Native American medicinal plants, chemical constituents of Osmorhiza chilensis and Clematis hirsutissima
by John Robert Kern

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Chemistry
Montana State University

Abstract:
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One compound, anemonin, 25, was isolated and characterized from Chematis hirsutissima.

[Chemical Diagrams not captured by OCR]

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To my parents

NATIVE AMERICAN MEDICINAL PLANTS. CHEMICAL CONSTITUENTS OF *OSMORHIZA CHILENSIS* AND *CLEMATIS HIRSUTISSIMA*

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JOHN ROBERT KERN

A thesis submitted in partial fulfillment
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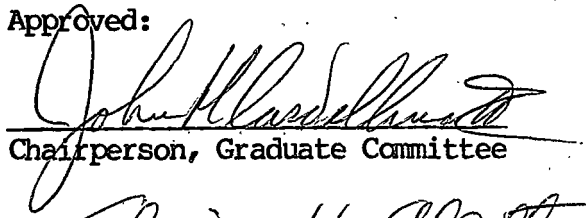
Approved:



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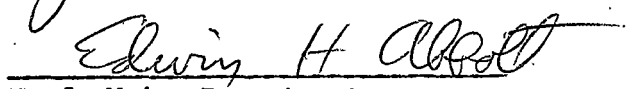

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Bozeman, Montana

June, 1982

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ABSTRACT

This thesis comprises an investigation into the chemical constituents of Osmorhiza chilensis (Mountain Sweet Cicely), a plant used as a medicinal herb, and Clematis hirsutissima (Sugar Bowls), employed as a horse restorative and medicinal herb by Native Americans of the Northwest Rocky Mountains.

Compounds isolated and characterized from Osmorhiza chilensis are: anethole, **20**, estragole, **21**, 3,4-dimethoxy eugenol, **22**, falcarindiol, **4**, and 3-O-methyl falcardiniol **18**.

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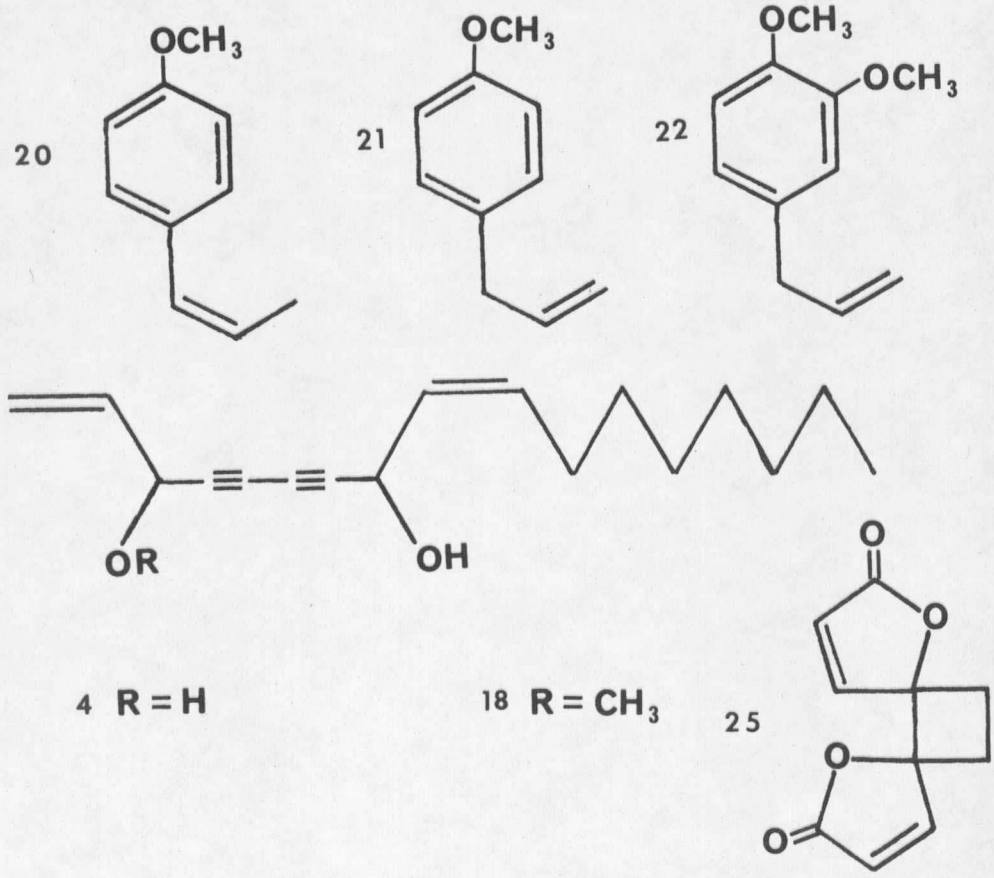


PART I

Osmorhiza chilensis

INTRODUCTION

1. Historical

Osmorhiza chilensis (Mountain Sweet Cicely), a small perennial herb indigenous to the temperate and mountainous regions of the Northern Hemisphere, was used by Flathead (1), Crow (2), Blackfoot (3) and Thompson (4) Indian nations for the treatment of colds, pneumonia, sore throats, toothaches and stomachaches. The roots of *Osmorhiza chilensis* were usually brewed into a tea which, when imbibed, provided soothing relief for these maladies.

Osmorhiza chilensis is a member of the family Umbelliferae, probably the first family of flowering plants to receive general recognition (5). This family is widely represented in the pharmacopoeias of many European, Asian and North American cultures. With the renewed interest today in the use of herbal medicines, a wide variety of umbelliferous plants are still in use as medicinal agents. Umbelliferous plants are aromatic plants, that is, almost all of them exhibit distinctive odors and flavors. Many umbelliferous plants are used as spices and herbs in cooking (see Table 1). A few, however, are poisonous - the most famous is *Conium maculatum* (Poison Hemlock), which, according to legend, caused the death of Socrates.

Table 1. Common Umbelliferous Plants and Their Uses. (6)

Species	Uses
Carrots (*Daucus carota* L.)	Food, Flavoring, Medicinal: Diuretic, Excitant, Stimulant. Jaundice, Drospy.
Parsnips (*Pastinaca sativa* L.)	Food, Medicinal: Tonic and Carminative.
Celery (*Apium graveolens* L.)	Food, Flavoring, Medicinal: Gout, Sciatic Pain, Diuretic, Sedative.
Caraway (*Carum carvi* L.)	Flavoring, Medicinal: Stimulant, Stomachic, Carminative, Diuretic, Scabies.
Parsley (*Petroselinum crispum* Miller)	Flavoring, Medicinal: Diuretic, Stimulant, Carminative.
Chervil (*Anthriscus cerefolium* L.)	Flavoring, Medicinal: Diuretic, Depurative (e.g., for cancer).
Fennel (*Foeniculum vulgare* Miller)	Flavoring, Medicinal: Stimulant, Tonic, Stomachic, Carminative.
Dill (*Anethum garveolens* L.)	Flavoring, Medicinal: Carminative, Diuretic, Stimulant.
Coriander (*Coriandrum sativium* L.)	Flavoring (Soap and Perfume), Medicinal: Carminative, Diuretic, Aphrodisiac, Nervous Disorders.

Table 1. Continued

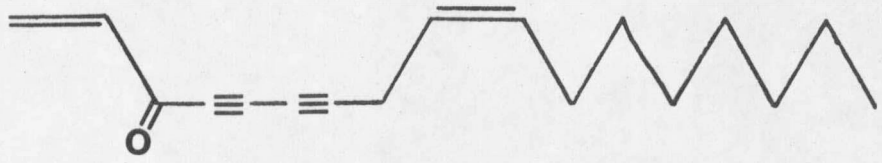
Cumin (*Cuminum cyminum* L.)	Flavoring, Medicinal: Antispasmotic, Astringent, Stimulant, Carminative, Stomachic, Diuretic, Emmenagogue.
Anise (*Pimpinella anisum* L.)	Flavoring, Medicinal: Aromatic, Stimulant, Carminative, Diaphoretic, Stimulate Secretion of milk.
Dog Parsley (*Aethusa cynapium* L.) (Dog Poison)	Medicinal: G. I. Tract Problems, Convulsions, Sedative.
Poison Hemlock (*Conium maculatum* L.)	Medicinal: Carminative, Diuretic, Aphrodisiac, Nervous Disorders.
Cowbane (*Cicuta virosa* L.)	Medicinal: Diuretic, Carminative.
Hemlock Water (*Oenanthe crocata* L.) Dropwort	Medicinal: Epilespy, Stupefying Fish.

2. Chemistry of Umbelliferous Plants

Umbelliferous plants, throughout the course of some 200 years of chemical investigations, have exhibited a wide variety of chemical constituents. The "aromaticity" of certain umbellifers is reflected in their essential oils. These essential oils contain monoterpenes, sesquiterpenes and phenylpropanoids. Another large class of natural products found in umbelliferous plants are coumarins. Coumarins have been isolated from over 160 umbellifer species. Approximately 125 of the 200 known coumarins have been isolated and characterized from umbelliferous plants (7). Triterpenes and saponins comprise another large class of compounds found in this family. Other important classes of compounds found in umbelliferous plants include the fatty seed oils, polyols, oligosaccharides, phenylpropanoids and flavonoids.

Naturally occurring acetylenes also represent a significant class of compounds in umbelliferous plants. Of the approximately 650 naturally occurring acetylenes (isolated from 15 different families of higher plants, as well as from algae and microorganisms), approximately 80 have been isolated from different species of the Umbelliferae (8).

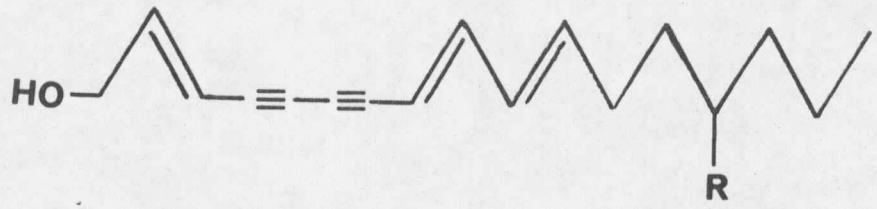
The majority of acetylenes isolated from umbelliferous plants are the C_{17}-chain compounds related to falcarinone **1**, isolated by Bohlmann (9) from Falcaria vulgaris in 1961. A limited number of C_{13}- and C_{15}-chain acetylenes have been isolated from several species (10).

1 

The first acetylenes isolated from umbelliferous plants were the toxic principals of Oenanthe crocata (hemlock water dropwort), oenanthotoxin **2a**, and Cicuta virosa (cowbane; water hemlock), cicutoxin **3a**, (11). Both these compounds exhibit similar pharmacological action, causing violent convulsions and death.

Both cicutoxin **3a** and oenanthotoxin **2a** are accompanied in the plant by the closely related cicutol **3b**, and oenanthotol **2a**, (11), both monoalcohols). It is of interest to note that both these monoalcohols exhibit no toxicity.

The majority of polyacetylenes which have been isolated are very unstable compounds. Usually colorless oils, they tend to decompose in



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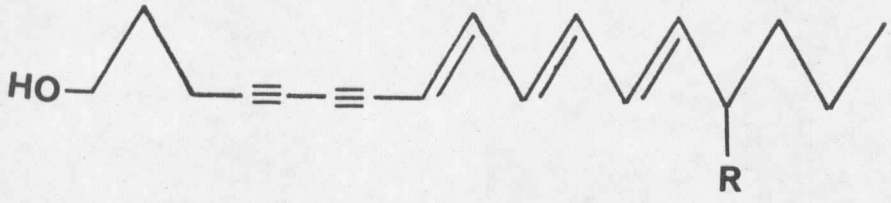


3a R = OH 3b R = H

air and are thermally unstable as well. Many are very difficult, if not impossible, to obtain in crystalline form.

The majority of research on naturally occurring acetylenes is due, in large part, to Sir E. R. H. Jones' group at Manchester and F. Bohlmann's group at Berlin.

3. Research Objectives

The initial objective of this thesis project was to identify and characterize chemical constituents from Native American medicinal plants. The plants were chosen on the basis of their medicinal properties as cited in the literature and a lack of thorough previous chemical studies on that particular species. The second objective was to screen extracts of the plants for anticancer and antimicrobial properties and, as individual metabolites were identified, to screen these also.

Osmorhiza chilensis was targeted because of these considerations: 1) it has reported medicinal properties and 2) a lack of thorough chemical studies on the genus Osmorhiza was indicated.

During the course of the project a known antifungal metabolite, falcarindiol, 4, was isolated. A study by Kemp (12) indicated that both alcohol moieties of falcarindiol were necessary for antifungal activity. Studies were then undertaken to determine the absolute configuration of the molecule. If the absolute configuration could be

established, other researchers might utilize that data to design a more stable antifungal compound.

Another objective undertaken, after work began, was to determine the amount of falcarindiol at various growth stages of the plant. The final objective was to characterize an apparently new compound isolated from the extracts Osmorhiza chilensis, 3-O-methylfalcarindiol.

RESULTS AND DISCUSSION

1. Falcarindiol

Isolation. A compound which exhibited interesting or unusual ^{1}H-NMR signals (Figure 1) was isolated from fraction 5 of the Florisil chromatography. Fraction 5 has been eluted with ethyl acetate-hexane (1:4), indicating that the components were moderately polar. The compound was then purified using silica gel chromatography and, finally, gel permeation chromatography with Sephadex LH-20. In both methods, the compound was the last fraction eluted from the column, indicating a fairly polar compound. The compound was ultimately obtained as a nearly colorless oil which comprised 9.4% of the total extract.

Characterization. The IR spectrum of this compound (Figure 2) exhibited a broad OH stretch at 3300 cm^{-1}, olefinic and aliphatic C-H stretches at 2950 and 2880 cm^{-1}, respectively, weak absorptions at 2150 and 2050 cm^{-1} indicating the possibility of acetylenic functionalities, a weak C=C band at 1630 cm^{-1}, 2 bands at 990 and 940 cm^{-1} indicative of a terminal vinylic moiety and a band at 740 cm^{-1} indicative of a *cis* double bond. The UV spectrum (Figure 3) of the compound exhibited 3 major bands with low molar extinction coefficients. This pattern suggested that the compound contained conjugated triple bonds. The molar extinction coefficients were

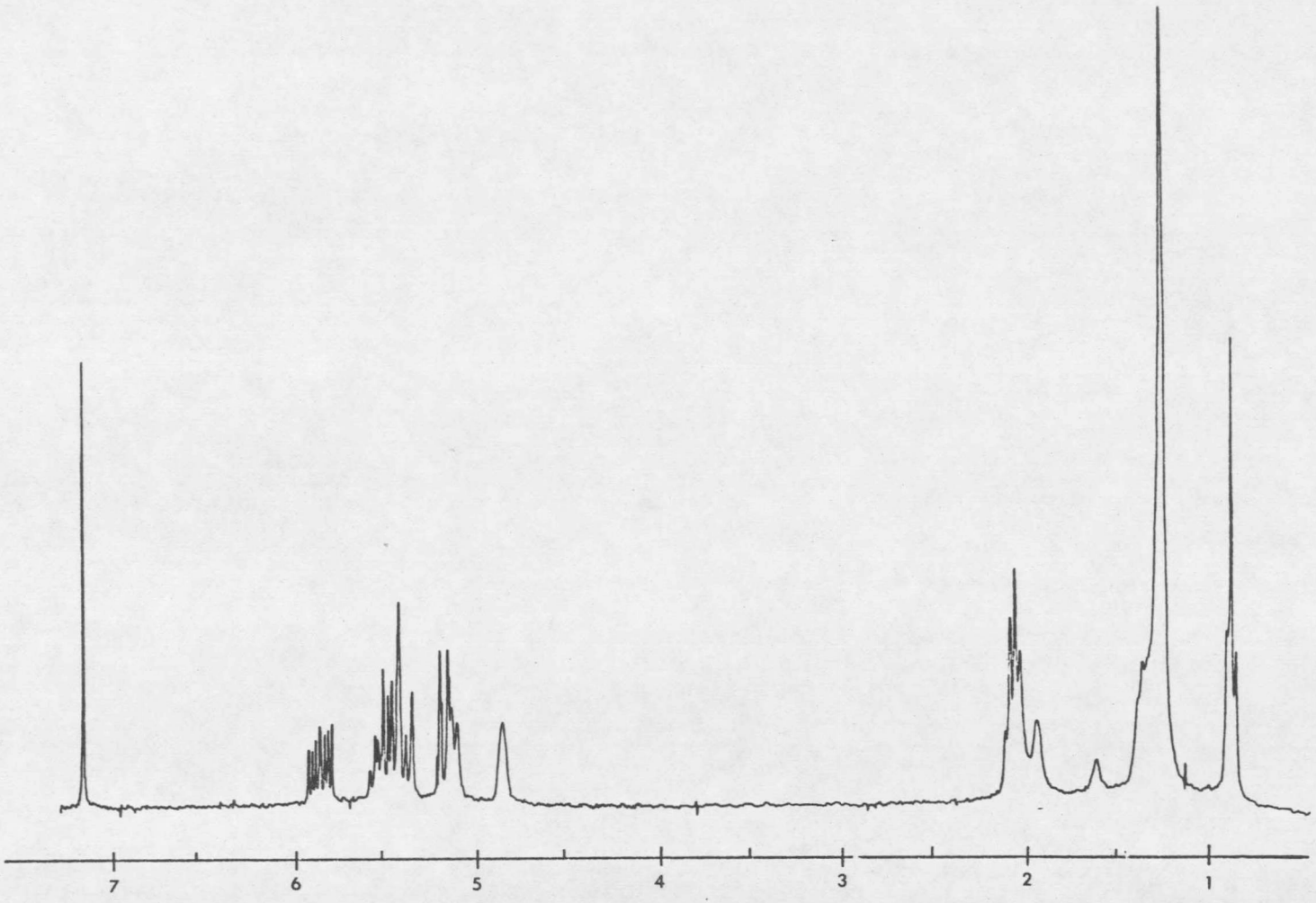


Figure 1. ^{1}H-NMR Spectrum of Falcarindiol.

