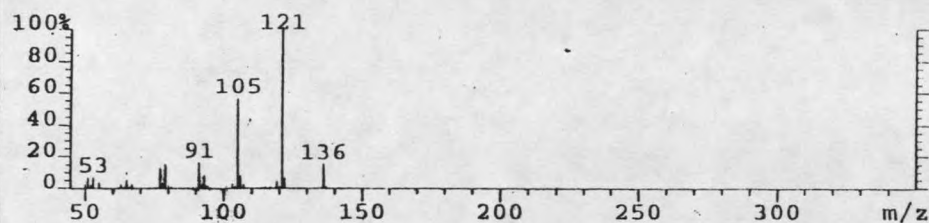
Normal Search: 29 of 100 Rank: Purity Mass Weighting: On Presearch Minimum: 20  
File: 2MAY97K Ident: 752\_754-730\_734 Mer Def 0.25 S:10 Acquired: 2-MAY-1997 16:47:26 +14:48  
70S EI+ Function: Magnet TIC: 252887



Rank: 1 NISTREP #3553 Purity: 859 Mixture: 900 Matched: 7 MF: C<sub>10</sub>H<sub>16</sub> MW: 136 CAS: 514-96-5 NIST: 122103  
Name: 1,3-Cyclohexadiene, 1,2,6,6-tetramethyl- SMILES: CC1=C(C)C(C)(C)CC=C1

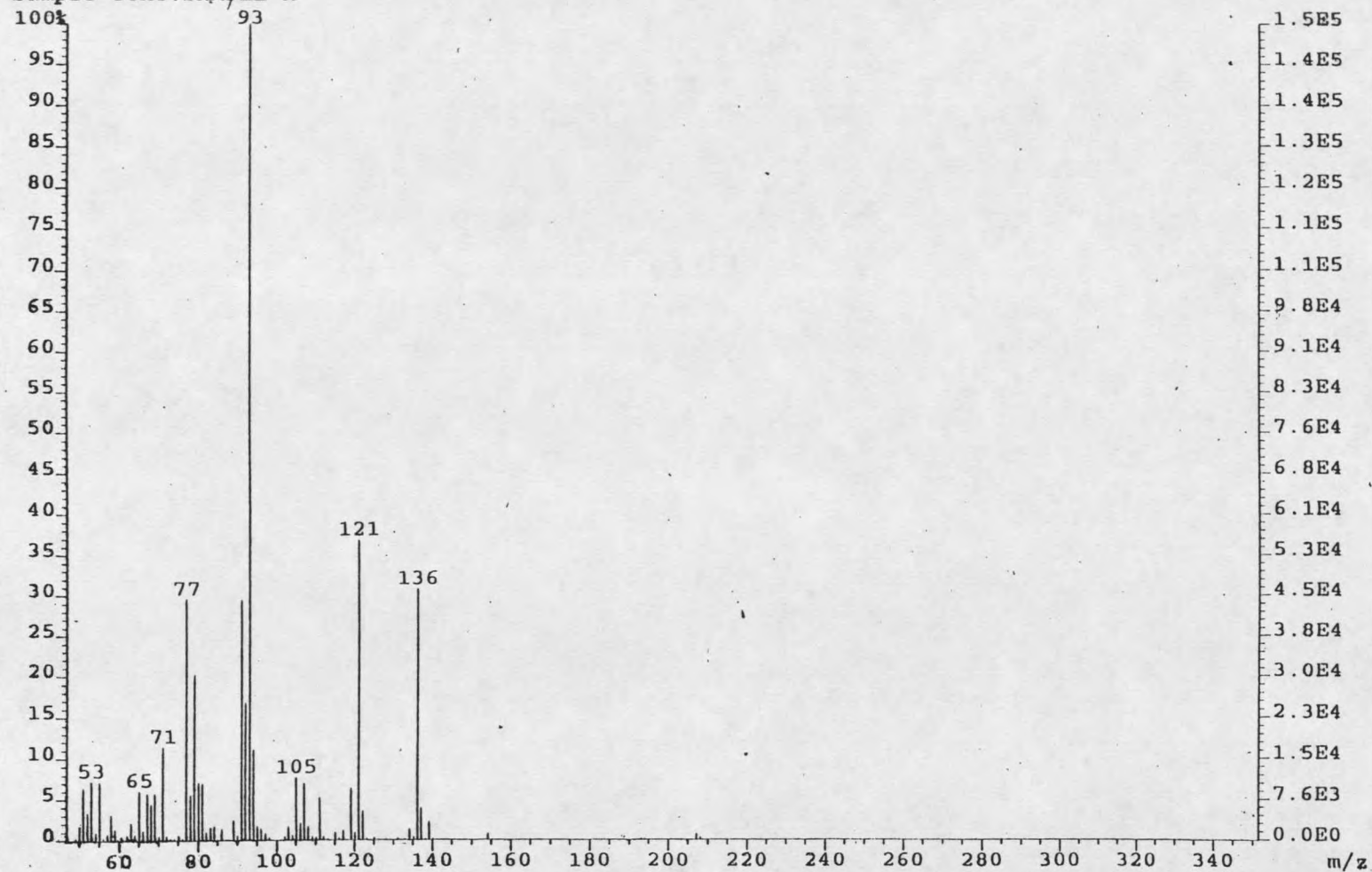


Rank: 2 NISTREP #3544 Purity: 847 Mixture: 886 Matched: 7 MF: C<sub>10</sub>H<sub>16</sub> MW: 136 CAS: 514-94-3 NIST: 122105  
Name: 1,3-Cyclohexadiene, 1,5,5,6-tetramethyl- SMILES: CC1C(=CC=CC1(C)C)C





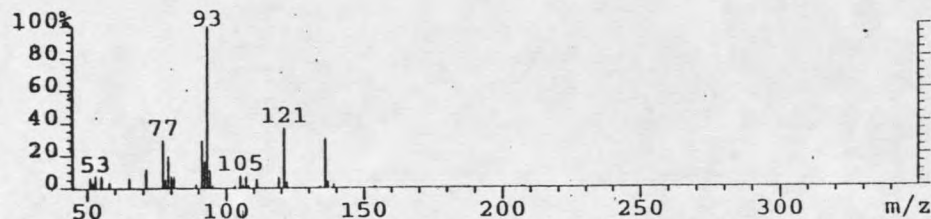
File: 2MAY97K Ident: 825\_828-813\_815 Mer Def 0.25 S:10 Acq: 3-MAY-1997 02:47:26 +15:52 Cal: 2MAY97  
70S EI+ Magnet BpM: 93 BpI: 151317 TIC: 660255 Flags: HALL  
Sample Text: SAMPLE K



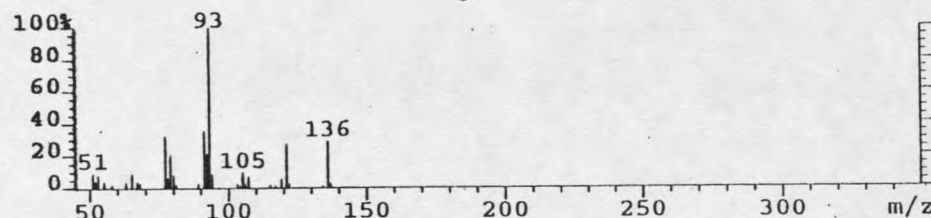
Libraries 1: NIST (62235) 2: NISTREP (12593)

| Peaks:   | 8  | 7   | 6   | 5    | 4    | 3    | 2     | 1     | 0     |
|----------|----|-----|-----|------|------|------|-------|-------|-------|
| Matches: | 33 | 100 | 239 | 751  | 1983 | 4032 | 7847  | 15878 | 43965 |
| Total:   | 33 | 133 | 372 | 1123 | 3106 | 7138 | 14985 | 30863 | 74828 |

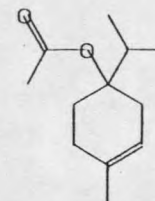
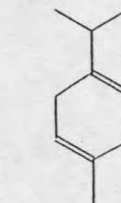
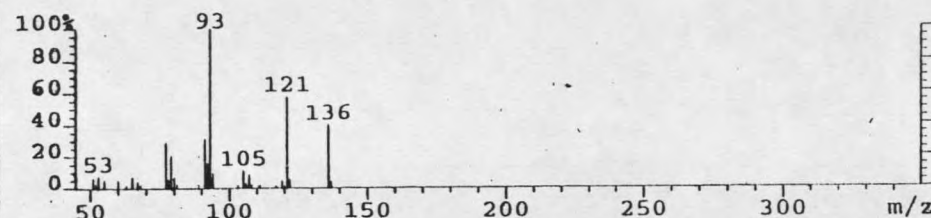
Normal Search: 32 of 33 Rank: Purity Mass Weighting: On Presearch Minimum: 20  
File: 2MAY97K Ident: 825\_828-813\_815 Mer Def 0.25 S:10 Acquired: 2-MAY-1997 16:47:26 +15:52  
70S EI+ Function: Magnet TIC: 660255



Rank: 1 NISTREP #3510 Purity: 967 Mixture: 976 Matched: 8 MF: C<sub>10</sub>H<sub>16</sub> MW: 136 CAS: 99-85-4 NIST: 79637  
Name: 1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)- SMILES: CC(C)C1=CCC(=CC1)C

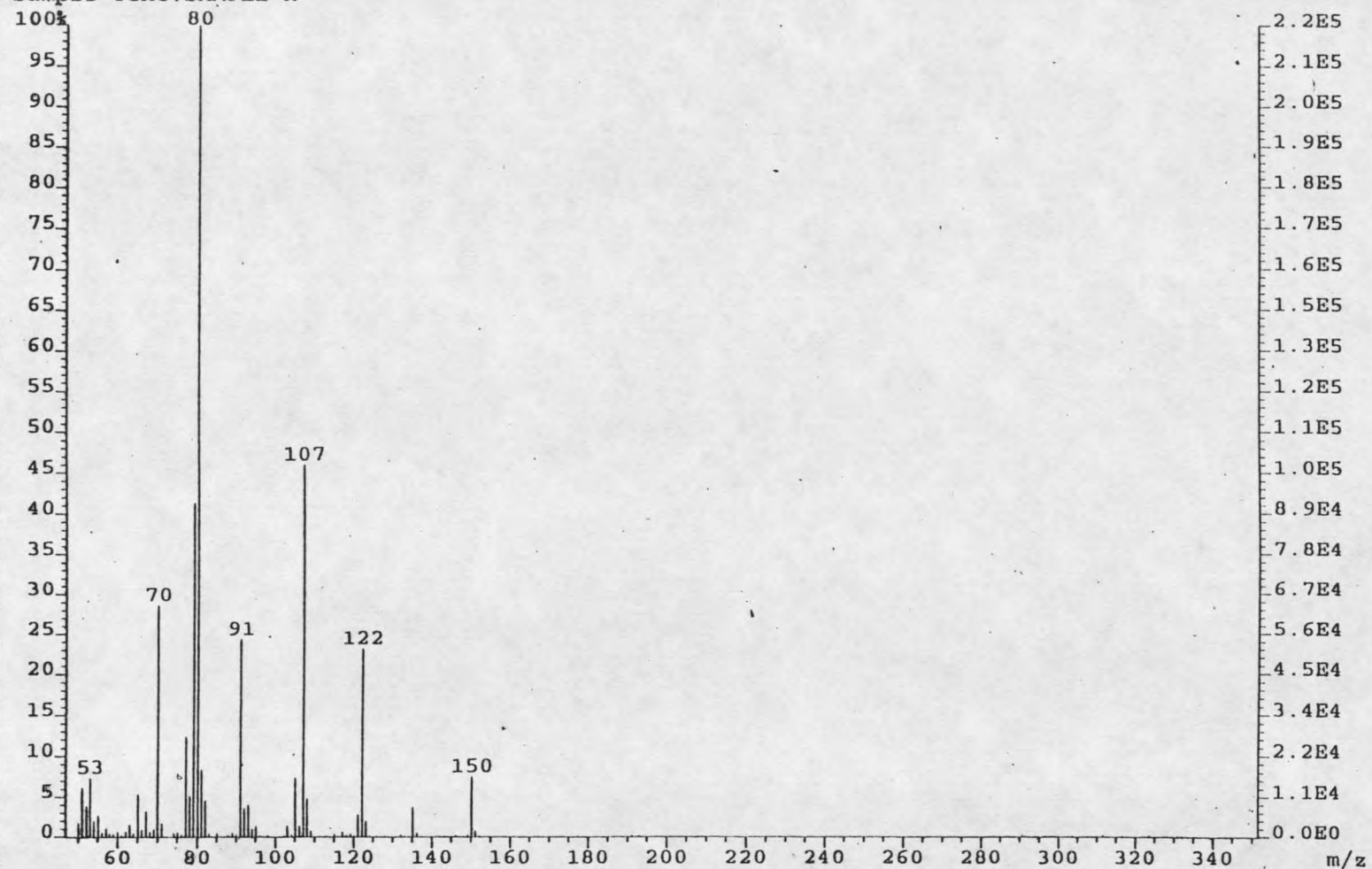


Rank: 2 NIST #21864 Purity: 957 Mixture: 964 Matched: 8 MF: C<sub>12</sub>H<sub>20</sub>O<sub>2</sub> MW: 196 CAS: 4821-04-9 NIST: 8648  
Name: 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, acetate | p-Menth-1-en-4-ol, acetate | Ter>





File: 2MAY97K Ident: 837\_841-845\_847 Mer Def 0.25 S:10 Acq: 3-MAY-1997 02:47:26 +16:02 Cal: 2MAY97>  
70S EI+ Magnet BpM:80 BpI:223635 TIC:867572 Flags:HALL  
Sample Text: SAMPLE K



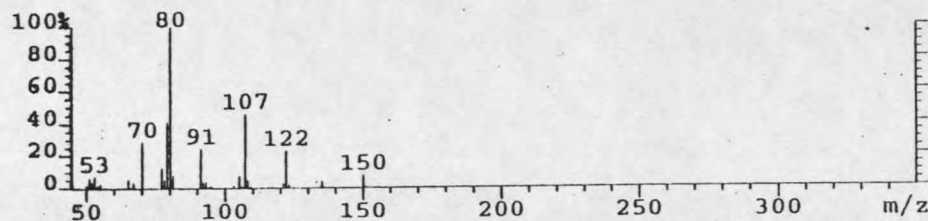
Libraries 1: NIST (62235) 2: NISTREP (12593)

|          |   |    |     |     |      |      |       |       |       |
|----------|---|----|-----|-----|------|------|-------|-------|-------|
| Peaks:   | 8 | 7  | 6   | 5   | 4    | 3    | 2     | 1     | 0     |
| Matches: | 3 | 13 | 124 | 564 | 1479 | 3600 | 7990  | 18459 | 42596 |
| Total:   | 3 | 16 | 140 | 704 | 2183 | 5783 | 13773 | 32232 | 74828 |

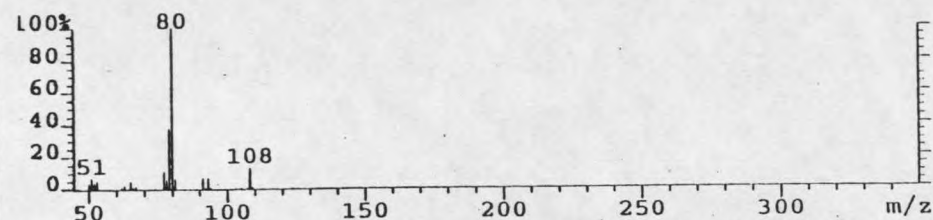
Normal Search: 3 of 140 Rank: Purity Mass Weighting: On Presearch Minimum: 20

File: 2MAY97K Ident: 837\_841-845\_847 Mer Def 0.25 S: 10 Acquired: 2-MAY-1997 16:47:26 +16:02

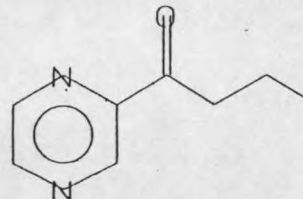
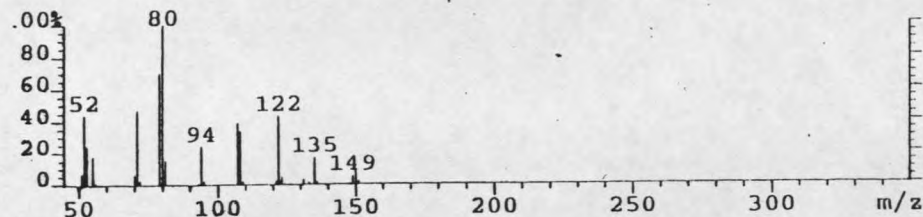
70S EI+ Function: Magnet TIC: 867572



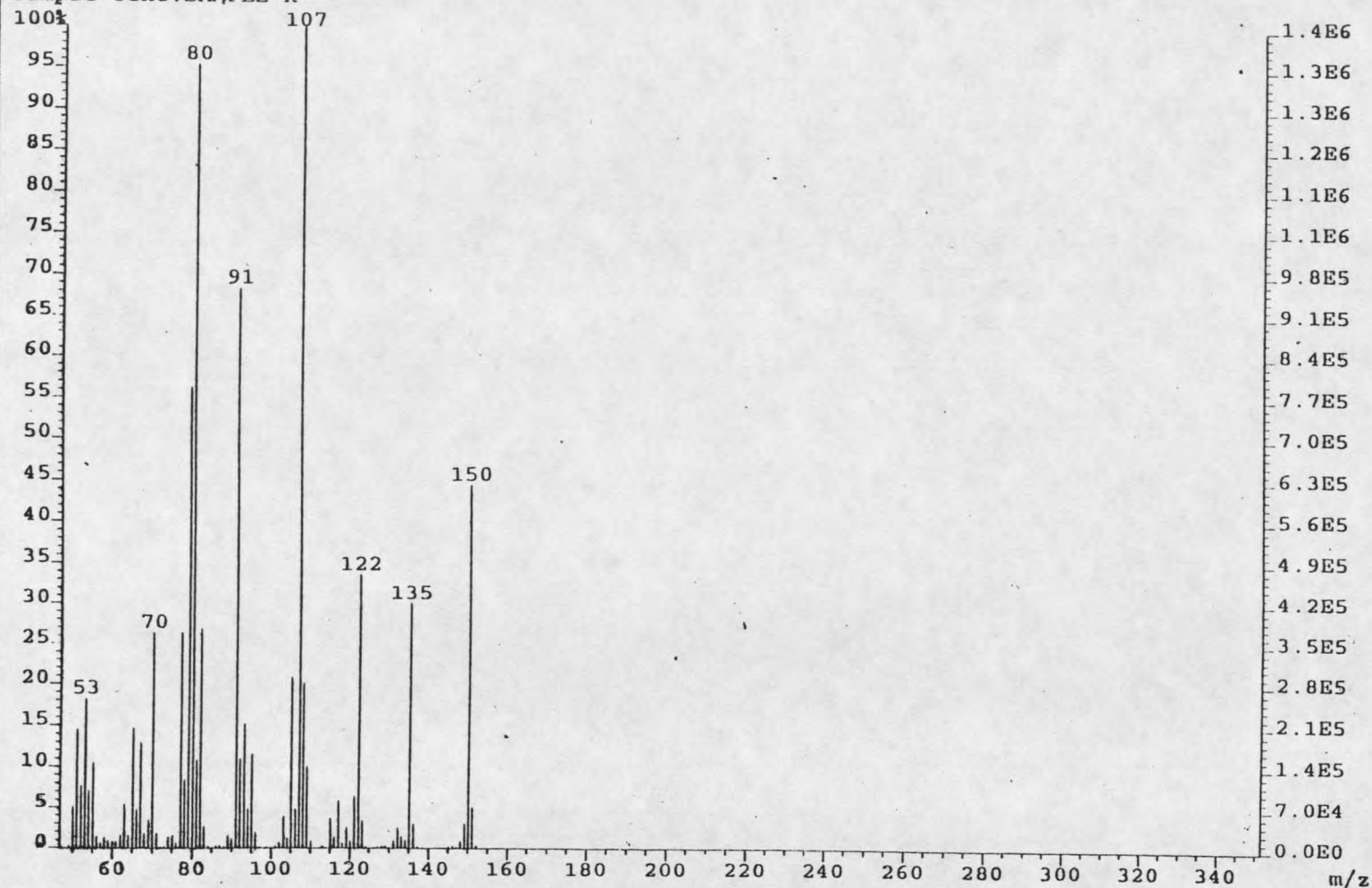
Rank: 1 NIST #2158 Purity: 712 Mixture: 789 Matched: 6 MF: C<sub>8</sub>H<sub>12</sub> MW: 108 CAS: 694-92-8 NIST: 60854  
Name: Bicyclo[2.2.1]hept-2-ene, 2-methyl- | 2-Norbornene, 2-methyl- | 2-Methyl-2-norbornene SMIL>



Rank: 2 NIST #9651 Purity: 682 Mixture: 723 Matched: 6 MF: C<sub>8</sub>H<sub>10</sub>N<sub>2</sub>O MW: 150 NIST: 108588  
Name: 1-(2-Pyrazinyl)butanone SMILES: CCCC(=O)c1ncncn1



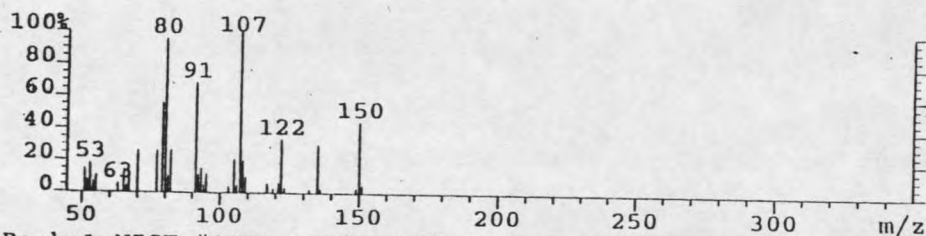
File: 2MAY97K Ident: 886\_890-899\_903 Mer Def 0.25 S:10 Acq: 3-MAY-1997 02:47:26 +16:45 Cal: 2MAY97>  
70S EI+ Magnet BpM:107 BpI:1400432 TIC:11315119 Flags: HALL  
Sample Text: SAMPLE K



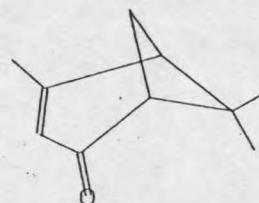
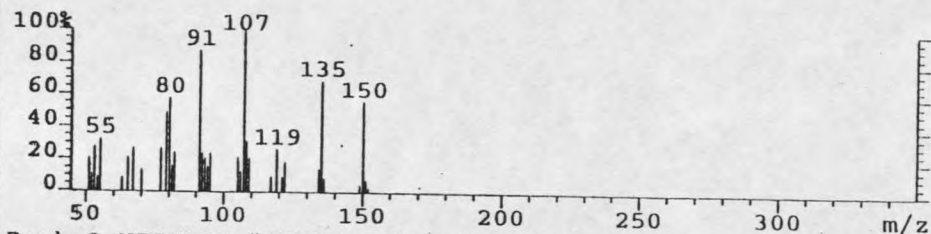
Libraries 1: NIST (62235) 2: NISTREP (12593)

|          |   |    |     |     |      |      |       |       |       |
|----------|---|----|-----|-----|------|------|-------|-------|-------|
| Peaks:   | 8 | 7  | 6   | 5   | 4    | 3    | 2     | 1     | 0     |
| Matches: | 3 | 12 | 112 | 510 | 1372 | 3339 | 7711  | 18680 | 43089 |
| Total:   | 3 | 15 | 127 | 637 | 2009 | 5348 | 13059 | 31739 | 74828 |

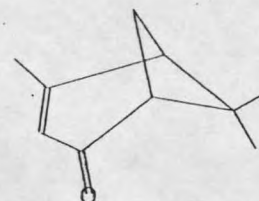
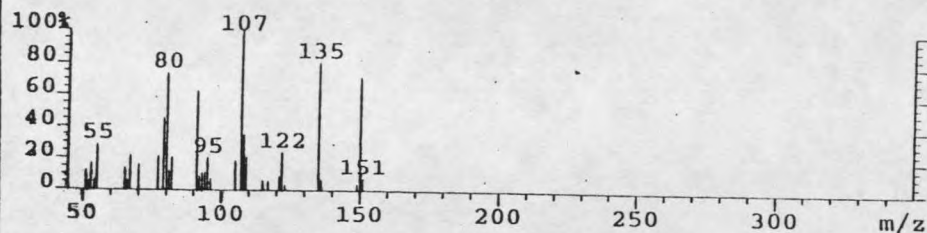
Normal Search: 29 of 127 Rank: Purity Mass Weighting: On Presearch Minimum: 20  
File: 2MAY97K Ident: 886\_890-899\_903 Mer Def 0.25 S: 10 Acquired: 2-MAY-1997 16:47:26 +16:45  
70S EI+ Function: Magnet TIC: 11315119



Rank: 1 NIST #9853 Purity: 817 Mixture: 818 Matched: 7 MF: C<sub>10</sub>H<sub>14</sub>O MW: 150 CAS: 18309-32-5 NIST: 38883  
Name: D-Verbenone | Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-, (1R)- | 2-Pinen-4-one, (1R), >

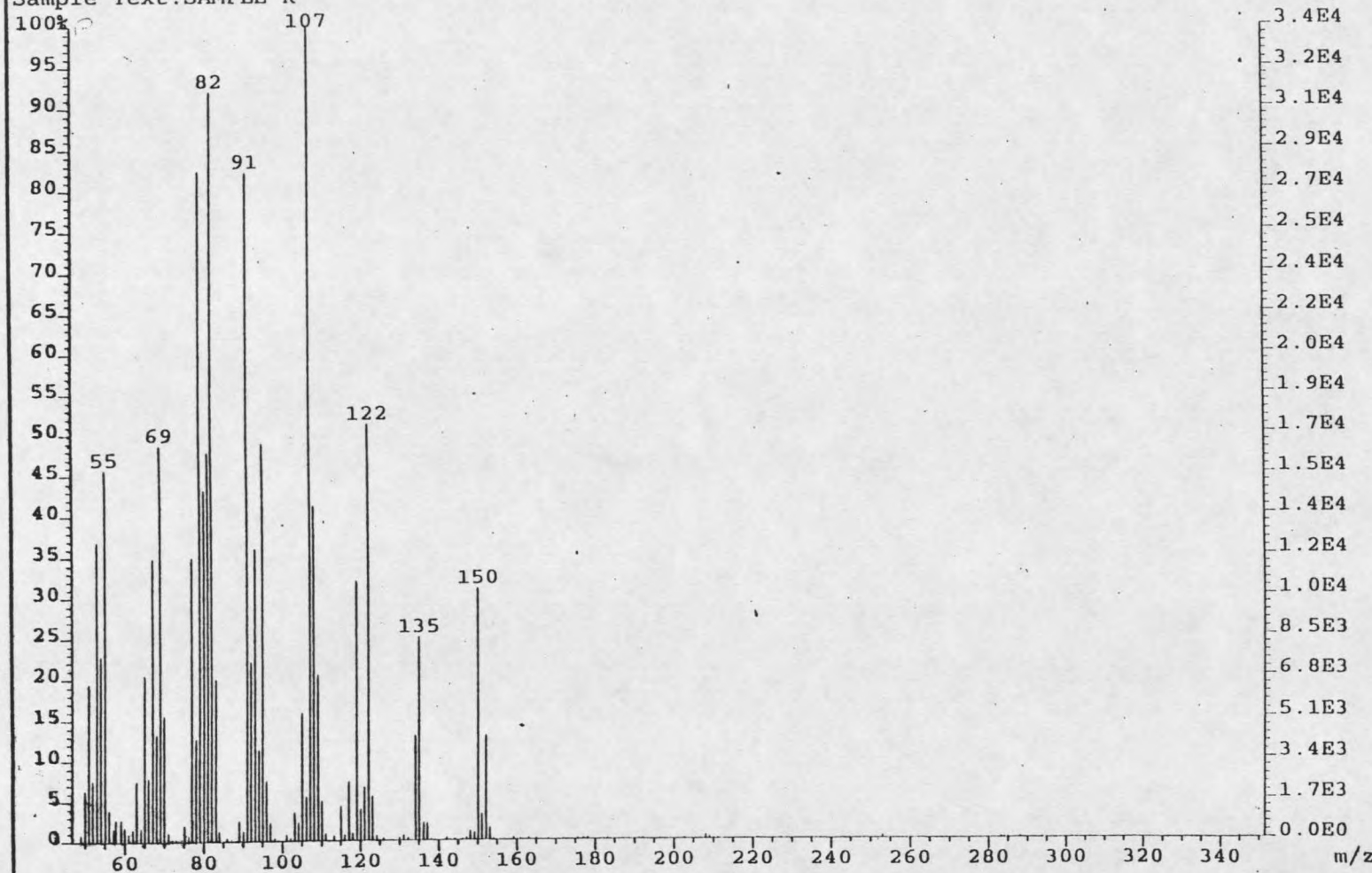


Rank: 2 NISTREP #4575 Purity: 814 Mixture: 816 Matched: 8 MF: C<sub>10</sub>H<sub>14</sub>O MW: 150 CAS: 80-57-9 NIST: 109296 >  
Name: Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl- SMILES: CC1=CC(=O)C2CC1C2(C)C





File: 2MAY97K Ident: 921\_924-931\_934 Mer Def 0.25 S:10 Acq: 3-MAY-1997 02:47:26 +17:14 Cal: 2MAY97>  
70S EI+ Magnet BpM:107 BpI:33940 TIC:431546 Flags: HALL  
Sample Text: SAMPLE K



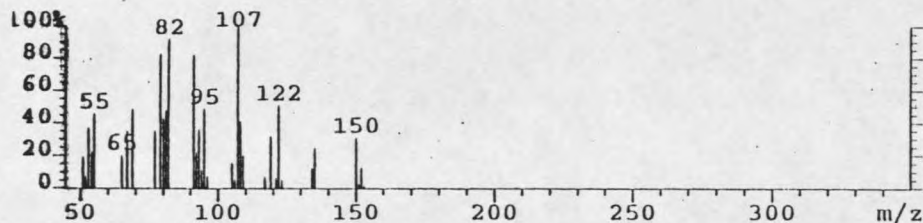
Libraries 1: NIST (62235) 2: NISTREP (12593)

|          |   |   |    |     |      |      |       |       |       |
|----------|---|---|----|-----|------|------|-------|-------|-------|
| Peaks:   | 8 | 7 | 6  | 5   | 4    | 3    | 2     | 1     | 0     |
| Matches: | 2 | 4 | 33 | 236 | 1153 | 3318 | 7539  | 15809 | 46734 |
| Total:   | 2 | 6 | 39 | 275 | 1428 | 4746 | 12285 | 28094 | 74828 |

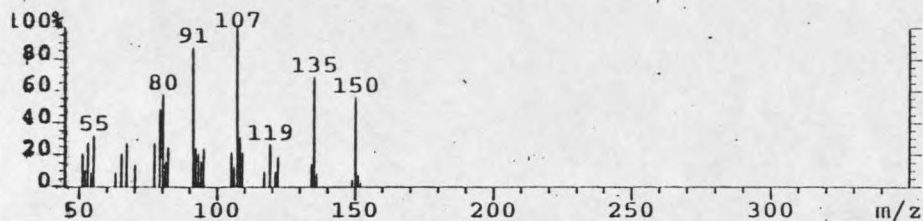
Normal Search: 14 of 39 Rank: Purity Mass Weighting: On Presearch Minimum: 20

File: 2MAY97K Ident: 921\_924-931\_934 Mer Def 0.25 S:10 Acquired: 2-MAY-1997 16:47:26 +17:14

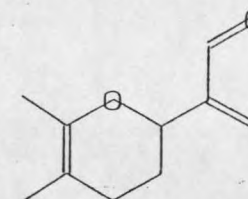
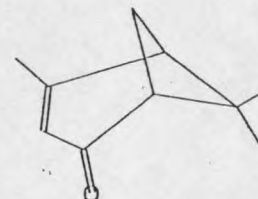
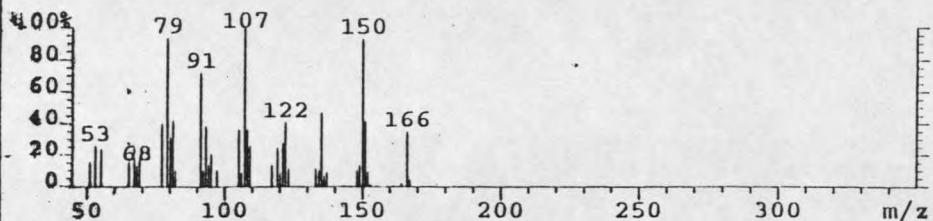
OS EI+ Function: Magnet TIC: 431546



Rank: 1 NIST #9853 Purity: 739 Mixture: 764 Matched: 8 MF: C<sub>10</sub>H<sub>14</sub>O MW: 150 CAS: 18309-32-5 NIST: 38883  
Name: D-Verbenone | Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-, (1R)- | 2-Pinen-4-one, (1R), >

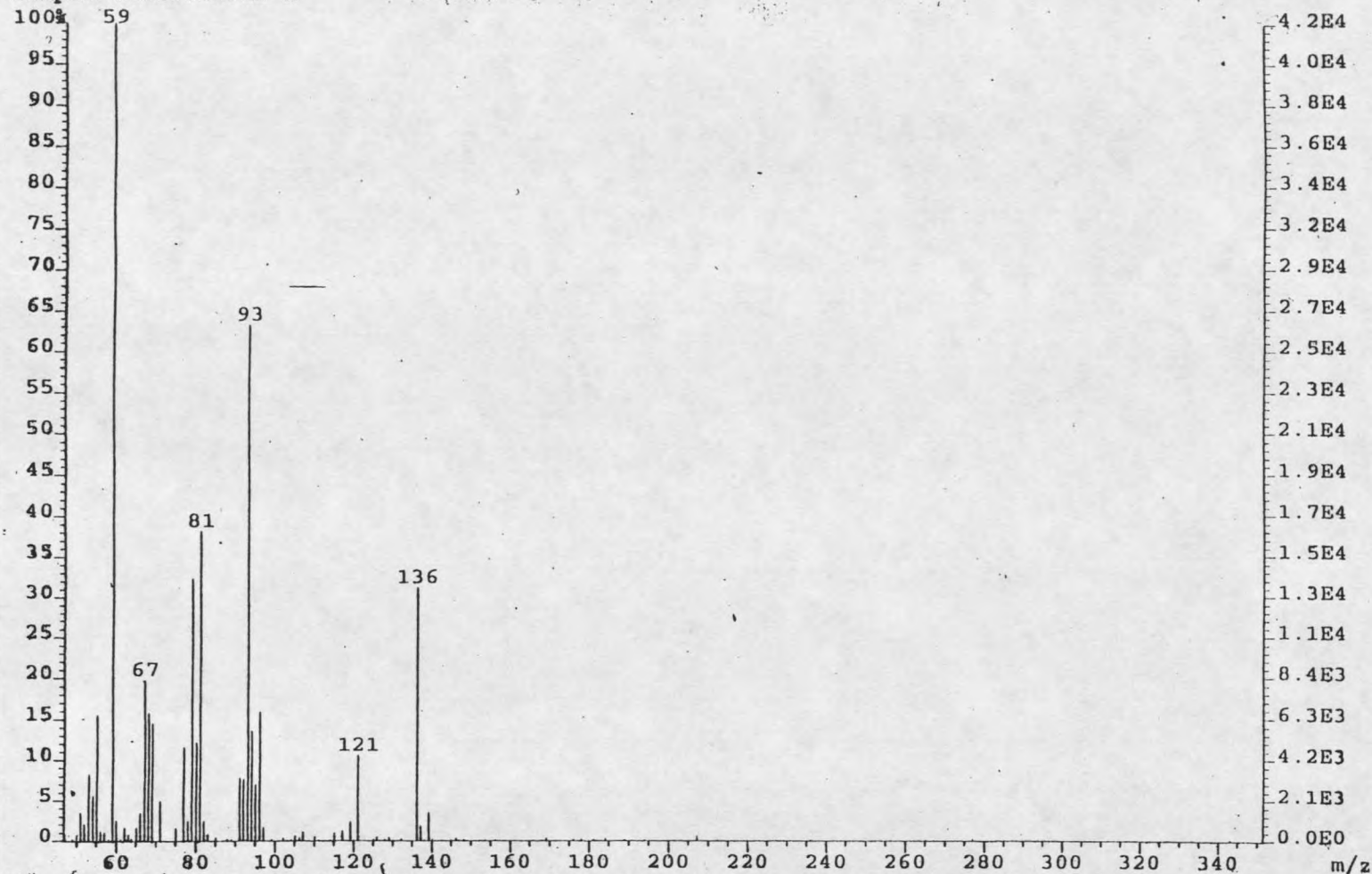


Rank: 2 NIST #13995 Purity: 659 Mixture: 663 Matched: 6 MF: C<sub>10</sub>H<sub>14</sub>O<sub>2</sub> MW: 166 NIST: 111150  
Name: 3,7-Epoxy-2-methylene-6-methyloct-6-en-1-al SMILES: CC1=C(C)OC(CC1)C(=C)C=O