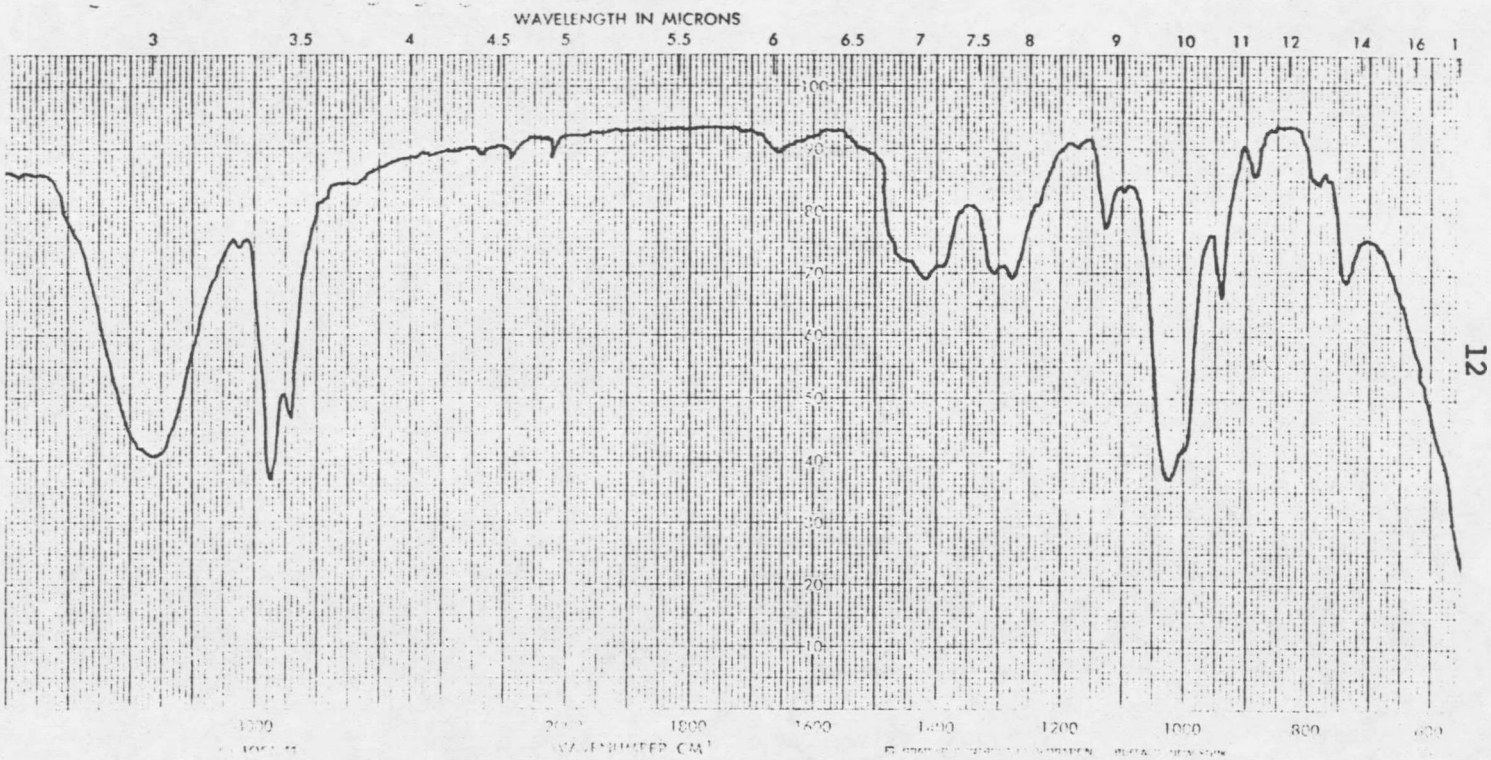


Figure 2. IR Spectrum of Falcarindiol.

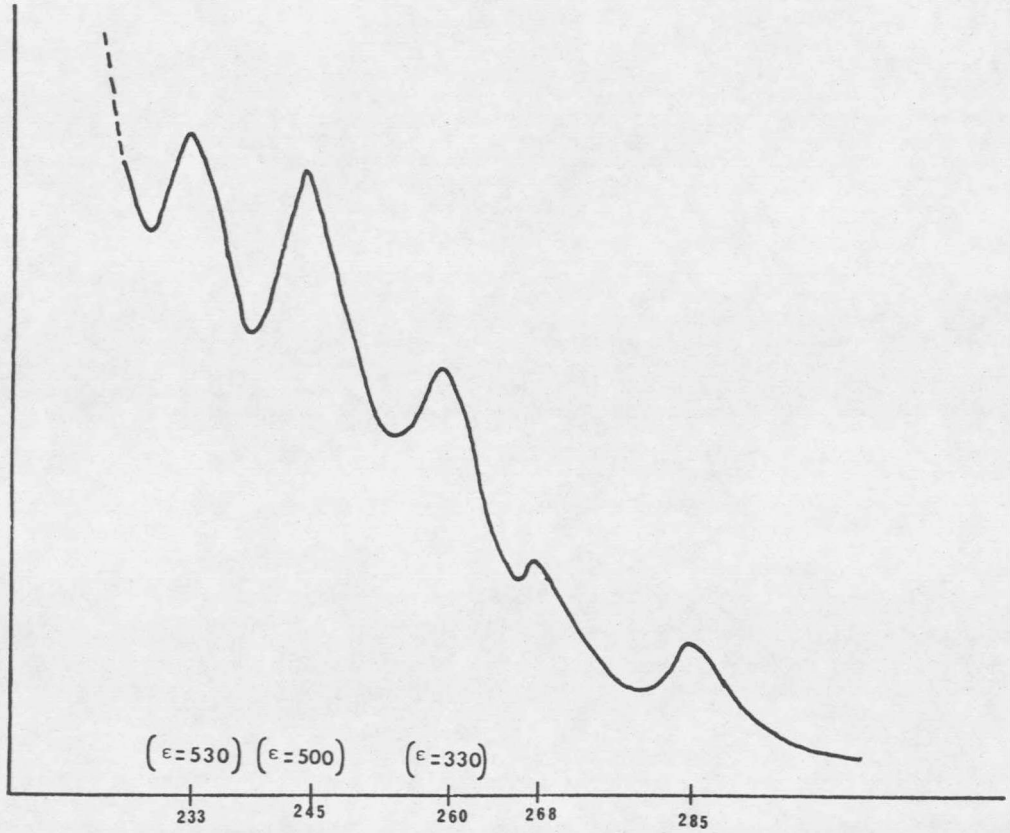
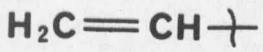


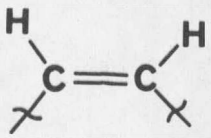
Figure 3. UV Spectrum of Falcarindiol.

calculated after the compound was identified.

The ^{1}H-NMR spectrum (Figure 1) of the compound exhibited a variety of signals in the olefinic region (δ 6.0-4.5), a multiplet at δ 2.1, a methylene envelope at δ 1.2 and a triplet at δ 0.9. Integration of the ^{1}H-NMR spectrum indicated the presence of seven hydrogens in the olefinic region. Supporting evidence from the IR spectrum indicated that: 1) the compound has terminal vinylic double bond (part structure **4a**) and 2) that the possibility of a <u>cis</u> double bond may exist (part structure **4b**). This would account for 5 of the 7



4a

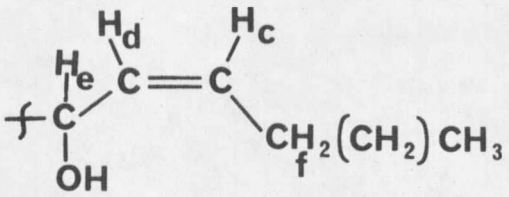


4b

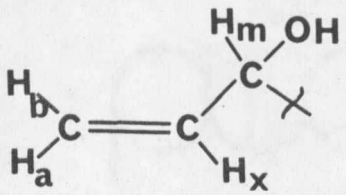
hydrogens in the olefinic region, leaving 2 hydrogens to be assigned. That the compound contained a hydroxyl group(s) was demonstrated by the IR band at 3300 cm^{-1}. The compound was acetylated to determine

the number of hydroxyl groups present in the molecule. The ^{1}H-NMR spectrum (Figure 4) of **5**, the acetylation product, exhibited 2 singlets at δ2.08 and 2.06, indicating that the parent compound contained 2 hydroxyl groups. A substantial downfield shift of 2 distinct doublets in the olefinic region, from δ4.8 and δ5.2 to δ5.8 and δ6.1, respectively, indicated that these were the two remaining protons unaccounted for and that they were on carbons bearing the hydroxyl moieties. At this time ^{1}H-NMR decoupling experiments were undertaken on the acetylated product and the parent compound. From these studies and supporting evidence, (IR, acetylation), part structures **4c** and **4d**, were proposed.

4c



4d



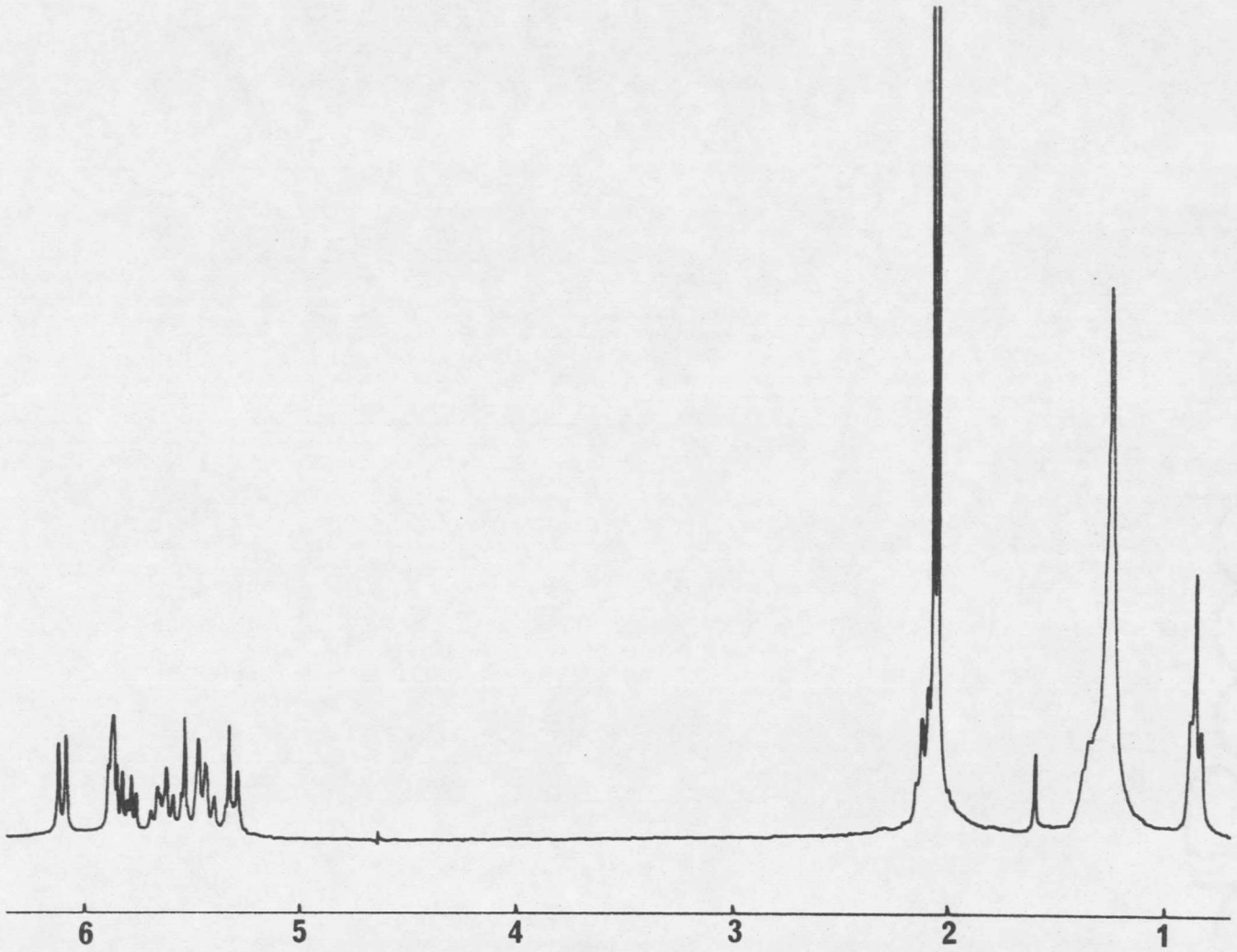


Figure 4. ^{1}H-NMR Spectrum of Acetylated Falcarindiol.

Further evidence for these part structures was ascertained by closer examination of the signals in the olefinic region (see Figure 5). An apparent ABMX pattern was observed for the protons of **4c**. The signal for Hx appeared as a doublet of doublet of doublets at δ5.80. Observed were <u>trans</u> (J_{BX} = 17 Hz), <u>cis</u> (J_{AX} = 10.8 Hz), and vicinal (J_{MX} = 7.7 Hz) couplings. The signal for Ha appeared as a doublet of doublets at δ5.25. Here <u>cis</u> (J_{AX} = 10.9 Hz) and geminal (J_{AB} = 4.5 Hz) couplings were observed. The signal for H_B appeared as a doublet of doublets at δ5.45. Observed were <u>trans</u> (J_{BX} = 17 Hz) and geminal (J_{BA} = 4.5 Hz) couplings. The signal for H_m appeared as a broad doublet at δ4.8. Here vicinal (J_{MX} = 7.7 Hz) coupling was observed.

For part structure **4d**, a doublet of triplets was observed at δ5.6 for H_C, (J = 10.8,6 Hz), a doublet of doublets at δ5.5 for H_D, (J = 10.8,8.5 Hz) and for the methine proton H_E, a doublet at δ5.15 (J = 8.5 Hz). The coupling constant of 10.8 Hz between the two olefinic protons corroborated the assignment of <u>cis</u> configuration.