File: 2MAY97K Ident: 968\_970-963\_965 Mer Def 0.25 S:10 Acq: 3-MAY-1997 02:47:26 +17:54 Cal: 2MAY97  
70S EI+ Magnet BpM: 59 BpI: 42071 TIC: 204922 Flags: HALL  
Sample Text: SAMPLE K



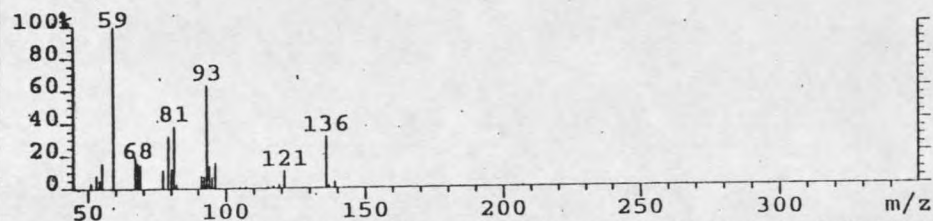
Libraries 1: NIST (62235) 2: NISTREP (12593)

|          |   |    |     |     |      |      |       |       |       |
|----------|---|----|-----|-----|------|------|-------|-------|-------|
| Peaks:   | 8 | 7  | 6   | 5   | 4    | 3    | 2     | 1     | 0     |
| Matches: | 0 | 25 | 121 | 370 | 1210 | 2749 | 6218  | 17347 | 46788 |
| Total:   | 0 | 25 | 146 | 516 | 1726 | 4475 | 10693 | 28040 | 74828 |

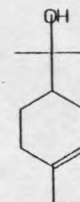
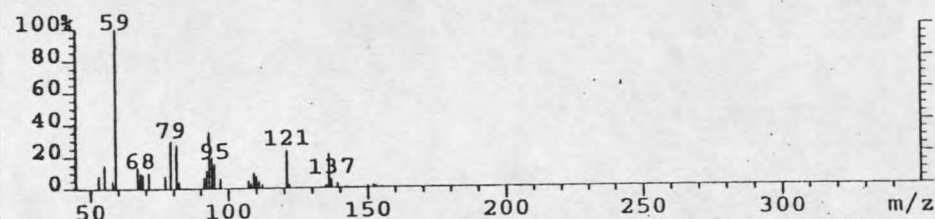
Normal Search: 2 of 25 Rank: Purity Mass Weighting: On Presearch Minimum: 20

File: 2MAY97K Ident: 968\_970-963\_965 Mer Def 0.25 S:10 Acquired: 2-MAY-1997 16:47:26 +17:54

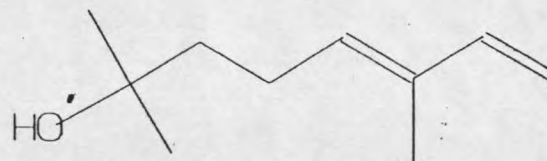
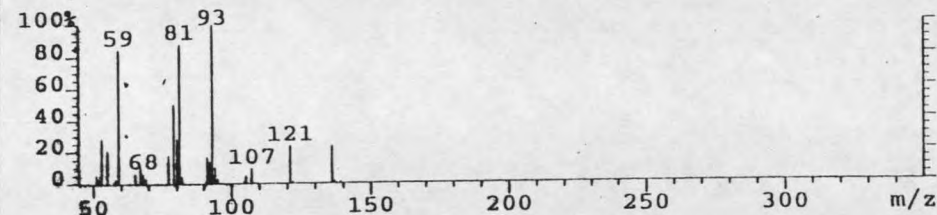
70S EI+ Function: Magnet TIC: 204922



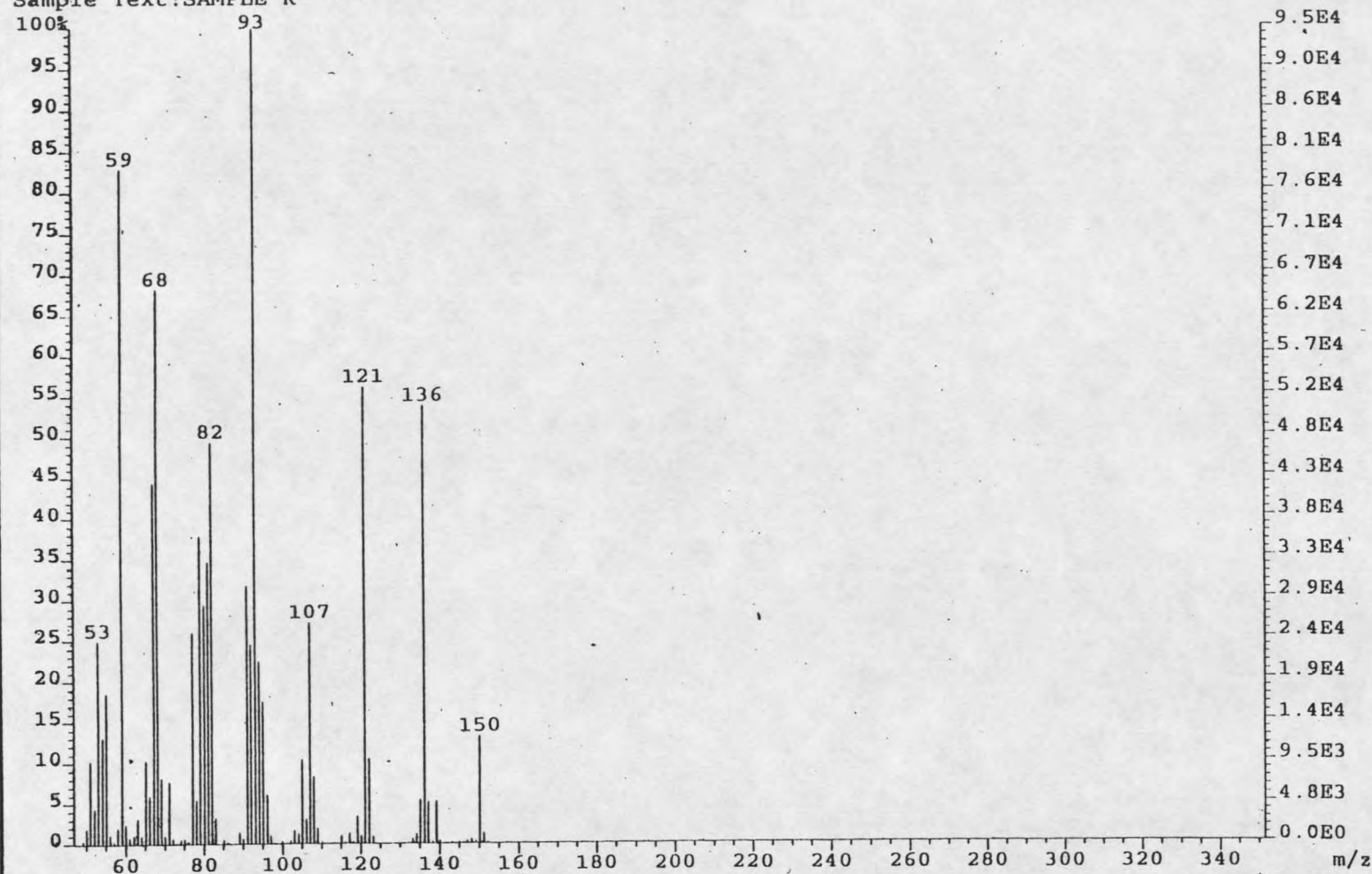
Rank: 1 NISTREP #4944 Purity: 856 Mixture: 880 Matched: 7 MF: C<sub>10</sub>H<sub>18</sub>O MW: 154 CAS: 98-55-5 NIST: 104187  
Name: 3-Cyclohexene-1-methanol, .alpha.,.alpha.4-trimethyl- SMILES: CC1=CCC(CC1)C(C)(C)O



Rank: 2 NIST #10929 Purity: 820 Mixture: 836 Matched: 7 MF: C<sub>10</sub>H<sub>18</sub>O MW: 154 CAS: 5986-38-9 NIST: 90779  
Name: 5,7-Octadien-2-ol, 2,6-dimethyl- | 2,6-Dimethyl-5,7-octadien-2-ol | Ocimenol SMILES: CC(C)=CC(C)C=CCO



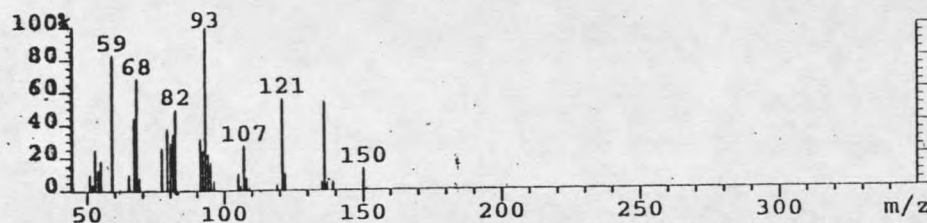
File: 2MAY97K Ident: 1016\_1020-1026\_1029 Mer Def 0.25 S: 10 Acq: 3-MAY-1997 02:47:26 +18:36 Cal: 2M>  
70S EI+ Magnet BpM: 93 BpI: 95083 TIC: 882405 Flags: HALL  
Sample Text: SAMPLE K



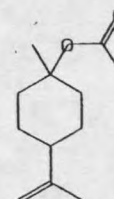
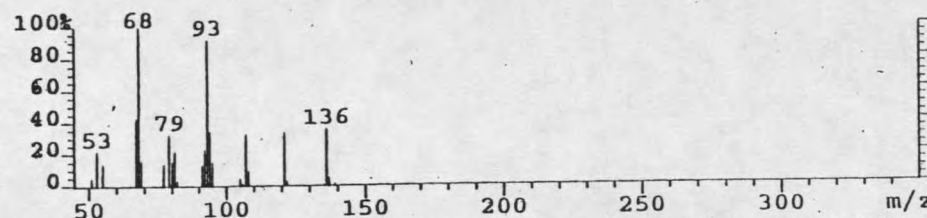
Libraries 1: NIST (62235) 2: NISTREP (12593)

| Peaks:   | 8 | 7  | 6   | 5   | 4    | 3    | 2     | 1     | 0     |
|----------|---|----|-----|-----|------|------|-------|-------|-------|
| Matches: | 1 | 39 | 184 | 459 | 1397 | 3063 | 6970  | 17279 | 45436 |
| Total:   | 1 | 40 | 224 | 683 | 2080 | 5143 | 12113 | 29392 | 74828 |

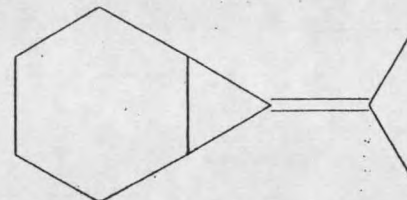
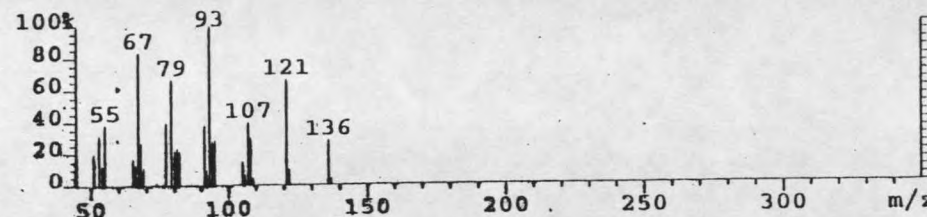
Normal Search: 26 of 40 Rank: Purity Mass Weighting: On Presearch Minimum: 20  
File: 2MAY97K Ident: 1016\_1020-1026\_1029 Mer Def 0.25 S:10 Acquired: 2-MAY-1997 16:47:26 +18:36  
708 EI+ Function: Magnet TIC: 882405



Rank: 1 NISTREP #7271 Purity: 740 Mixture: 849 Matched: 7 MF: C<sub>12</sub>H<sub>20</sub>O<sub>2</sub> MW: 196 CAS: 10198-23-9 NIST: 86  
Name: Cyclohexanol, 1-methyl-4-(1-methylethenyl)-, acetate SMILES: CC(=O)OC1(C)CCC(CC1)C(C)=C

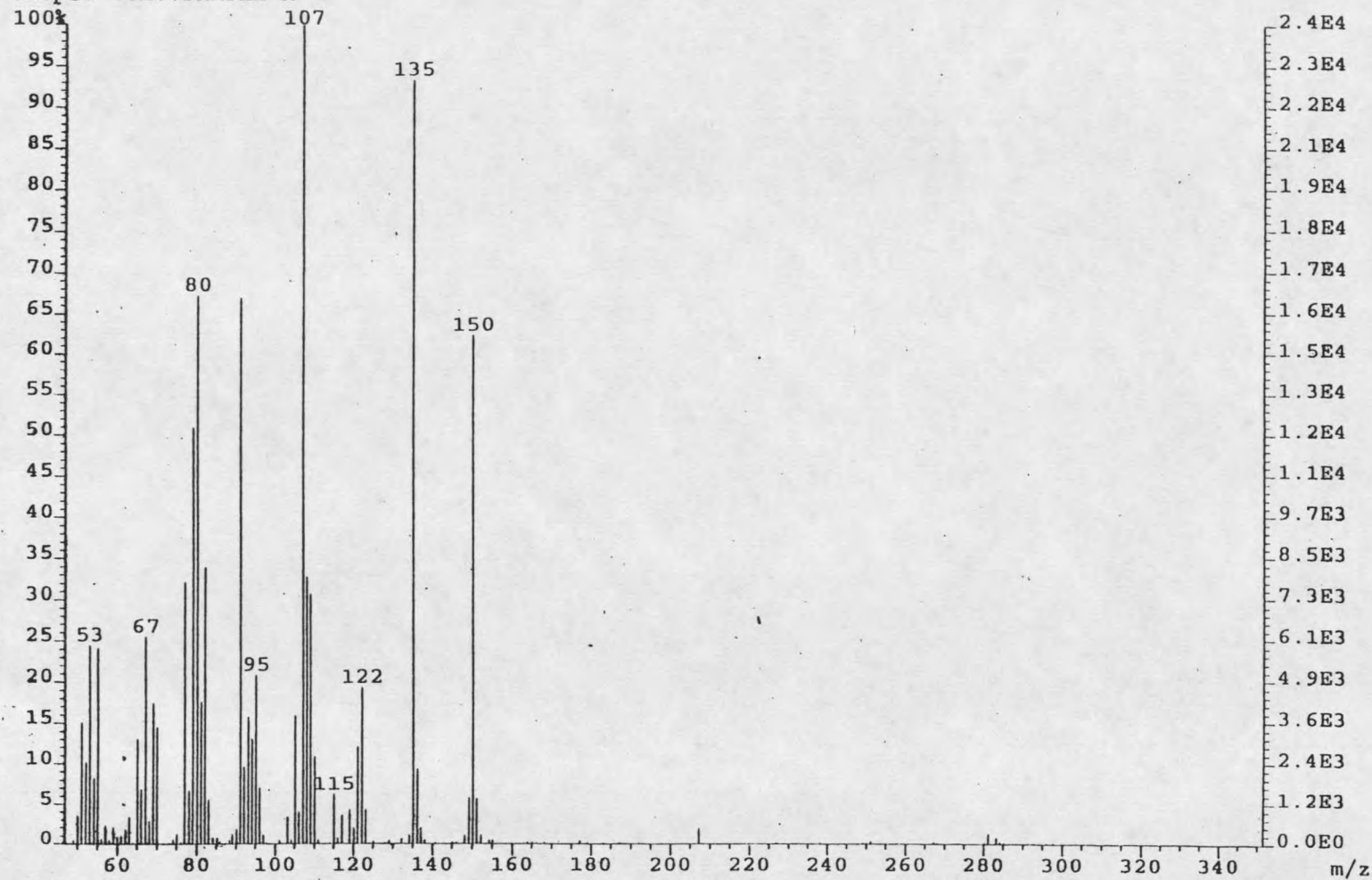


Rank: 2 NIST #6672 Purity: 717 Mixture: 820 Matched: 7 MF: C<sub>10</sub>H<sub>16</sub> MW: 136 CAS: 53282-47-6 NIST: 3316  
Name: Bicyclo[4.1.0]heptane, 7-(1-methylethylidene)- SMILES: CC(C)=C1C2CCCC12





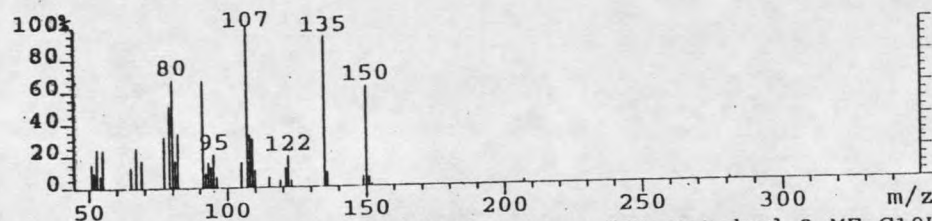
File: 2MAY97K Ident: 1054 1057-1046 1049 Mer Def 0.25 S: 10 Acq: 03-MAY-1997 02:47:26 +19:09 Cal: 2M  
70S EI+ Magnet BpM: 107 BpI: 24278 TIC: 238357 Flags: HALL  
Sample Text: SAMPLE K



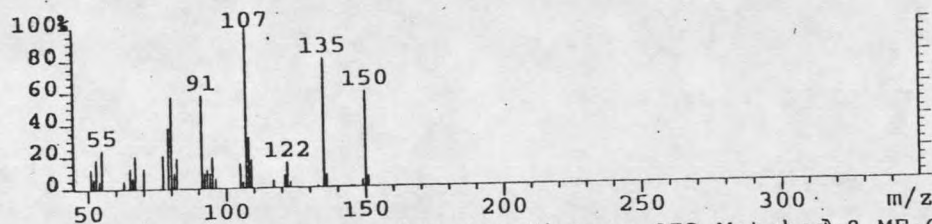
Libraries 1: NIST (62235) 2: NISTREP (12593)

| Peaks:   | 8 | 7  | 6   | 5   | 4    | 3    | 2     | 1     | 0     |
|----------|---|----|-----|-----|------|------|-------|-------|-------|
| Matches: | 5 | 10 | 96  | 469 | 1277 | 3354 | 7590  | 17705 | 44322 |
| Total:   | 5 | 15 | 111 | 580 | 1857 | 5211 | 12801 | 30506 | 74828 |

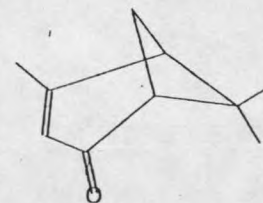
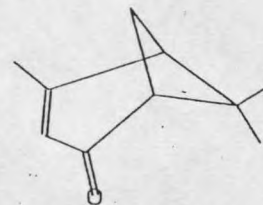
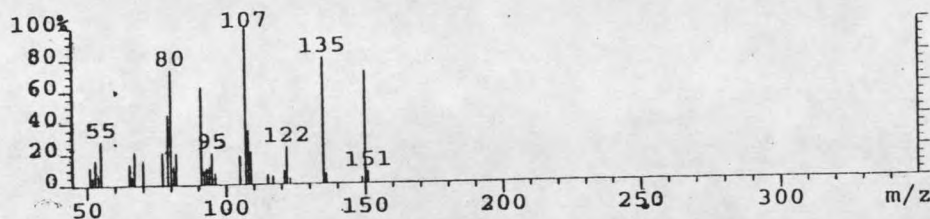
Normal Search: 29 of 111 Rank: Purity Mass Weighting: On Presearch Minimum: 20  
File: 2MAY97K Ident: 1054\_1057-1046\_1049 Mer Def 0.25 S: 10 Acquired: 2-MAY-1997 16:47:26 +19:09  
70S EI+ Function: Magnet TIC: 238357



Rank: 1 NIST #9851 Purity: 969 Mixture: 978 Matched: 8 MF: C<sub>10</sub>H<sub>14</sub>O MW: 150 CAS: 80-57-9 NIST: 4631  
Name: Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl- | 2-Pinen-4-one | Berbenone SMILES: CC1=CC(>

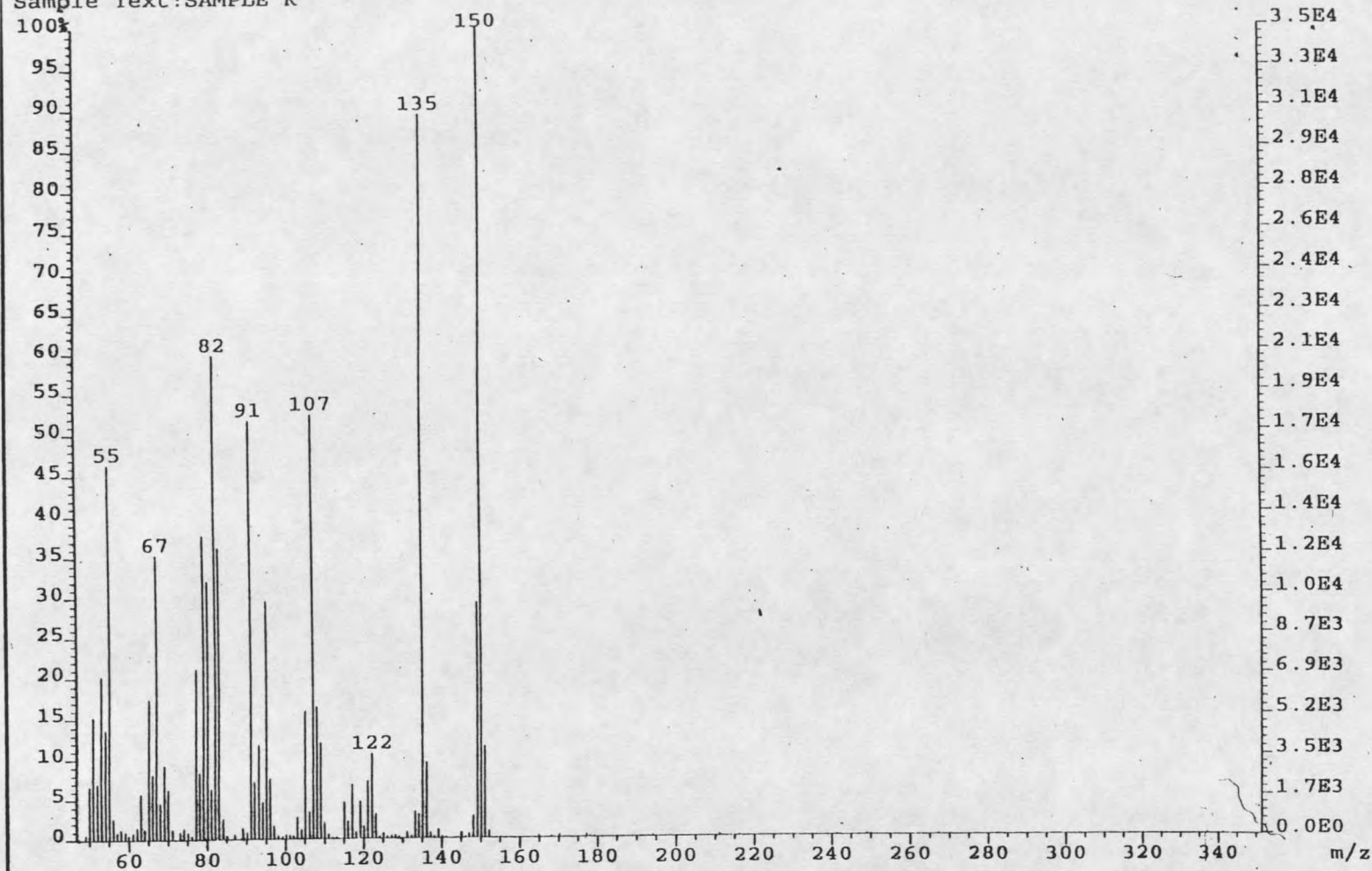


Rank: 2 NISTREP #4575 Purity: 964 Mixture: 972 Matched: 8 MF: C<sub>10</sub>H<sub>14</sub>O MW: 150 CAS: 80-57-9 NIST: 109296  
Name: Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl- SMILES: CC1=CC(=O)C2CC1C2(C)C





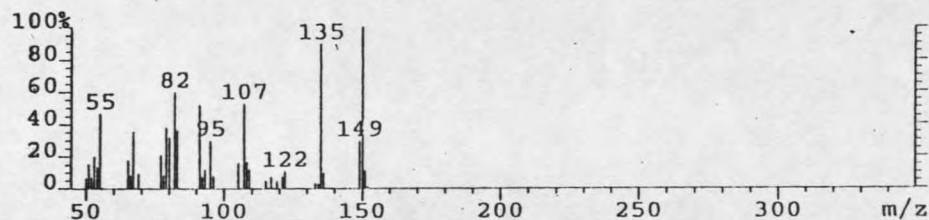
File: 2MAY97K Ident: 1112 1117-1126 1132 Mer Def 0.25 S:10 Acq: 13-MAY-1997 02:47:26 +19:59 Cal: 2M>  
70S EI+ Magnet BpM:150 BpI:34651 TIC:327583 Flags: HALL  
Sample Text: SAMPLE K



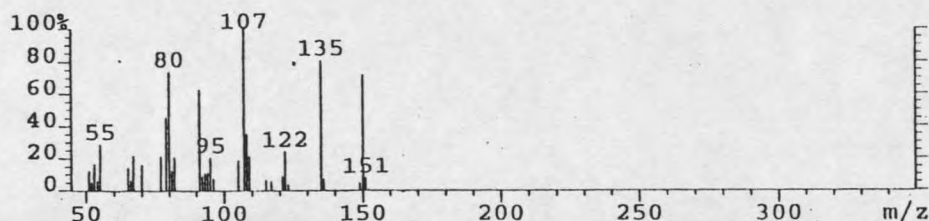
Libraries 1: NIST (62235) 2: NISTREP (12593)

| Peaks:   | 8 | 7 | 6  | 5   | 4    | 3    | 2     | 1     | 0     |
|----------|---|---|----|-----|------|------|-------|-------|-------|
| Matches: | 0 | 3 | 30 | 252 | 1353 | 3604 | 9072  | 20080 | 40434 |
| Total:   | 0 | 3 | 33 | 285 | 1638 | 5242 | 14314 | 34394 | 74828 |

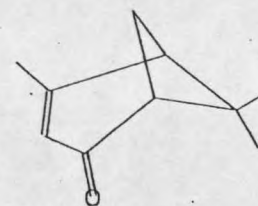
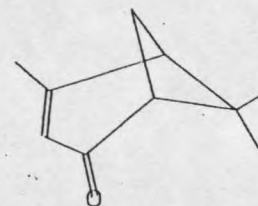
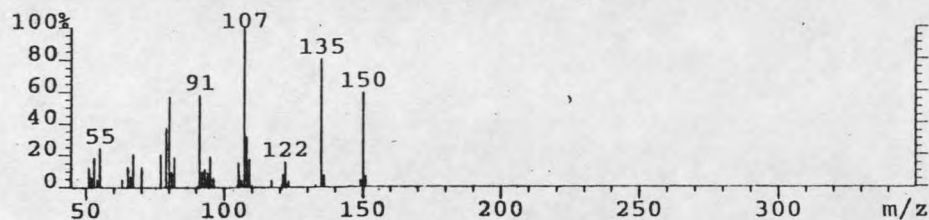
Normal Search: 7 of 33 Rank: Purity Mass Weighting: On Presearch Minimum: 20  
File: 2MAY97K Ident: 1112\_1117-1126\_1132 Mer Def 0.25 S:10 Acquired: 2-MAY-1997 16:47:26 +19:59  
70S EI+ Function: Magnet TIC: 327583



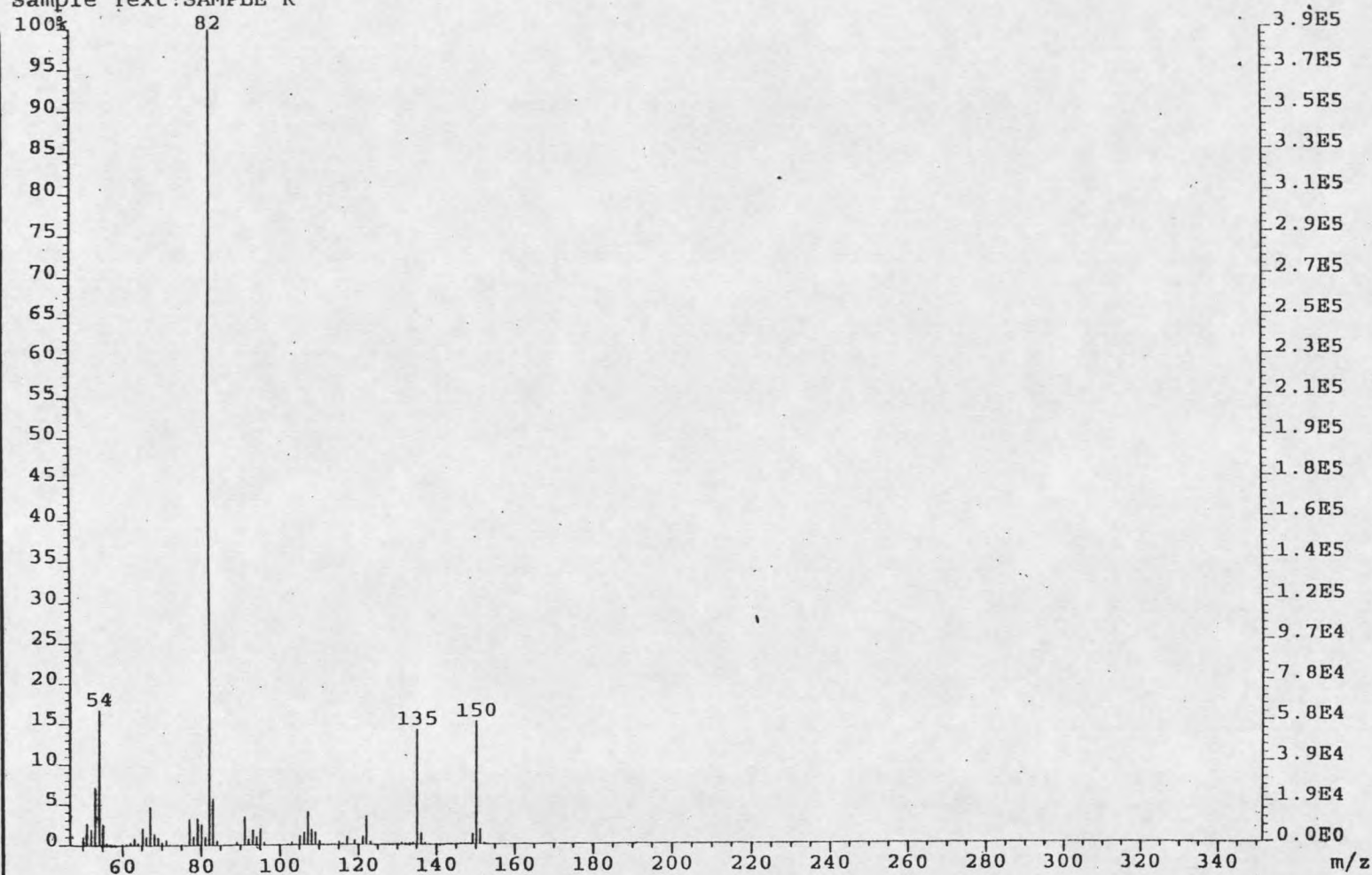
Rank: 1 NISTREP #4575 Purity: 777 Mixture: 800 Matched: 6 MF: C<sub>10</sub>H<sub>14</sub>O MW: 150 CAS: 80-57-9 NIST: 109296  
Name: Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl- SMILES: CC1=CC(=O)C2CC1C2(C)C



Rank: 2 NIST #9851 Purity: 766 Mixture: 789 Matched: 6 MF: C<sub>10</sub>H<sub>14</sub>O MW: 150 CAS: 80-57-9 NIST: 4631  
Name: Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl- | 2-Pinen-4-one | Berbenone SMILES: CC1=CC(=O)C2CC1C2(C)C



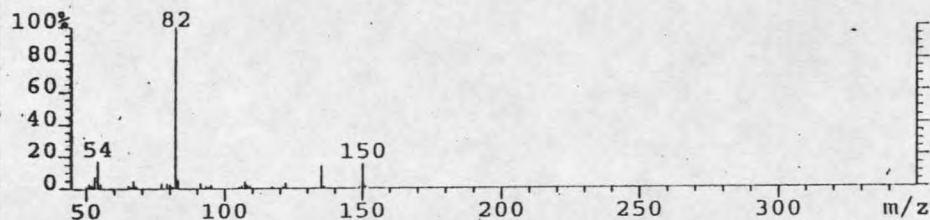
File: 2MAY97K Ident: 1175-1179-1165-1169 Mer Def 0.25 S: 10 Acq: 03-MAY-1997 02:47:26 +20:53 Cal: 2M  
70S EI+ Magnet BpM: 82 BpI: 389151 TIC: 879630 Flags: HALL  
Sample Text: SAMPLE K



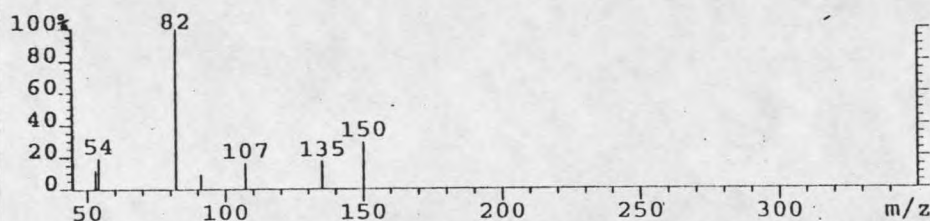
Libraries 1: NIST (62235) 2: NISTREP (12593)

|          |   |   |    |     |     |      |       |       |       |
|----------|---|---|----|-----|-----|------|-------|-------|-------|
| Peaks:   | 8 | 7 | 6  | 5   | 4   | 3    | 2     | 1     | 0     |
| Matches: | 0 | 2 | 15 | 132 | 664 | 2721 | 7319  | 18329 | 45646 |
| Total:   | 0 | 2 | 17 | 149 | 813 | 3534 | 10853 | 29182 | 74828 |