A multiplet at δ2.05, partially overlapped by a broad OH singlet, integrated for the 2 hydrogens of a methylene adjacent to an olefin. Irradiation of this signal (H_F) simplified H_C to a doublet. The other hydroxyl moiety exhibited a broad singlet at δ1.6. A large methylene envelope at δ1.2 integrated for 10 hydrogens, indicating a chain of 5 methylene groups. A triplet at δ0.9 integrated to 3 hydrogens,

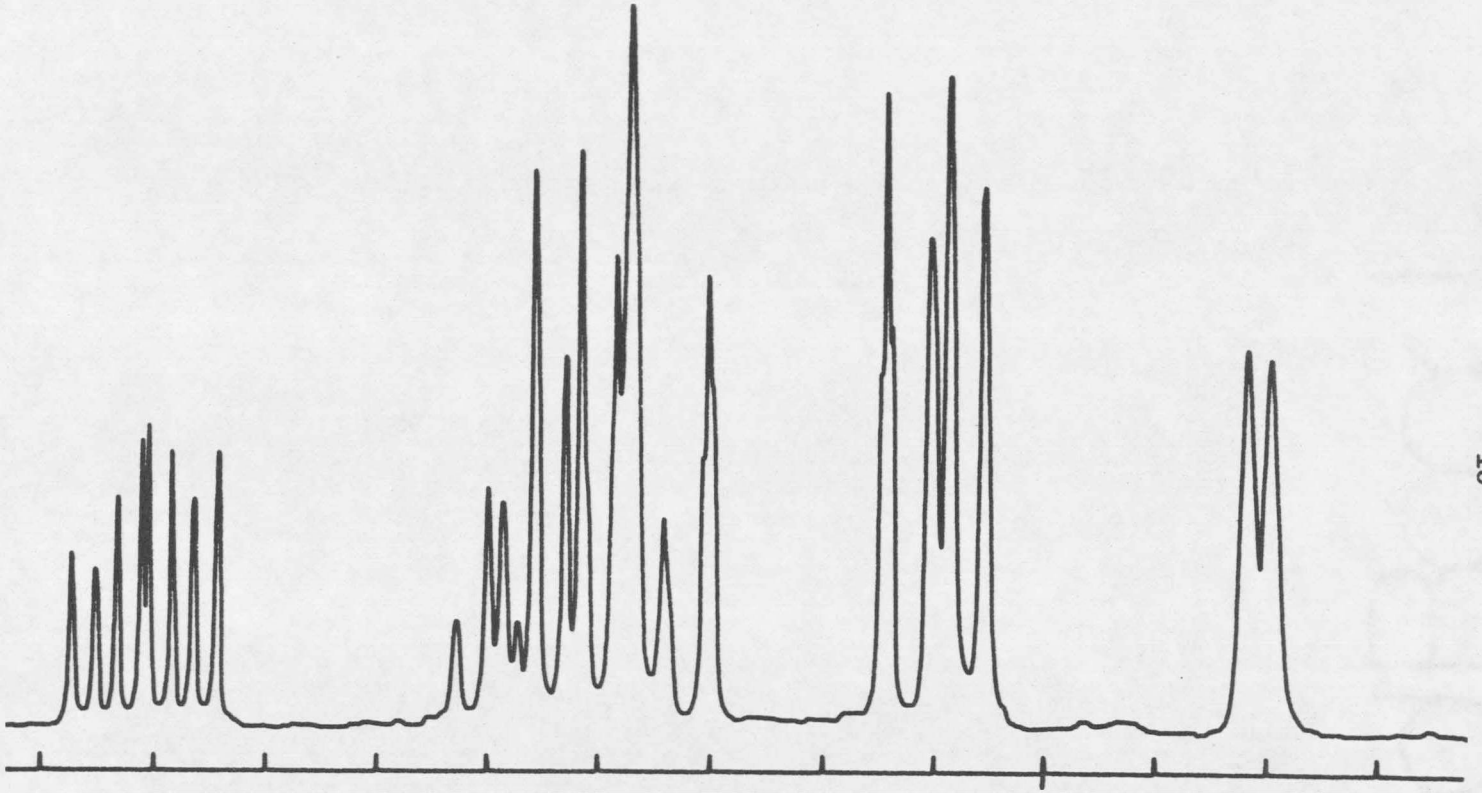
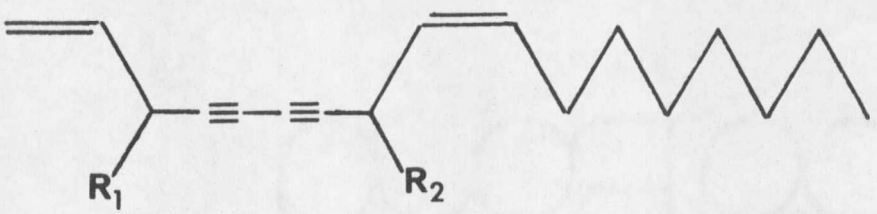


Figure 5. ^{1}H-NMR Spectrum of Olefinic Region of Falcarindiol.

indicating that this compound contained only 1 terminal methyl group.

The ^{13}C-NMR spectrum (Figure 6) exhibited the expected chemical shifts for part structures **4c** and **4d**. Signals were observed for the four olefinic carbons, the 2 methine carbons bearing hydroxyl groups, 6 methylene carbons and one methyl carbon. In addition, four singlets were observed between δ80 and δ67. These signals were assigned as the four carbons of a conjugated triple bond system. The only possibility of placing conjugated triple bonds was between the part structures **4c** and **4d**. In doing so the structure **4** was generated. Assignment of the chemical shifts for each carbon appears in Table 2.

To verify the assignment of the 2 alkyne linkages the parent compound (assigned structure **4**), was catalytically hydrogenated (5% Pd



4 $R_1 = R_2 = OH$ **5** $R_1 = R_2 = O\,Ac$

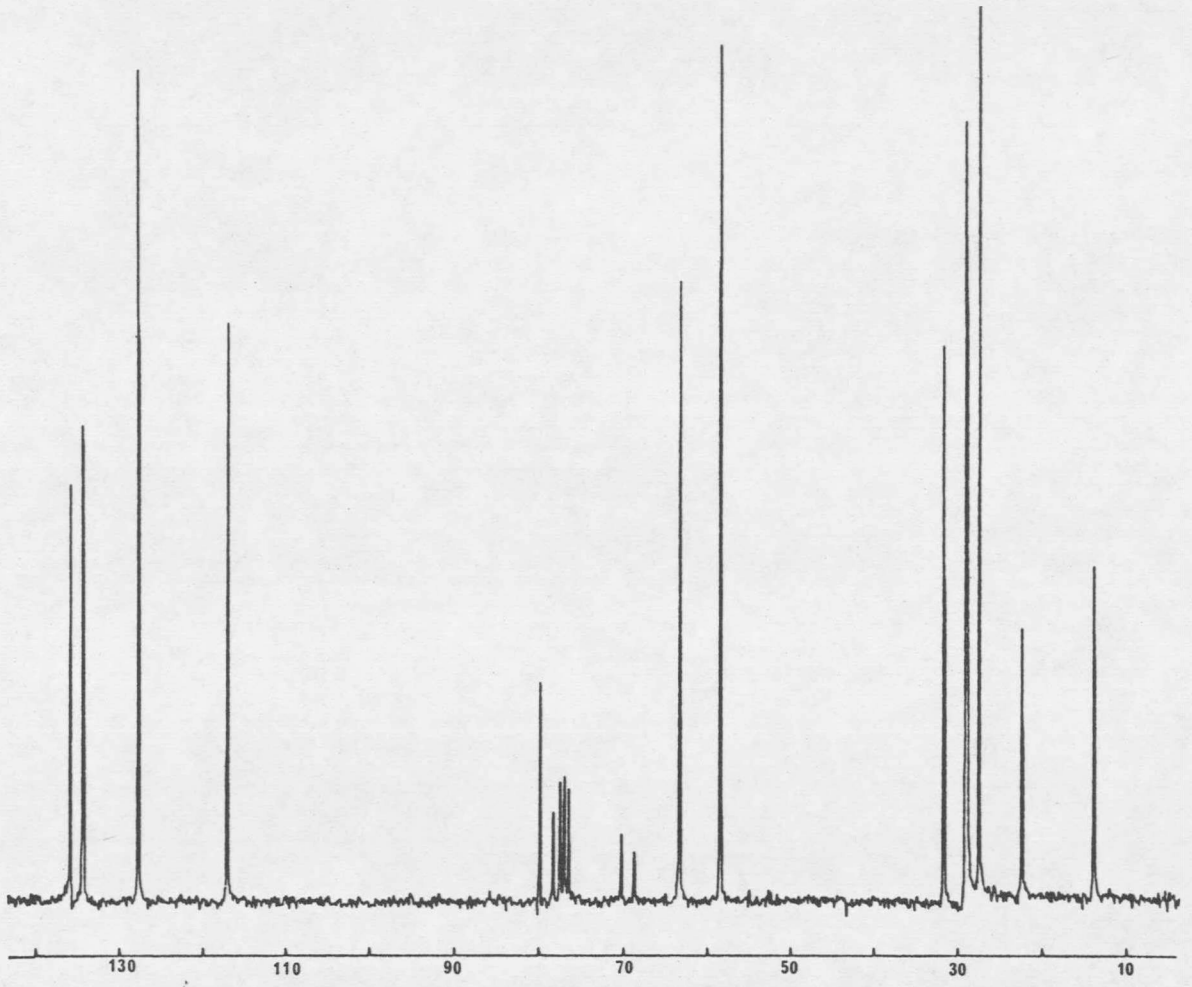


Figure 6. ^{13}C-NMR Spectrum of Falcarindiol.

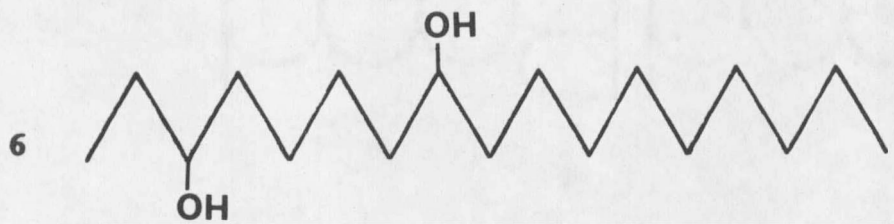
Table 2. ^{13}C-NMR Chemical Shift Assignments, Falcarindiol and 3-O-methylfalcarindiol.

Carbon No.	Falcarindiol 4	3-O-methylfalcarindiol 18
1	117.0, t	118.5, t
2	135.8, d	133.9, d
3	63.2, d	71.8, d
4	78.2, s[c]	76.4, s[c]
5	70.1, s[c]	71.3, s[c]
6	68.6, s[c]	68.7, s[c]
7	79.8, s[c]	79.3, s[c]
8	58.4, d	58.5, d
9	134.3, d	134.5, d
10	127.6, d	127.8, d
11	28.9, t[a]	28.9, t[b]
12	27.5, t[a]	28.9, t[b]
13	27.5, t[a]	27.5, t[b]
14	29.1, t[a]	29.1, t[b]
15	31.6, t	31.6, t
16	22.5, t	22.4, t
17	13.9, q	13.9, q
18	-	55.6, q

[a]Assignments interchangeable; [b]assignments interchangeable; [c]based on the assignment of Hearn (13).

on charcoal). Filtration of the reaction mixture and evaporation for the solvent yielded the dodecahydro-derivative, **6**, as white crystals, m.p. 90-1°C.

The ^{13}C-NMR spectrum of **6** exhibited signals at δ71.8 and δ73.1 for the 2 methine carbons bearing hydroxyl groups, signals for 13 methylene carbons and 2 methyl carbons. The absence of the other peaks between δ80 and δ67 in the ^{13}C-NMR spectrum of **6** did indeed provide supporting evidence for the presence of the acetylenic linkages. Combustion analysis of **6** indicated the molecular formula $C_{17}H_{36}O_2$ and permitted inferral of $C_{17}H_{24}O_2$ for **4**.



6

Compound **6**, 3,8-heptadecadiol, was oxidized using Jones reagent (14) to the corresponding diketone, **7**, colorless crystals, m.p. 69-70°C. In order to give more supporting evidence that the compound was

a C_{17}-chain compound and to fix absolutely the location of the carbonyls, a mass spectrum of **7** was obtained. The molecular ion was observed at m/z 268 ($C_{17}H_{32}O_2$). Fragment ions were observed m/z 239, 211, 155 and 127 (see Figure 7). Because of this and the preceeding data on the parent compound, the assignment of structure **4**, was confirmed. Compound **4** was found to be identical with falcarindiol, first isolated by F. Bohlmann (15) from Falcaria vulgaris and subsequently identified by other research groups (see Table 4).

M^+ 268 $C_{17}H_{32}O_2$

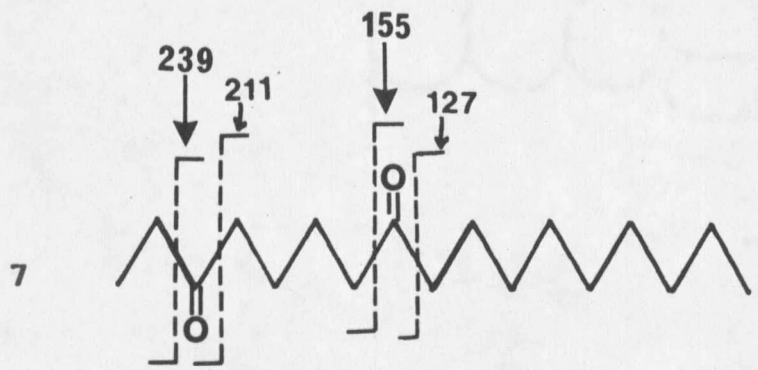
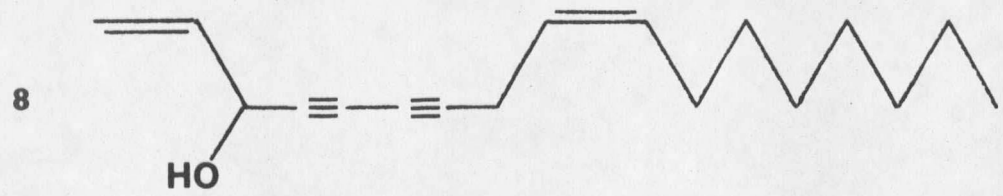


Figure 7. Mass Spectral Fragmentation of 3,8-heptadecadione

Derivatives of Falcarindiol. An excellent study by Kemp (12), (Table 3), comparing the antifungal activities of falcarindiol **4** and falcarinol, **8**, showed that the diol was required for antifungal activity. It is interesting to recall here that the toxic principals of Oenanthe crocata and Cicuta virosa exhibited the same pattern towards toxicity, that is, the diol was required for toxicity. This seems to point to the possibility that there is a similarity in recognition sites towards antifungal activity and toxicity. Because of this, it was felt that the absolute configuration of the alcohol moieties might be of interest and, if a crystalline derivative of **4** could be prepared for an x-ray crystallographic study, some conclusions regarding the antifungal properties and configuration of the molecule might be deduced.

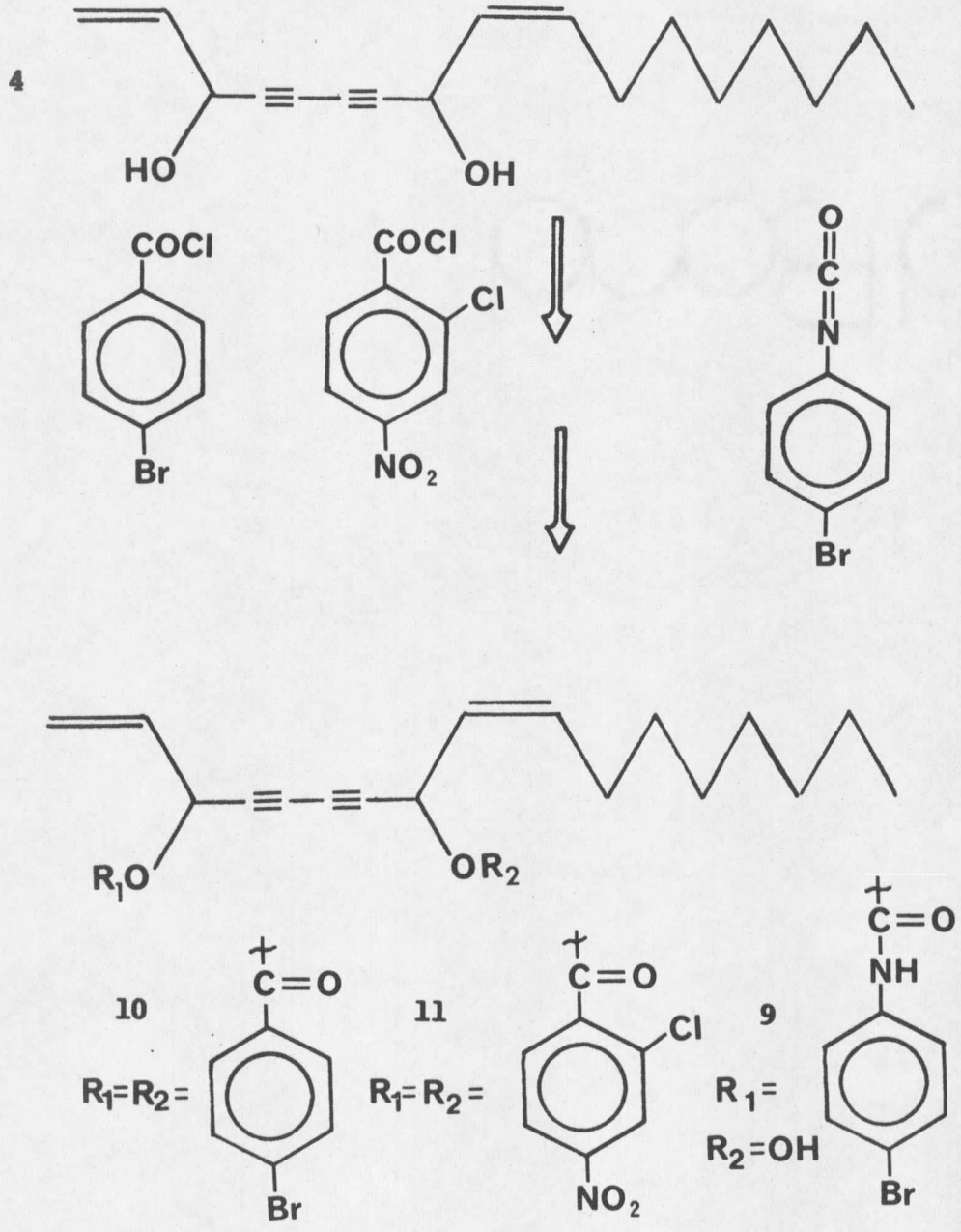


Since **4** exists as an oil and repeated attempts at obtaining it in crystalline form failed, one phenyl urethane and two benzoate derivatives were synthesized.

Table 3. Activity of Falcarinol and Falcarindiol in Spore Germination Tests. Germination as a Percentage of Control (12).

Fungus	Falcarinol		Falcarindiol	
	100 ppm	10 ppm	100 ppm	10 ppm
Alternaria brassicicola	100	100	0	100
Botrytis cinerea	100	100	35	100
Septoria nodorum	54	100	0	48
Ascochyta fabae	100	100	0	100
Glomerella cingulata	100	100	0	100
Fusarium culmorum	100	100	0	100
Aspergillus niger	100	100	0	100

Falcarindiol was treated with 2 equivalents of each derivatizing agent, 1) p-bromobenzoyl chloride, 2) 2-chloro-4-nitro-benzoyl chloride, 3) p-bromo phenyl isocyanate, as described in the experimental section (see Scheme I). In each case, the products **9, 10, 11,** isolated were obtained as a colorless oils, and repeated attempts at crystallization failed. The p-bromophenyl urethane of falcarindiol **9** indicated an incomplete reaction, due to the fact that only the C_8 hydroxyl moiety was derivatized. (This was deduced from



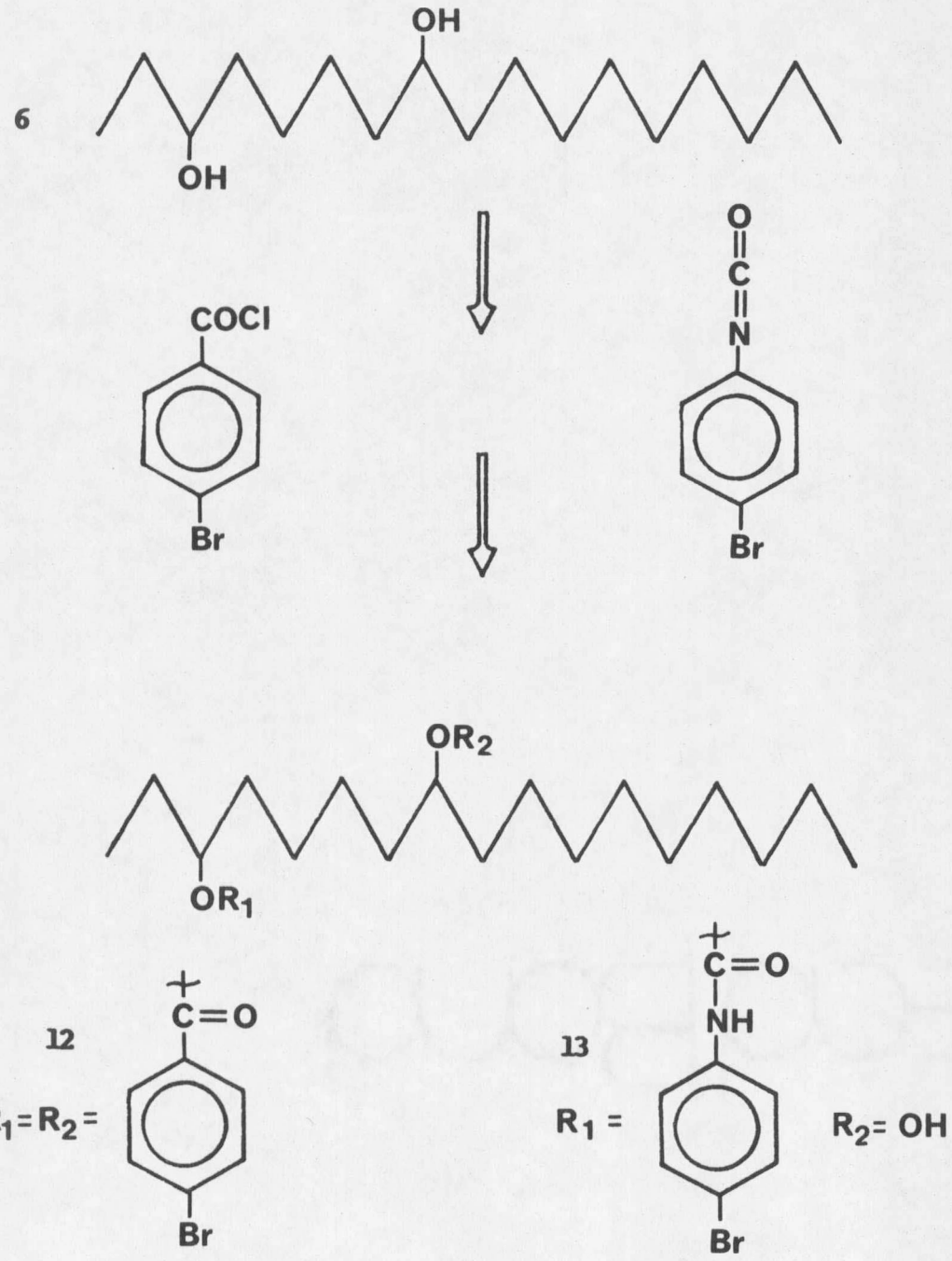
Scheme I. Derivatives of Falcarindiol

the ^{1}H-NMR spectrum of **9** which exhibited the same doublet for the C_3 methine proton, δ4.8, as that of falcarindiol.

Since if was possible to obtain crystals of reduced falcarindiol, **6**, it was decided to pursue derivatives of this compound in order to provide crystals for an x-ray study. Even though this would not be the parent compound, it was felt that if a crystalline derivative could be obtained and an x-ray study were successful, the absolute configuration of the alcohol moieties could still be established.

3,8 Heptadecadiol, **6**, was treated with 2 equivalents of each derivatizing agent, 1) p-bromobenzoyl chloride and 2) p-bromophenyl isocyanate, according to the procedures presented in the experimental section (Scheme II). The bis-p-bromobenzoate, **12** was obtained as a colorless oil and repeated attempts at crystallization failed. The p-bromophenyl urethane, **13**, was isolated as a white solid, m.p. 109-110°C. Repeated attempts at obtaining suitable crystals of **13** have thus far failed, but efforts are continuing.

Substituent effects on the alcoholic methine protons of falcarindiol. The preparation of the derivatives of falcarindiol gave the opportunity to observe the effects of various organic substituents on the methine protons of falcarindiol. Figure 8 shows the olefinic region of the ^{1}H-NMR spectrum for the parent compound falcarindiol **4**, the diacetate **5**, and the bis-2-chloro-4-nitrobenzoate **11**. In the



Scheme II. Derivatives of 3,8-heptadecadiol.

parent compound 4, the chemical shifts of the methine protons of C_3 and C_8 were observed to be δ4.8 and δ5.18, respectively. For the diacetate 5, the deshielding effects of the acetate moieties were reflected by a downfield shift of C_3 and C_8 to δ5.8 and δ6.1, respectively. The more strongly deshielding 2-chloro-4-nitrobenzoate moieties not only shift the methine protons of C_3 and C_8 to δ6.2 and δ6.4, but the olefinic protons are all effected somewhat and shifted approximately 0.1-0.2 ppm downfield.

Antifungal properties of falcarindiol. The antifungal activity of falcarindiol has been documented by several groups (16-19). The elegant study by Garrod, Lewis and Coxon (16) demonstrated a gradient distribution of 4 in the rhizomes of the carrot (Daucus carota), the heaviest concentration residing in the outer tissue layers. As mentioned earlier, Kemp (19) has done a comparative study of the antifungal activities of 4 vs. 8, suggesting that both hydroxyls of 4 are essential for antifungal activity. A study by Muir (20) states that 4 exhibits a marked specificity for dermatophyte fungi and acts by inhibiting spore germination.

In order to determine whether falcarindiol was present in the roots of Osmorhiza chilensis at other times of the year an extraction of roots collected in mid summer was undertaken. The rhizomes of O. chilensis were extracted and chromatographed in the same manner the

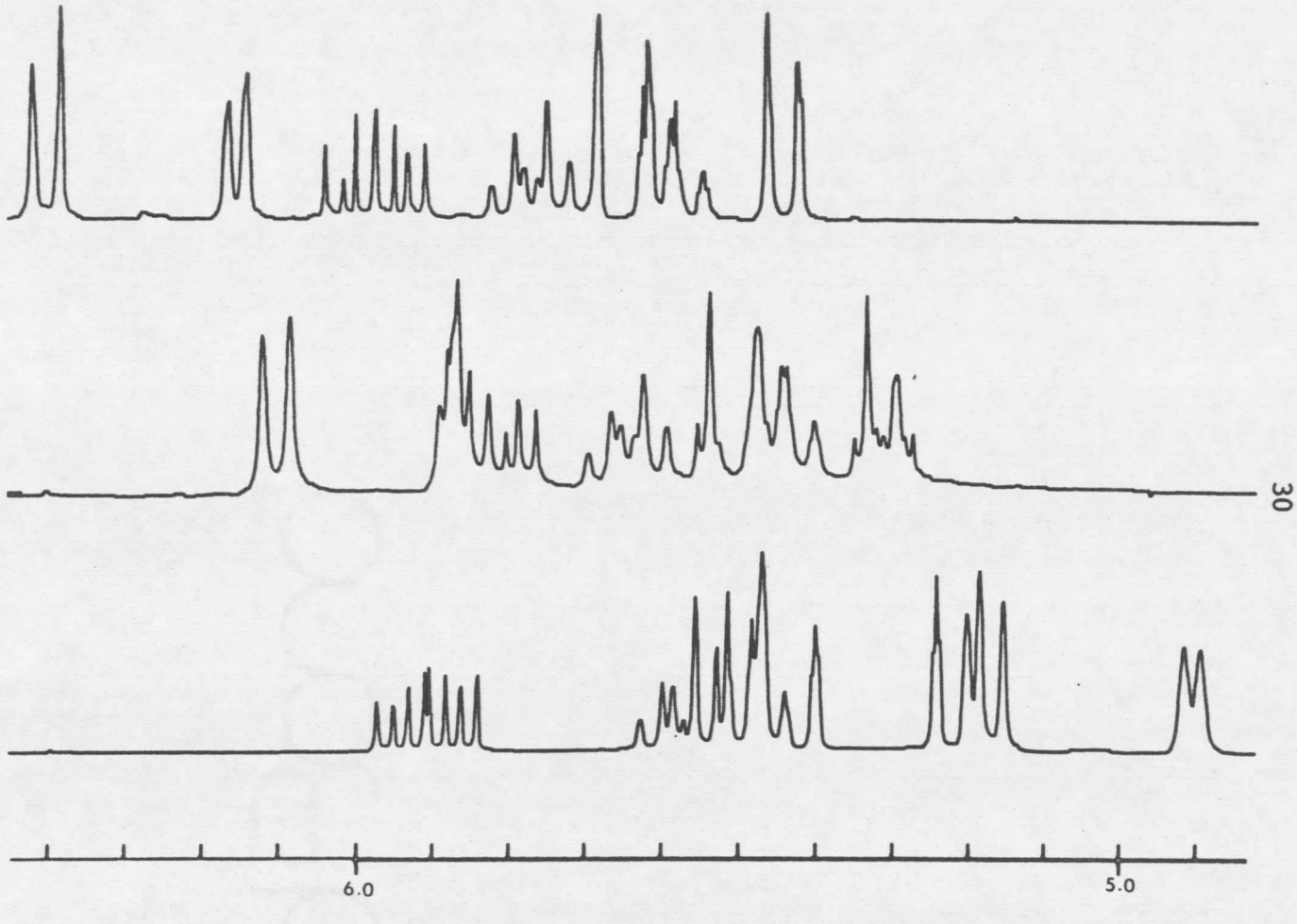


Figure 8. Substituent Effects on the Olefinic Proton Signals of Falcarindiol.

autumn collection. ^{1}H-NMR of all fractions were obtained. Since no trace of 4 could be found from the summer collection, it was inferred that the high concentration of falcarindiol in the fall collections serves to protect the plant from pathogens during the senescent, dormant and bloom phases of its growth cycle. To our knowledge this is the highest known concentration (0.21% of fresh weight) of falcarindiol yet found in an umbelliferous plant (see Table 4).

Biogenesis of falcarindiol. The majority of natural acetylenes all contain an unbranched chain of carbon atoms which can be derived from unsaturated carboxylic acids with even numbers of carbon atoms. The C_{17} chain of falcarinone, 1, and related compounds implies that these compounds are derived from C_{18} acids. The discovery of a non-conjugated ene-acetylene C_{18} acid, crepenynic acid, 16, by Mikolajczak (21) provided additional insight into the biosynthesis of C_{17} polyacetylenes. A plausible scheme for the biosynthesis of C_{17} polyacteylenes is presented in Figure 9 (22). Linoleic acid, 15, is formed by a dehydrogenation reaction from oleic acid, 14. Another dehydrogenation at C_6 results in crepenynic acid, 16. The next step may incorporate 2 distinct dehydrogenations, one forming the C_4-C_5 triple bond and the other the insertion of the Δ_{17} double bond to give compound 17. The next step would be oxidation at allylic centers to give either the diketone or the diol falcarindiol, 4.

Table 4. Percentage of Falcarindiol Found in Umbelliferous Plants.

Plant	% Falcarindiol (fresh wt.)	Reference
Daucus carota	5.6×10^{-4}	(16)
Falcaria vulgaris	5.2×10^{-4}	(15)
Daucus carota	9.0×10^{-4}	(17)
Aegopodium podagraria	2.2×10^{-2}	(12)
Peucedanum oreoselinum	3.5×10^{-1}*	(18)
Opopanax chironium	3.0×10^{-4}	(19)
Osmorhiza chilensis	2.1×10^{-1}	This work

* % reported is for dry weight of plant

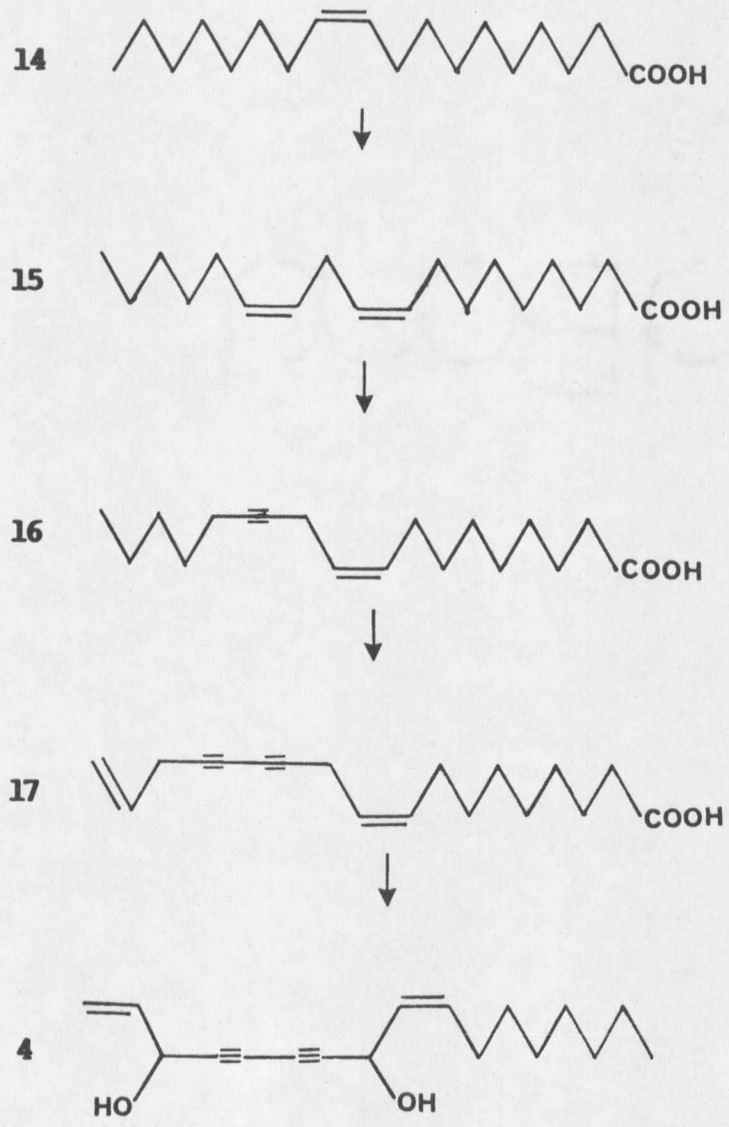


Figure 9. Proposed Biogenesis of Falcarindiol (22).