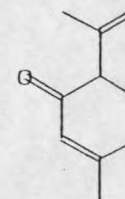
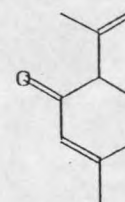
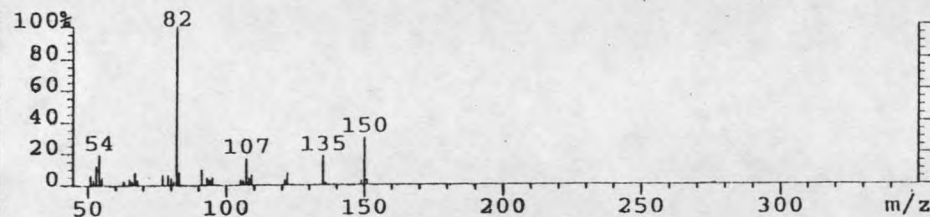
Normal Search: 11 of 149 Rank: Purity Mass Weighting: On Presearch Minimum: 20  
File: 2MAY97K Ident: 1175\_1179-1165\_1169 Mer Def 0.25 S: 10 Acquired: 2-MAY-1997 16:47:26 +20:53  
70S EI+ Function: Magnet TIC: 879630



Rank: 1 NIST #9822 Purity: 940 Mixture: 953 Matched: 6 MF: C<sub>10</sub>H<sub>14</sub>O MW: 150 CAS: 529-01-1 NIST: 21438  
Name: 2-Cyclohexen-1-one, 3-methyl-6-(1-methylethenyl)- | p-Mentha-1,8-dien-3-one | Isopiperiten>

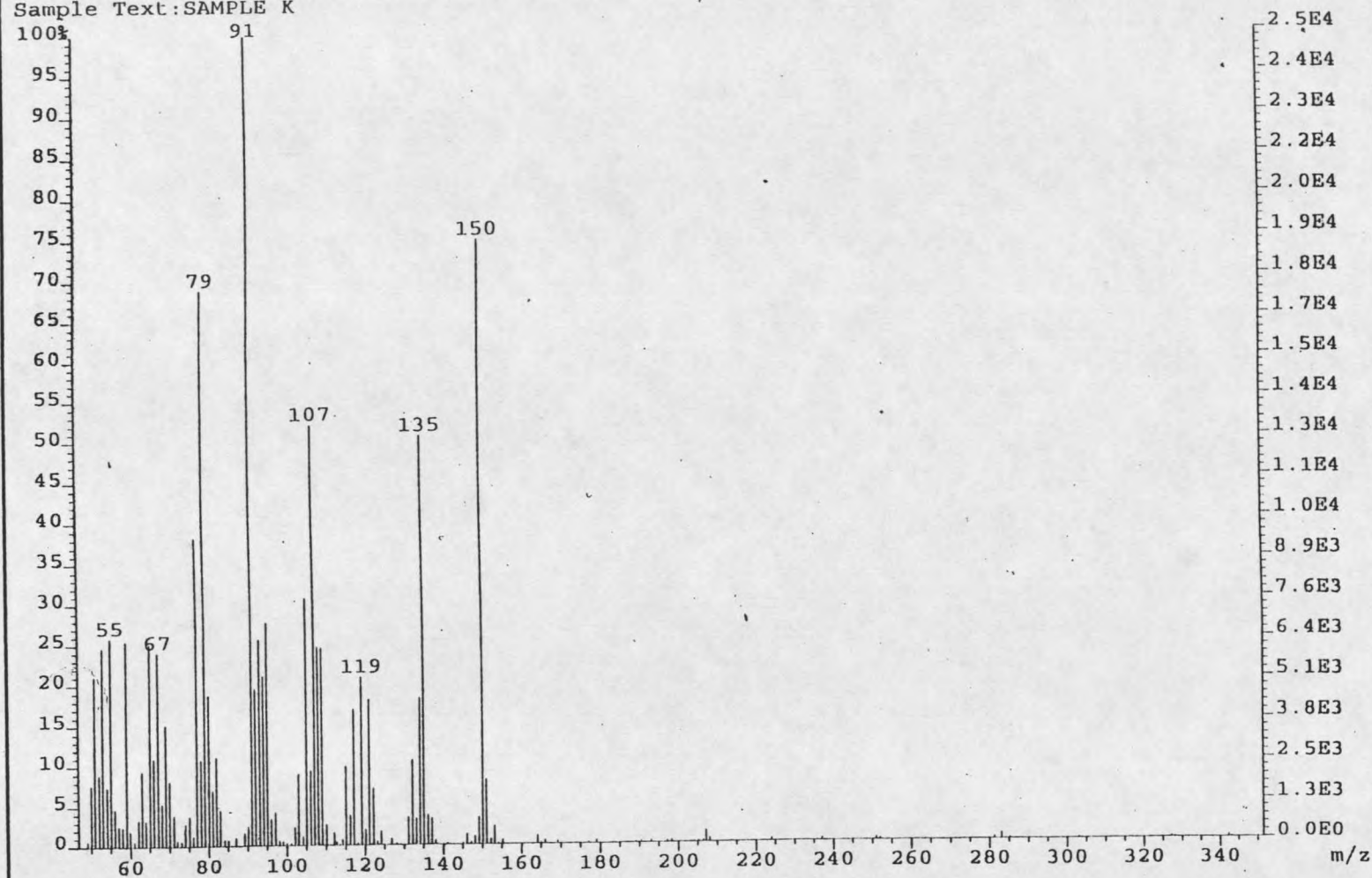


Rank: 2 NIST #9860 Purity: 937 Mixture: 937 Matched: 7 MF: C<sub>10</sub>H<sub>14</sub>O MW: 150 CAS: 16750-82-6 NIST: 4627  
Name: 2-Cyclohexen-1-one, 3-methyl-6-(1-methylethenyl)-, (S)- | p-Mentha-1,8-dien-3-one, (+)- | >





File: 2MAY97K Ident: 1320 1322-1312 1315 Mer Def 0.25 S: 10 Acq: 3-MAY-1997 02:47:26 +22:57 Cal: 2M>  
70S EI+ Magnet BpM: 91 BpI: 25428 TIC: 269411 Flags: HALL  
Sample Text: SAMPLE K



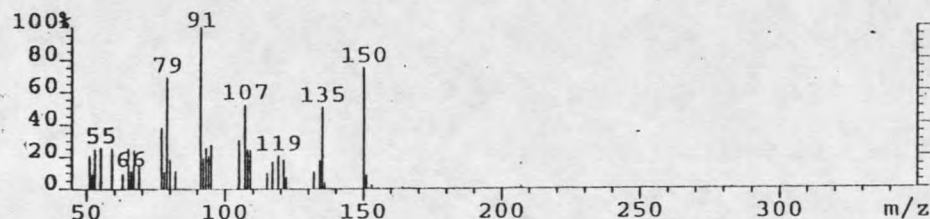
Libraries 1: NIST (62235) 2: NISTREP (12593)

| Peaks:   | 8 | 7  | 6   | 5   | 4    | 3    | 2     | 1     | 0     |
|----------|---|----|-----|-----|------|------|-------|-------|-------|
| Matches: | 3 | 20 | 146 | 591 | 1754 | 3872 | 8634  | 15273 | 44535 |
| Total:   | 3 | 23 | 169 | 760 | 2514 | 6386 | 15020 | 30293 | 74828 |

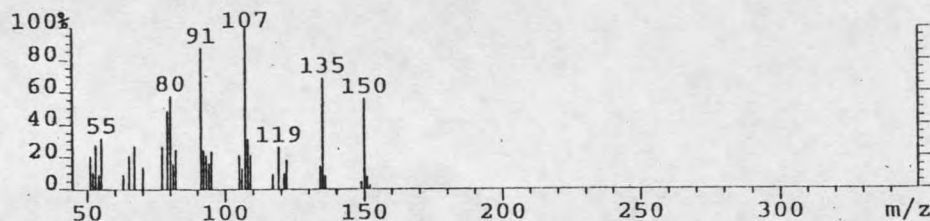
Normal Search: 9 of 23 Rank: Purity Mass Weighting: On Presearch Minimum: 20

File: 2MAY97K Ident: 1320\_1322-1312\_1315 Mer Def 0.25 S: 10 Acquired: 2-MAY-1997 16:47:26 +22:57

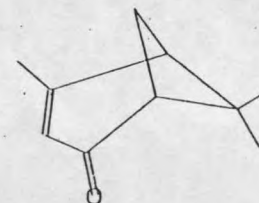
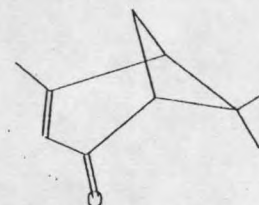
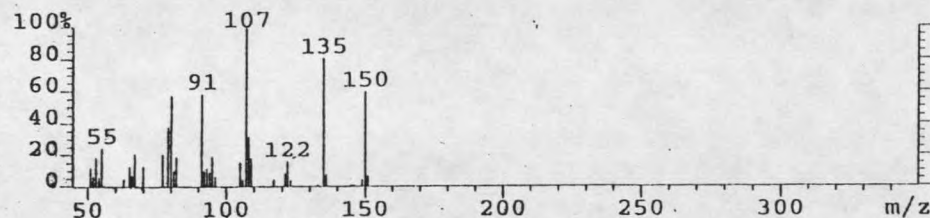
70S EI+ Function: Magnet TIC: 269411



Rank: 1 NIST #9853 Purity: 822 Mixture: 844 Matched: 7 MF: C<sub>10</sub>H<sub>14</sub>O MW: 150 CAS: 18309-32-5 NIST: 38883  
Name: D-Verbenone | Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-, (1R)- | 2-Pinen-4-one, (1R), >

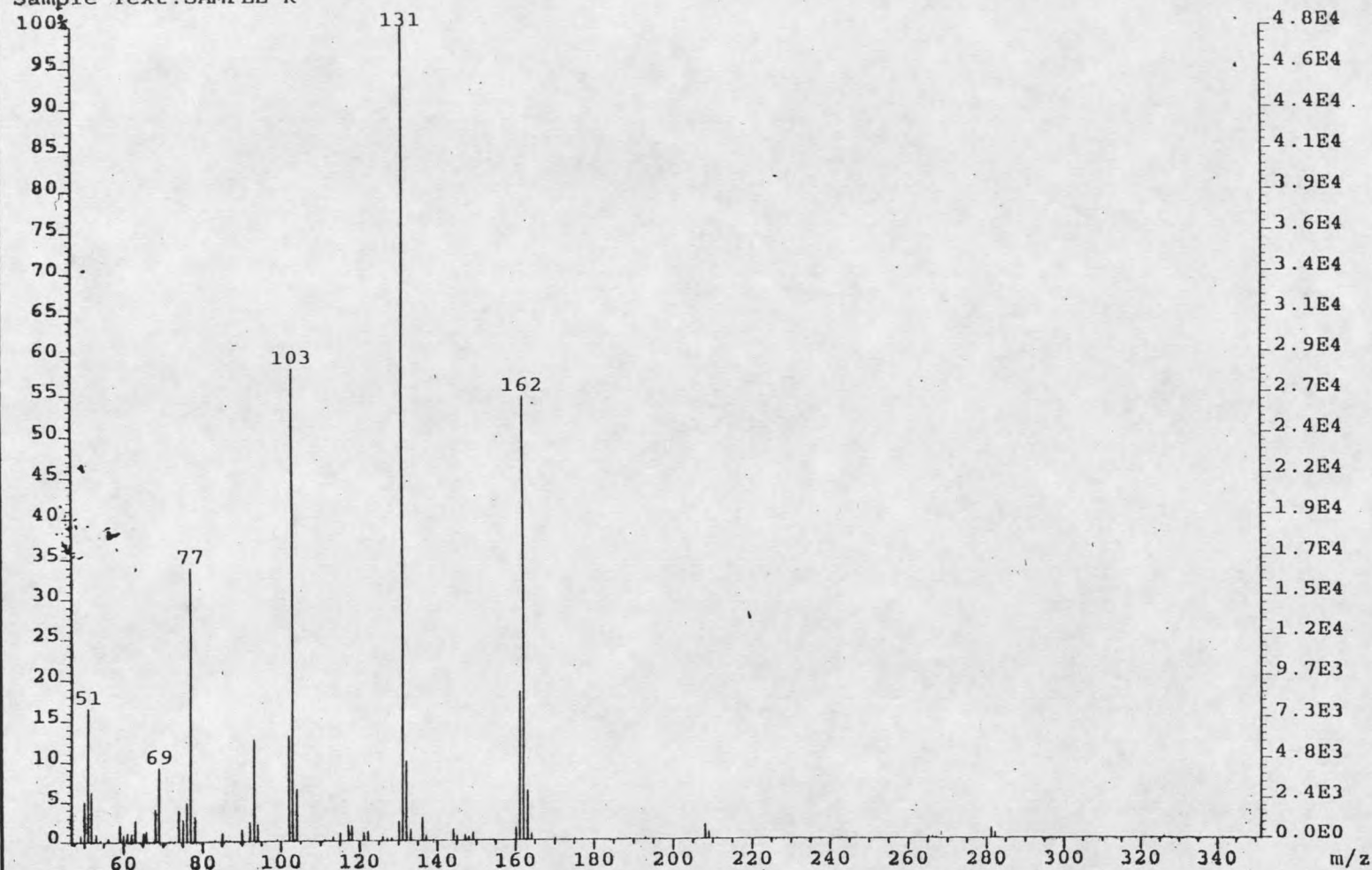


Rank: 2 NIST #9851 Purity: 718 Mixture: 755 Matched: 7 MF: C<sub>10</sub>H<sub>14</sub>O MW: 150 CAS: 80-57-9 NIST: 4631  
Name: Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl- | 2-Pinen-4-one | Berbenone SMILES: CC1=CC(>





File: 2MAY97K Ident: 1380 1383-1388 Mer Def 0.25 S:10 Acq: 3-MAY-1997 02:47:26 +23:49 Cal: 2MAY97K>  
70S EI+ Magnet BpM:131 BpI:48425 TIC:205399 Flags: HALL  
Sample Text: SAMPLE K



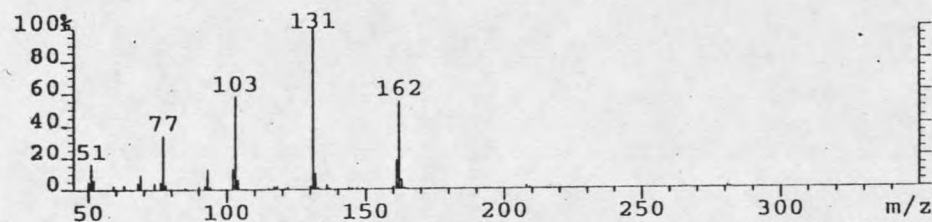
Libraries 1: NIST (62235) 2: NISTREP (12593)

| Peaks:   | 8 | 7  | 6  | 5   | 4   | 3    | 2    | 1     | 0     |
|----------|---|----|----|-----|-----|------|------|-------|-------|
| Matches: | 7 | 21 | 61 | 147 | 483 | 1820 | 6557 | 20530 | 45202 |
| Total:   | 7 | 28 | 89 | 236 | 719 | 2539 | 9096 | 29626 | 74828 |

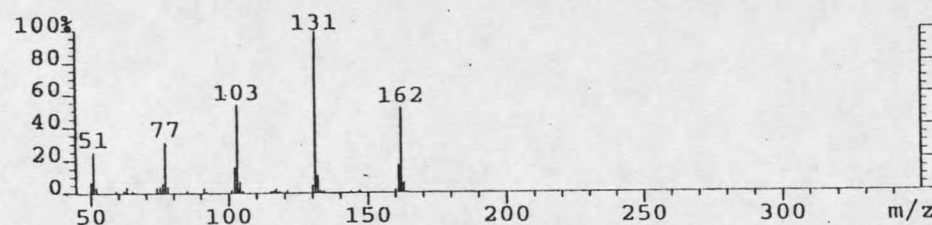
Normal Search: 18 of 28 Rank: Purity Mass Weighting: On Presearch Minimum: 20

File: 2MAY97K Ident: 1380\_1383-1388 Mer Def 0.25 S:10 Acquired: 2-MAY-1997 16:47:26 +23:49

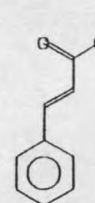
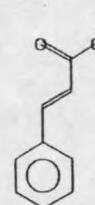
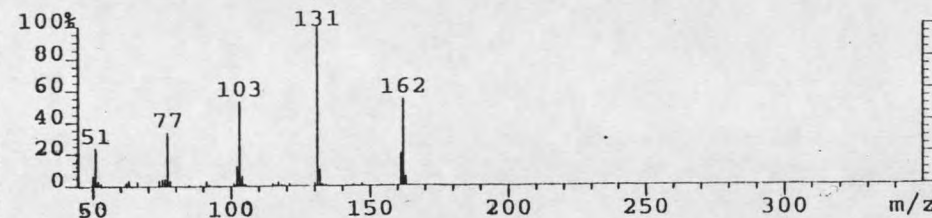
70S EI+ Function: Magnet TIC: 205399



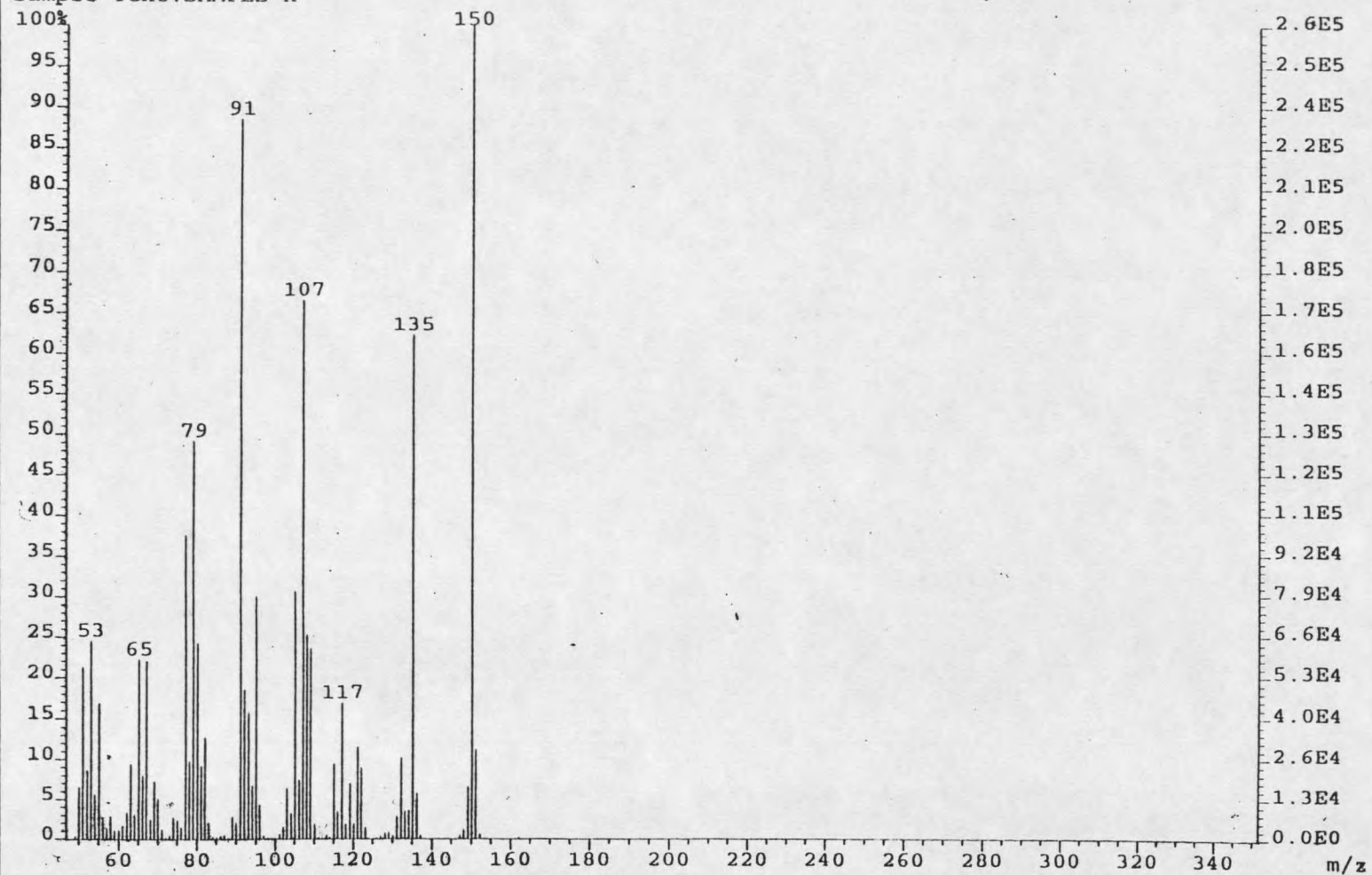
Rank: 1 NIST #12901 Purity: 983 Mixture: 994 Matched: 8 MF: C<sub>10</sub>H<sub>10</sub>O<sub>2</sub> MW: 162 CAS: 103-26-4 NIST: 63065  
Name: 2-Propenoic acid, 3-phenyl-, methyl ester | Cinnamic acid, methyl ester | Methyl cinnamate>



Rank: 2 NISTREP #5434 Purity: 983 Mixture: 994 Matched: 8 MF: C<sub>10</sub>H<sub>10</sub>O<sub>2</sub> MW: 162 CAS: 103-26-4 NIST: 1098>  
Name: 2-Propenoic acid, 3-phenyl-, methyl ester SMILES: COC(=O)C=Cc1ccccc1



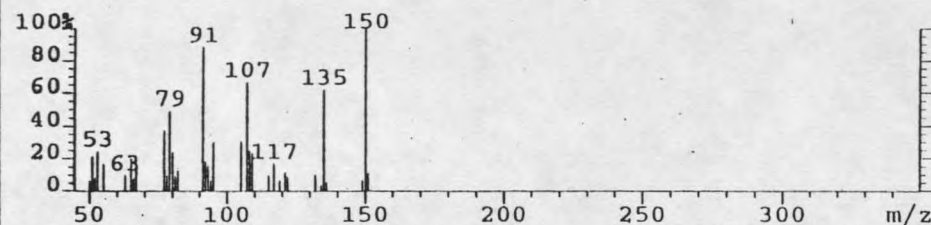
File: 2MAY97K Ident: 1401\_1406-1363\_1368 Mer Def 0.25 S:10 Acq: 3-MAY-1997 02:47:26 +24:08 Cal: 2M  
70S EI+ Magnet BpM: 150 BpI: 263570 TIC: 2494700 Flags: HALL  
Sample Text: SAMPLE K



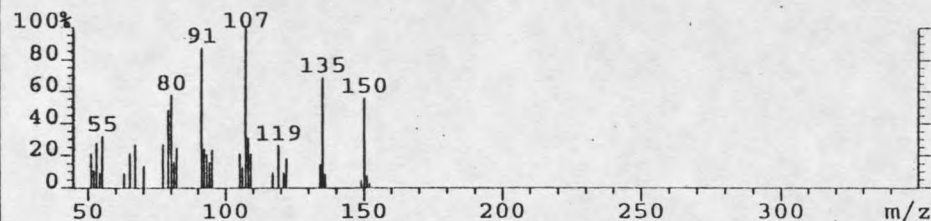
Libraries 1: NIST (62235) 2: NISTREP (12593)

|          |   |    |     |     |      |      |       |       |       |
|----------|---|----|-----|-----|------|------|-------|-------|-------|
| Peaks:   | 8 | 7  | 6   | 5   | 4    | 3    | 2     | 1     | 0     |
| Matches: | 4 | 16 | 128 | 496 | 1524 | 3855 | 8740  | 16079 | 43986 |
| Total:   | 4 | 20 | 148 | 644 | 2168 | 6023 | 14763 | 30842 | 74828 |

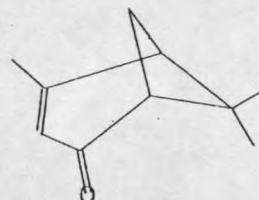
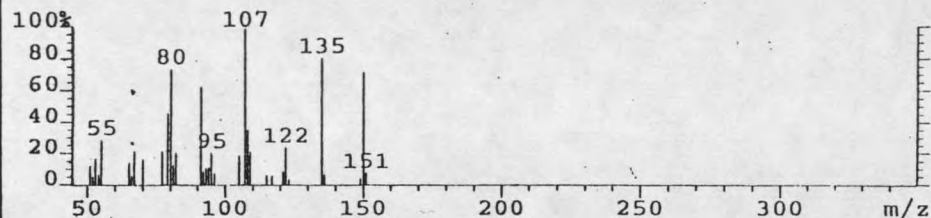
Normal Search: 10 of 20 Rank: Purity Mass Weighting: On Presearch Minimum: 20  
File: 2MAY97K Ident: 1401\_1406-1363\_1368 Mer Def 0.25 S: 10 Acquired: 2-MAY-1997 16:47:26 +24:08  
70S EI+ Function: Magnet TIC: 2494700



Rank: 1 NIST #9853 Purity: 835 Mixture: 843 Matched: 7 MF: C<sub>10</sub>H<sub>14</sub>O MW: 150 CAS: 18309-32-5 NIST: 38883  
Name: D-Verbenone | Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-, (1R)- | 2-Pinen-4-one, (1R),>

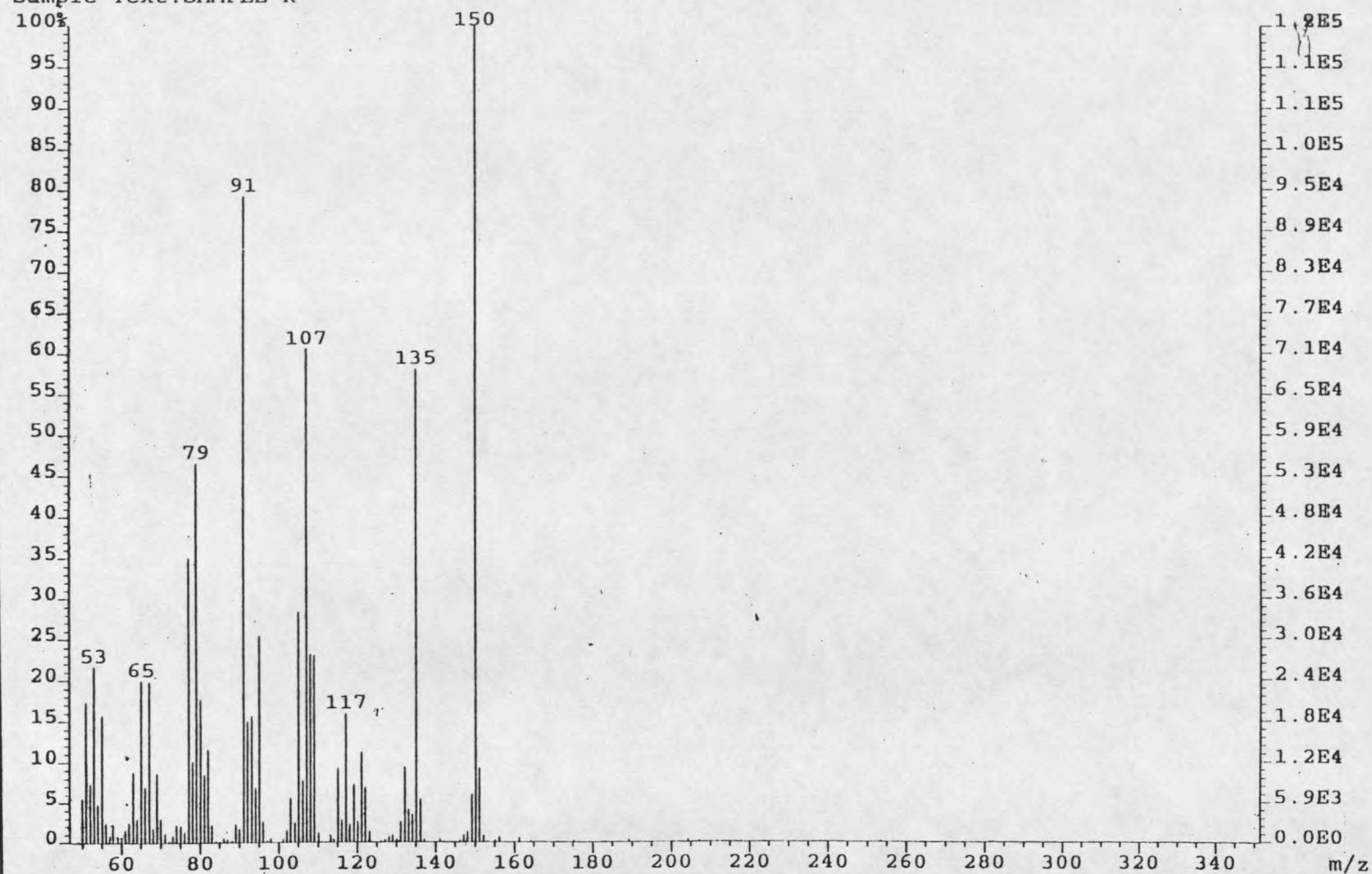


Rank: 2 NISTREP #4575 Purity: 833 Mixture: 840 Matched: 7 MF: C<sub>10</sub>H<sub>14</sub>O MW: 150 CAS: 80-57-9 NIST: 109296  
Name: Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl- SMILES: CC1=CC(=O)C2CC1C2(C)C





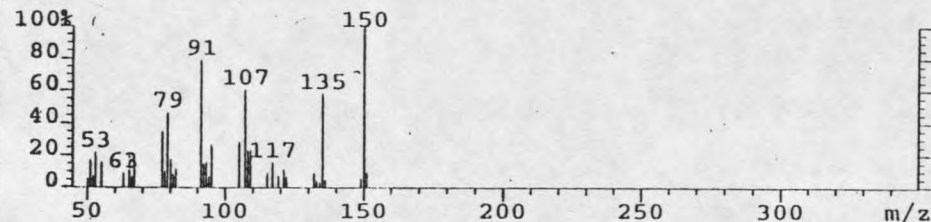
File: 2MAY97K Ident: 1445\_1448-1434\_1438 Mer Def 0.25 S:10 Acq: 3-MAY-1997 02:47:26 +24:45 Cal: 2M>  
70S EI+ Magnet BpM: 150 BpI: 118883 TIC: 1037524 Flags: HALL  
Sample Text: SAMPLE K



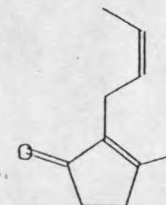
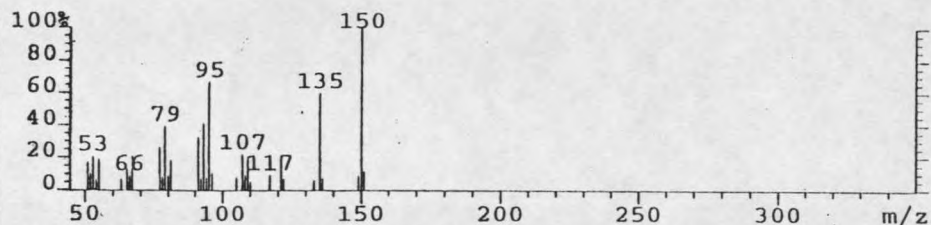
Libraries 1: NIST (62235) 2: NISTREP (12593)

|          |   |    |     |     |      |      |       |       |       |
|----------|---|----|-----|-----|------|------|-------|-------|-------|
| Peaks:   | 8 | 7  | 6   | 5   | 4    | 3    | 2     | 1     | 0     |
| Matches: | 2 | 24 | 185 | 636 | 1700 | 3950 | 8901  | 15959 | 43471 |
| Total:   | 2 | 26 | 211 | 847 | 2547 | 6497 | 15398 | 31357 | 74828 |

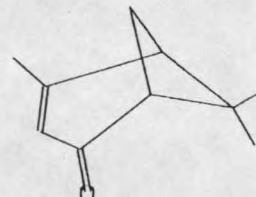
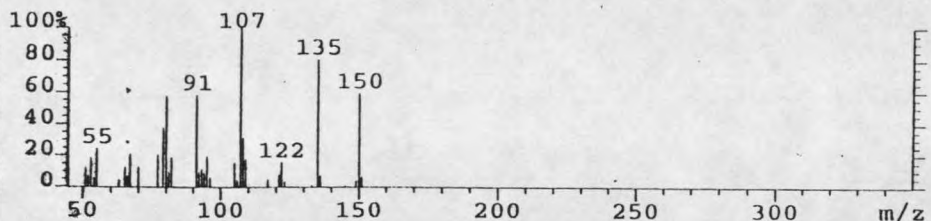
Normal Search: 12 of 26 Rank: Purity Mass Weighting: On Presearch Minimum: 20  
File: 2MAY97K Ident: 1445\_1448-1434\_1438 Mer Def 0.25 S:10 Acquired: 2-MAY-1997 16:47:26 +24:45  
70S EI+ Function: Magnet TIC: 1037524



Rank: 1 NIST #9796 Purity: 803 Mixture: 812 Matched: 7 MF: C<sub>10</sub>H<sub>14</sub>O MW: 150 CAS: 17190-71-5 NIST: 29825  
Name: 2-Cyclopenten-1-one, 2-(2-butenyl)-3-methyl-, (Z)- | (Z)-Cinerone | Cinerone SMILES: CC=CC1>

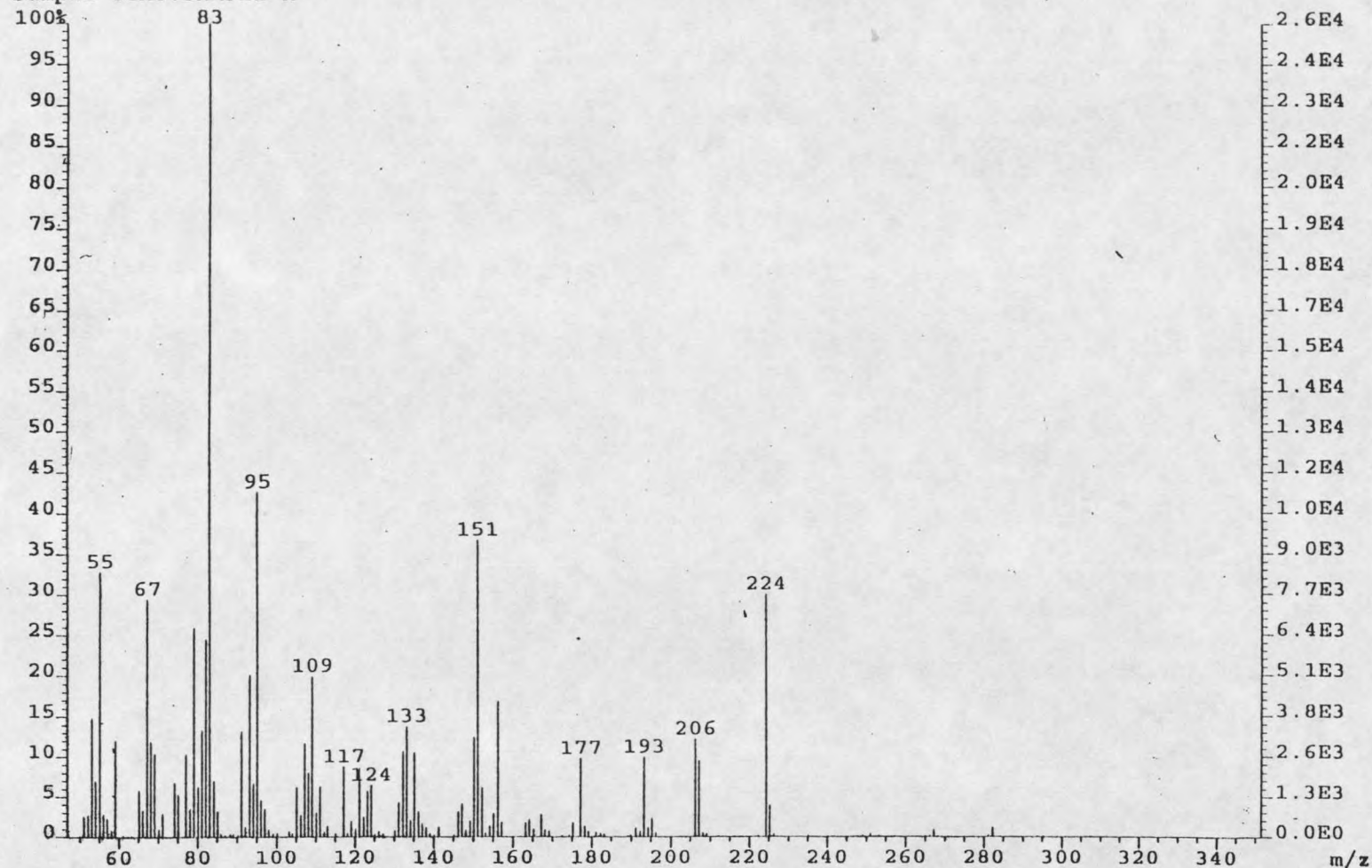


Rank: 2 NIST #9851 Purity: 796 Mixture: 804 Matched: 7 MF: C<sub>10</sub>H<sub>14</sub>O MW: 150 CAS: 80-57-9 NIST: 4631  
Name: Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl- | 2-Pinen-4-one | Berbenone SMILES: CC1=CC(>





File:2MAY97K Ident:1867\_1871-1857\_1862 Mer Def 0.25 S:10 Acq: 3-MAY-1997 02:47:26 +30:49 Cal:2M>  
70S EI+ Magnet BpM:83 BpI:25598 TIC:201112 Flags:HALL  
Sample Text:SAMPLE K

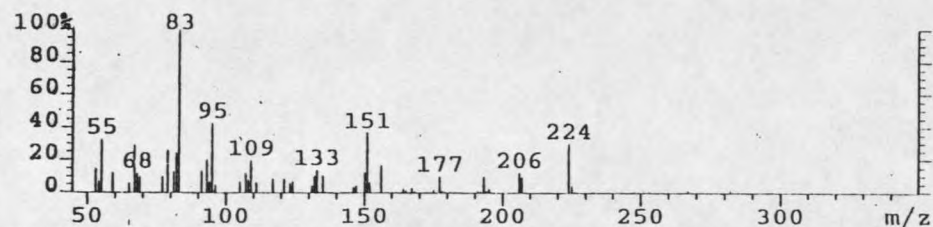


Libraries 1: NIST (62235) 2: NISTREP (12593)

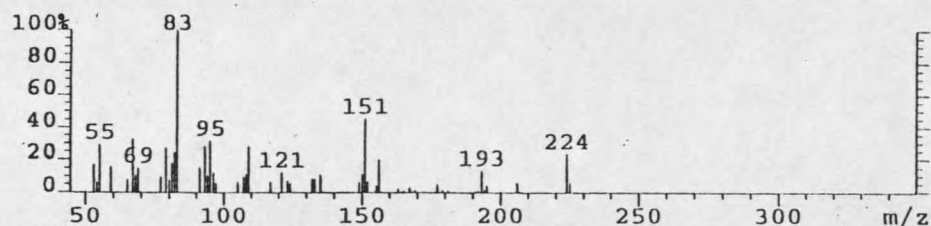
|          |   |   |   |    |     |      |      |       |       |
|----------|---|---|---|----|-----|------|------|-------|-------|
| Peaks:   | 8 | 7 | 6 | 5  | 4   | 3    | 2    | 1     | 0     |
| Matches: | 0 | 2 | 0 | 24 | 349 | 1743 | 5363 | 17155 | 50192 |
| Total:   | 0 | 2 | 2 | 26 | 375 | 2118 | 7481 | 24636 | 74828 |

Normal Search: 3 of 26 Rank: Purity Mass Weighting: On Presearch Minimum: 20

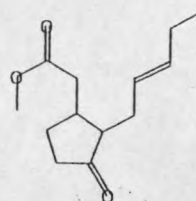
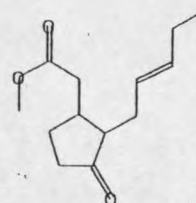
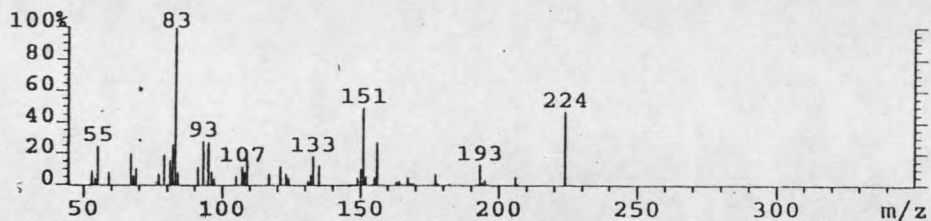
File: 2MAY97K Ident: 1867\_1871-1857\_1862 Mer Def 0.25 S: 10 Acquired: 2-MAY-1997 16:47:26 +30:49  
70S EI+ Function: Magnet TIC: 201112



Rank: 1 NIST #28650 Purity: 933 Mixture: 939 Matched: 7 MF: C<sub>13</sub>H<sub>20</sub>O<sub>3</sub> MW: 224 CAS: 1211-29-6 NIST: 10715  
Name: Methyl jasmonate | Cyclopentaneacetic acid, 3-oxo-trans-2-(cis-2-pentenyl), methyl ester |>



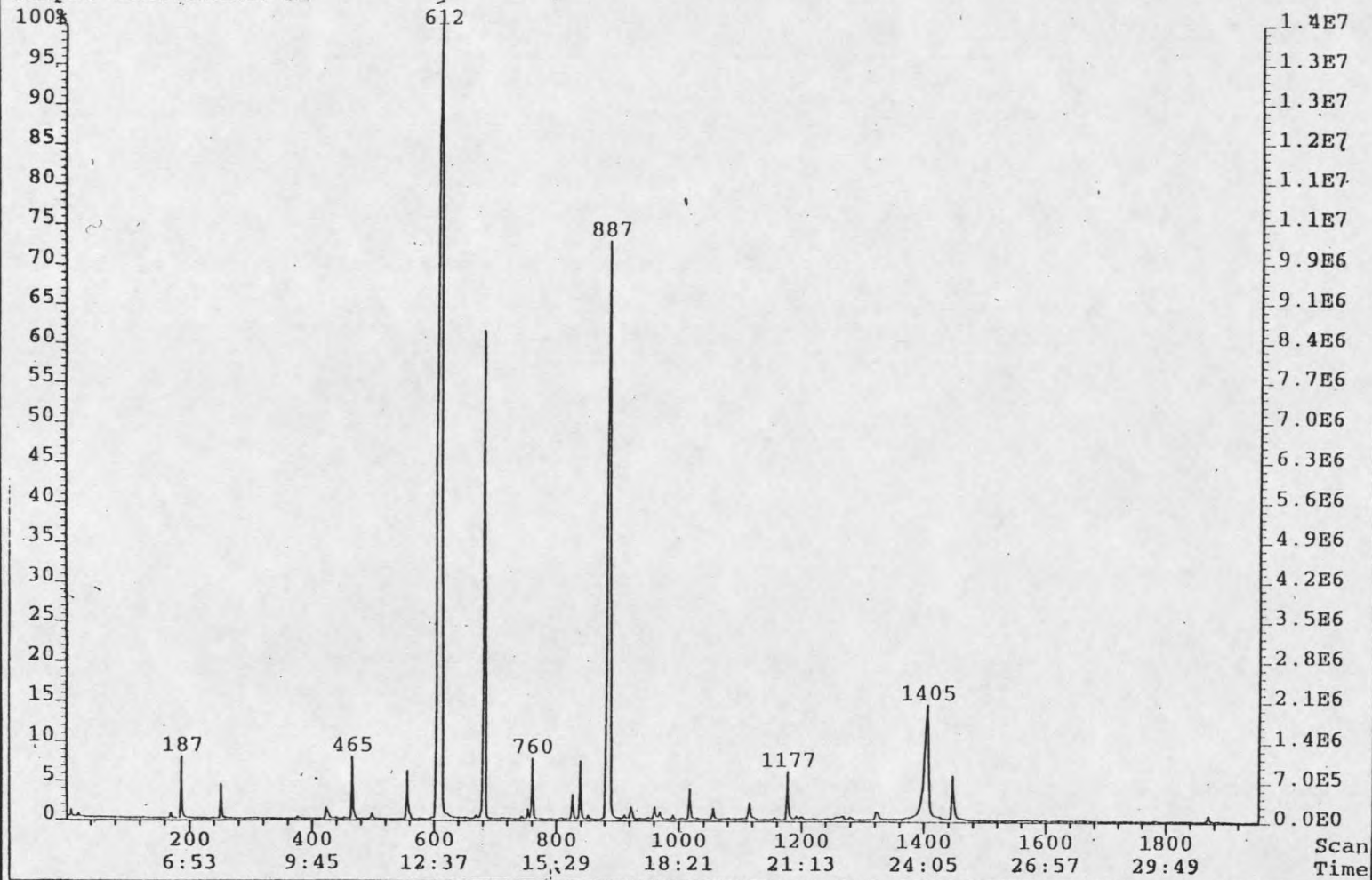
Rank: 2 NISTREP #8469 Purity: 904 Mixture: 913 Matched: 7 MF: C<sub>13</sub>H<sub>20</sub>O<sub>3</sub> MW: 224 CAS: 1211-29-6 NIST: 109  
Name: Methyl jasmonate SMILES: CCC=CCC1C(CCC1=O)CC(=O)OC



File: 2MAY97K #1-1953 Acq: 3-MAY-1997 03:55:07 GC EI+ Magnet 70S

TIC (+RP) S:11 Exp:GC-MSLR

Sample Text: SAMPLE L1



MS\_USER:CHR\_DEFAULT.LIS 5-MAY-1997 08:03

Listing of peaks detected for -

data file 2MAY97K

data ident TIC (+RP) S:11 PKD(3,3,3,0.10%,0.0,0.00%,F,T)

Axis display range X\_SCAN (1.00, 1953.00)

Normalising intensity 1.40738E+07 at buffer index 612

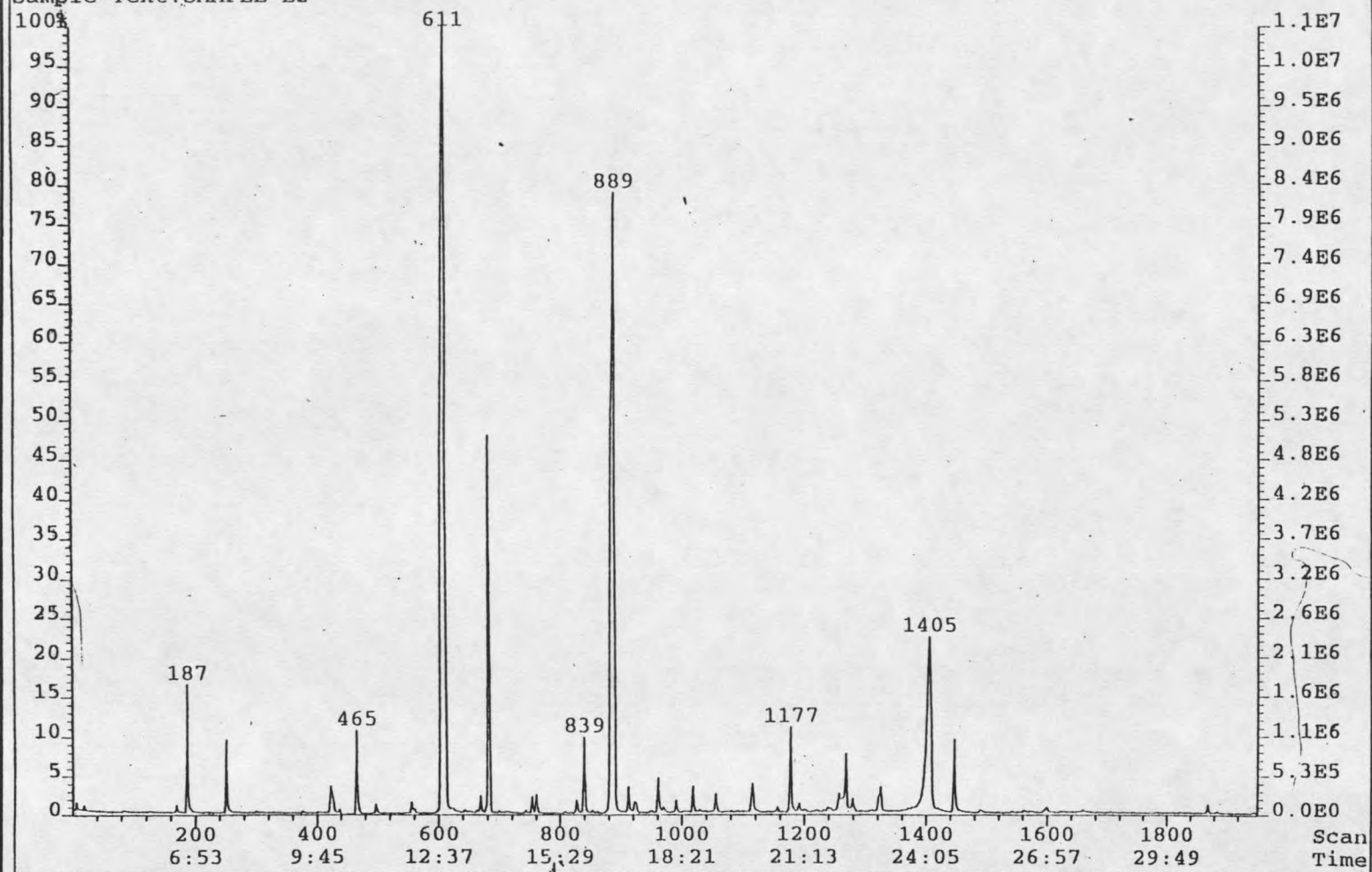
Height threshold 2.00% of maximum value 1.40468E+07

| SCAN AT | TIME AT | ABS         | REL       | ABS         | REL     | PEAK BASELINE START  | PEAK BASELINE END    |
|---------|---------|-------------|-----------|-------------|---------|----------------------|----------------------|
| MAX HT  | MAX HT  | HEIGHT      | HEIGHT(%) | AREA        | AREA(%) | X_SCAN, INTENSITY    | X_SCAN, INTENSITY    |
| 187     | 6:41    | 1.10362E+06 | 7.86      | 3.11161E+06 | 2.80    | 184.00, 4.28040E+04  | 193.65, 4.28040E+04  |
| 250     | 7:36    | 6.31956E+05 | 4.50      | 1.90778E+06 | 1.71    | 248.00, 3.02640E+04  | 265.00, 3.02640E+04  |
| 465     | 10:41   | 1.12443E+06 | 8.00      | 3.45077E+06 | 3.10    | 462.01, 2.62480E+04  | 473.00, 2.62480E+04  |
| 555     | 11:58   | 8.70844E+05 | 6.20      | 2.99373E+06 | 2.69    | 552.00, 2.70984E+04  | 564.80, 2.70984E+04  |
| 612     | 12:47   | 1.40468E+07 | 100.00    | 1.11269E+08 | 100.00  | 598.00, 2.70750E+04  | 618.00, 2.70750E+04  |
| 682     | 13:47   | 8.63977E+06 | 61.51     | 3.18433E+07 | 28.62   | 675.00, 3.60120E+04  | 690.00, 3.60120E+04  |
| 760     | 14:54   | 1.09038E+06 | 7.76      | 3.33445E+06 | 3.00    | 756.00, 3.40850E+04  | 766.89, 3.40850E+04  |
| 826     | 15:51   | 4.34425E+05 | 3.09      | 1.43287E+06 | 1.29    | 823.00, 3.76735E+04  | 832.01, 3.76735E+04  |
| 839     | 16:02   | 1.04583E+06 | 7.45      | 3.14128E+06 | 2.82    | 835.03, 3.33640E+04  | 846.00, 3.33640E+04  |
| 887     | 16:44   | 1.02085E+07 | 72.68     | 5.95962E+07 | 53.56   | 877.14, 3.93440E+04  | 900.00, 3.93440E+04  |
| 1017    | 18:36   | 5.44466E+05 | 3.88      | 1.86174E+06 | 1.67    | 1014.00, 4.18410E+04 | 1024.00, 4.18410E+04 |
| 1115    | 20:00   | 3.07310E+05 | 2.19      | 1.41357E+06 | 1.27    | 1110.00, 3.40540E+04 | 1122.00, 3.40540E+04 |
| 1177    | 20:53   | 8.59240E+05 | 6.12      | 2.94682E+06 | 2.65    | 1173.00, 3.85150E+04 | 1186.00, 3.85150E+04 |
| 1405    | 24:09   | 1.99134E+06 | 14.18     | 1.75539E+07 | 15.78   | 1382.00, 8.21920E+04 | 1417.00, 8.21920E+04 |
| 1446    | 24:45   | 7.90053E+05 | 5.62      | 3.39909E+06 | 3.05    | 1442.01, 4.96120E+04 | 1457.72, 4.96120E+04 |

File: 2MAY97K #1-1952 Acq: 3-MAY-1997 05:02:37 GC EI+ Magnet 70S

TIC (+RP) S:12 Exp:GC-MSLR

Sample Text: SAMPLE L2





MS\_USER:CHR\_DEFAULT.LIS 5-MAY-1997 08:03

Listing of peaks detected for -

data file 2MAY97K

data ident TIC (+RP) S:12 PKD(3,3,3,0.10%,0.0,0.00%,F,T)

axis display range X\_SCAN (1.00, 1952.00)

Normalising intensity 1.05575E+07 at buffer index 611

Height threshold 2.00% of maximum value 1.05304E+07

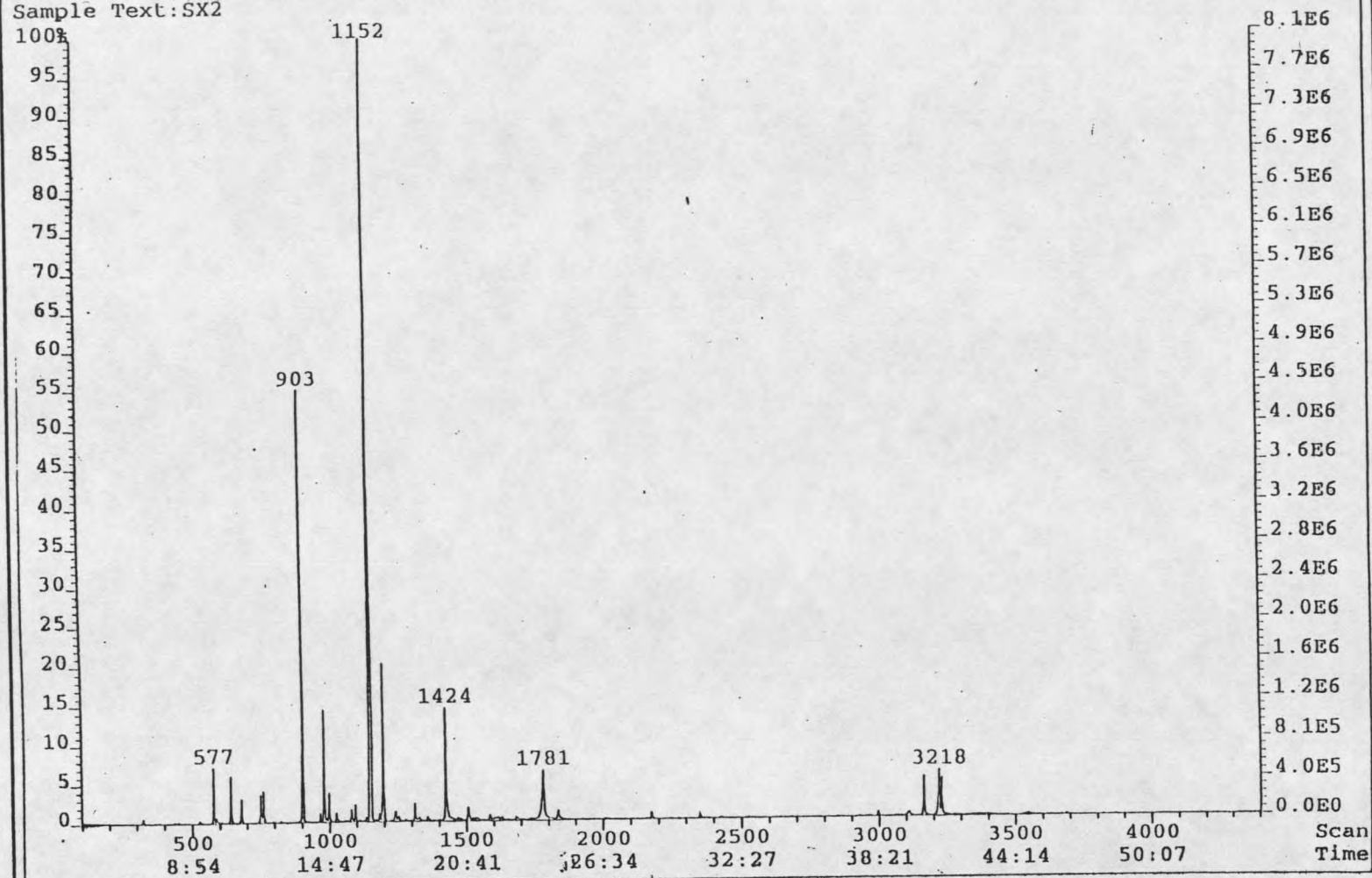
| SCAN AT | TIME AT | ABS         | REL       | ABS         | REL     | PEAK BASELINE START  | PEAK BASELINE END    |
|---------|---------|-------------|-----------|-------------|---------|----------------------|----------------------|
| MAX HT  | MAX HT  | HEIGHT      | HEIGHT(%) | AREA        | AREA(%) | X_SCAN, INTENSITY    | X_SCAN, INTENSITY    |
| 187     | 6:42    | 1.72667E+06 | 16.40     | 4.63778E+06 | 5.61    | 180.00, 3.59550E+04  | 196.00, 3.59550E+04  |
| 251     | 7:37    | 9.83933E+05 | 9.34      | 2.72532E+06 | 3.30    | 247.00, 3.86530E+04  | 260.66, 3.86530E+04  |
| 423     | 10:05   | 3.77745E+05 | 3.59      | 2.09424E+06 | 2.53    | 419.00, 2.99910E+04  | 435.00, 2.99910E+04  |
| 465     | 10:41   | 1.12885E+06 | 10.72     | 3.51808E+06 | 4.26    | 462.00, 1.36710E+04  | 474.00, 1.36710E+04  |
| 611     | 12:46   | 1.05304E+07 | 100.00    | 8.26748E+07 | 100.00  | 598.00, 2.71270E+04  | 620.00, 2.71270E+04  |
| 668     | 13:35   | 2.39520E+05 | 2.27      | 8.30663E+05 | 1.00    | 665.00, 2.63660E+04  | 674.00, 2.63660E+04  |
| 682     | 13:47   | 5.04748E+06 | 47.93     | 1.74971E+07 | 21.16   | 674.00, 2.63660E+04  | 688.00, 2.63660E+04  |
| 753     | 14:49   | 2.34507E+05 | 2.23      | 6.83311E+05 | 0.83    | 750.00, 2.46710E+04  | 757.00, 2.46710E+04  |
| 760     | 14:55   | 2.54801E+05 | 2.42      | 8.10956E+05 | 0.98    | 757.00, 2.46710E+04  | 765.00, 2.46710E+04  |
| 839     | 16:03   | 1.02638E+06 | 9.75      | 3.28698E+06 | 3.98    | 833.00, 2.26635E+04  | 846.00, 2.26635E+04  |
| 889     | 16:46   | 8.30554E+06 | 78.87     | 5.60078E+07 | 67.74   | 873.00, 2.66390E+04  | 899.00, 2.66390E+04  |
| 912     | 17:05   | 3.61869E+05 | 3.44      | 1.26487E+06 | 1.53    | 907.00, 2.66390E+04  | 918.00, 2.66390E+04  |
| 961     | 17:47   | 4.80180E+05 | 4.56      | 1.66641E+06 | 2.02    | 957.00, 3.21590E+04  | 967.00, 3.21590E+04  |
| 1018    | 18:37   | 3.61097E+05 | 3.43      | 1.12265E+06 | 1.36    | 1014.00, 3.96440E+04 | 1022.97, 3.96440E+04 |
| 1055    | 19:08   | 2.62439E+05 | 2.49      | 8.93293E+05 | 1.08    | 1052.00, 3.32100E+04 | 1061.00, 3.32100E+04 |
| 1115    | 20:00   | 4.01647E+05 | 3.81      | 2.02559E+06 | 2.45    | 1110.00, 3.05690E+04 | 1129.00, 3.05690E+04 |
| 1177    | 20:53   | 1.14458E+06 | 10.87     | 3.93194E+06 | 4.76    | 1170.00, 3.43320E+04 | 1187.00, 3.43320E+04 |
| 1257    | 22:02   | 2.44949E+05 | 2.33      | 9.40403E+05 | 1.14    | 1250.00, 5.74410E+04 | 1259.00, 5.74410E+04 |
| 1268    | 22:12   | 7.39010E+05 | 7.02      | 2.69742E+06 | 3.26    | 1263.00, 8.98400E+04 | 1275.00, 8.98400E+04 |
| 1325    | 23:01   | 3.40582E+05 | 3.23      | 1.82559E+06 | 2.21    | 1318.00, 4.65970E+04 | 1333.00, 4.65970E+04 |
| 1405    | 24:09   | 2.34851E+06 | 22.30     | 2.26455E+07 | 27.39   | 1385.00, 3.99860E+04 | 1417.00, 3.99860E+04 |
| 1446    | 24:45   | 9.80508E+05 | 9.31      | 3.45394E+06 | 4.18    | 1440.00, 3.99860E+04 | 1455.00, 3.99860E+04 |



File: 17OCT97A #1-4498 Acq: 19-OCT-1997 09:11:47 GC EI+ Magnet 70S

TIC (+RP) S: 35 Exp: DUNKEL

Sample Text: SX2



MS\_USER:CHR\_DEFAULT.LIS 2-DEC-1997 09:08

Listing of peaks detected for -

data file 17OCT97A

data ident TIC (+RP) S:35 PKD(5,3,5,0.10%,0.0,0.00%,F,T)

Axis display range X\_SCAN (100.00, 4400.00)

Normalising intensity 8.09222E+06 at buffer index 1152

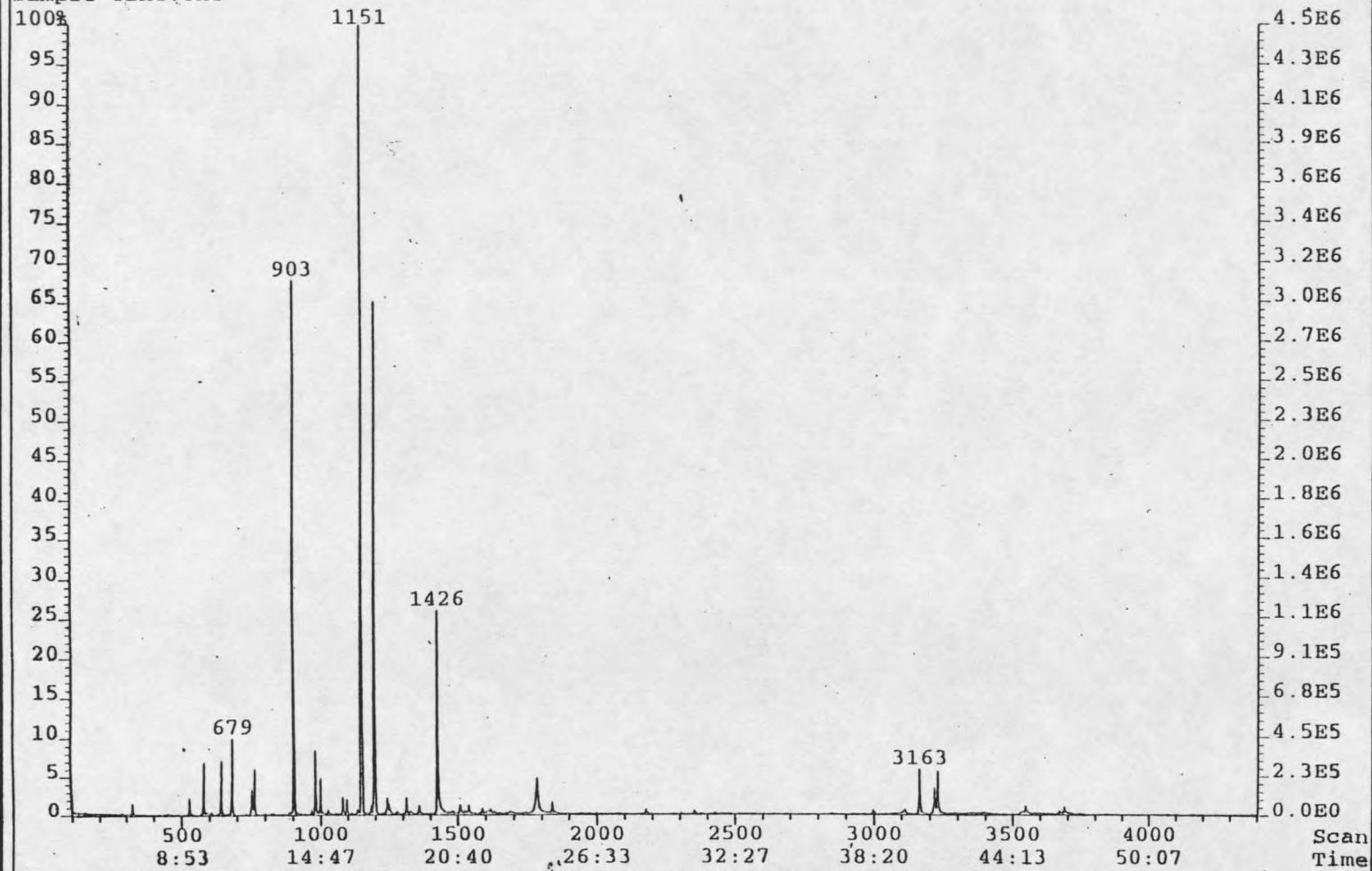
Height threshold 0.50% of maximum value 8.07959E+06

| SCAN AT<br>MAX HT | TIME AT<br>MAX HT | ABS<br>HEIGHT | REL<br>HEIGHT(%) | ABS<br>AREA | REL<br>AREA(%) | PEAK BASELINE START<br>X_SCAN, INTENSITY | PEAK BASELINE END<br>X_SCAN, INTENSITY |
|-------------------|-------------------|---------------|------------------|-------------|----------------|------------------------------------------|----------------------------------------|
| 577               | 9:48              | 5.77966E+05   | 7.15             | 1.80409E+06 | 4.53           | 573.00, 4.60400E+03                      | 584.00, 4.60400E+03                    |
| 639               | 10:32             | 4.93635E+05   | 6.11             | 1.78645E+06 | 4.48           | 635.02, 4.50700E+03                      | 646.67, 4.50700E+03                    |
| 678               | 11:00             | 2.52220E+05   | 3.12             | 8.76035E+05 | 2.20           | 674.00, 5.85875E+03                      | 684.73, 5.85875E+03                    |
| 748               | 11:49             | 2.91435E+05   | 3.61             | 1.18901E+06 | 2.98           | 744.00, 4.74900E+03                      | 756.00, 4.74900E+03                    |
| 903               | 13:39             | 4.43998E+06   | 54.95            | 1.63557E+07 | 41.05          | 897.06, 9.78713E+03                      | 908.89, 9.78713E+03                    |
| 979               | 14:33             | 1.15922E+06   | 14.35            | 4.12688E+06 | 10.36          | 974.04, 7.81100E+03                      | 986.00, 7.81100E+03                    |
| 1093              | 15:53             | 1.78103E+05   | 2.20             | 6.60687E+05 | 1.66           | 1090.00, 5.62600E+03                     | 1100.00, 5.62600E+03                   |
| 1152              | 16:35             | 8.07959E+06   | 100.00           | 3.98416E+07 | 100.00         | 1141.00, 1.26265E+04                     | 1158.49, 1.26265E+04                   |
| 1196              | 17:06             | 1.62095E+06   | 20.06            | 6.80897E+06 | 17.09          | 1186.00, 1.17975E+04                     | 1203.85, 1.17975E+04                   |
| 1241              | 17:38             | 9.74160E+04   | 1.21             | 5.50104E+05 | 1.38           | 1235.00, 1.56500E+04                     | 1245.00, 1.56500E+04                   |
| 1311              | 18:27             | 1.80700E+05   | 2.24             | 6.64168E+05 | 1.67           | 1307.00, 1.03280E+04                     | 1317.00, 1.03280E+04                   |
| 1424              | 19:47             | 1.15367E+06   | 14.28            | 5.51431E+06 | 13.84          | 1419.14, 2.65720E+04                     | 1438.00, 2.65720E+04                   |
| 1505              | 20:44             | 1.38463E+05   | 1.71             | 6.92798E+05 | 1.74           | 1501.00, 1.11920E+04                     | 1512.00, 1.11920E+04                   |
| 1781              | 23:59             | 4.77471E+05   | 5.91             | 4.02600E+06 | 10.11          | 1769.00, 5.41700E+04                     | 1790.00, 5.41700E+04                   |
| 1835              | 24:37             | 9.00810E+04   | 1.11             | 4.56591E+05 | 1.15           | 1831.00, 1.96080E+04                     | 1842.00, 1.96080E+04                   |
| 3162              | 40:15             | 4.06928E+05   | 5.04             | 1.76897E+06 | 4.44           | 3157.00, 1.73420E+04                     | 3170.00, 1.73420E+04                   |
| 3218              | 40:55             | 4.62487E+05   | 5.72             | 2.36127E+06 | 5.93           | 3210.00, 2.76910E+04                     | 3223.00, 2.76910E+04                   |
| 3228              | 41:02             | 3.77690E+05   | 4.67             | 1.60890E+06 | 4.04           | 3223.00, 2.76910E+04                     | 3236.00, 2.76910E+04                   |