Pharmacological Screens. The dichloromethane and water soluble extracts of Osmorhiza chilensis exhibited no toxicity towards bacteria deficient in DNA repair activity,nor did falcarindiol itself. Because of the previous research on the antifungal activity of falcarindiol (as described earlier), no antifungal screens were done on falcarindiol.

Summary. A major constituent of the rhizomes of Osmorhiza chilensis was isolated, characterized and identified as falcarindiol, 4. Attempts were made to obtain a crystalline derivatives of 4 for x-ray crystallographic studies. Substituent effects on methine protons were discussed. A correlation between the amounts of 4 found in autumn and summer plants was presented. A plausible biosynthetic pathway for 4 was reviewed.

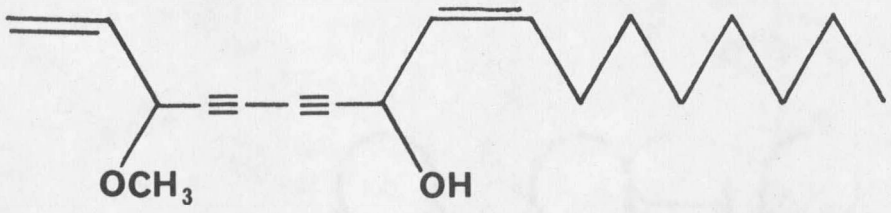
2. 3-O-Methylfalcarindiol

Isolation. From less polar chromatography fractions a compound related to falcarindiol was isolated. This compound was isolated twice, using two separate isolation schemes presented in the experimental section. In the most efficient scheme, fraction 3 from the Florisil chromatography of the crude dichloromethane soluble extracts of the rhizomes of Osmorhiza chilensis (fall collection) was permeated through Sephadex LH-20. The seventh of ten fractions, 162 mg (0.036% fresh weight), colorless oil, was identified as the target

compound.

Characterization. The ^{1}H-NMR spectrum (Figure 10), revealed a few differences from that of falcarindiol (Figure 1); the presence of a three proton singlet at δ3.38 suggested a methoxyl functionality. A diamagnetic shift of the C_3 proton (of falcarindiol) from δ4.92 to δ4.58 in this compound suggested that the methoxyl group was attached at C_3. The rest of the ^{1}H-NMR spectrum of this compound exhibited virtually identical splittings and chemical shifts as that of falcarindiol. The ^{13}C-NMR spectrum of this compound supported a molecular formula of $C_{18}H_{26}O_2$, and was very similiar to the ^{13}C-NMR of falcarindiol, with the exception of a signal at δ55.6 for a methoxyl carbon and a substantial shift of the methine carbon at C_3 from δ63.2 in falcarindiol to δ71.8. These data supported structure **18**, 3-O-methylfalcarindiol, as being the target compound.

18



Discussion. 3-O-Methylfalcarindiol was obtained as a highly unstable colorless oil. ^{1}H-NMR and ^{13}C-NMR data were acquired within the first week after isolation. Unfortunately, the compound

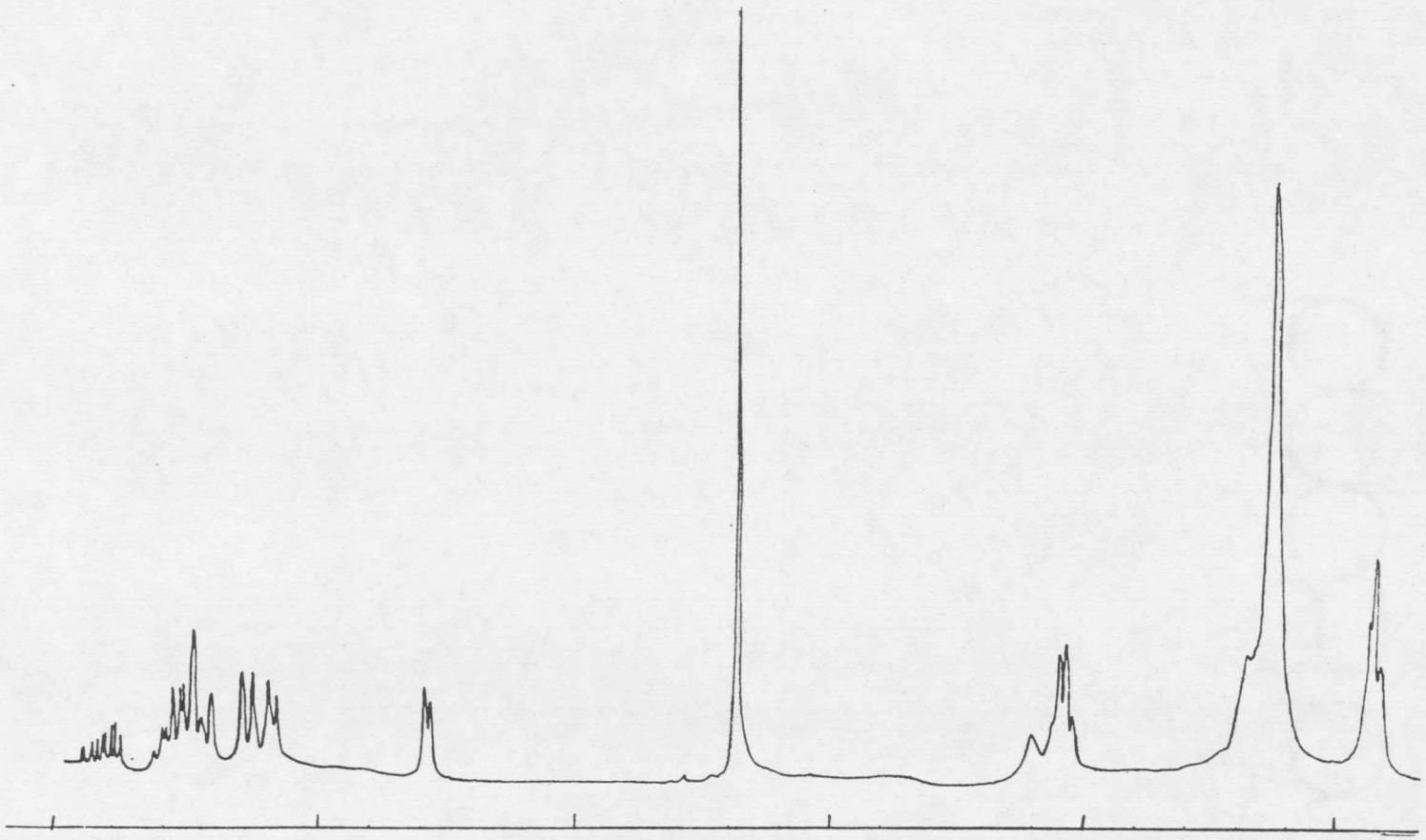
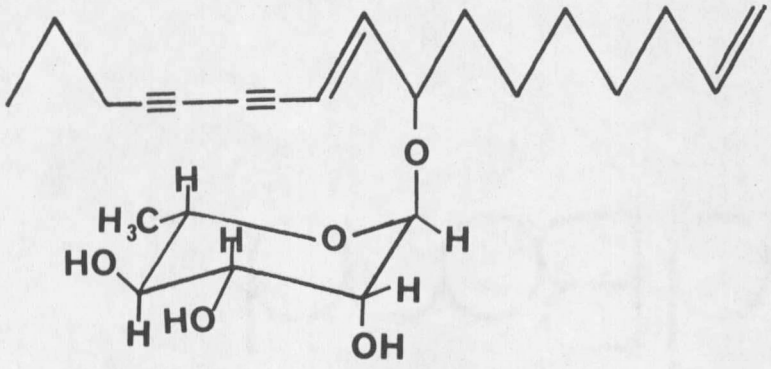


Figure 10. ^{1}H-NMR Spectrum of 3-O-methylfalcarindiol.

decomposed before UV data could be obtained to support the proposed structure, **18**.

That **18** is an artifact cannot, at this time be ruled out. The extraction of the roots with methanol could have led to alcoholysis of a glycoside linkage to C_3 of falcarindiol. A C_{10} rhamnoside of a related acetylenic alcohol **19** from Serratula gmelini has been reported (23). The authors stated that alcoholysis with traces of toluene p-sulfonic acid yielded a mixture of a polyacetylenic alcohol, a polyacetylenic ethyl ether and an ethyl rhamnoside.

19



The possibility that **18** is an artifact could be resolved if another extraction procedure omitting methanol were employed.

Summary. A new compound, 3-O-methylfalcarindiol, **18**, was isolated from the dichloromethane soluble extracts of the rhizomes of

Osmorhiza chilensis collected in the fall. The compound was extremely unstable and decomposed within 2 weeks. The possibility that **18** is an artifact was discussed.

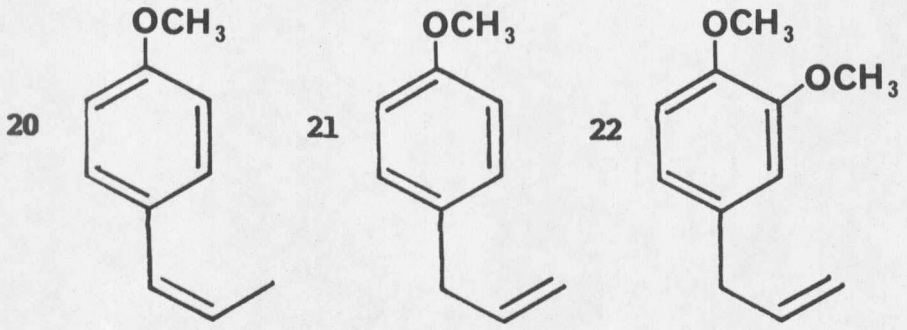
3. The Phenylpropanoids

Introduction. A wide variety of phenylpropanoid compounds are found in umbelliferous plants. Usually, these compounds are found in the essential oil fractions of the fruits or rhizomes. Many of these phenylpropanoids exhibit a characteristic odor and are primarily responsible for the flavor associated with the plant. There are five classes of phenylpropanoids which are classified according to the hydroxylation (or methoxylation) pattern of the benzene ring. (i.e., 4-OH;3,4 di-OH; 3,4,5-tri-OH; 2,4,5-tri-OH; 2,3,4,5-tetra-OH). (24).

Isolation. Three phenylpropanoid compounds were isolated from the less polar fractions (2,3,4) from the Florisil chromatography of the dichloromethane soluble extracts of Osmorhiza chilensis (fall collection). These three fractions accounted for 50% of the crude extract (or 1.1% of the fresh weight).

Fraction 2 contained a mixture of the regioisomers anethole, **20**, and estragole, **21**. An attempt at separation of these compounds by distillation failed due to the proximity of their boiling points. Fraction 3 contained a mixture of **20**, **21** and 3,4-dimethoxy eugenol, **22**. This fraction was subjected to adsorption (silica gel) and gel

permeation (Sephadex LH-20) chromatography. Compound **22** was isolated in pure form from the final fraction of the gel permeation chromatography.



Characterization. The ^{1}H-NMR spectra of **20** and **22** (Figures 11 and 12) exhibited basically the same chemical shifts. Compounds **20** and **21** exhibited an AA'-BB' splitting pattern which typifies para-disubstituted benzenes, whereas compound **22**, exhibited the expected splitting pattern for a 1,2,4-trisubstituted benzene. The pattern for the olefinic protons of **20** and **22** were the same. A multiplet at δ6.3 was assigned as the M proton of an apparent $ABMX_2$ system. A multiplet at 5.1 was assigned as the A and B protons and a doublet at δ3.85 was assigned as the X protons of this system. The olefinic protons of **21** appeared as a doublet at δ7.7 for the hydrogen

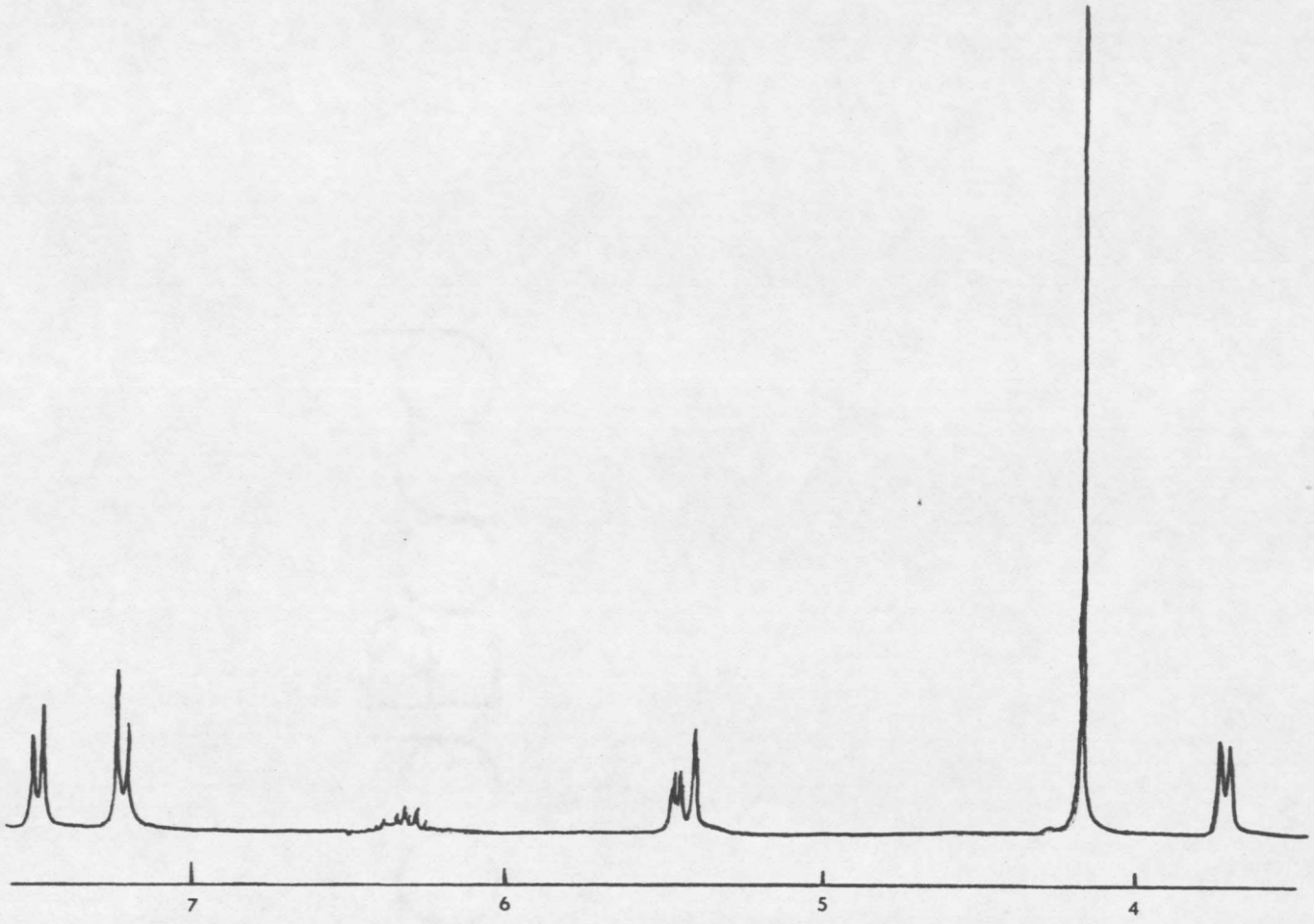


Figure 11. ^{1}H-NMR Spectrum of Estragole.

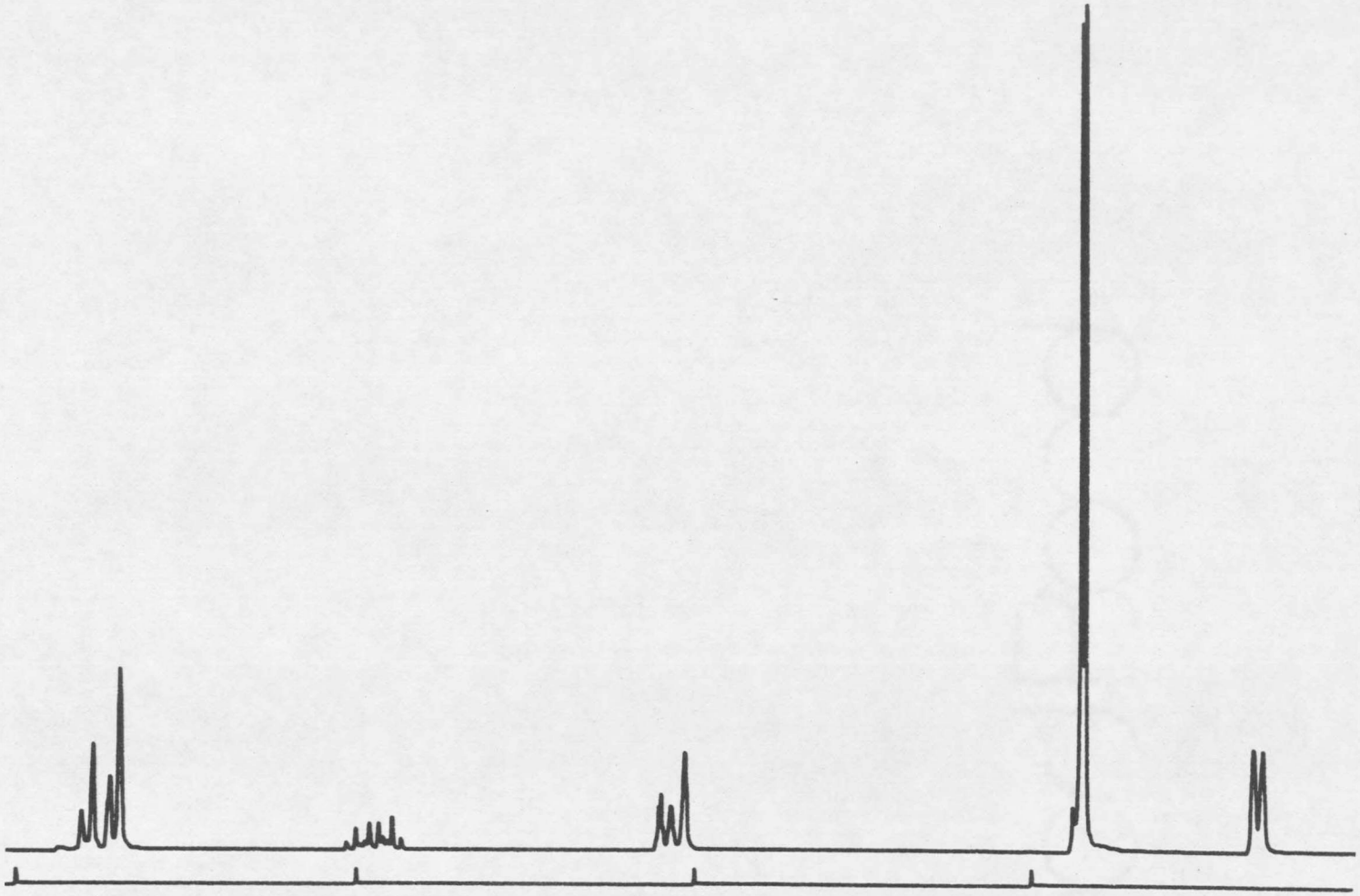


Figure 12. ^{1}H-NMR Spectrum of 3,4-dimethoxy Eugenol.

in conjugation with the benzene ring and as a multiplet at δ7.2 for the other hydrogen. The terminal methyl group of this compound appeared as a doublet of doublets at δ1.9.

The methoxyl hydrogens appeared as a singlet at δ4.18 for compounds **20** and **21** and as 2 singlets at δ3.85 and δ3.83 for **22**. The IR spectra of **20** and **21** exhibited the expected signals for the olefinic C-H stretches and p-disubstituted benzenes. Compound **22** exhibited a pattern similar to **20**, with the exception of the benzene substitution patterns.

Summary. The phenylpropanoids anethole **20**, estragole **21** and 3,4-dimethoxyeugenol **22**, were identified as major constituents of the dichloromethane soluble extracts of the rhizomes of Osmorhiza chilensis collected in the fall.

A total absence of these compounds in the rhizomes of Osmorhiza chilensis collected in mid-summer was noted, though the characteristic aroma was observed in the fruits and seeds.

The folk medicinal use of Osmorhiza chilensis might be due, in large measure, to these types of compounds. When the rhizomes of Osmorhiza chilensis are brewed into a tea, these compounds are released and provide a soothing licorice-flavored drink.

EXPERIMENTAL

1. Physical Measurements

The NMR spectra were obtained on a Bruker WM-250 MHz FT NMR spectrometer using $CDCl_3$ as solvent and internal standard; data are reported as δ units (ppm), relative to tetra methylsilane ($\delta = 0$). The IR spectra were measured on a Beckman IR 20 and are reported in cm^{-1}. UV spectra were recorded on either Cary 14 or Varian 634 UV spectrometers. Mass spectra were obtained on a Varian CH-5 mass spectrometer or at the Midwest Center for Mass Spectrometry on a Kratos MS 50 high resolution mass spectrometer. Column chromatography was monitored with an ISCO UA-5 UV monitor operating at 254 and 280 nm. HPLC was performed with a Beckman-Altex component system (model 110A pump and model 153 UV monitor operating at 254 nm). Melting points were determined with a Mel-Temp apparatus and are uncorrected. Combustion analysis were performed by Galbraith Laboratories. The packings used in column chromatography are described in Table 5.

2. Pharmacological Screening

Assays for toxicity to bacteria deficient in DNA repair capacity were performed by Dr. Samuel Rogers of this department. Antimicrobial activity was determined in the following manner. Sterile paper discs were dipped in a dichloromethane or water solution of the test

Table 5. Chromatography Packing Material.

Material	Mesh	Manufacturer
Florisil	60-200	Floridin Corp.
Silica Gel	70-230	EM Reagents
Sephadex LH-20	25-100 μ	Pharmacia Fine Chemicals
Sephadex LH-60	40-120 μ	Pharmacia Fine Chemicals
Bio-Beads SX-8	200-400	Bio-Rad Laboratories

compound and placed on agar plates. The agar plates were then sprayed with the test organism and incubated at room temperature from 24 to 48 hours. The zone of inhibition was measured and reported in millimeters (radius from edge of the disc). Test organisms used were Fusarium solani, Rhodotorula glutinus, Pseudomonas syringae, and Corynebacterium michiganense.

3. Collection and Identification of Plant Material

The plant materials, Osmorhiza chilensis and Clematis hirsuitissima, were identified by Mr. Patrick Plantenburg, Department of Biology, Montana State University.

The rhizomes of Osmorhiza chilensis were collected in November

1980 near the Fish Hatchery on Bridger Mountain Drive, 2 miles north of Bozeman, Montana. The collection site was located approximately ¼ mile down Fish Hatchery Road from Bridger Mountain Road. The plants were found growing in shaded ditches alongside the road.

The second collection of Osmorhiza chilensis was at the Montana State University Agricultural Experimental Station at Red Bluff Montana in July 1981. The plants were collected along the banks of Spring Creek. Access to Spring Creek can be obtained by taking the dirt road across from the Red Bluff Ranch and traveling approximately 2 miles in a south-east direction.

4. Extraction and Initial Separations - Osmorhiza chilensis

Extraction - Fall Collection. The rhizomes (836 g fresh weight) of Osmorhiza chilensis were ground in a Waring blender and steeped for 24 hours in methanol (x2). The methanolic extracts were removed by suction filtration and evaporated, in vacuo, to a brown syrup. The ground rhizomes were then steeped in dichloromethane for 24 hours (x2). The reduced methanolic extracts were suspended in 200 ml H_2O and equilibrated with the dichloromethane extracts. The combined dichloromethane phase was evaporated, in vacuo, to a brown syrup, 18.6 g, (2.2% of the fresh weight).

Extraction - Summer Collection. The rhizomes of Osmorhiza chilensis (322 g fresh weight) were ground in a Waring blender and

steeped for 24 hours in methanol (x2). The methanolic extracts were evaporated, *in vacuo*, to a yellow syrup. The ground rhizomes were then steeped in dichloromethane for 24 hours (x2). The reduced methanolic extracts were suspended in 50 ml H_2O and equilibrated with the dichloromethane extracts. The dichloromethane phase was evaporated, *in vacuo*, to a pale yellow oil, 1.27 g (0.4% of the fresh weight).

Initial Separation of the Dichloromethane Extract - Fall Collection. 8.0 g of the crude dichloromethane soluble extracts werechromatographed on Florisil (200 g, 4.5 x 60 cm column). Elution commenced with hexane and proceeded through a series of solvent combinations of gradually increasing polarity (hexane-ethyl acetate-methanol); fourteen fractions were collected. A typical separation is detailed in Table 6.

Initial Separation of the Dichloromethane Extract - Summer Collection. The dichloromethane extract, 1.27 g, was chromatographed on Florisil (80 g, 4.5 x 24 cm column). Elution commenced with hexane and proceeded through a series of solvent combinations of gradually increasing polarity (hexane-ethyl acetate-methanol); thirteen fractions were collected. There were no phenylpropanoids detected and neither falcarindiol, **4**, nor 3-O-methylfalcarindiol, **18**, were detected in any fraction from this extract.

Table 6. Florisil Chromatography of the Dichloromethane Soluble Extracts of Osmorhiza Chilensis.

Fraction Number	ml Eluent	Eluent	Wt (g)	Major Chemical Constituents
1	500	Hexane	0.222	Sesquiterpenes? hydrocarbons
2	700	Hexane-Ethyl Acetate (24:1)	1.50	Phenylpropanoids: anthehole, estragole
3	100	Hexane-Ethyl Acetate (24:1)	1.38	Phenylpropanoids: 3,4-dimethoxy eugenol
4	400	Hexane-Ethyl Acetate (23:2)	1.03	Phenylpropanoids: 3-O-methyl falcarindiol
5	1100	Hexane-Ethyl Acetate (4:1) & (3:2)	2.54	Polyacetylenes, falcarindiol, 3-O-methyl falcarindiol
6	200	Hexane-Ethyl Acetate (3:2)	0.056	Polyacetylenes
7	250	Hexane-Ethyl Acetate (1:4)	0.095	Unsaturated fatty acids
8	750	Ethyl Acetate	0.074	Unsaturated fatty acids
9	750	Ethyl Acetate Methanol (24:1)	0.077	Saturated fatty acids
10	500	Ethyl Acetate Methanol (47:3)	0.090	Saturated fatty acids
11	500	Ethyl Acetate Methanol (4:1)	0.051	Unidentified
12	500	Ethyl Acetate Methanol (4:1) & (1:3)	0.028	Unidentified
13	250	Ethyl Acetate Methanol (1:3)	0.122	Unidentified
14	300	Methanol	0.222	Unidentified

5. Anethole and Estragole

Isolation from the Dichloromethane Extract. Fraction 2, 1.5 g, from the Florisil chromatography was distilled, in vacuo, (1.3 mm Hg) and a fraction, 1.0 g, b.p. 32-35°C, was collected. This fraction was identified as a mixture of the regioisomers anethole, **20**, and estragole, **21**, both colorless oils, $bp_{2.3}$ 81-81.5°C (25) and bp_{12} 95-96°C (26), respectively.

Characterization - Estragole and Anethole.

IR ν_{max}^{neat} 3100,2850,1650,1625,1590,1520,1250,1180,1050,990,920, 810 cm^{-1}; ^{1}H-NMR ($CDCl_3$)(estragole): δ7.50(2H, dd, J = 8.0, 1.2); 7.20(2H, dd, J = 8.0, 1.2); 6.40(1H, m); 5.5(1H, m); 5.45(1H, m); 4.18(3H, s); 3.75(2H, d, J = 6.3). ^{1}H-NMR ($CDCl_3$)(Anethole): δ7.70(1H, d, J = 8); 7.50(2H, dd, J = 8.0, 1.2); 7.20(2H, dd, J = 8.0,1.2); 6.40(1H, m); 4.18(3H, s); 1.90(3H, dd, J = 8, 1.2).

6. 3,4-Dimethoxy Eugenol

Isolation. Fraction 3, 2.6 g from the Florisil chromatography was rechromatographed on silica gel (200 g, 4.5 x 60 cm column) using dichloromethane-hexane (4:1) as the eluent; six fractions were collected. Fraction 5, 63 mg, was permeated through Sephadex LH-20 (120 x 2 cm column) with dichloromethane-methanol (1:1) as the eluent. The seventh of seven fractions was identified as 3,4-dimethoxy eugenol, **22**, 27 mg colorless oil.

Characterization. IR $\nu_{max}^{CHCl_3}$ 3100,1650,1600,1040,990,920, 810 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 6.80(1H, d, J = 8.5,1.3); 6.72(1H, d, J = 8.5); 6.70(1H, d, J = 1.3); 5.8(1H, ddt, J = 17.1,11.8,6.6); 5.1(1H, dd, J = 17.1,2.5); 5.05(1H, dd, J = 11.8,2.5); 3.85(3H, s); 3.83(3H, s); 3.30(2H, d, J = 6.6).

7. Falcarindiol

Isolation. Fraction 5, 2.55 g, from the Florisil chromatography was rechromatographed on silica gel (200 g, 4.5 x 60 cm column) using cyclohexane-dichloromethane (1:4) as the eluent; four fraction were collected. Fraction 4, 1.699 g, was permeated through Sephadex LH-20 (195 x 2.5 cm column) with dichloromethane-methanol (1:1) as the eluent. The seventh of seven fractions obtained was identified as falcarindiol, 4, 754 mg colorless oil.

Characterization. IR $\nu_{max}^{CHCl_3}$ 3325,2950,2880,2150,2050, 1630,990,940,740 cm^{-1} UV: λ_{max}^{EtOH} 260 nm(ϵ = 330), 245(500), 233(530); ^{1}H-NMR ($CDCl_3$): δ 5.80(1H, ddd, J = 17,10.8,7.7); 5.6(1H, dt, J = 10.8,6); 5.5(1H, dd, J = 10.8,8.5); 5.45(1H, dd, J = 17,4.5); 5.25(1H, dd, J = 10.8,4.5); 5.15(1H, d, J = 8.5); 4.8(1H, d, J = 7.7); 2.05(2H, m); 2.0(O-H, br s); 1.6(O-H, br s); 1.3(10H, br); 0.9(3H, br t, J = 6.5). ^{13}C-NMR ($CDCl_3$): δ135.8(t), 134.3(d), 127.6(d), 117.0(t), 79.8(s), 78.2(s), 70.1(s), 68.6(s), 63.2(d), 58.4(d), 31.6(t),

29.1(t), 28.9(t), 27.5(t), 27.5(t), 22.5(t), 13.9(q).

8. Acetylation of falcarindiol.

30 mg of falcarindiol was acetylated with 5 ml acetic anhydride in 5 ml dry pyridine at 40-50°C for 2 hours. Excess reagent and solvent were removed, in vacuo. The residue, 42 mg, was chromatographed on silica gel (10 g, 1.5 x 60 cm column) using hexane-diethyl ether (4:1) as the eluent; Fraction 2, 32 mg (81% yield) was identified as the diacetate of falcarindiol **5**, a colorless oil. IR: $\nu_{max}^{CHCl_3}$ 2200,1750,980,940,700 cm^{-1}; UV: λ_{max}^{EtOH} 262 nm (ϵ = 403), 248(680), 235(725); ^{1}H-NMR ($CDCl_3$): δ 6.20(1H, d, J = 9.0); 5.90(1H, d, J = 8.8); 5.8(1H, ddd, J = 17.6,10.0,8.8); 5.65(1H, dt, J = 10.0,6.5); 5.5(1H, dd, J = 10.0,9.0); 5.45(1H, dd, J = 17.6,4.2); 5.30(1H, dd, J = 10.0,4.2); 2.1(2H, m); 2.08(3H, s); 2.06(3H, s); 1.3(10H, br m); 0.9(3H, t, J = 6.6).