File:17OCT97A #1-4499 Acq:18-OCT-1997 23:52:42 GC EI+ Magnet 70S

TIC (+RP) S:27 Exp:DUNKEL

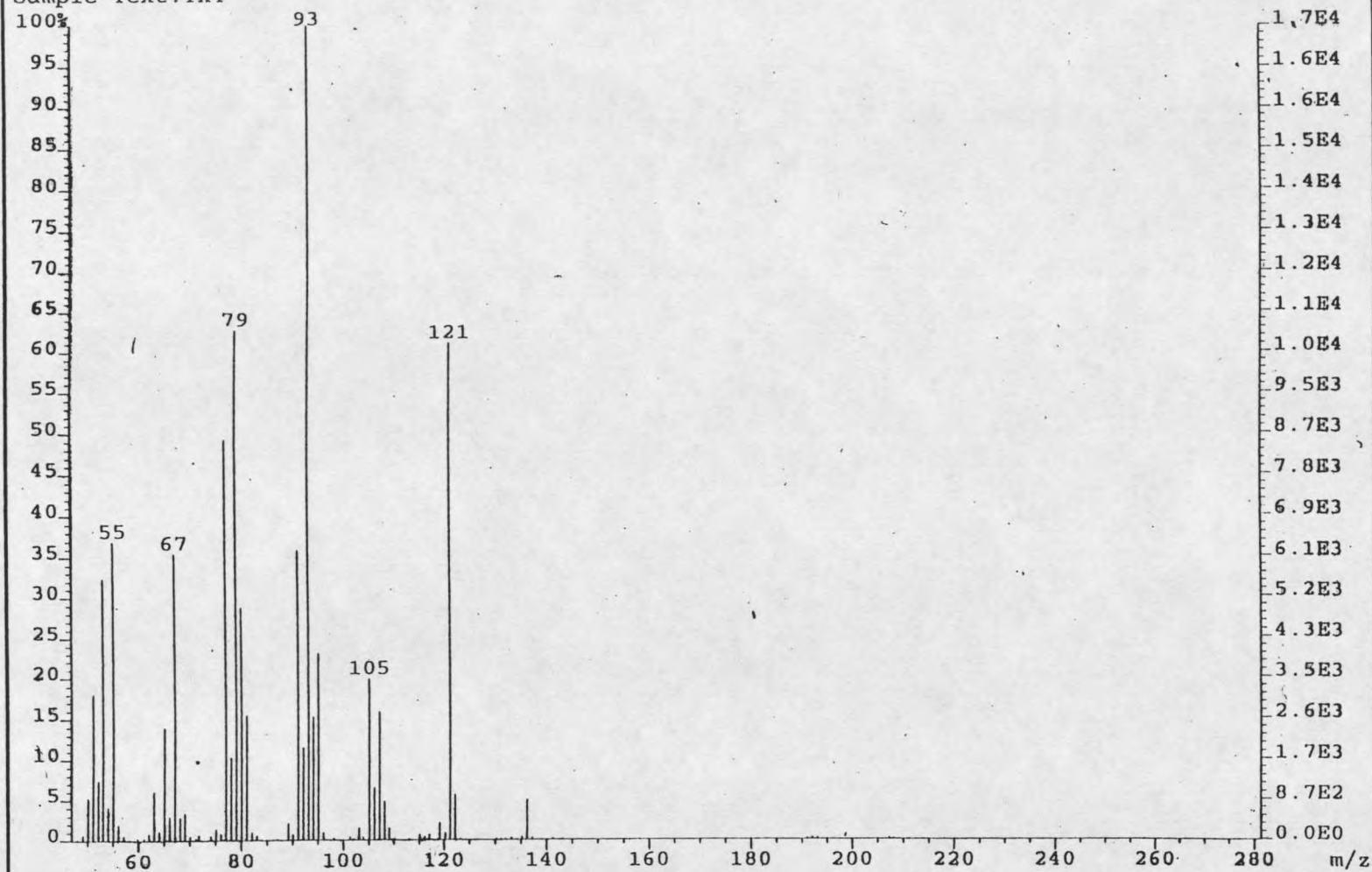
Sample Text:TX4



MS\_USER:CHR\_DEFAULT.LIS 2-DEC-1997 09:06  
 Listing of peaks detected for -  
 data file 17OCT97A  
 data ident TIC (+RP) S:27. PKD(5,3,5,0.10%,0.0,0.00%,F,T)  
 Axis display range X\_SCAN (100.00, 4400.00)  
 Normalising intensity 4.54790E+06 at buffer index 1151  
 Height threshold 0.50% of maximum value 4.53833E+06

| SCAN AT | TIME AT | ABS         | REL       | ABS         | REL     | PEAK BASELINE START  | PEAK BASELINE END    |
|---------|---------|-------------|-----------|-------------|---------|----------------------|----------------------|
| MAX HT  | MAX HT  | HEIGHT      | HEIGHT(%) | AREA        | AREA(%) | X_SCAN, INTENSITY    | X_SCAN, INTENSITY    |
| 641     | 10:33   | 3.14727E+05 | 6.93      | 1.05791E+06 | 5.69    | 637.00, 7.08600E+03  | 647.85, 7.08600E+03  |
| 679     | 11:00   | 4.45091E+05 | 9.81      | 1.53902E+06 | 8.28    | 675.00, 6.74000E+03  | 687.00, 6.74000E+03  |
| 760     | 11:57   | 2.68034E+05 | 5.91      | 9.68838E+05 | 5.21    | 757.11, 6.15900E+03  | 767.00, 6.15900E+03  |
| 903     | 13:38   | 3.07465E+06 | 67.75     | 1.13448E+07 | 61.03   | 898.06, 8.75894E+03  | 911.93, 8.75894E+03  |
| 980     | 14:33   | 3.74366E+05 | 8.25      | 1.31284E+06 | 7.06    | 976.00, 7.48800E+03  | 986.00, 7.48800E+03  |
| 999     | 14:46   | 2.10620E+05 | 4.64      | 7.52693E+05 | 4.05    | 995.00, 7.37800E+03  | 1005.00, 7.37800E+03 |
| 1079    | 15:43   | 1.00167E+05 | 2.21      | 3.95203E+05 | 2.13    | 1076.00, 9.78600E+03 | 1086.00, 9.78600E+03 |
| 1151    | 16:33   | 4.53833E+06 | 100.00    | 1.85876E+07 | 100.00  | 1142.02, 9.57350E+03 | 1159.00, 9.57350E+03 |
| 1198    | 17:07   | 2.95371E+06 | 65.08     | 1.17532E+07 | 63.23   | 1190.00, 1.10710E+04 | 1206.00, 1.10710E+04 |
| 1426    | 19:48   | 1.16318E+06 | 25.63     | 5.46839E+06 | 29.42   | 1420.00, 1.74980E+04 | 1437.00, 1.74980E+04 |
| 1782    | 23:59   | 1.79610E+05 | 3.96      | 1.39723E+06 | 7.52    | 1776.00, 3.72990E+04 | 1792.00, 3.72990E+04 |
| 3163    | 40:15   | 2.58117E+05 | 5.69      | 1.10870E+06 | 5.96    | 3158.00, 1.22490E+04 | 3170.00, 1.22490E+04 |
| 3216    | 40:53   | 1.35300E+05 | 2.98      | 5.75063E+05 | 3.09    | 3211.00, 2.65470E+04 | 3220.69, 2.65470E+04 |
| 3227    | 41:00   | 2.30536E+05 | 5.08      | 9.79768E+05 | 5.27    | 3223.01, 2.42000E+04 | 3235.00, 2.42000E+04 |

File:17OCT97A Ident:575\_582-553\_564 Mer Def 0.25 S:27 Acq:18-OCT-1997 23:52:42 +9:49 Cal:17OCT97  
70S EI+ Magnet BpM:93 BpI:17344 TIC:116137 Flags:HALL  
Sample Text:TX4

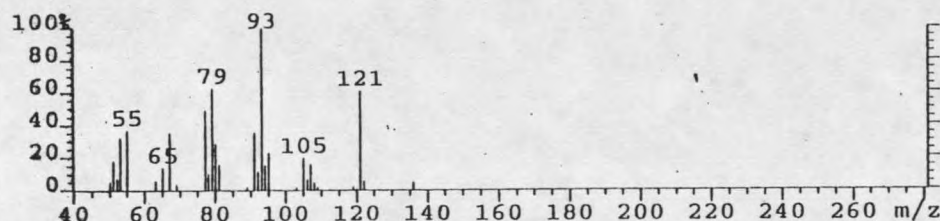




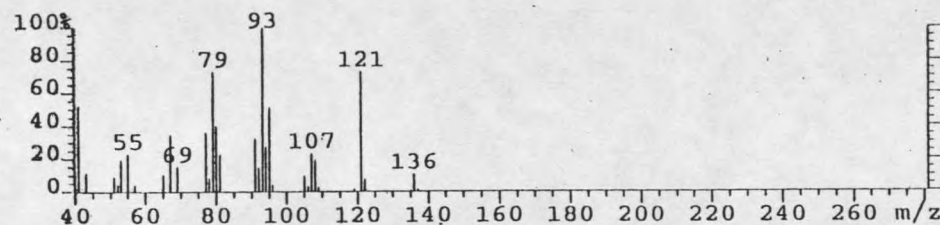
Libraries 1: NIST (62235) 2: NISTREP (12593)

| Peaks:   | 8 | 7  | 6   | 5   | 4    | 3    | 2     | 1     | 0     |
|----------|---|----|-----|-----|------|------|-------|-------|-------|
| Matches: | 3 | 46 | 237 | 680 | 1840 | 4359 | 9668  | 20122 | 37873 |
| Total:   | 3 | 49 | 286 | 966 | 2806 | 7165 | 16833 | 36955 | 74828 |

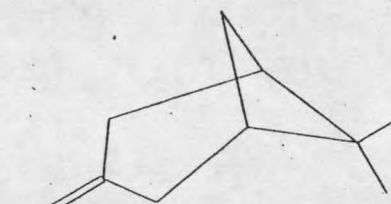
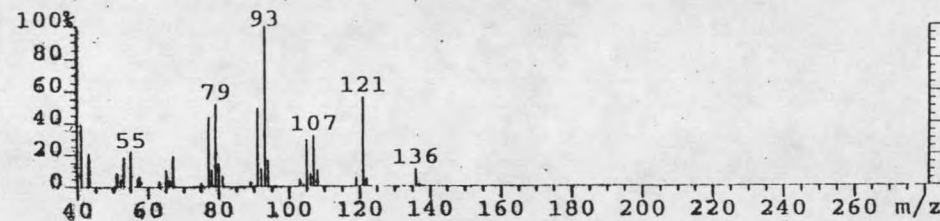
Normal Search: 48 of 49 Rank: Purity Mass Weighting: On Presearch Minimum: 20  
File:17OCT97A Ident:575\_582-553\_564 Mer Def 0.25 S:27 Acquired:17-OCT-1997 15:52:42 +9:49  
70S EI+ Function:Magnet TIC:116137



Rank:1 NIST #6619 Purity:890 Mixture:891 Matched:7 MF:C10H16 MW:136 CAS:16022-04-1 NIST:3321  
Name:Bicyclo[3.1.1]heptane, 6,6-dimethyl-3-methylene- | Norpinane, 6,6-dimethyl-3-methylene- SM:

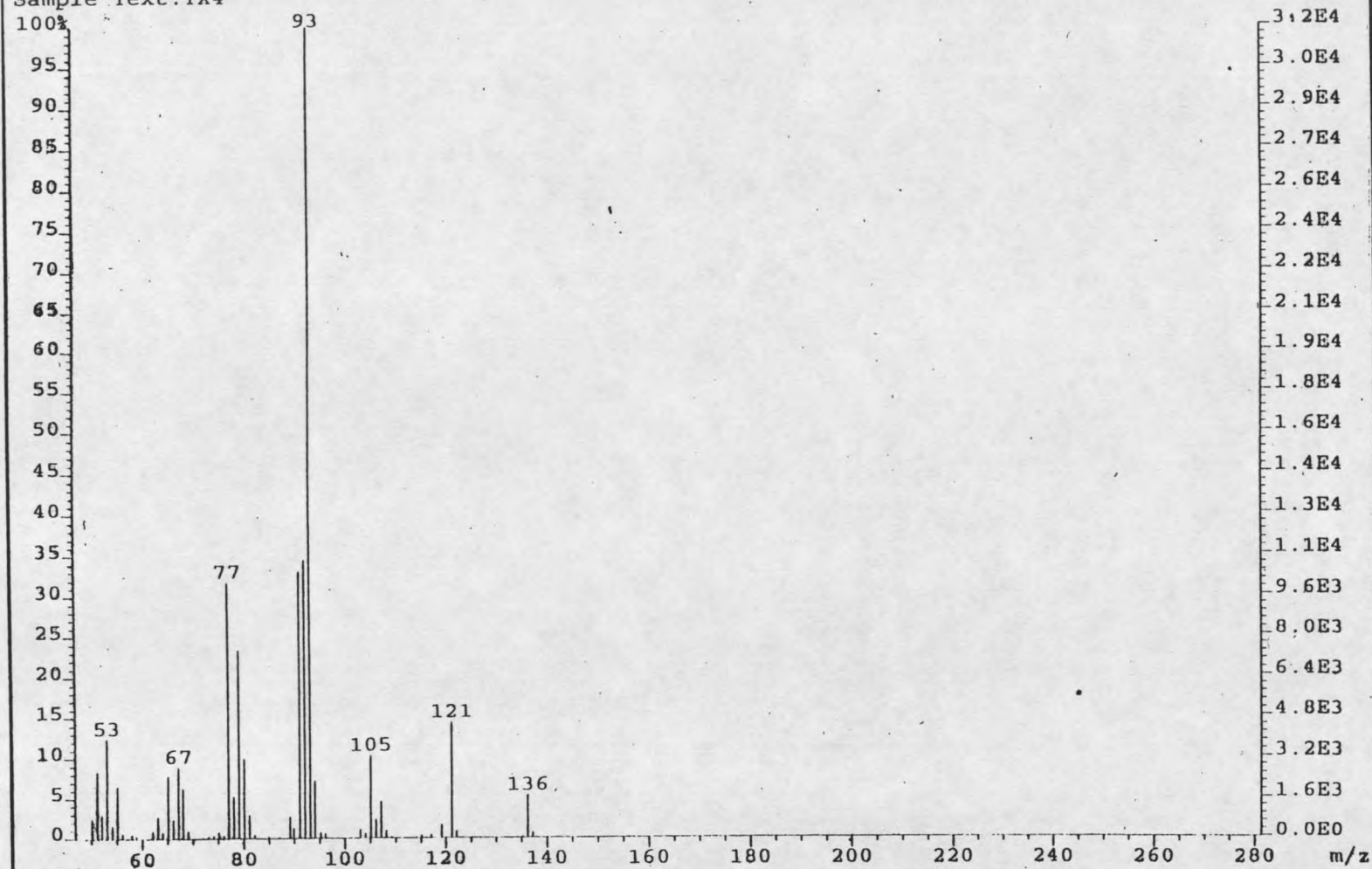


Rank:2 NIST #6699 Purity:887 Mixture:906 Matched:7 MF:C10H16 MW:136 CAS:29548-02-5 NIST:61693  
Name:1,3,6-Heptatriene, 2,5,5-trimethyl- SMILES:CC(=C)C=CC(C)(C)C=C





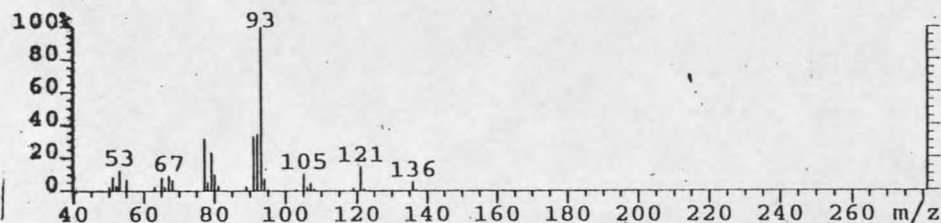
File:17OCT97A Ident:638\_646-654\_664 Mer Def 0.25 S:27 Acq:18-OCT-1997 23:52:42 +10:34 Cal:17OCT>  
70S EI+ Magnet BpM:93 BpI:31991 TIC:119455 Flags:HALL  
Sample Text:TX4



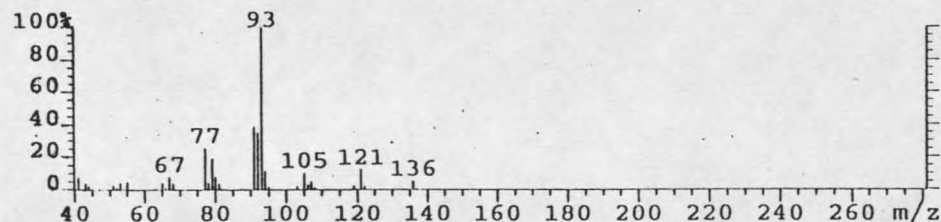
Libraries 1: NIST (62235) 2: NISTREP (12593)

| Peaks:   | 8  | 7   | 6   | 5   | 4    | 3    | 2     | 1     | 0     |
|----------|----|-----|-----|-----|------|------|-------|-------|-------|
| Matches: | 27 | 86  | 209 | 584 | 1538 | 3888 | 8274  | 14503 | 45719 |
| Total:   | 27 | 113 | 322 | 906 | 2444 | 6332 | 14606 | 29109 | 74828 |

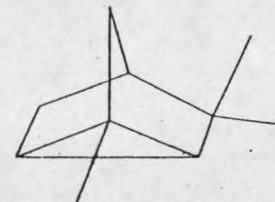
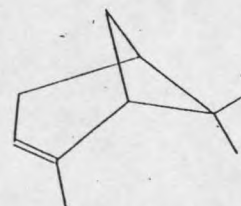
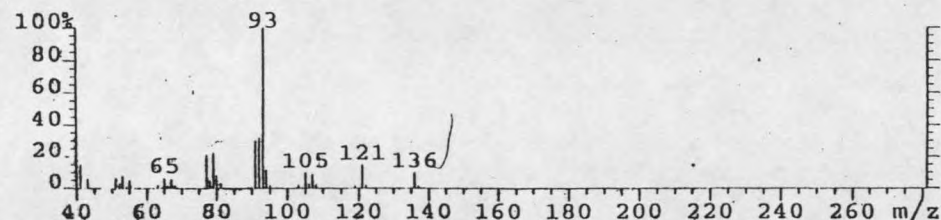
Normal Search: 26 of 27 Rank: Purity Mass Weighting: On Presearch Minimum: 20  
File: 17OCT97A Ident: 638 646-654 664 Mer Def 0.25 S: 27 Acquired: 17-OCT-1997 15:52:42 +10:34  
70S EI+ Function: Magnet TIC: 119455



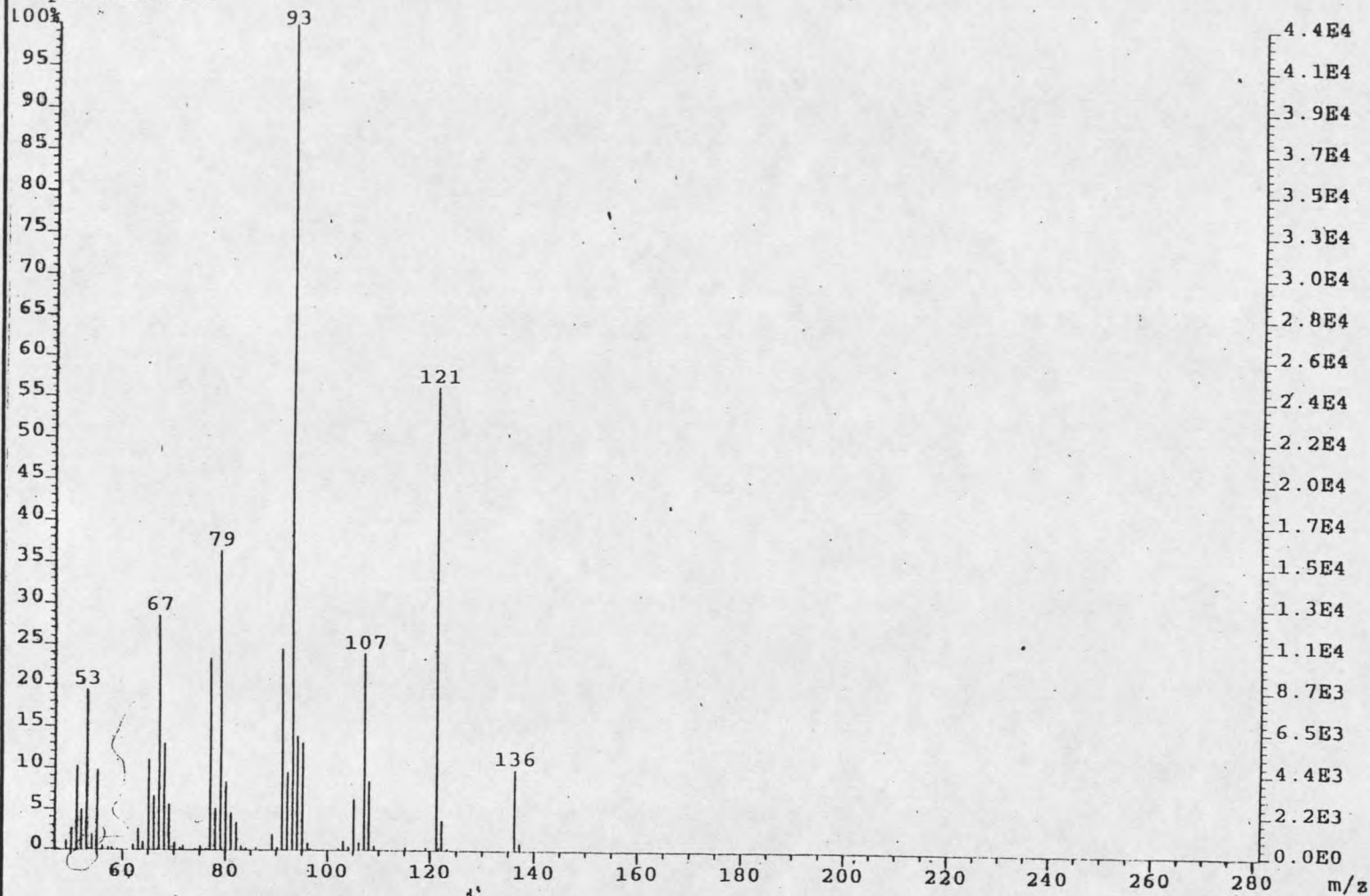
Rank: 1 NISTREP #3573 Purity: 984 Mixture: 985 Matched: 8 MF: C<sub>10</sub>H<sub>16</sub> MW: 136 CAS: 80-56-8 NIST: 77618  
Name: .alpha.-Pinene SMILES: CC1=CCC2CC1C2(C)C



Rank: 2 NISTREP #3509 Purity: 975 Mixture: 976 Matched: 8 MF: C<sub>10</sub>H<sub>16</sub> MW: 136 CAS: 488-97-1 NIST: 118832  
Name: Tricyclo[2.2.1.0<sup>2,6</sup>]heptane, 1,3,3-trimethyl- SMILES: CC1(C)C3CC2C1C2(C)C3



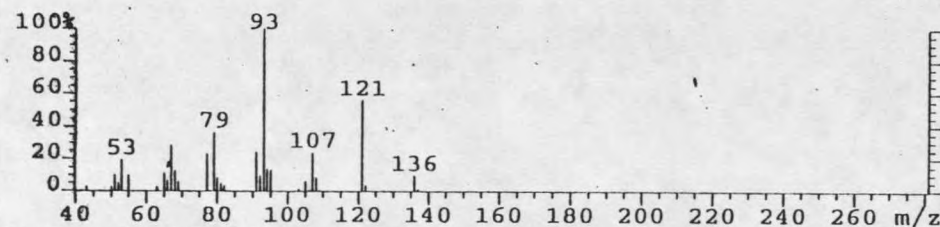
File:17OCT97A Ident:676\_682-696\_707 Mer Def 0.25 S:27 Acq:18-OCT-1997 23:52:42 +11:00 Cal:17001  
70S EI+ Magnet BpM:93 BpI:43567 TIC:214139 Flags:HALL  
Sample Text:TX4



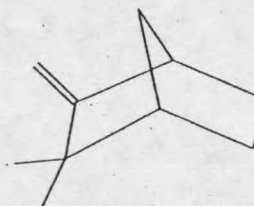
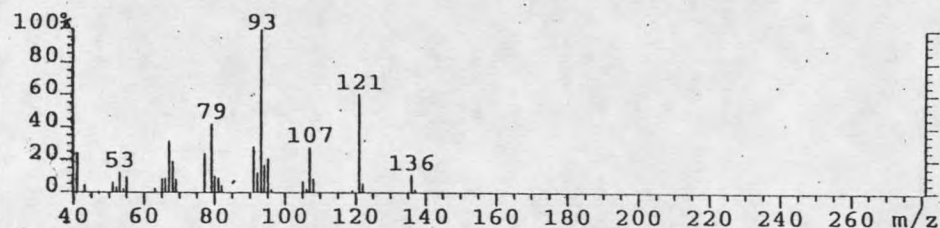
Libraries 1: NIST (62235) 2: NISTREP (12593)

| Peaks:   | 8  | 7   | 6   | 5    | 4    | 3    | 2     | 1     | 0     |
|----------|----|-----|-----|------|------|------|-------|-------|-------|
| Matches: | 17 | 92  | 314 | 712  | 1440 | 3373 | 7767  | 16426 | 44687 |
| Total:   | 17 | 109 | 423 | 1135 | 2575 | 5948 | 13715 | 30141 | 74828 |

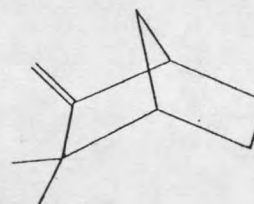
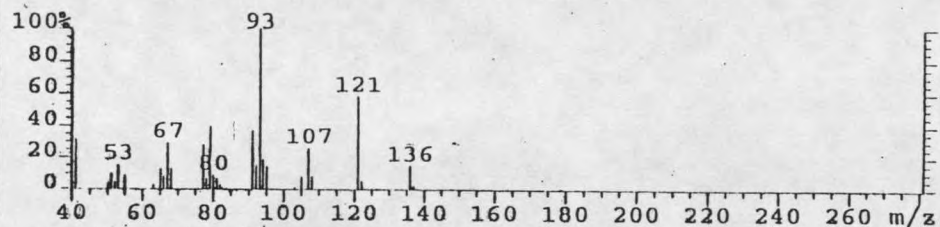
Normal Search: 97 of 109 Rank: Purity Mass Weighting: On Presearch Minimum: 20  
File:17OCT97A Ident:676\_682-696\_707 Mer Def 0.25 S:27 Acquired:17-OCT-1997.15:52:42 +11:00  
70S EI+ Function:Magnet TIC:214139



Rank:1 NISTREP #3530 Purity:976 Mixture:976 Matched:8 MF:C10H16 MW:136 CAS:79-92-5 NIST:43971  
Name:Camphene SMILES:CC2(C)C1CCC(C1)C2=C



Rank:2 NISTREP #3531 Purity:963 Mixture:964 Matched:8 MF:C10H16 MW:136 CAS:79-92-5 NIST:114291  
Name:Camphene SMILES:CC2(C)C1CCC(C1)C2=C

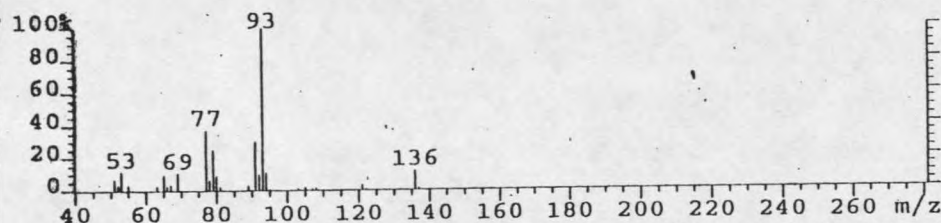




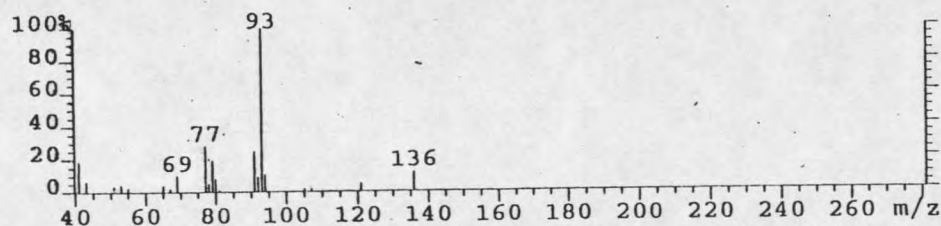
Libraries 1: NIST (62235) 2: NISTREP (12593)

| Peaks:   | 8 | 7  | 6   | 5   | 4    | 3    | 2     | 1     | 0     |
|----------|---|----|-----|-----|------|------|-------|-------|-------|
| Matches: | 4 | 38 | 153 | 453 | 1420 | 3622 | 7037  | 18236 | 43865 |
| Total:   | 4 | 42 | 195 | 648 | 2068 | 5690 | 12727 | 30963 | 74828 |

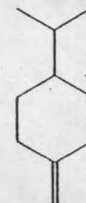
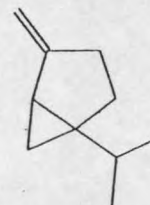
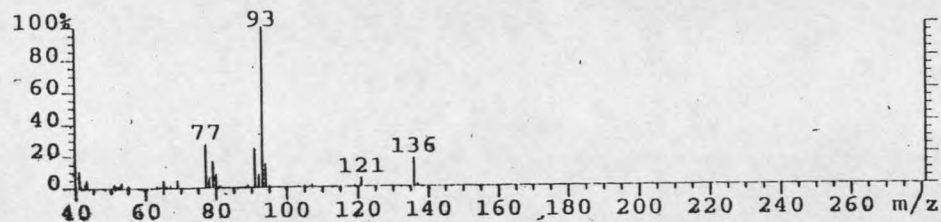
Normal Search: 37 of 42 Rank: Purity Mass Weighting: On Presearch Minimum: 20  
File:17OCT97A Ident:748\_751-738\_741 Mer Def 0.25 S:27 Acquired:17-OCT-1997 15:52:42 +11:50  
70S EI+ Function:Magnet TIC:119115



Rank:1 NISTREP #3516 Purity:970 Mixture:973 Matched:7 MF:C10H16 MW:136 CAS:3387-41-5 NIST:3315  
Name:Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)- SMILES:CC(C)C12CCC(=C)C1C2



Rank:2 NIST #6641 Purity:969 Mixture:969 Matched:8 MF:C10H16 MW:136 CAS:555-10-2 NIST:3307  
Name:.beta.-Phellandrene | Cyclohexene, 3-methylene-6-(1-methylethyl)- | p-Mentha-1(7),2-diene :



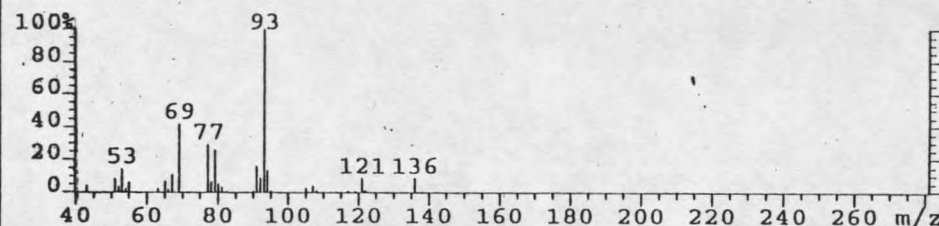
Libraries 1: NIST (62235) 2: NISTREP (12593)

| Peaks:   | 8 | 7  | 6   | 5   | 4    | 3    | 2     | 1     | 0     |
|----------|---|----|-----|-----|------|------|-------|-------|-------|
| Matches: | 5 | 68 | 141 | 449 | 1359 | 3740 | 8540  | 19245 | 41281 |
| Total:   | 5 | 73 | 214 | 663 | 2022 | 5762 | 14302 | 33547 | 74828 |

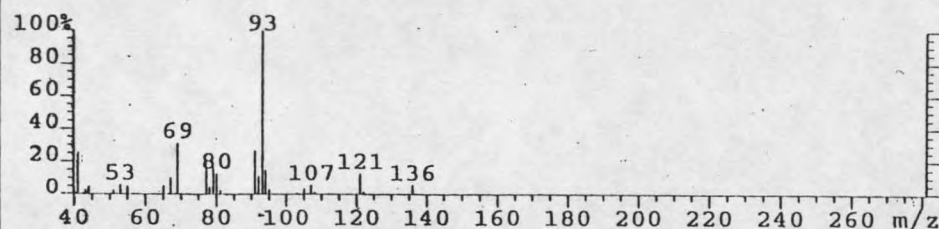
Normal Search: 71 of 73 Rank: Purity Mass Weighting: On Presearch Minimum: 20

File:17OCT97A Ident:754\_756-739\_742 Mer Def 0.25 S:27 Acquired:17-OCT-1997 15:52:42 +11:54

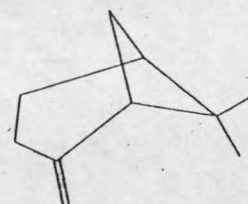
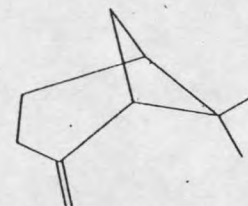
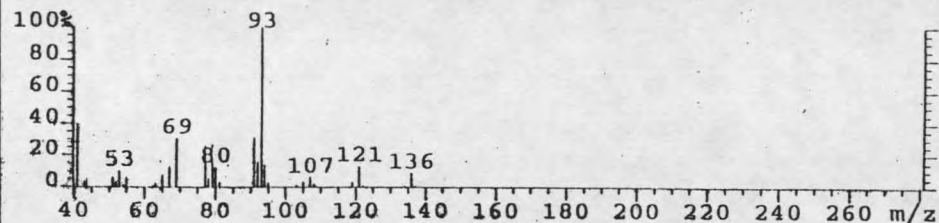
70S EI+ Function:Magnet TIC:40701



Rank:1 NISTREP #3569 Purity:944 Mixture:945 Matched:7 MF:C10H16 MW:136 CAS:127-91-3 NIST:77617  
Name:.beta.-Pinene SMILES:CC1(C)C2CCC(=C)C1C2

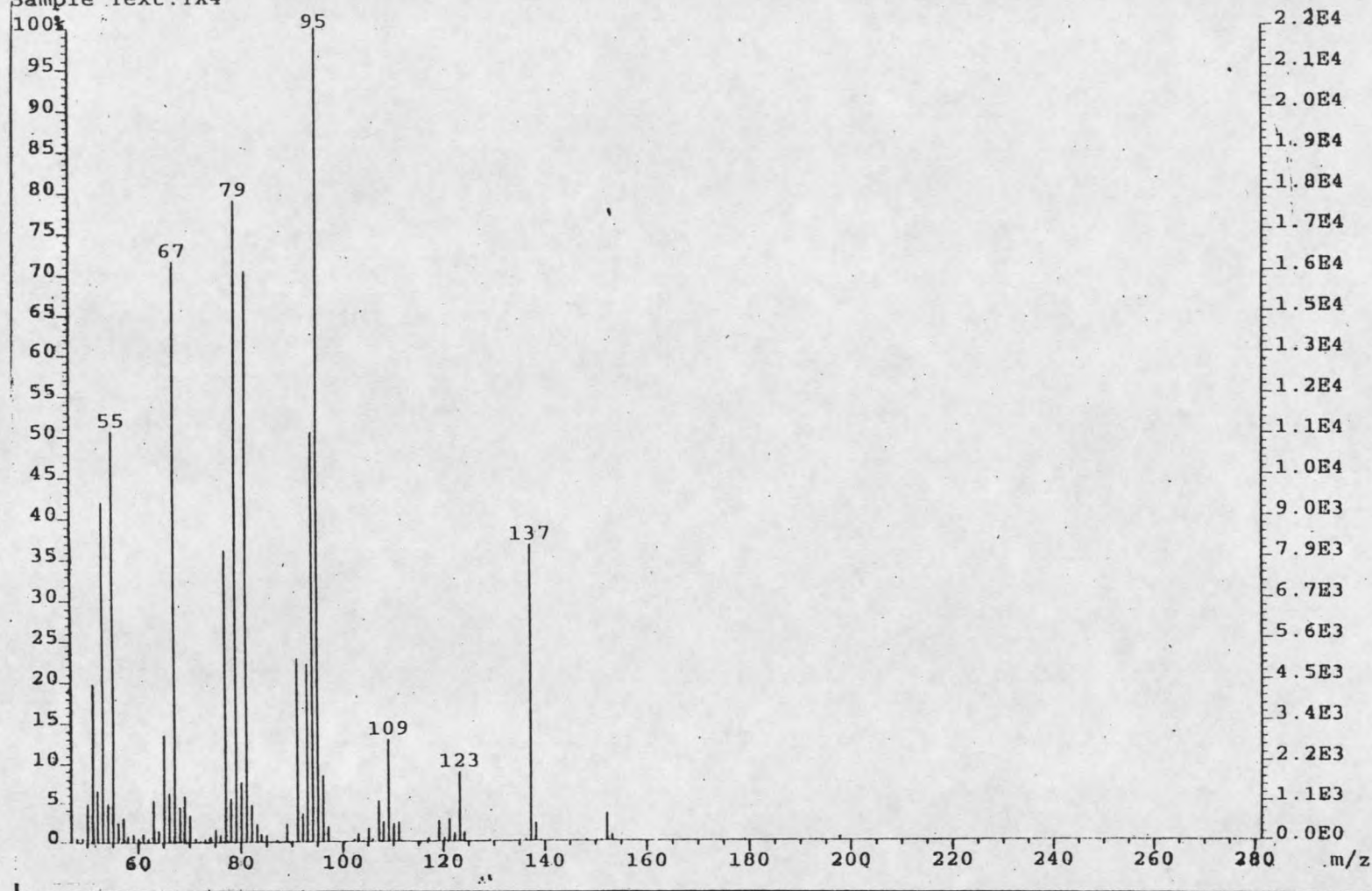


Rank:2 NIST #6602 Purity:919 Mixture:919 Matched:7 MF:C10H16 MW:136 CAS:18172-67-3 NIST:113186  
Name:Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)- | 2(10)-Pinene, (1S,5S)-(-)- | (-):