9. Catalytic Hydrogenation of Falcarindiol.

Falcarindiol, 32 gm, was hydrogenated over palladium on charcoal (5%) in 10 ml ethanol for 45 minutes at 30 psi, using a Parr hydrogenator. Filtration of the reaction mixture and evaporation of the solvent gave a white solid. Recrystallization from diethyl ether-hexane gave **6**, 30.5 mg (95% yield), colorless crystals, mp 90-91°C; ^{1}H-NMR ($CDCl_3$): δ 0.8(3H, t), 0.9(3H, t), 1.2(18H, m), 1.4(8H, m),

2.35(2H, br s), 3.55(2H, m); ^{13}C-NMR ($CDCl_3$): δ9.6(q), 13.9(q), 22.5(t), 25.5(t), 25.6(t), 29.2(t), 29.3(t), 29.5(3C,t), 30.0(t), 31.7(t), 36.7(t), 37.3(t), 37.4(t), 71.8(d), 73.1(d). Analysis; Calculated for $C_{17}H_{36}O_2$: C, 74.94; H, 13.32. Found: C, 74.88; H, 13.46.

10. Oxidation of 3,8-heptadecadiol.

3,8-heptadecadiol, 6, 19mg, was oxidized with Jones reagent (13). The reaction was carried out by the addition of the reagent to a stirred acetone solution of the diol maintained at 15-20°C. The reaction mixture was extracted with dichloromethane and the dichloromethane layer was evaporated, in vacuo, to a white solid. Recrystallization from diethylether-hexane gave 7, 19 mg (100% yield) colorless crystals, mp 69.5-70°C. IR: $\nu_{max}^{CHCl_3}$ 2950,1730,1450 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ2.4(8H, m); 1.5(8H, m); 2(12H, m); 0.90(3H, t, J = 6.6); 0.85(3H, t, J = 6.5).

bis-p-Bromobenzoate of Falcarindiol. The bis-p-bromobenzoate of falcarindiol was obtained by reacting 150 mg of falcarindiol, and 198 mg of p-bromobenzoyl chloride in 5 ml pyridine at 40-50°C for 24 hours. 270 mg of a brown solid was obtained after evaporation of the solvent. The product was permeated through Sephadex LH-20 (195 x 2.5 cm column); seven fractions were collected. Fraction 5, 146 mg, was rechromatographed on silica gel (20 g, 1 x 60 cm column) using hexane-

diethyl ether (4:1) as the eluent. Six fractions were collected; Fraction 2, 136 mg, (38% yield) afforded **10**, a colorless oil. ^{1}H-NMR ($CDCl_3$): δ 7.90(4H, dd, J = 8.3,1.1); 7.58(4H, dd, J = 8.3, 1.1); 6.35(1H, d, J = 9.0); 6.15(1H, d, J = 7.5); 5.90(1H, ddd, J = 17.3,12.3,7.5); 5.70(1H, dt, J = 12.3,6); 5.65(1H, dd, J = 12.3,9.0); 5.55(1H, dd, J = 17.3,4.5); 5.40(1H, dd, 12.3,4.5); 2.1(2H,br m); 1.2(10H, br m); 0.9(3H, br t, J = 7.0).

bis-2-Chloro-4-nitrobenzoate of Falcarindiol. 4.0 g of 2-chloro-4-nitro benzoic acid were refluxed with 10 ml of thionyl chloride for 30 minutes. A portion of the product, 2-chloro-4-nitro benzoyl chloride, 169 mg, was then reacted with 100 mg of falcarindiol in 5 ml pyridine at 30-40°C for 2 hours. The residue after evaporation of solvent was chromatographed on silica gel (20 g, 1 x 60 cm column) using hexane-diethyl ether (4:1) as the eluent. 2 fractions were collected; Fraction 2, 128 g (55% yield) afforded **11**, a colorless oil; ^{1}H-NMR ($CDCl_3$): δ 8.3(2H, d, J = 3.5); 8.15(2H, dd, J = 8.0,1.5); 7.95(2H, dd, J = 8.0,1); 6.4(1H, d, J = 9.8); 6.15(1H, d, J = 7.0); 5.90(1H, ddd, J = 17.5,10.5,7.0); 5.80(1H, dt, J = 12.6); 5.75(1H, dd, J = 12, 6.3); 5.65(1H, dd, 17.5,4); 5.43(1H, d, J = 10.5,4); 2.1(2H, br m); 1.3(10H, br m); 0.9(3H, t, J = 6.0).

p-Bromophenylurethane of Falcarindiol. The p-bromophenyl-urethane of falcarindiol was obtained by reacting 100 mg of p-

bromophenyl isocyanate with 50 mg falcarindiol in 5 ml pyridine at 50°C for 2 hours, followed by stirring at room temperature overnight. After evaporation of solvent, the reaction products, were permeated through Bio-Beads SX-8, (120 x 2 cm column), using dichloromethane-cyclohexane (3:2) as the eluent: eight fractions were collected; Fraction 4 was identified as the mono p-bromophenyl urethane of falcarindiol, 9, 51 mg (41% yield), a colorless oil. ^{1}H-NMR ($CDCl_3$): δ7.4(2H, dd, J = 9.4,1.8); 7.25(2H, dd, J = 9.4,1.8); 6.9(N-H, s); 6.15(1H, d, J = 8.1); 5.9(1H, ddd, J = 17,11,7); 5.70(1H, dt, J = 11.8,6.5); 5.60(1H, dd, J = 11.8,7.2); 5.50(1H, dd, J = 17,3.5); 5.1(1H, dd, J = 11, 3.5); 4.9(1H, d, J = 7); 2.1(2H, m); 1.2(10H, m); 0.9(3H, t, J = 6.0).

Bis-p-bromo benzoate of 3,8-heptadecadiol. The bis p-bromo-benzoate of dodecahydrofalcarindiol was prepared by reacting 47 mg of p-bromobenzoyl chloride with 21 mg of 3,8-heptadecadiol, 6, in 5 ml pyridine at 40-50°C for 3 hours. The product was chromatographed on silica gel (20 g, 1 x 60 cm column) using dichloromethane-hexane (1:1) as the eluent. Nine fractions were collected; the fourth fraction was identified as the bis-p-bromobenzoate of 3,8-heptadecadiol, 12, 15.9 mg, (33% yield), colorless oil; ^{1}H-NMR ($CDCl_3$): δ7.9(4H, dd, J = 9.4,1.3); 7.55(4H, dd, J = 9.4,1.3); 5.0(2H, m); 1.4(8H, m); 1.2(18H, m); 0.9(3H, t, J = 6.0); 0.83(3H, t, J = 6.5).

p-Bromophenylurethane of 3,8-heptadecadiol. The p-bromophenyl urethane of dodecahydrofalcarindiol was obtained by reacting 60 mg of p-bromophenyl isocyante with 40 mg of 3,8-heptadecadiol in 5 ml pyridine at 40-50°C for 2 hours, then stirring the mixture at room temperature overnight. The reaction products were suspended in hot dichloromethane and filtered to removed any impurities. The products were then permeated through Sephadex LH-20 (2 x 120 cm column), using dichloromethane-methanol (1:1) as the eluent. Four fractions were collected; the second fraction was identified as the mono-p-bromophenylurethane of 3,8-heptadecadiol, **13**, 33.5 mg (36% yield) white solid, mp. 109-110°C; ^{1}H-NMR ($CDCl_3$): δ 7.4(2H, dd, J = 9.0,2.1); 7.25(2H, dd, J = 9.0,2.1); 6.6(N-H, s); 4.8(1H, m); 3.69(1H, br m); 2.4(OH, br s); 1.5(4H, m); 1.2(22H, m); 0.9(3H, t, J = 6.0); 0.85(3H, t, J = 6.5).

11. 3-O-Methylfalcarindiol

Isolation from the Dichloromethane Soluble Extracts. Fraction 5, 2.55 g from the Florisil chromatography was rechromatographed on silica gel (200 g, 4.5 x 60 cm column) using cyclohexane-dichloromethane (1:4) as the eluent; four fractions were collected. Fraction 4, 1.66 g was permeated through Sephadex LH-20 (195 x 2.5 cm column) with dichloromethane-methanol (1:1) as the eluent. Fraction

5, 274 mg was permeated through Bio-Beads SX-8 using dichloromethane-cyclohexanol (3:2) as the eluent. Fraction 2, 214 mg was permeated once more through Sephadex LH-20 (195 x 2.5 cm column). Fraction 1, 162 mg (2% of total extract) a colorless oil, was identified as 3-O-methyl falcarindiol **18**.

Compound **18** was also isolated at a later date using only 2 chromatographic steps from the crude dichloromethane soluble extracts. The crude dichloromethane soluble extracts were chromatographed on Florisil (200 g, 4.5 x 60 cm column), the third fraction from this chromatography was permeated through Sephdex LH-20 (195 x 2.5 cm column) using dichloromethane-methanol (1:1) as the eluent. The third fraction 160 mg afforded 3-O-methylfalcarindiol.

Characterization. IR: $\nu_{max}^{CHCl_3}$ 3400,2150 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ5.80(1H, ddd, J = 17,11.5,7); 60(1H, dt, J = 11.5,6); 5(1H, dd, J = 11.5,8.5); 45(1H, dd, J = 17,4); 30(1H, dd, J = 11.5,4); 18(1H, d, J = 8.5); 58(1H, d, J = 7); 38(3H, s); 10(2H, m); 2(O-H, br s); 25(10H, br m); 85(3H, br, t, J = 6.5); ^{13}C-NMR: δ134.5(d), 133.9(d), 127.8(d), 118.5(t), 79.3(s), 76.4(s), 71.8(d), 71.3(s), 68.7(s), 58.5(d), 55.6(q), 31.6(t), 29.1(t), 28.9(t), 28.9(t), 27.5(t), 22.4(t), 13.9(q).

Part II

Clematis hirsutissima

INTRODUCTION

1. Historical

Clematis hirsutissima is an erect perennial herb of the family Ranunculaceae. It is commonly found in open grassland slopes and in meadows, ranging from northern New Mexico through the Rocky Mountains to British Columbia. Clematis hirsutissima was commonly employed as a horse restorative or stimulant by the Nez Perce Indians in the Oregon Territory (27). The use of horse stimulants and horse medicines was a common occurrence among the many Native American nations. The majority of horse stimulants employed by the Indians were used to increase the stamina and speed of the horse. In contrast, Clematis hirsutissima was utilized as a type of smelling salt to restore horses that were exhausted from racing, hunting or warfare (28).

The following is an excerpt of C. A. Geyer's account of the use of Clematis hirsutissima by the Nez Perce (30).

> "At a horse-racing of the Nez Perces Indians, I witnessed the application of this root. It happened that several horses were run nearly to death so that they fell down during the heat of the day. As soon as such an accident happened, an Indian put a piece of the root (the outer coat scraped off) into the nostrils of the animal. The effect was surprising, the creature sprang up under convulsions, was brought to the river and bathed, and I found several which had been so treated, afterward grazing with the herd, apparently without having sustained any injury."

The Teton Sioux used Clematis hirsutissima as a snuff, prepared from the dried and powdered root, to stimulate tired horses when hard

pressed by the enemy (30). The Flathead Indians of Western Montana used Clematis hirsutissima as an itch medicine and as as a remedy for headaches (27). Clematis hirsutissima is sometimes referred to as the "headache weed" (31).

2. Research Objectives

Clematis hirsutissima was chosen as a target plant because of the interesting, if varied, medicinal uses of the plant. From the data gathered in the literature, it appeared that Clematis hirsutissima could possibly contain a compound or compounds that exhibited their effects on the central nervous system. The plant was also chosen due to a lack of thorough previous chemical studies on the genus Clematis.

The major goal of this part of the thesis project was to isolate and characterize the active compound or compounds responsible for the stimulating effect on horses.

RESULTS AND DISCUSSION

1, Anemonin

Isolation. Specimens of Clematis hirsutissima were collected in July from grassland slopes near Bozeman, Montana. Only a small collection of the plant was obtained, due to the inavailability of the plant at that time.

The rhizomes of Clematis hirsutissima were ground and extracted successively with methanol and dichloromethane. Because central nervous system agents were the goal of this work, an alkaloid extraction was employed. The dichloromethane soluble extracts were washed with 0.1 M hydrochloric acid. The hydrochloric acid soluble extracts were made basic (~pH 10) with potassium carbonate. The basic solution was then extracted with diethyl ether and upon evaporation of the diethyl ether a small residue (5.2 mg) was obtained. The ^{1}H-NMR of this fraction was of no interest, showing only a large methylene envelope at δ 1.2. This extract was analyzed for the presence of alkaloids by using thin layer chromatography, followed by spraying of the TLC plates with two alkaloid detecting reagents, Dragendorff's (32a) and Mayer's (32b) reagents. Both tests for the presence of alkaloids were negative.

The remaining dichloromethane extract was washed with water and, upon evaporation of solvent, gave 846 mg of a light brown syrup (0.5%

of the fresh weight). During the course of this extraction it was noted that the investigator was suffering from nausea and severe headaches. The crude dichloromethane extract exhibited these same effects.

The dichloromethane extract was permeated through Sephadex LH-20 and 13 fractions were collected. Fractions 10 and 11 (31.2 mg) were combined and permeated once more through Sephadex LH-20, then through Bio-Beads SX-8. Final purification of the target compound was achieved by HPLC using an Altex Ultrasphere-Cyano column (10 mm x 25 cm). 5.5 mg of a white solid was obtained and recrystallized from ethanol, mp. 150-151°C.

Characterization. The ^{1}H-NMR spectrum of this compound, (Figure 13) at first glance seemed rather simplistic. Only 3 distinct signals were seen, two doublets at δ7.7 and δ6.1 and a symmetrical multiplet of 16 peaks at δ2.4. ^{1}H-NMR decoupling experiments established that the doublets were coupled to one another (J = 5.7 Hz) but not to the multiplet at δ2.4. Integration of the ^{1}H-NMR spectrum gave a ratio of 1:1:2 for the three signals. The ^{13}C-NMR of the compound (Figure 14) exhibited five signals, a singlet at δ173 suggested a carbonyl carbon, probably an ester, doublets at δ153 and δ121 were assigned as two olefinic carbons, a singlet at δ90 for a quaternary sp_3 carbon and a

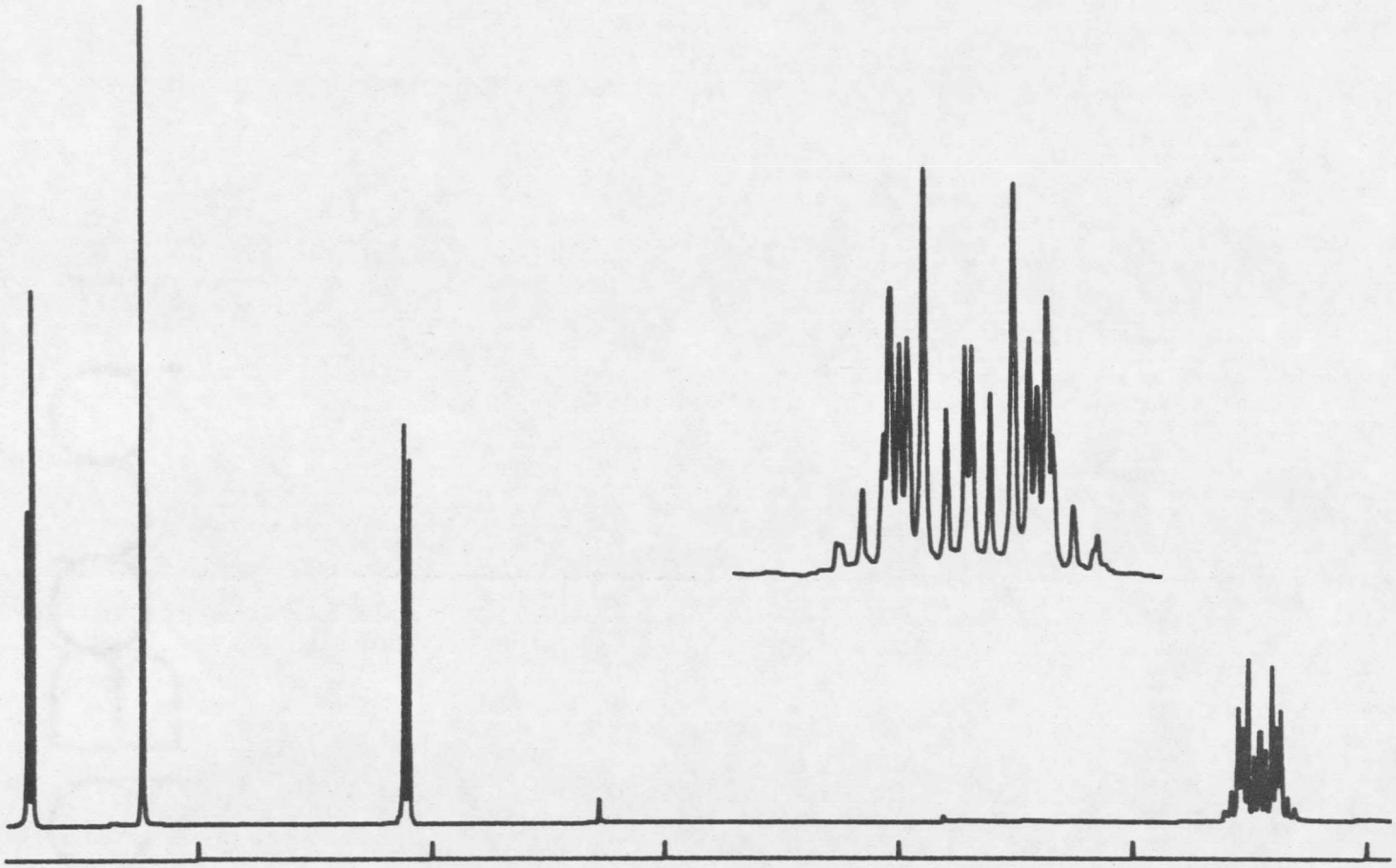


Figure 13. ^{1}H-NMR Spectrum of Anemonin.