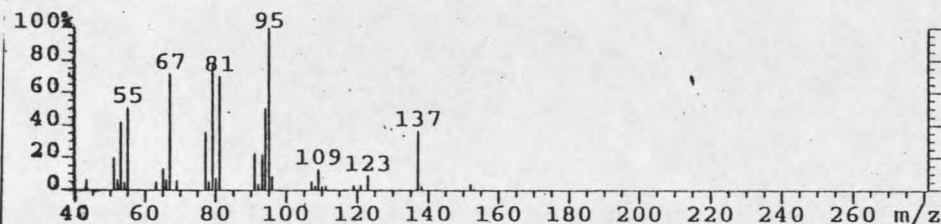
File:17OCT97A Ident:759\_763-769\_774 Mer Def 0.25 S:27 Acq:18-OCT-1997 23:52:42 +11:58 Cal:17OCT  
70S EI+ Magnet BpM:95 BpI:22487 TIC:175438 Flags:HALL  
Sample Text:TX4



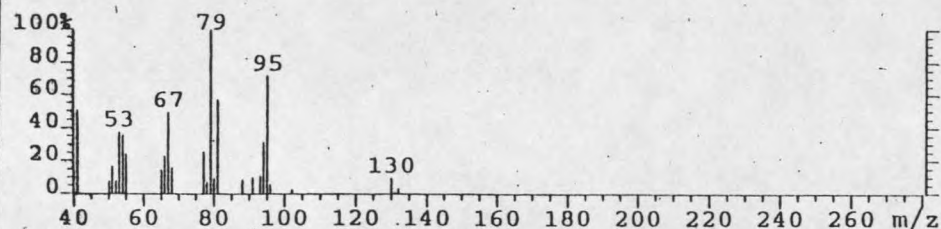
Libraries 1: NIST (62235) 2: NISTREP (12593)

| Peaks:   | 8 | 7  | 6   | 5   | 4    | 3    | 2     | 1     | 0     |
|----------|---|----|-----|-----|------|------|-------|-------|-------|
| Matches: | 1 | 11 | 173 | 735 | 1946 | 3800 | 8246  | 21635 | 38281 |
| Total:   | 1 | 12 | 185 | 920 | 2866 | 6666 | 14912 | 36547 | 74828 |

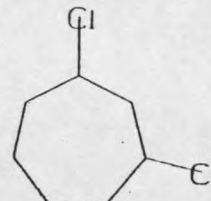
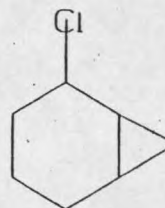
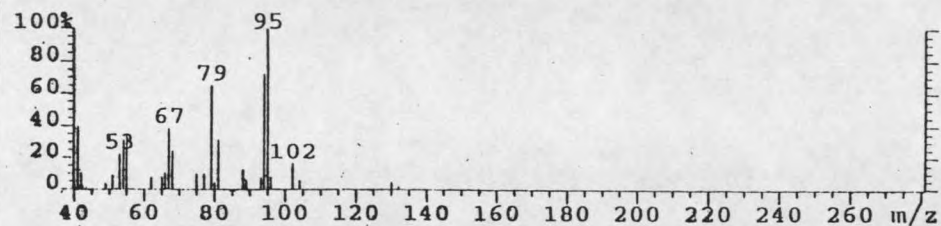
Normal Search: 137 of 185 Rank: Purity Mass Weighting: On Presearch Minimum: 20  
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70S EI+ Function:Magnet TIC:175438



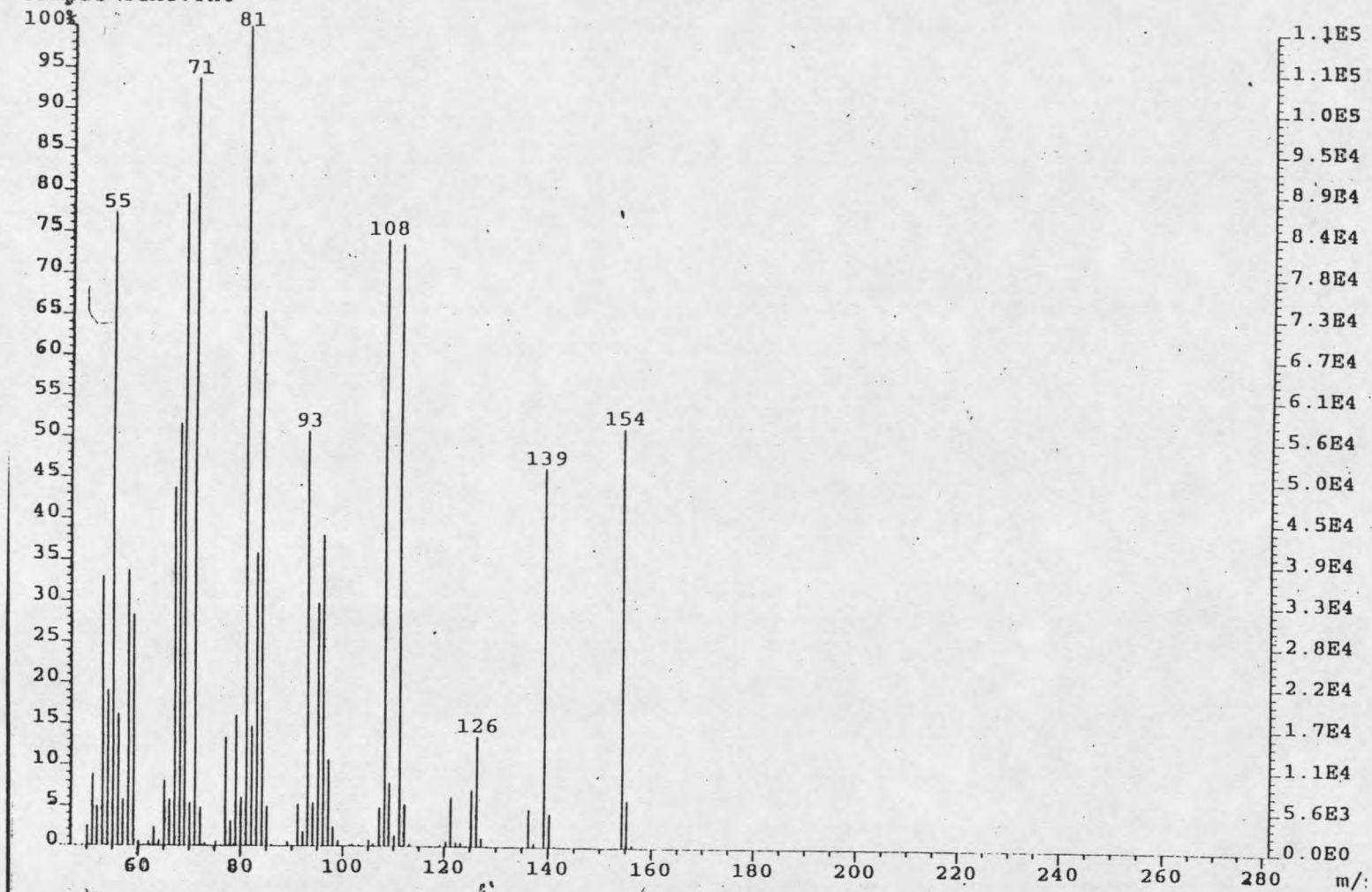
Rank:1 NIST #5397 Purity:771 Mixture:832 Matched:6 MF:C7H11Cl MW:130 CAS:34825-90-6 NIST:26735  
Name:Bicyclo[4.1.0]heptane, 2-chloro- SMILES:ClC1CCCC2CC12



Rank:2 NIST #13859 Purity:724 Mixture:795 Matched:6 MF:C7H12Cl2 MW:166 CAS:32718-94-8 NIST:5835  
Name:Cycloheptane, 1,3-dichloro-, cis- SMILES:ClC1CCCC(Cl)C1



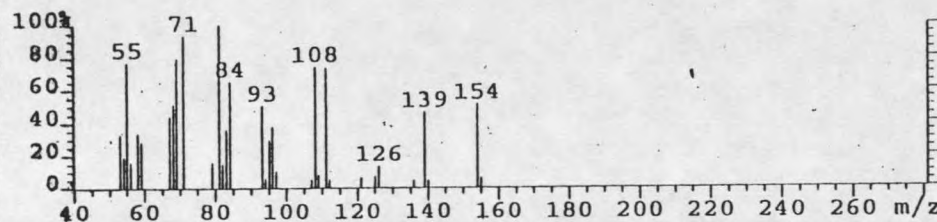
File:17OCT97A Ident:900\_907-930\_943 Mer Def 0.25 S:27 Acq:18-OCT-1997 23:52:42 +13:38 Cal:17OCT97  
70S EI+ Magnet BpM:81 BpI:111644 TIC:1408889 Flags:HALL  
Sample Text:TX4



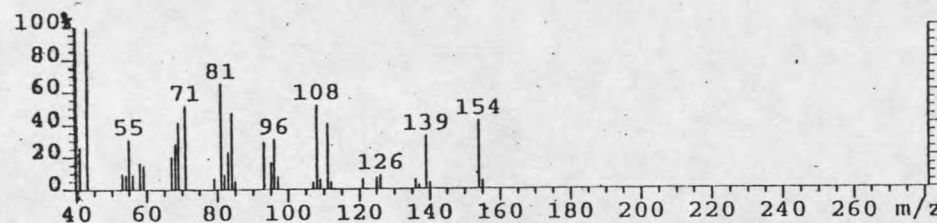
Libraries 1: NIST (62235) 2: NISTREP (12593)

| Peaks:   | 8 | 7 | 6  | 5   | 4    | 3    | 2     | 1     | 0     |
|----------|---|---|----|-----|------|------|-------|-------|-------|
| Matches: | 0 | 9 | 12 | 140 | 878  | 3466 | 8870  | 17276 | 44177 |
| Total:   | 0 | 9 | 21 | 161 | 1039 | 4505 | 13375 | 30651 | 74828 |

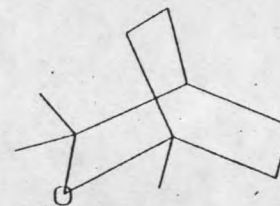
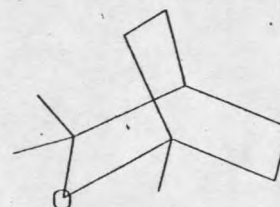
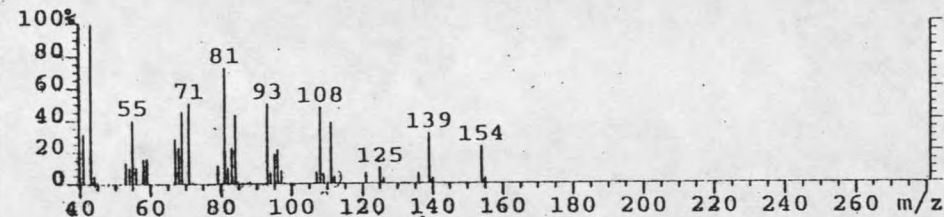
Normal Search: 19 of 21 Rank: Purity Mass Weighting: On Presearch Minimum: 20  
File: 17OCT97A Ident: 900\_907-930\_943 Mer Def 0.25 S: 27 Acquired: 17-OCT-1997 15:52:42 +13:38  
70S EI+ Function: Magnet TIC: 1408889



Rank: 1 NISTREP #4854 Purity: 800 Mixture: 800 Matched: 7 MF: C<sub>10</sub>H<sub>18</sub>O MW: 154 CAS: 470-82-6 NIST: 43952  
Name: Eucalyptol SMILES: CC12CCC(CC1)C(C)(C)O2

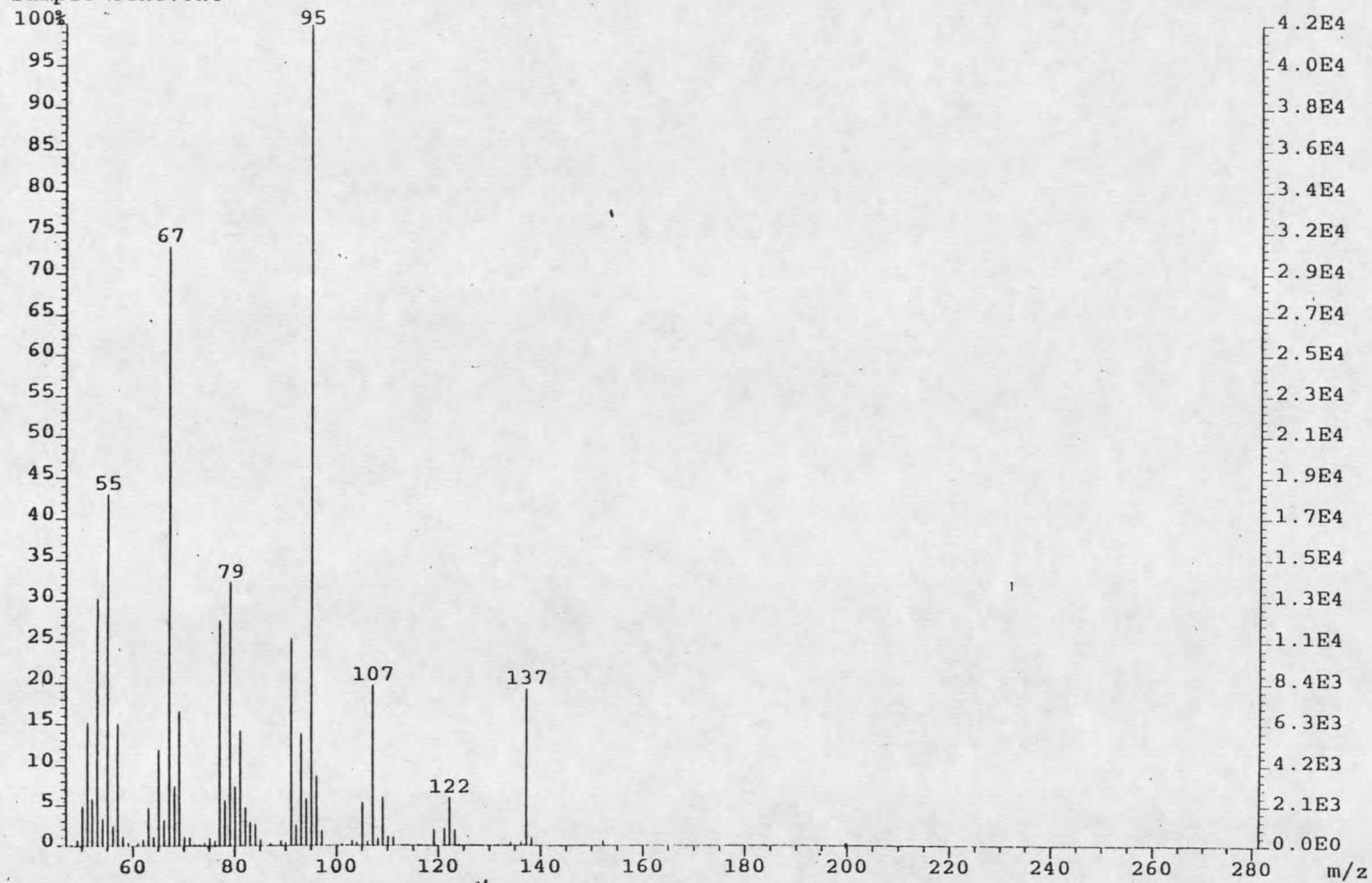


Rank: 2 NISTREP #4853 Purity: 791 Mixture: 791 Matched: 7 MF: C<sub>10</sub>H<sub>18</sub>O MW: 154 CAS: 470-82-6 NIST: 77593  
Name: Eucalyptol SMILES: CC12CCC(CC1)C(C)(C)O2





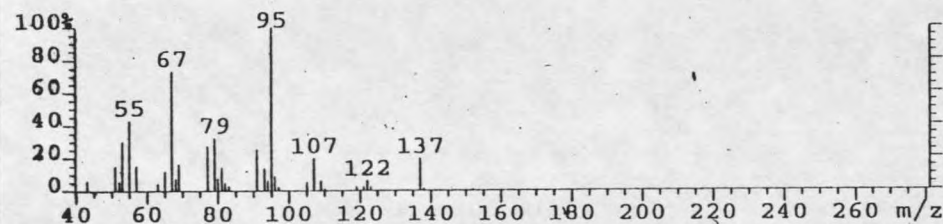
File:17OCT97A Ident:978\_982-943\_951 Mer Def 0.25 S:27 Acq:18-OCT-1997 23:52:42 +14:33 Cal:17OCT>  
70S EI+ Magnet BpM:95 BpI:42076 TIC:247316 Flags:HALL  
Sample Text:TX4



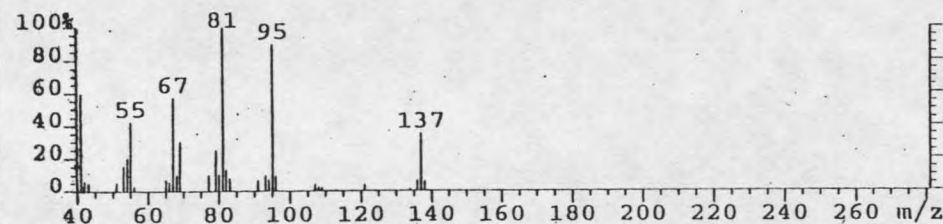
Libraries 1: NIST (62235) 2: NISTREP (12593)

| Peaks:   | 8 | 7  | 6   | 5   | 4    | 3    | 2     | 1     | 0     |
|----------|---|----|-----|-----|------|------|-------|-------|-------|
| Matches: | 2 | 20 | 135 | 749 | 2201 | 4287 | 10688 | 20249 | 36497 |
| Total:   | 2 | 22 | 157 | 906 | 3107 | 7394 | 18082 | 38331 | 74828 |

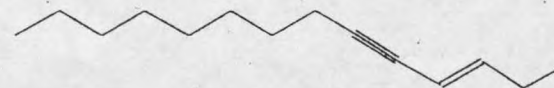
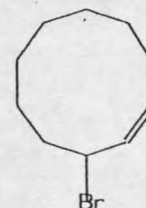
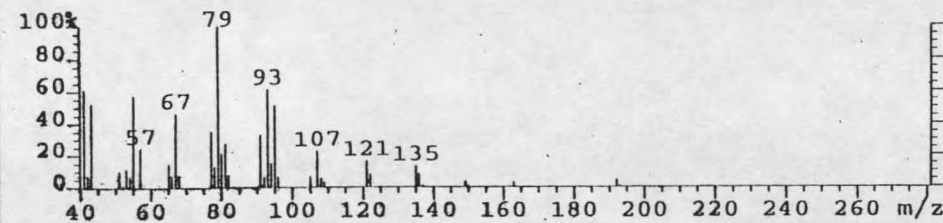
Normal Search: 17 of 22 Rank: Purity Mass Weighting: On Presearch Minimum: 20  
 File:17OCT97A Ident:978\_982-943\_951 Mer Def 0.25 S:27 Acquired:17-OCT-1997 15:52:42 +14:33  
 70S EI+ Function:Magnet TIC:247316



Rank:1 NIST #26651 Purity:605 Mixture:614 Matched:7 MF:C10H17Br MW:216 CAS:56325-56-5 NIST:6106  
 Name:Cyclodecene, 3-bromo- SMILES:BrC1CCCCCCCC=C1

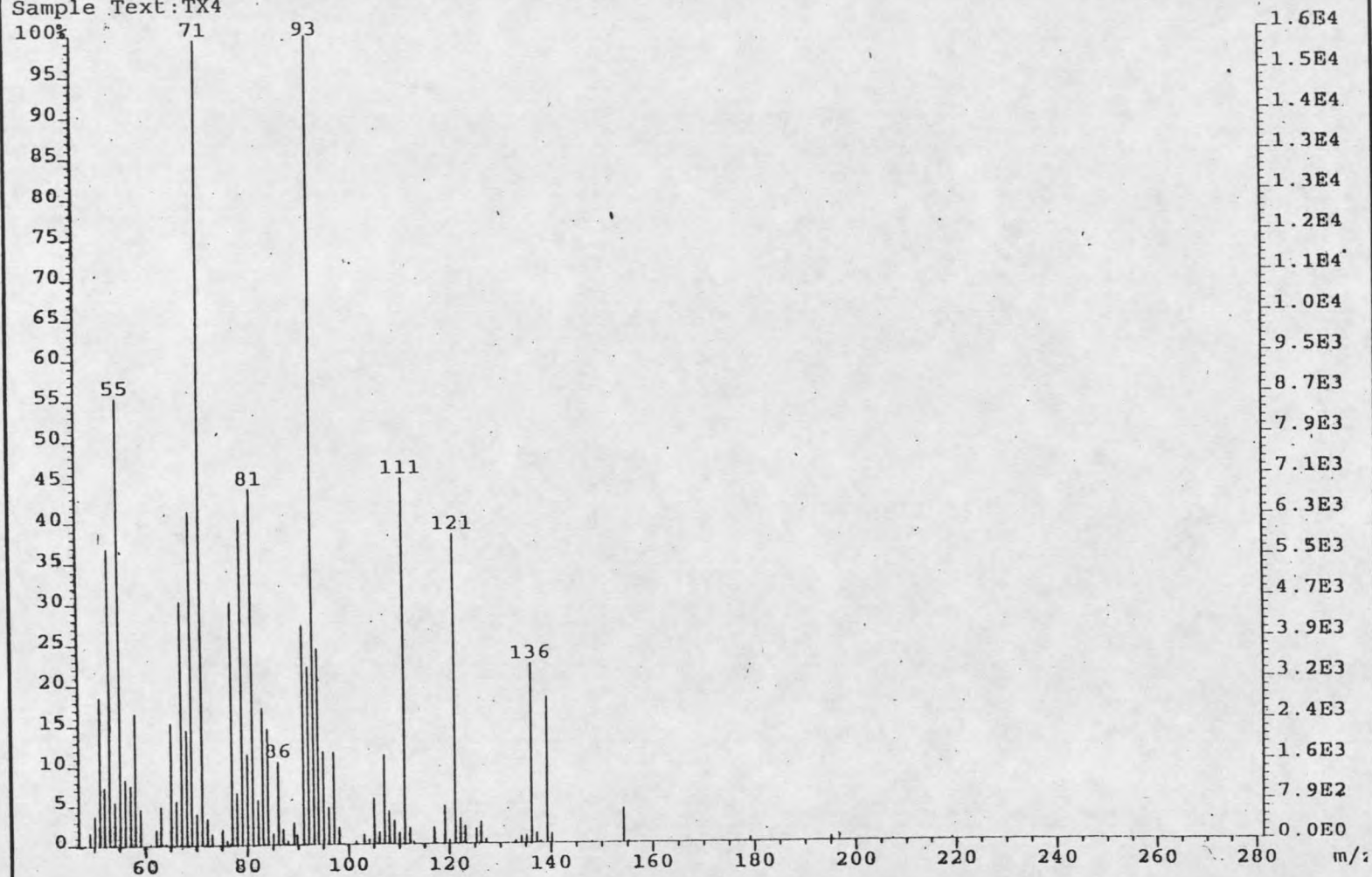


Rank:2 NIST #20849 Purity:497 Mixture:504 Matched:7 MF:C14H24 MW:192 CAS:74744-44-8 NIST:63020  
 Name:3-Tetradecen-5-yne, (E)- SMILES:CCCCCCCC#CC=CCC





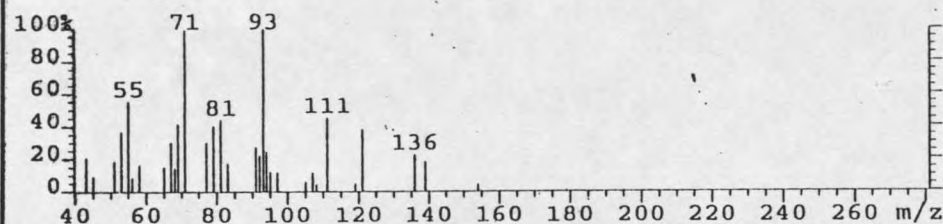
File:17OCT97A Ident:997\_999-950\_954 Mer Def 0.25 S:27 Acq:18-OCT-1997 23:52:42 +14:45 Cal:17OCT>  
70S EI+ Magnet BpM:93 BpI:15787 TIC:158776 Flags:HALL  
Sample Text:TX4



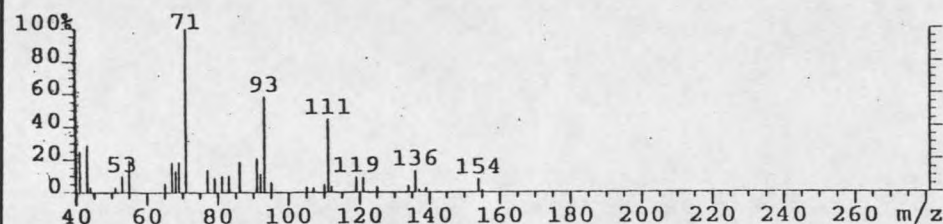
Libraries 1: NIST (62235) 2: NISTREP (12593)

|          |   |   |     |     |      |      |       |       |       |
|----------|---|---|-----|-----|------|------|-------|-------|-------|
| Peaks:   | 8 | 7 | 6   | 5   | 4    | 3    | 2     | 1     | 0     |
| Matches: | 0 | 6 | 118 | 334 | 1126 | 4266 | 12611 | 22878 | 33489 |
| Total:   | 0 | 6 | 124 | 458 | 1584 | 5850 | 18461 | 41339 | 74828 |

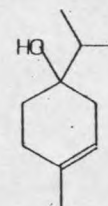
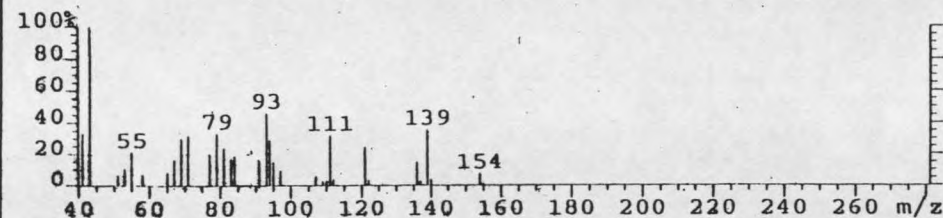
Normal Search: 109 of 124 Rank: Purity Mass Weighting: On Presearch Minimum: 20  
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70S EI+ Function:Magnet TIC:158776



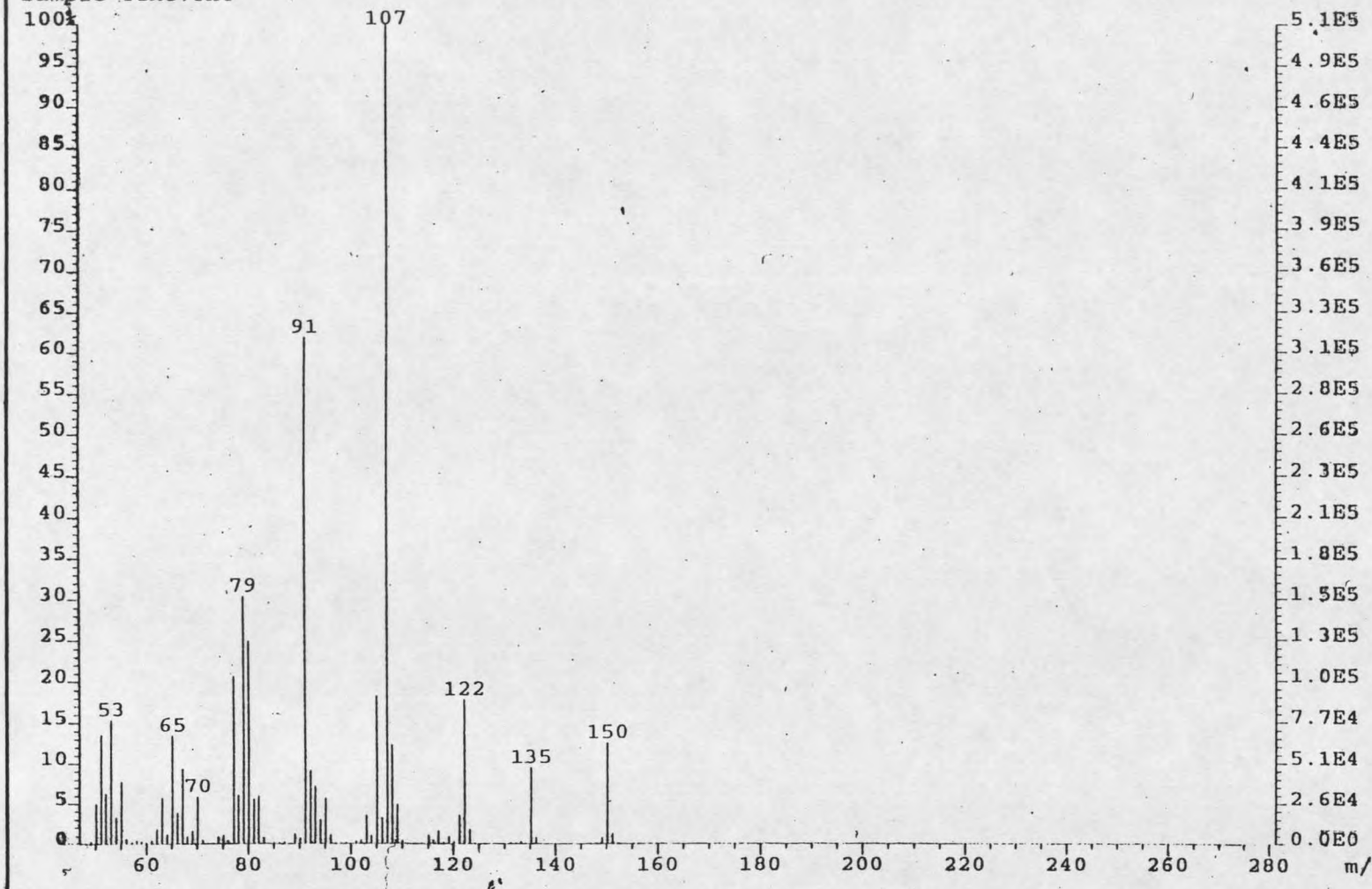
Rank:1 NISTREP #4870 Purity:764 Mixture:787 Matched:6 MF:C10H18O MW:154 CAS:562-74-3 NIST:77586>  
Name:3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)- SMILES:CC(C)C1(O)CCC(=CC1)C



Rank:2 NIST #10889 Purity:603 Mixture:618 Matched:6 MF:C10H18O MW:154 CAS:29803-81-4 NIST:10704>  
Name:2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, trans- SMILES:CC(C)C1CCC(C)(O)C=C1



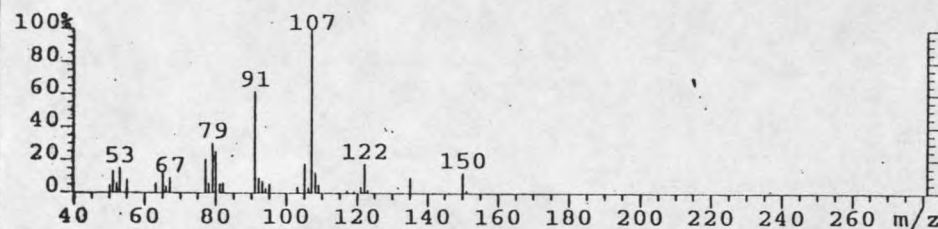
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70S EI+ Magnet BpM:107 BpI:514889 TIC:2507006 Flags:HALL  
Sample Text:TX4



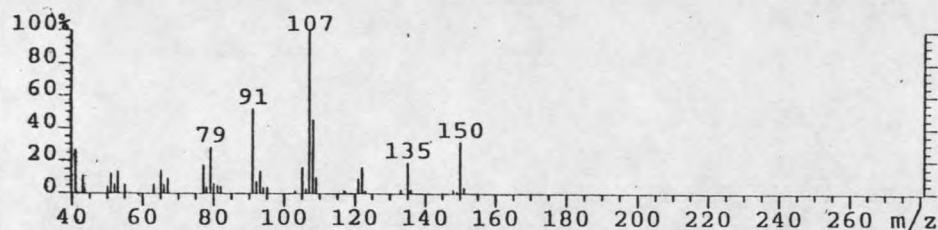
Libraries 1: NIST (62235) 2: NISTREP (12593)

|          |   |    |     |      |      |      |       |       |       |
|----------|---|----|-----|------|------|------|-------|-------|-------|
| Peaks:   | 8 | 7  | 6   | 5    | 4    | 3    | 2     | 1     | 0     |
| Matches: | 5 | 36 | 188 | 780  | 1780 | 4065 | 7469  | 12614 | 47891 |
| Total:   | 5 | 41 | 229 | 1009 | 2789 | 6854 | 14323 | 26937 | 74828 |

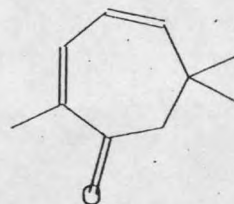
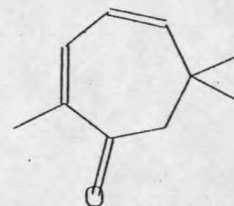
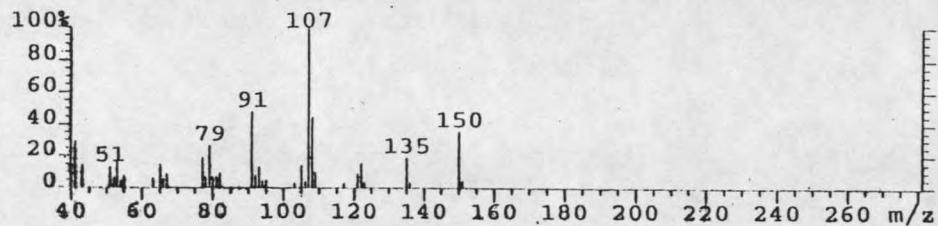
Normal Search: 36 of 41 Rank: Purity Mass Weighting: On Presearch Minimum: 20  
File:17OCT97A Ident:1146 1152-1118 1125 Mer Def 0.25 S:27 Acquired:17-OCT-1997 15:52:42 +16:32 >  
70S EI+ Function:Magnet TIC:2507006



Rank:1 NISTREP #4565 Purity:863 Mixture:863 Matched:8 MF:C10H14O MW:150 CAS:503-93-5 NIST:12209,  
Name:2,4-Cycloheptadien-1-one, 2,6,6-trimethyl- SMILES:CC1=CC=CC(C)(C)CC1=O

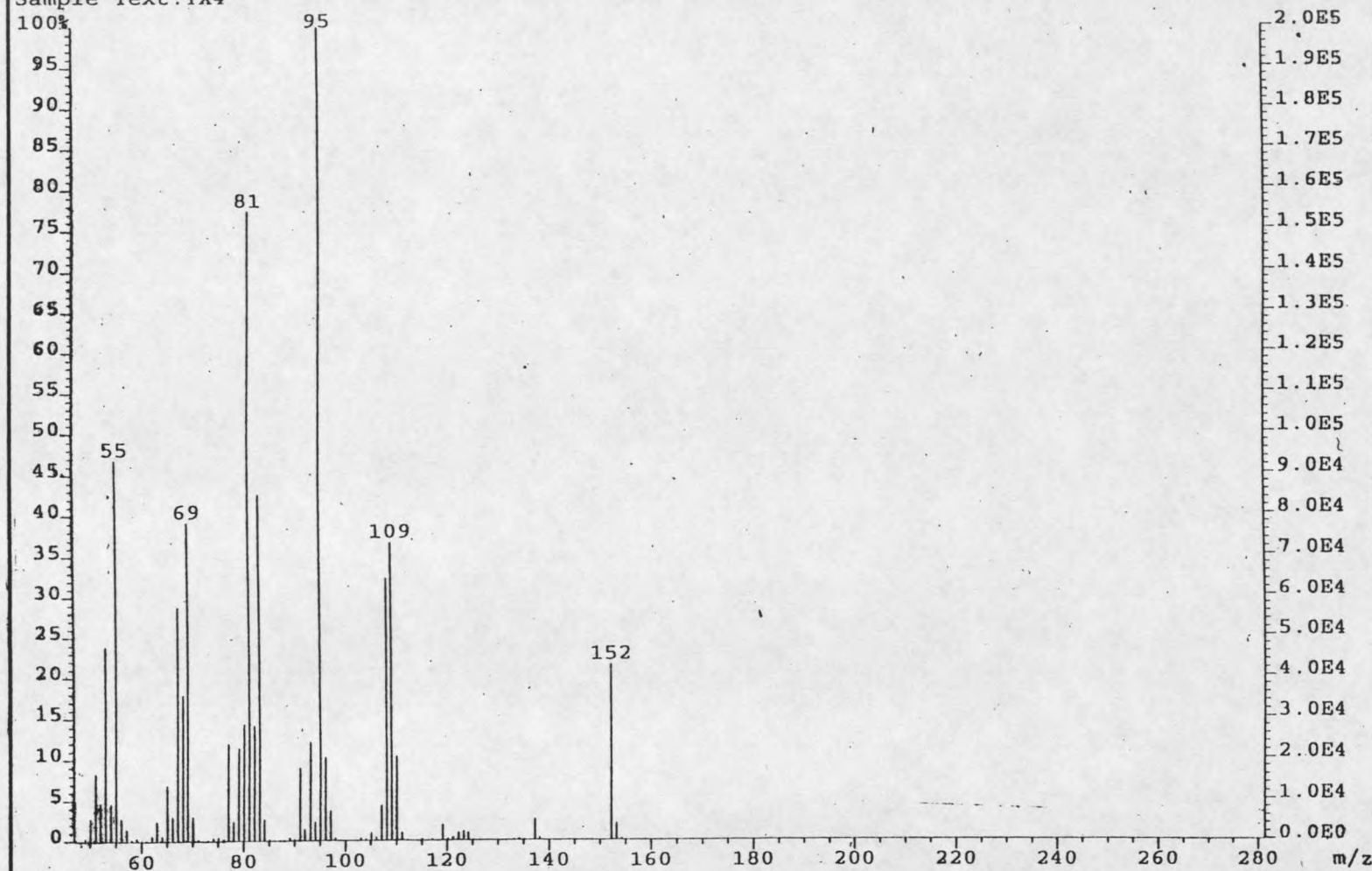


Rank:2 NIST #9827 Purity:844 Mixture:845 Matched:7 MF:C10H14O MW:150 CAS:503-93-5 NIST:4629  
Name:2,4-Cycloheptadien-1-one, 2,6,6-trimethyl- | Eucarvone SMILES:CC1=CC=CC(C)(C)CC1=O





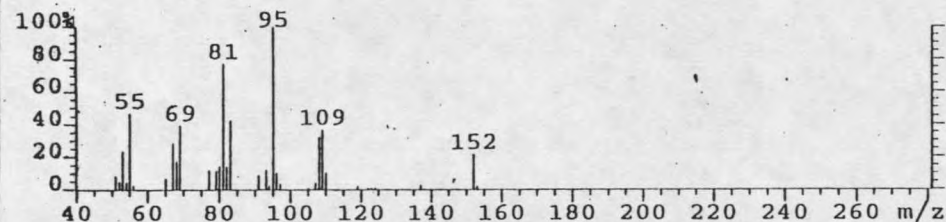
File:17OCT97A Ident:1194 1202-1212 1220 Mer Def 0.25 S:27 Acq:18-OCT-1997 23:52:42 +17:07 Cal:1>  
70S EI+ Magnet BpM:95 BpI:199231 TIC:1273439 Flags:HALL  
Sample Text:TX4



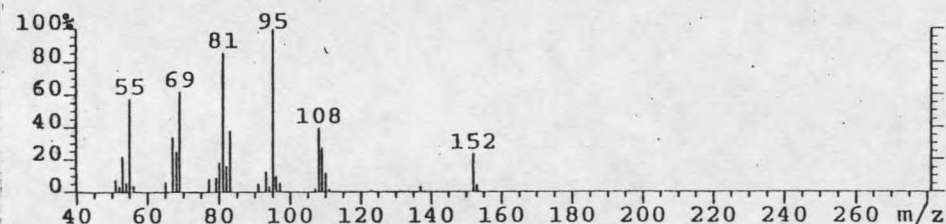
Libraries 1: NIST (62235) 2: NISTREP (12593)

|          |   |    |     |      |      |      |       |       |       |
|----------|---|----|-----|------|------|------|-------|-------|-------|
| Peaks:   | 8 | 7  | 6   | 5    | 4    | 3    | 2     | 1     | 0     |
| Matches: | 7 | 77 | 375 | 920  | 1790 | 4025 | 6532  | 14299 | 46803 |
| Total:   | 7 | 84 | 459 | 1379 | 3169 | 7194 | 13726 | 28025 | 74828 |

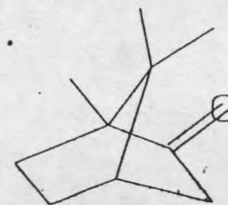
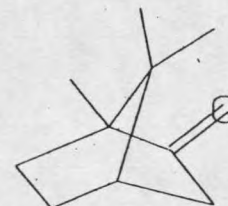
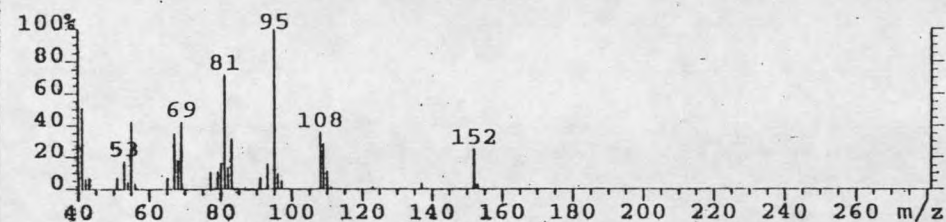
Normal Search: 78 of 84 Rank: Purity Mass Weighting: On Presearch Minimum: 20  
File:17OCT97A Ident:1194\_1202-1212\_1220 Mer Def 0.25 S:27 Acquired:17-OCT-1997 15:52:42 +17:07 >  
70S EI+ Function:Magnet TIC:1273439



Rank:1 NISTREP #4725 Purity:960 Mixture:960 Matched:8 MF:C10H16O MW:152 CAS:464-48-2 NIST:97024 >  
Name:Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)- SMILES:CC1(C)C2CCC1(C)C(=O)C2

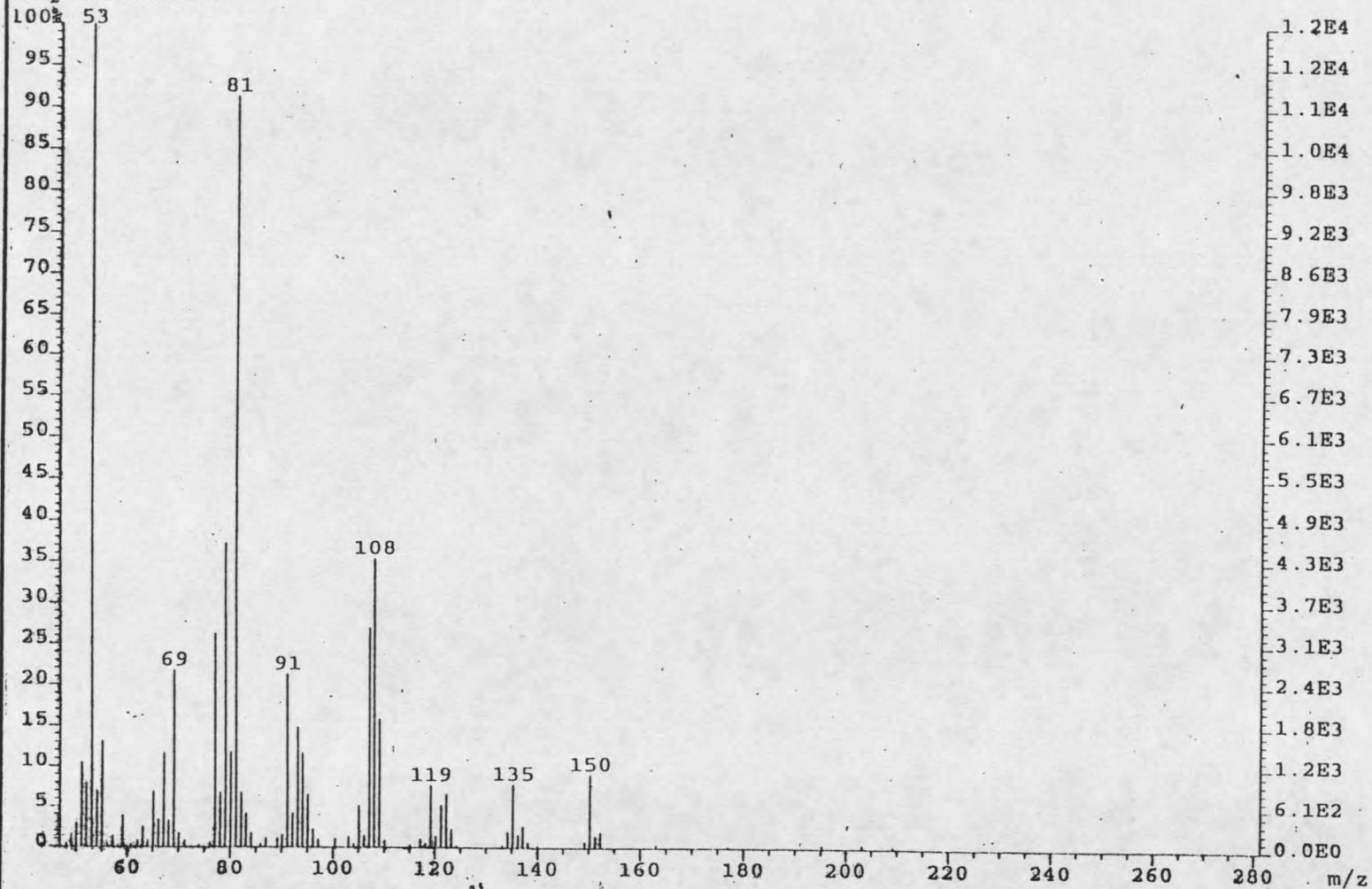


Rank:2 NIST #10338 Purity:937 Mixture:939 Matched:8 MF:C10H16O MW:152 CAS:464-48-2 NIST:114690  
Name:Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)- | (-)-Alcanfor | (-)-Camphor SMILES:CC





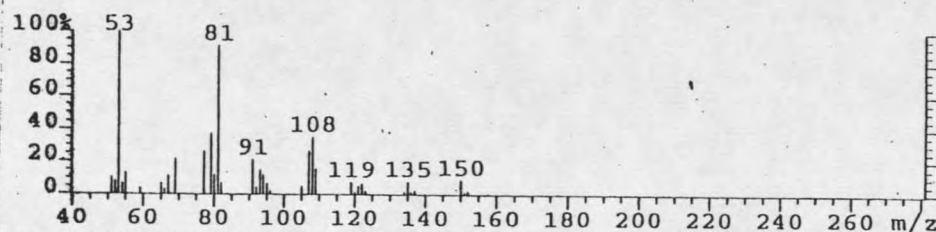
File:17OCT97A Ident:1241 1243-1226 1231 Mer Def 0.25 S:27 Acq:18-OCT-1997 23:52:42 +17:38 Cal:1>  
70S EI+ Magnet BpM:53 BpI:12226 TIC:74088 Flags:HALL  
Sample Text:TX4



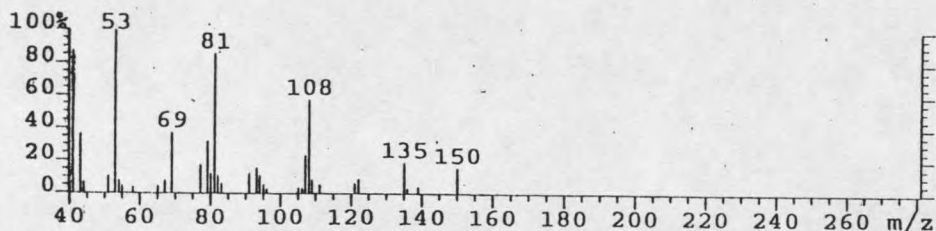
Libraries 1: NIST (62235) 2: NISTREP (12593)

| Peaks:   | 8 | 7  | 6   | 5   | 4    | 3    | 2     | 1     | 0     |
|----------|---|----|-----|-----|------|------|-------|-------|-------|
| Matches: | 0 | 10 | 149 | 575 | 1380 | 3360 | 8281  | 20733 | 40340 |
| Total:   | 0 | 10 | 159 | 734 | 2114 | 5474 | 13755 | 34488 | 74828 |

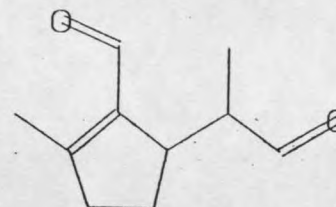
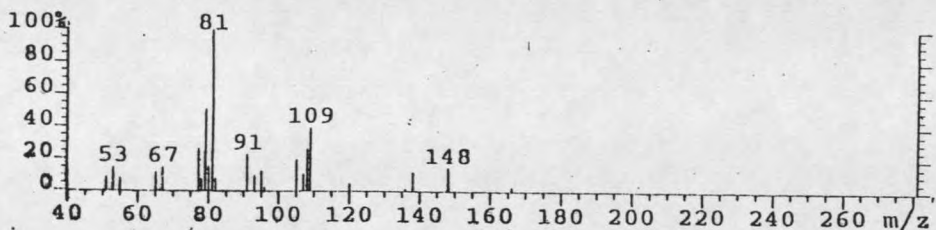
Normal Search: 38 of 159 Rank: Purity Mass Weighting: On Presearch Minimum: 20  
File: 17OCT97A Ident: 1241\_1243-1226\_1231 Mer Def 0.25 S: 27 Acquired: 17-OCT-1997 15:52:42 +17:38 >  
70S EI+ Function: Magnet TIC: 74088



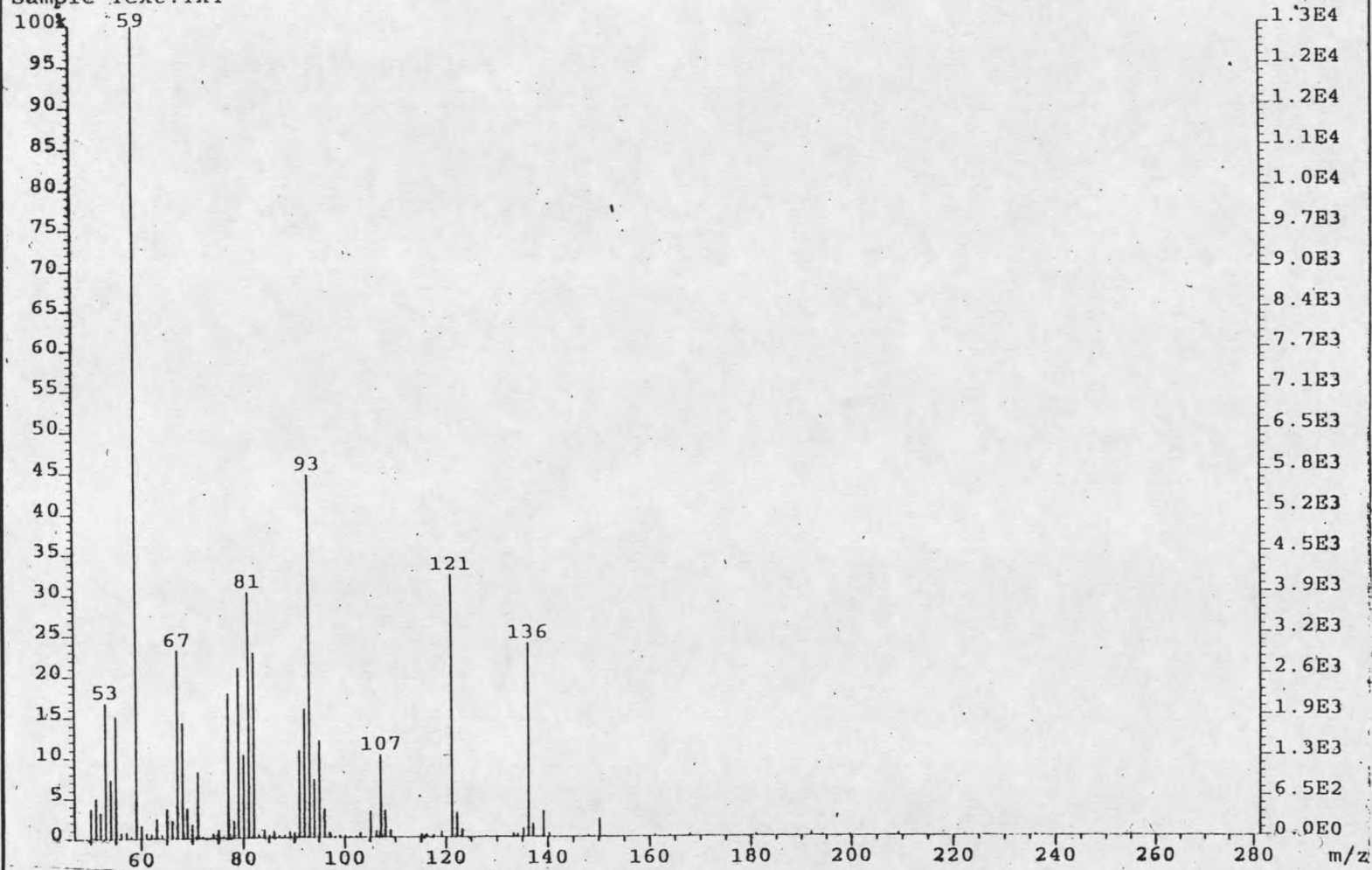
Rank: 1 NIST #9819 Purity: 771 Mixture: 779 Matched: 6 MF: C<sub>10</sub>H<sub>14</sub>O MW: 150 CAS: 16812-40-1 NIST: 107019 >  
Name: Bicyclo[3.3.1]heptan-3-one, 6,6-dimethyl-2-methylene- | Bicyclo[3.1.1]heptan-3-one, 6,6-di-



Rank: 2 NIST #14013 Purity: 636 Mixture: 670 Matched: 6 MF: C<sub>10</sub>H<sub>14</sub>O<sub>2</sub> MW: 166 CAS: 75332-42-2 NIST: 6612 >  
Name: 2-Cyclopentene-1-acetaldehyde, 2-formyl-.alpha.,3-dimethyl- SMILES: CC(C=O)C1CCC(=C1C=O)C



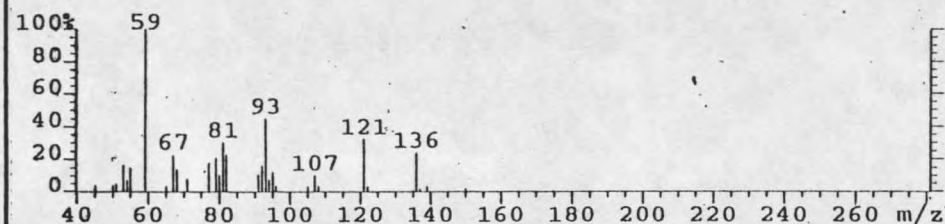
File:17OCT97A Ident:1310 1314-1289 1292 Mer Def 0.25 S:27 Acq:18-OCT-1997 23:52:42 +18:27 Cal:1>  
70S EI+ Magnet BpM:59 BpI:12904 TIC:68243 Flags:HALL  
Sample Text:TX4



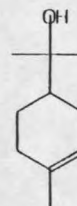
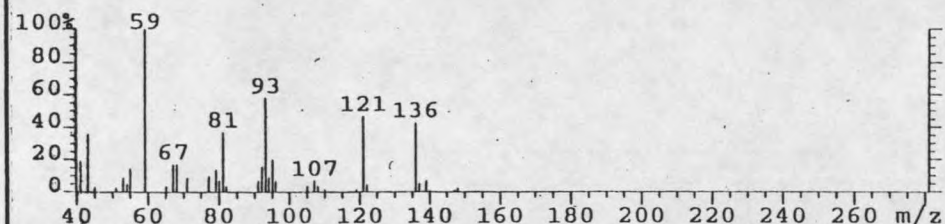
Libraries 1: NIST (62235) 2: NISTREP (12593)

|          |   |    |     |     |      |      |       |       |       |
|----------|---|----|-----|-----|------|------|-------|-------|-------|
| Peaks:   | 8 | 7  | 6   | 5   | 4    | 3    | 2     | 1     | 0     |
| Matches: | 1 | 25 | 154 | 460 | 1725 | 3573 | 7250  | 17591 | 44049 |
| Total:   | 1 | 26 | 180 | 640 | 2365 | 5938 | 13188 | 30779 | 74828 |

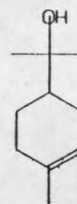
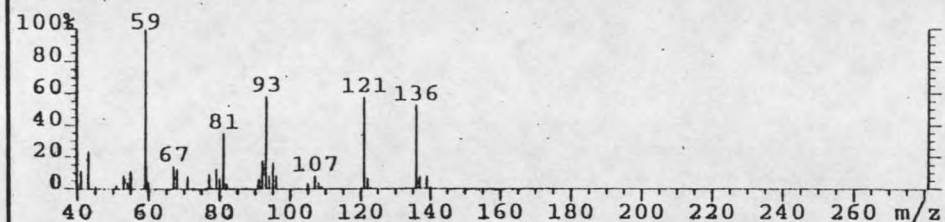
Normal Search: 24 of 26 Rank: Purity Mass Weighting: On Presearch Minimum: 20  
File:17OCT97A Ident:1310\_1314-1289\_1292 Mer Def 0.25 S:27 Acquired:17-OCT-1997 15:52:42 +18:27 >  
70S EI+ Function:Magnet TIC:68243



Rank:1 NIST #10948 Purity:877 Mixture:878 Matched:7 MF:C10H18O MW:154 CAS:10482-56-1 NIST:36610>  
Name:3-Cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl-, (S)- | p-Menth-1-en-8-ol, (S)-(-)->

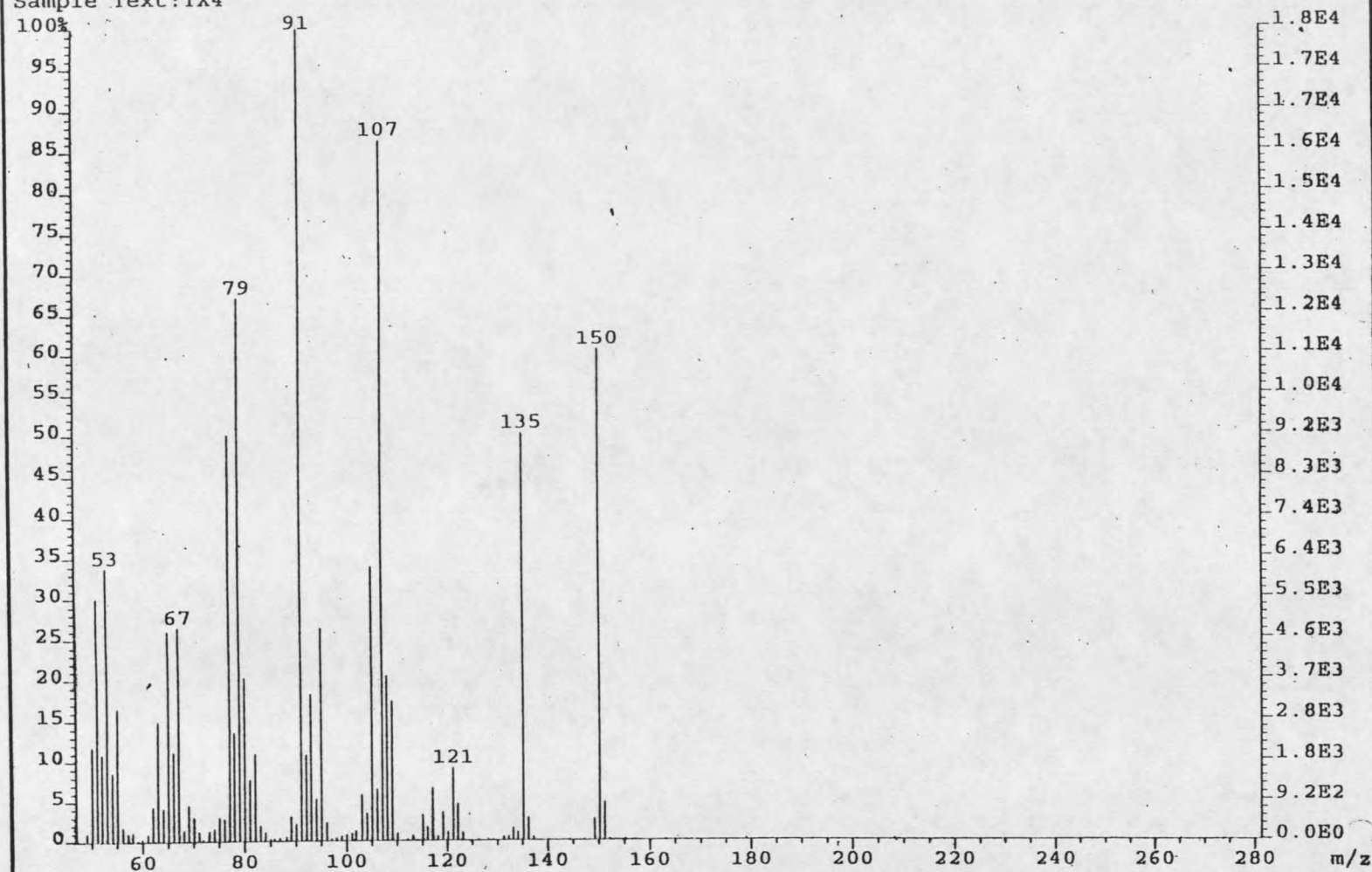


Rank:2 NISTREP #4902 Purity:852 Mixture:854 Matched:7 MF:C10H18O MW:154 CAS:10482-56-1 NIST:439>  
Name:3-Cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl-, (S)- SMILES:CC1=CCC(CC1)C(C)(C)O





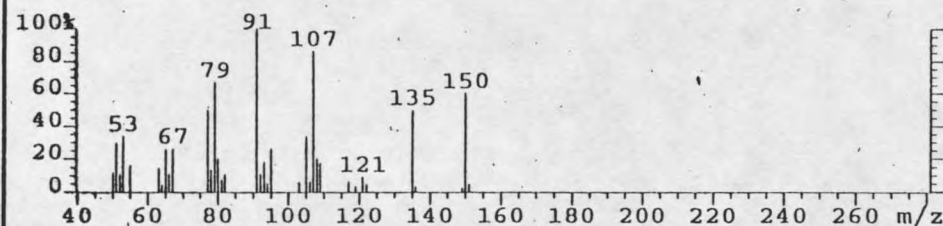
File:17OCT97A Ident:1778 1785-1723 1734 Mer Def 0.25 S:27 Acq:18-OCT-1997 23:52:42 +23:59 Cal:1>  
70S EI+ Magnet BpM:91 BpI:18387 TIC:168294 Flags:HALL  
Sample Text:TX4



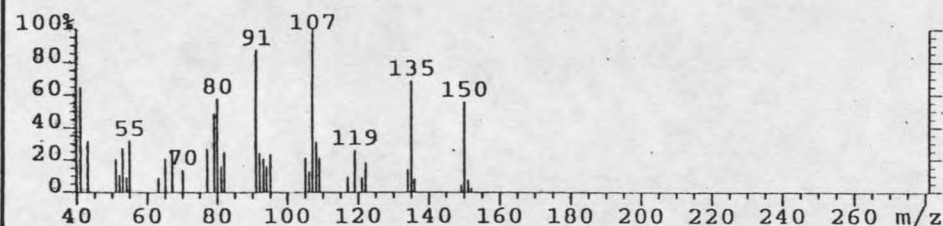
Libraries 1: NIST (62235) 2: NISTREP (12593)

|          |   |    |     |     |      |      |       |       |       |
|----------|---|----|-----|-----|------|------|-------|-------|-------|
| Peaks:   | 8 | 7  | 6   | 5   | 4    | 3    | 2     | 1     | 0     |
| Matches: | 2 | 12 | 129 | 485 | 1558 | 4139 | 9114  | 15780 | 43609 |
| Total:   | 2 | 14 | 143 | 628 | 2186 | 6325 | 15439 | 31219 | 74828 |

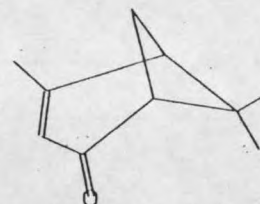
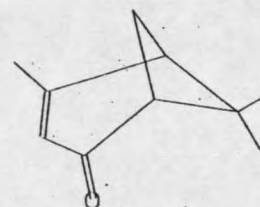
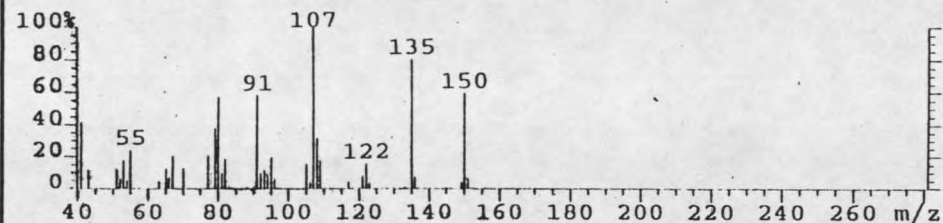
Normal Search: 99 of 143 Rank: Purity Mass Weighting: On Presearch Minimum: 20  
File:17OCT97A Ident:1778\_1785-1723\_1734 Mer Def 0.25 S:27 Acquired:17-OCT-1997 15:52:42 +23:59 >  
70S EI+ Function:Magnet TIC:168294



Rank:1 NIST #9853 Purity:828 Mixture:835 Matched:6 MF:C10H14O MW:150 CAS:18309-32-5 NIST:38883  
Name:D-Verbenone | Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-, (1R)- | 2-Pinen-4-one, (1R), >

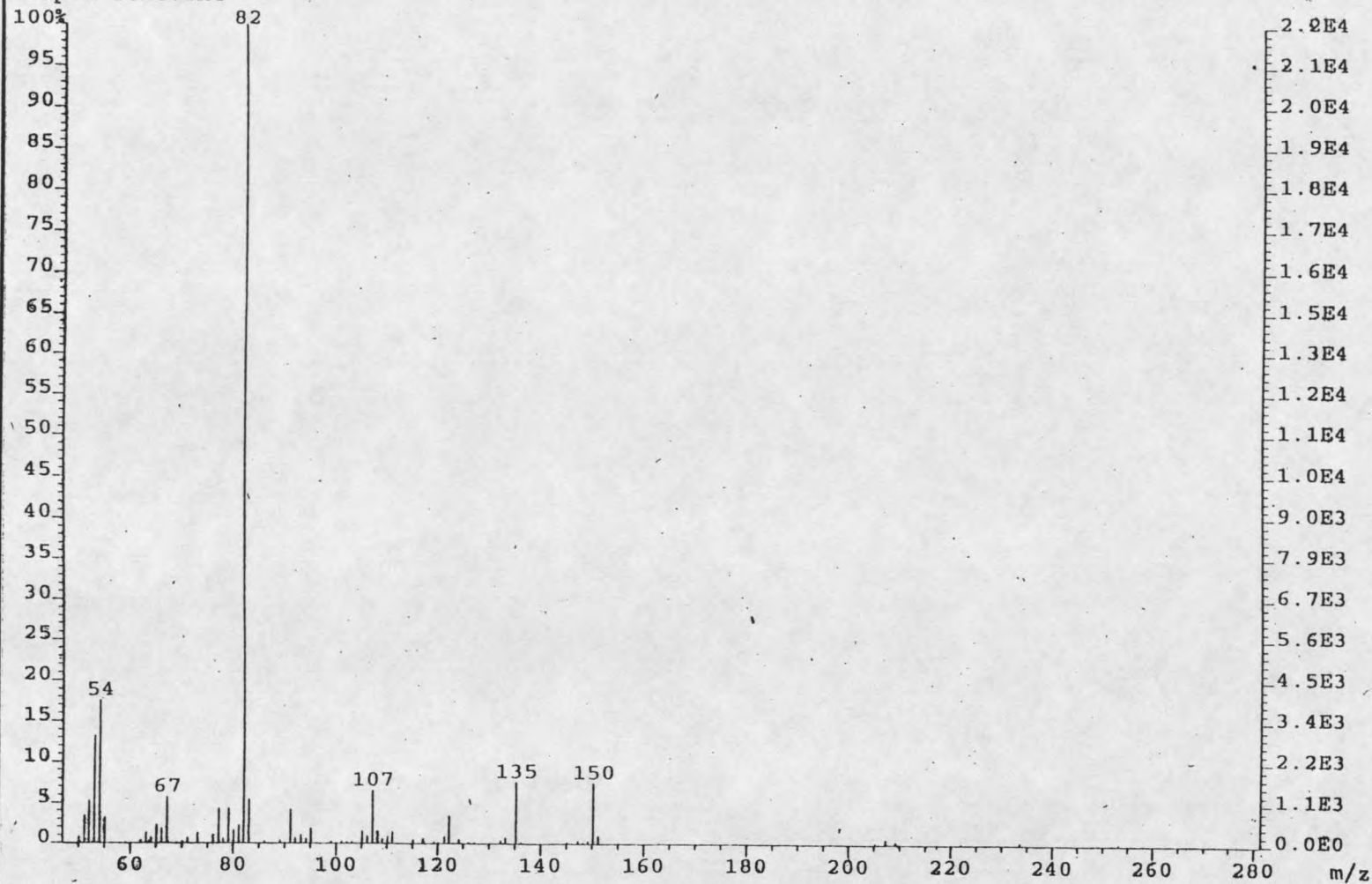


Rank:2 NIST #9851 Purity:797 Mixture:802 Matched:7 MF:C10H14O MW:150 CAS:80-57-9 NIST:4631  
Name:Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl- | 2-Pinen-4-one | Berbenone SMILES:CC1=CC(>





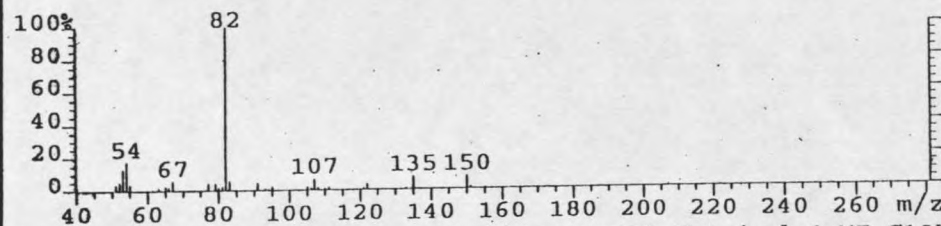
File:17OCT97A Ident:1506 1508-1489 1498 Mer Def 0.25 S:27 Acq:18-OCT-1997 23:52:42 +20:45 Cal:1  
70S EI+ Magnet BpM:82 BpI:22492 TIC:51722 Flags:HALL  
Sample Text:TX4



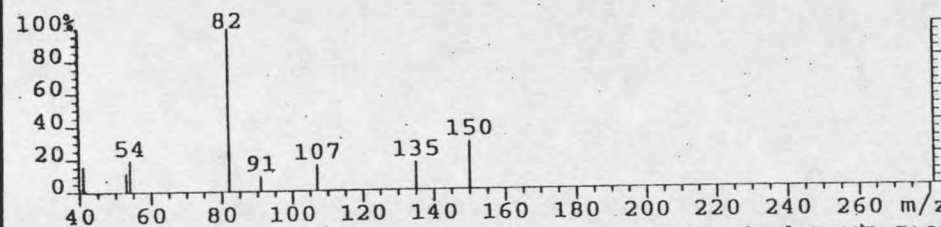
Libraries 1: NIST (62235) 2: NISTREP (12593)

|          |   |   |    |     |      |      |       |       |       |
|----------|---|---|----|-----|------|------|-------|-------|-------|
| Peaks:   | 8 | 7 | 6  | 5   | 4    | 3    | 2     | 1     | 0     |
| Matches: | 0 | 2 | 32 | 270 | 1066 | 3403 | 8008  | 19202 | 42845 |
| Total:   | 0 | 2 | 34 | 304 | 1370 | 4773 | 12781 | 31983 | 74828 |

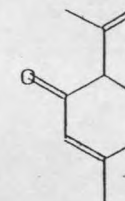
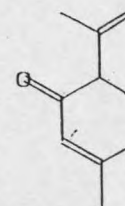
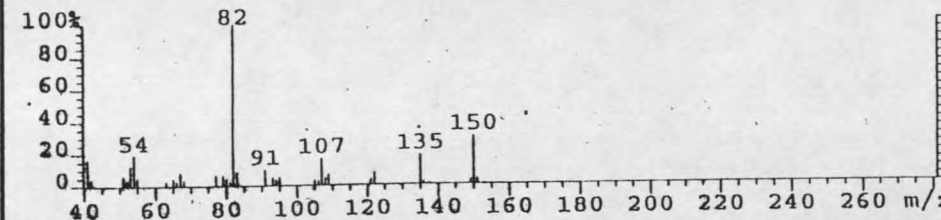
Normal Search: 3 of 34 Rank: Purity Mass Weighting: On Presearch Minimum: 20  
File:17OCT97A Ident:1506\_1508-1489\_1498 Mer Def 0.25 S:27 Acquired:17-OCT-1997 15:52:42 +20:45 >  
70S EI+ Function:Magnet TIC:51722



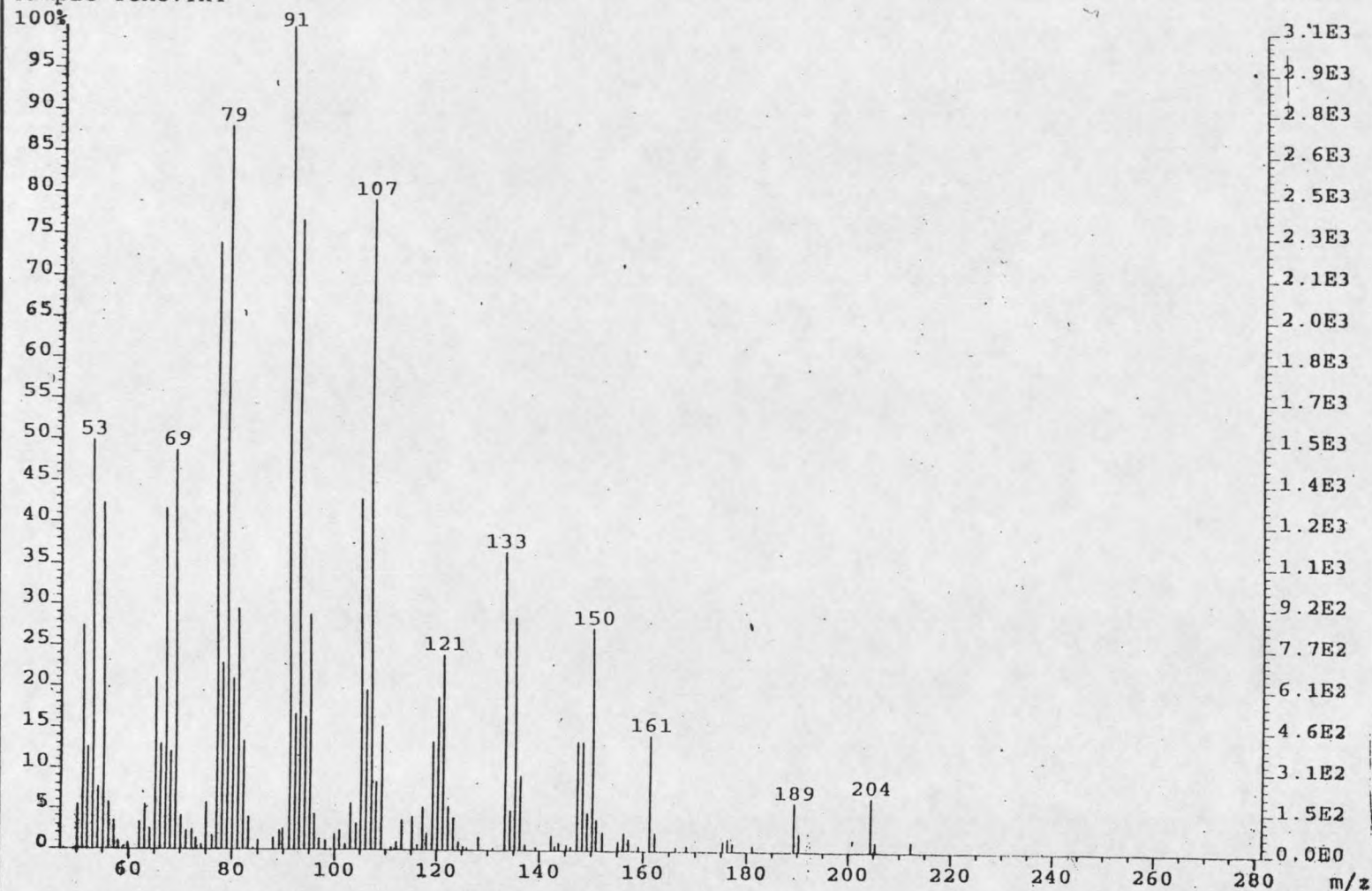
Rank:1 NIST #9822 Purity:885 Mixture:901 Matched:6 MF:C10H14O MW:150 CAS:529-01-1 NIST:21438  
Name:2-Cyclohexen-1-one, 3-methyl-6-(1-methylethenyl)- | p-Mentha-1,8-dien-3-one | Isopiperiten>



Rank:2 NIST #9860 Purity:881 Mixture:881 Matched:7 MF:C10H14O MW:150 CAS:16750-82-6 NIST:4627  
Name:2-Cyclohexen-1-one, 3-methyl-6-(1-methylethenyl)-, (S)- | p-Mentha-1,8-dien-3-one, (+)- | >



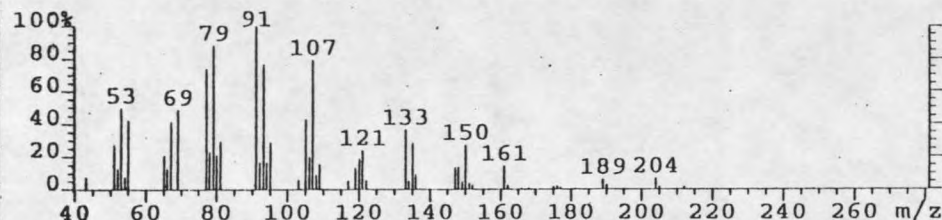
File:17OCT97A Ident:1834 1840-1862 1880 Mer Def 0.25 S:27 Acq:18-OCT-1997 23:52:42 +24:38 Cal:1  
70S EI+ Magnet BpM:91 BpI:3067 TIC:39909 Flags:HALL  
Sample Text:TX4



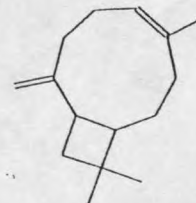
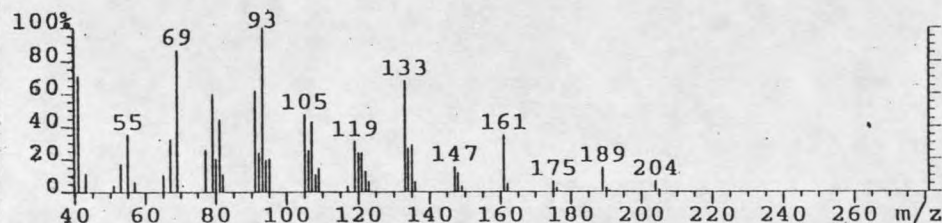
Libraries 1: NIST (62235) 2: NISTREP (12593)

|          |   |    |     |     |      |      |       |       |       |
|----------|---|----|-----|-----|------|------|-------|-------|-------|
| Peaks:   | 8 | 7  | 6   | 5   | 4    | 3    | 2     | 1     | 0     |
| Matches: | 5 | 42 | 228 | 714 | 1862 | 4088 | 8727  | 19804 | 39358 |
| Total:   | 5 | 47 | 275 | 989 | 2851 | 6939 | 15666 | 35470 | 74828 |

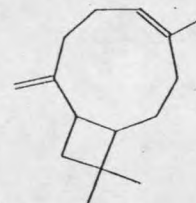
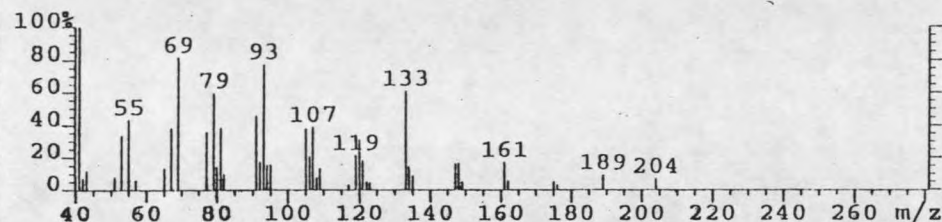
Normal Search: 42 of 47 Rank: Purity Mass Weighting: On Presearch Minimum: 20  
File:17OCT97A Ident:1834\_1840-1862\_1880 Mer Def 0.25 S:27 Acquired:17-OCT-1997 15:52:42 +24:38 >  
70S EI+ Function:Magnet TIC:39909



Rank:1 NIST #23962 Purity:711 Mixture:738 Matched:7 MF:C15H24 MW:204 CAS:13877-93-5 NIST:9205  
Name:Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene- | Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-

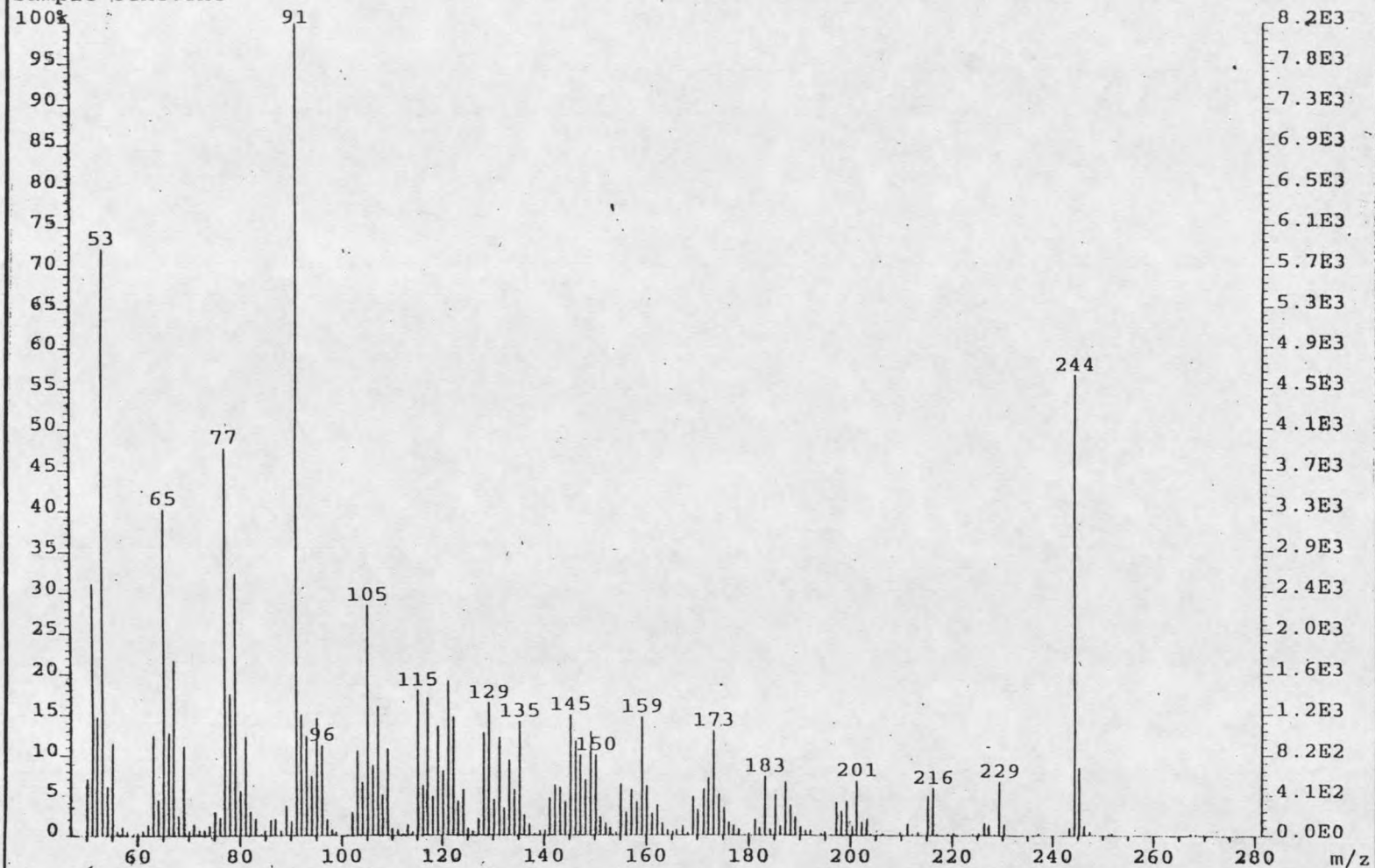


Rank:2 NISTREP #7699 Purity:687 Mixture:715 Matched:7 MF:C15H24 MW:204 CAS:87-44-5 NIST:121982  
Name:Caryophyllene SMILES:CC2=CCCC(=C)C1CC(C)(C)C1CC2





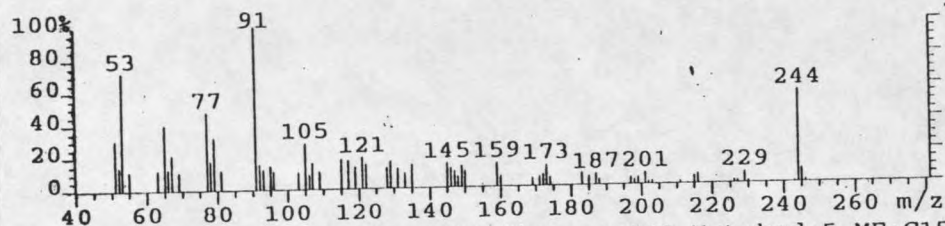
File:17OCT97A Ident:3158 3169-3128 3140 Mer Def 0.25 S:27 Acq:18-OCT-1997 23:52:42 +40:16 Cal:1>  
70S EI+ Magnet BpM:91 BpI:8159 TIC:98220 Flags:HALL  
Sample Text:TX4



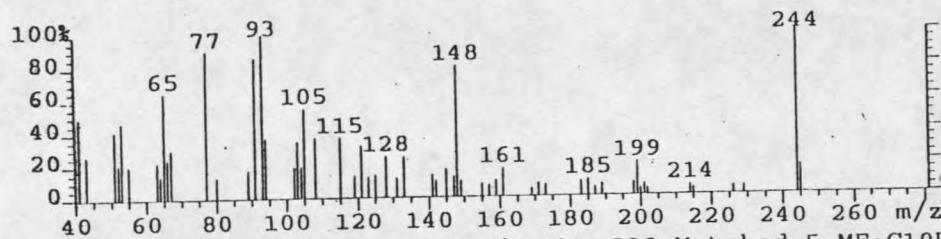
Libraries 1: NIST (62235) 2: NISTREP (12593)

|          |   |   |   |     |      |      |       |       |       |
|----------|---|---|---|-----|------|------|-------|-------|-------|
| Peaks:   | 8 | 7 | 6 | 5   | 4    | 3    | 2     | 1     | 0     |
| Matches: | 0 | 0 | 1 | 134 | 933  | 3166 | 8804  | 17015 | 44775 |
| Total:   | 0 | 0 | 1 | 135 | 1068 | 4234 | 13038 | 30053 | 74828 |

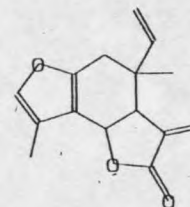
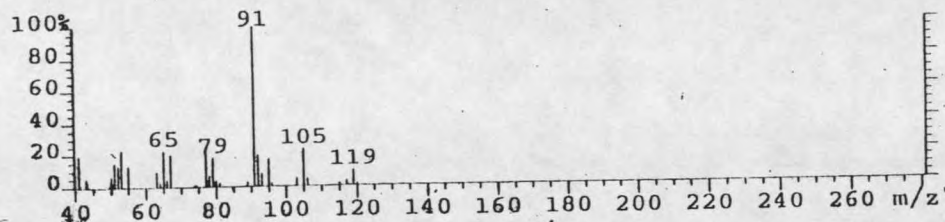
Normal Search: 39 of 135 Rank: Purity Mass Weighting: On Presearch Minimum: 20  
File: 17OCT97A Ident: 3158\_3169-3128\_3140 Mer Def 0.25 S: 27 Acquired: 17-OCT-1997 15:52:42 +40:16 >  
70S EI+ Function: Magnet TIC: 98220



Rank: 1 NIST #32760 Purity: 564 Mixture: 648 Matched: 5 MF: C<sub>15</sub>H<sub>16</sub>O<sub>3</sub> MW: 244 CAS: 957-66-4 NIST: 11813  
Name: Benzo[1,2-b:3,4-b']difuran-2(3H)-one, 4-ethenyl-3a,4,5,8b-tetrahydro-4,8-dimethyl-3-methyl:

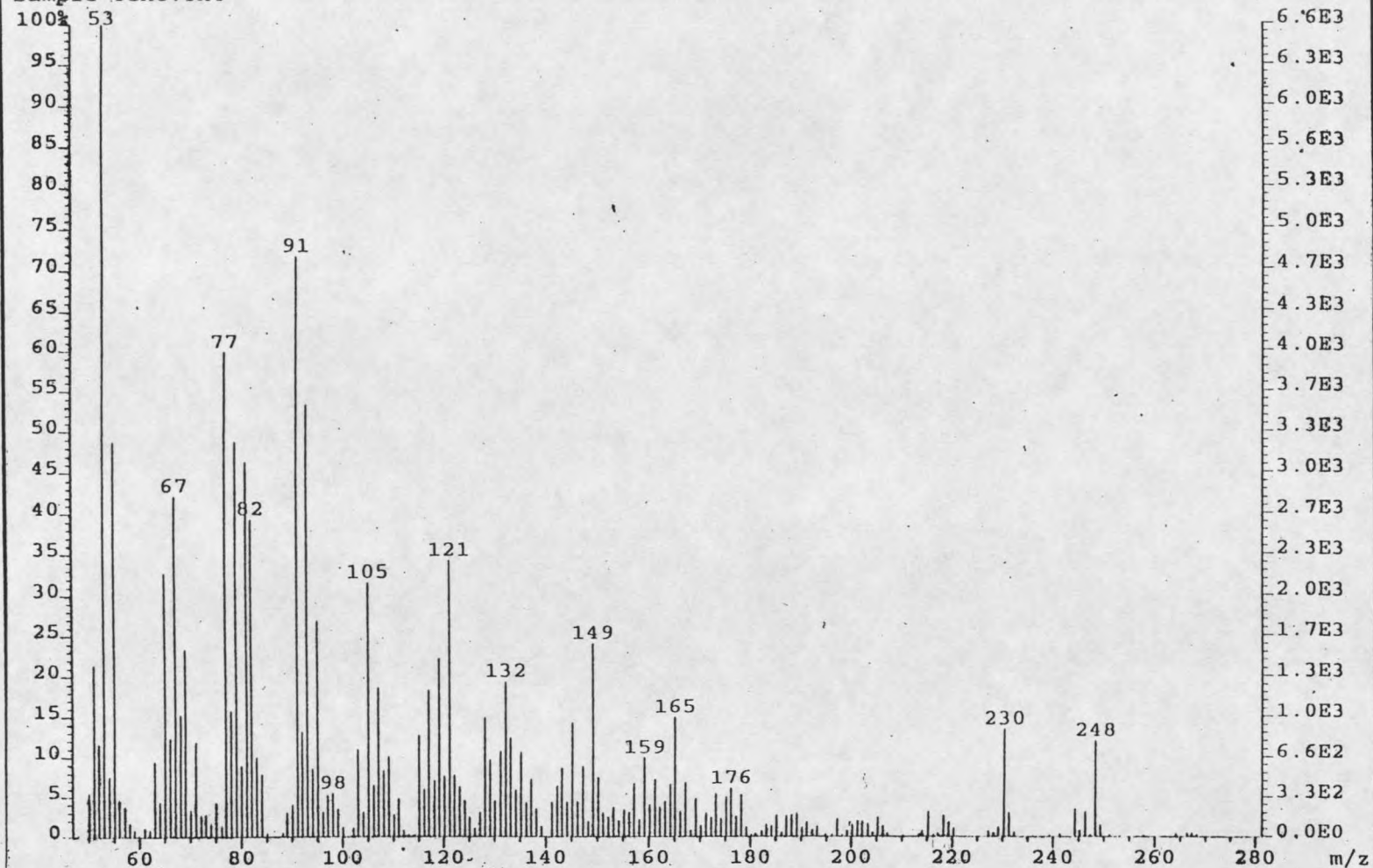


Rank: 2 NIST #6199 Purity: 501 Mixture: 829 Matched: 5 MF: C<sub>10</sub>H<sub>14</sub> MW: 134 CAS: 53963-03-4 NIST: 114825  
Name: 1,5-Decadiyne SMILES: CCCCC#CCCC#C





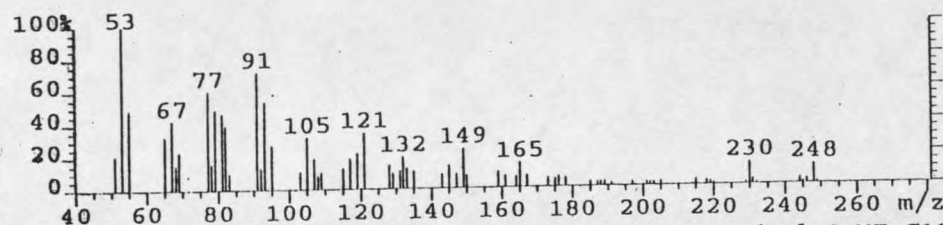
File:17OCT97A Ident:3212 3218-3180 3198 Mer Def 0.25 S:27 Acq:18-OCT-1997 23:52:42 +40:52 Cal:1>  
70S EI+ Magnet BpM:53 BpI:6645 TIC:97788 Flags:HALL  
Sample Text:TX4



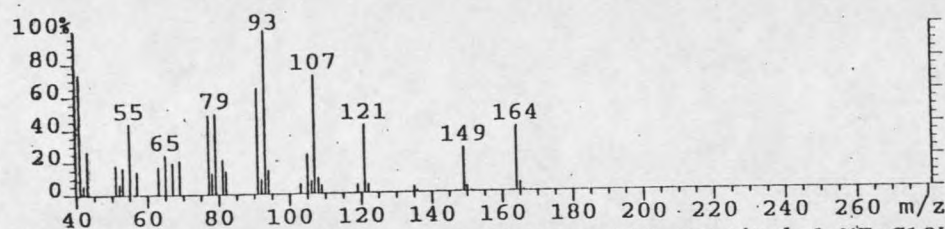
Libraries 1: NIST (62235) 2: NISTREP (12593)

|          |   |   |    |     |      |      |       |       |       |
|----------|---|---|----|-----|------|------|-------|-------|-------|
| Peaks:   | 8 | 7 | 6  | 5   | 4    | 3    | 2     | 1     | 0     |
| Matches: | 0 | 0 | 60 | 270 | 1268 | 3473 | 8990  | 17406 | 43361 |
| Total:   | 0 | 0 | 60 | 330 | 1598 | 5071 | 14061 | 31467 | 74828 |

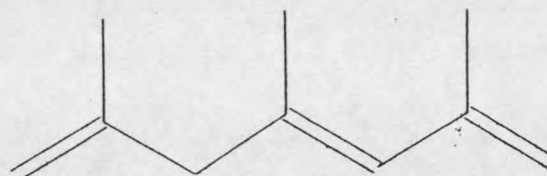
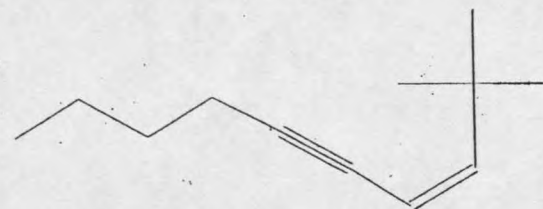
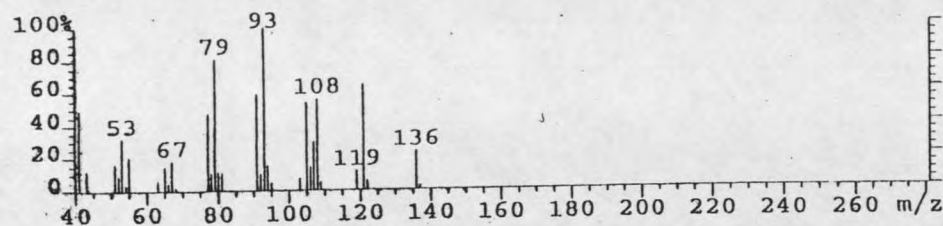
Normal Search: 43 of 60 Rank: Purity Mass Weighting: On Presearch Minimum: 20  
File: 17OCT97A Ident: 3212\_3218-3180\_3198 Mer Def 0.25 S: 27 Acquired: 17-OCT-1997 15:52:42 +40:52  
70S EI+ Function: Magnet TIC: 97788



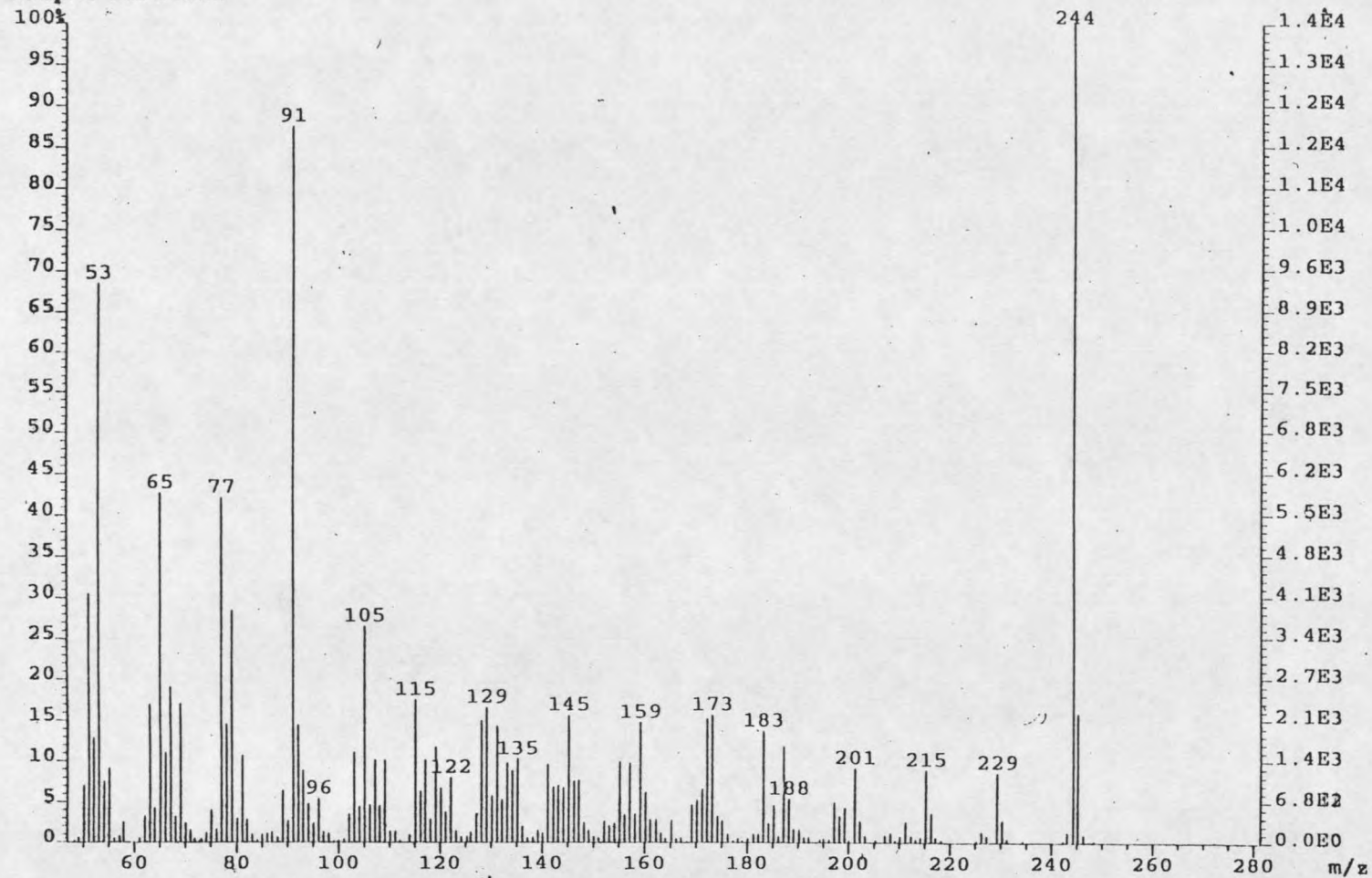
Rank: 1 NIST #13607 Purity: 490 Mixture: 568 Matched: 6 MF: C<sub>12</sub>H<sub>20</sub> MW: 164 CAS: 55638-49-8 NIST: 26697  
Name: 3-Decen-5-yne, 2,2-dimethyl-, (Z)- SMILES: CCCC#CC=CC(C)(C)C



Rank: 2 NIST #6600 Purity: 468 Mixture: 591 Matched: 6 MF: C<sub>10</sub>H<sub>16</sub> MW: 136 NIST: 113093  
Name: 2,4,6-Trimethyl-1,3,6-heptatriene SMILES: CC(=C)CC(C)=CC(C)=C



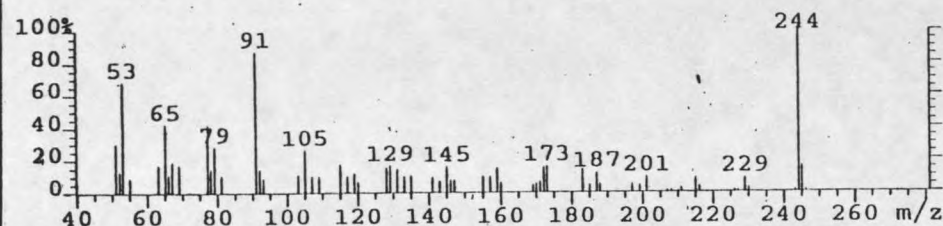
File:17OCT97A Ident:3225\_3230-3259\_3276 Mer Def 0.25 S:27 Acq:18-OCT-1997 23:52:42 +41:01 Cal:1>  
70S EI+ Magnet BpM:244 BpI:13699 TIC:161931 Flags:HALL  
Sample Text:TX4



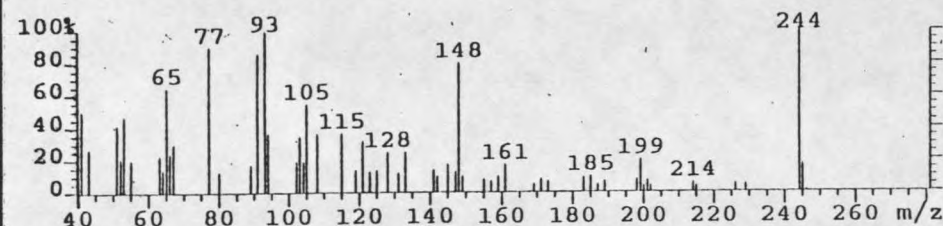
Libraries 1: NIST (62235) 2: NISTREP (12593)

|          |   |   |   |    |     |      |       |       |       |
|----------|---|---|---|----|-----|------|-------|-------|-------|
| Peaks:   | 8 | 7 | 6 | 5  | 4   | 3    | 2     | 1     | 0     |
| Matches: | 0 | 0 | 0 | 11 | 273 | 2296 | 8004  | 16324 | 47920 |
| Total:   | 0 | 0 | 0 | 11 | 284 | 2580 | 10584 | 26908 | 74828 |

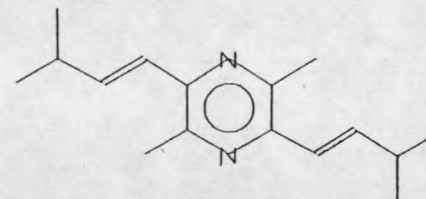
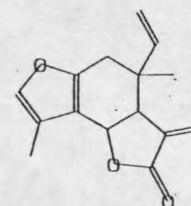
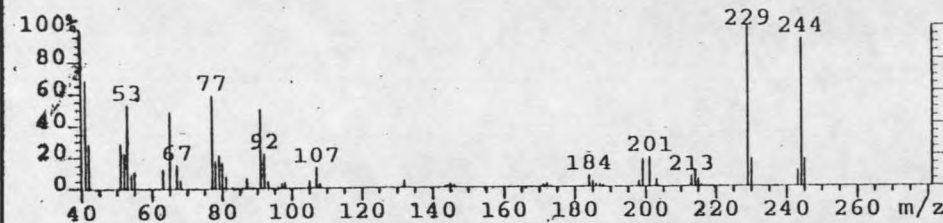
Normal Search: 21 of 284 Rank: Purity Mass Weighting: On Presearch Minimum: 20  
File:17OCT97A Ident:3225\_3230-3259\_3276 Mer Def 0.25 S:27 Acquired:17-OCT-1997 15:52:42 +41:01  
70S EI+ Function:Magnet TIC:161931



Rank:1 NIST #32760 Purity:580 Mixture:620 Matched:5 MF:C15H16O3 MW:244 CAS:957-66-4 NIST:11813  
Name:Benzo[1,2-b:3,4-b']difuran-2(3H)-one, 4-ethenyl-3a,4,5,8b-tetrahydro-4,8-dimethyl-3-methyl:



Rank:2 NIST #32791 Purity:509 Mixture:563 Matched:4 MF:C16H24N2 MW:244 NIST:115601  
Name:Pyrazine, 2,5-dimethyl-3,6-bis(3-methyl-1-butenyl) - SMILES:CC(C)C=Cc1nc(C)c(C=CC(C)C)nc1C



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