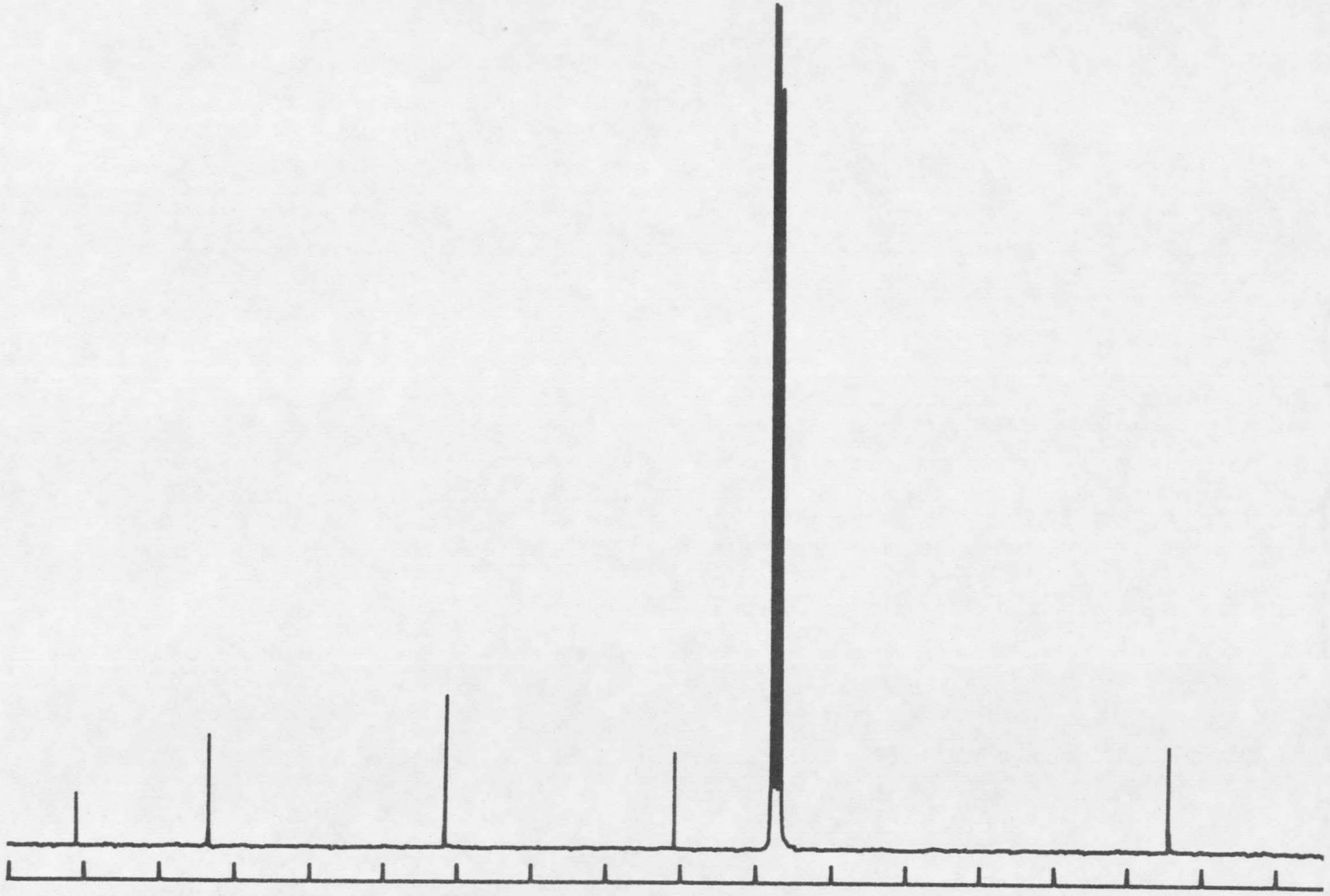
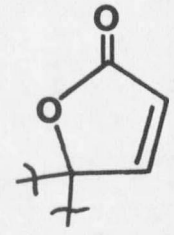


Figure 14. ^{13}C-NMR Spectrum of Anemonin.

triplet at δ23 for a methylene carbon. The IR spectrum (Figure 15) exhibited a strong band at 1790 cm^{-1}. This indicated that the compound could be an αβ-unsaturated-γ-lactone. The UV spectrum of the compound (Figure 16) exhibited a maximum at 221 nm (ε = 13,700), which supported the idea that an αβ-unsaturated-γ-lactone. From these data, part structure **23** was generated.

The symmetrical multiplet at δ2.4 can be explained by placing two methylene moieties between two αβ-unsaturated-γ-lactone rings. That is, a dimer of an αβ-unsaturated-γ-lactone. Only two feasible structures for the compound could be generated from this idea and from the supporting data, compounds **24** and **25**.

23



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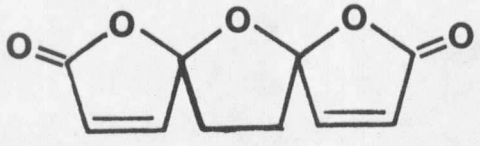

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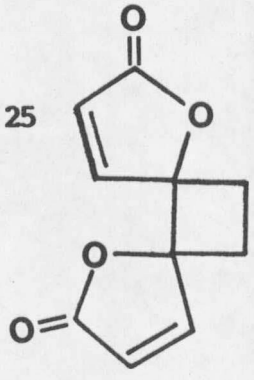


Mass spectrometry of the compound gave a molecular ion at m/z 192 ($C_{10}H_8O_4$), which supported structure **25**. The compound was identified as anemonin, **25**, (33), a compound derived from the dimerization of

Figure 15.

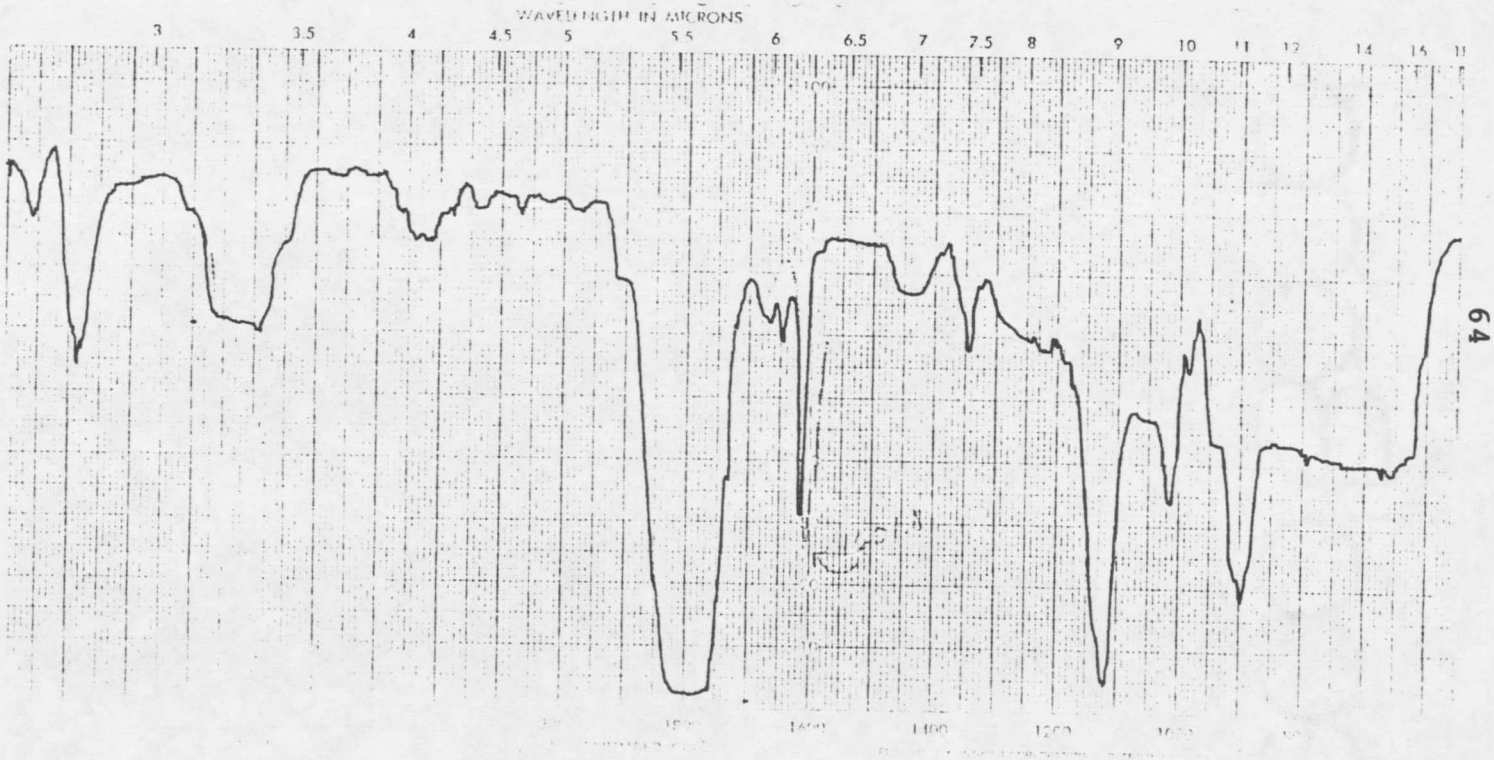


Figure 15. IR Spectrum of Anemonin.

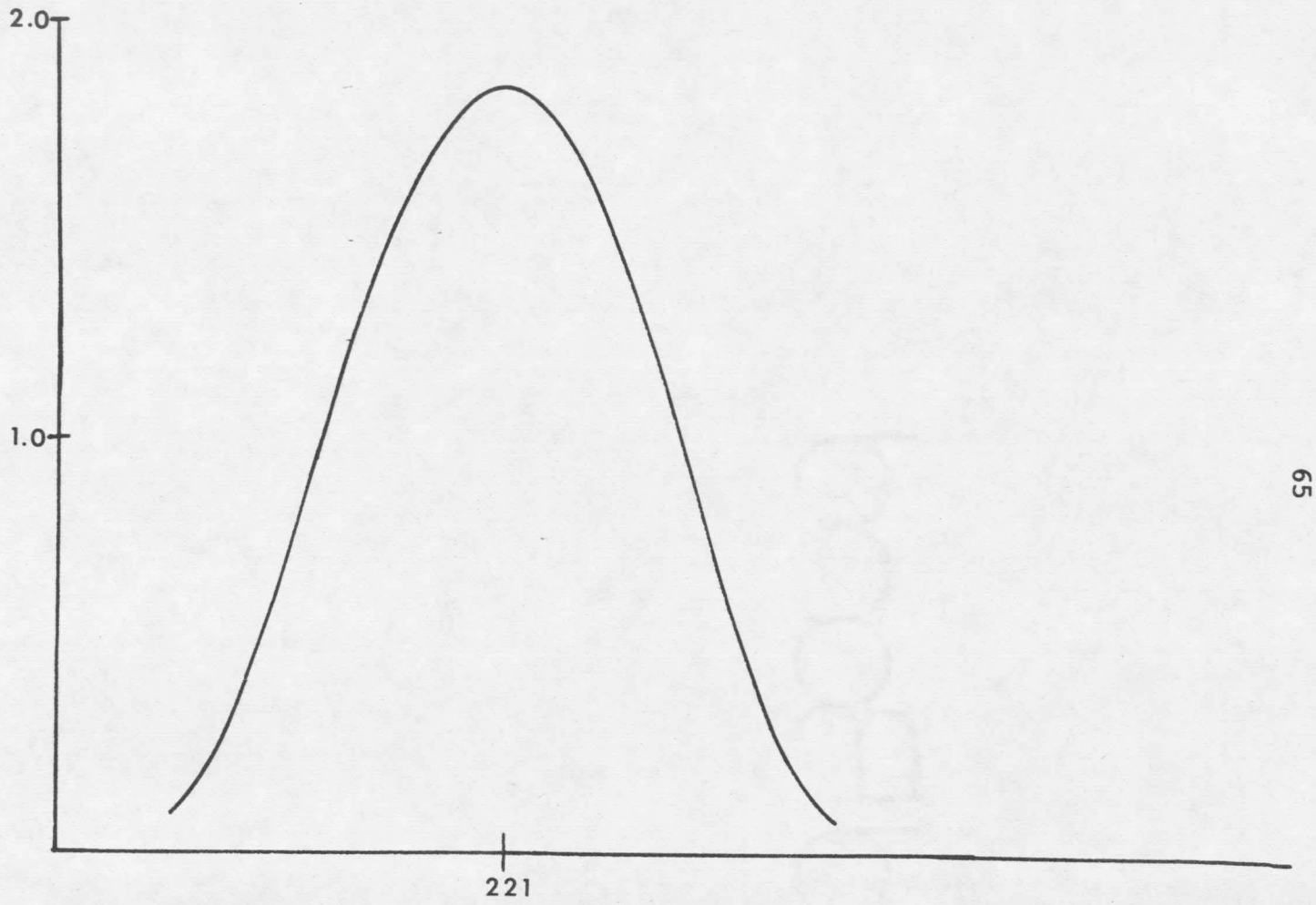
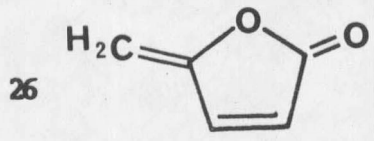


Figure 16. UV Spectrum of Anemonin.

protoanamonin **26** (34), an extremely disagreeable blistering agent.

It has long been known that many of the plants belonging to the family Ranunculaceae (Buttercup) cause erythema and blistering of the skin (35). One causative agent has been shown to be protoanemonin, **26**, isolated and characterized by Yasuhiko and Fujita (1922) (36) and Kipping (1935) (37). This compound is a water soluble oil.



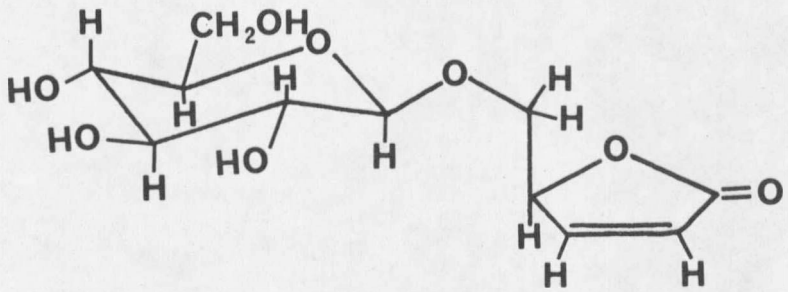
26

Anemonin **25** was first isolated in 1792 by Heyer (38), making it one of the earliest natural products isolated from plants. Hill and Heyningen (39) showed that protoanemonin is enzymically released from a glucosidic precursor upon crushing of the plant tissue. They suggested that the structure of the precursor was ranunculin, **27**.

They were able to isolate ranunculin as a crystalline substance which was stable both as a solid and in aqueous solution. They showed that ranunculin released D-glucose and protoanemonin upon treatment with alkali solution. Further proof that protoanemonin exists as a

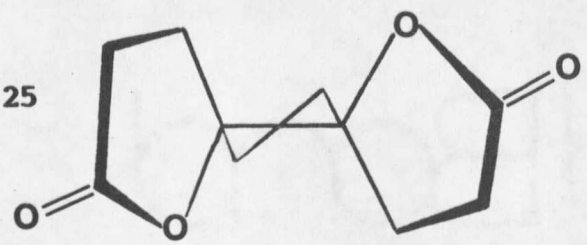
glucoside in the plant was established by Boll (40) in a study of the absolute configuration of ranunculin. Boll also stated that the glucoside was the β-anomer of D-glucose.

27



Moriarty and Karle (41) investigated anemonin by x-ray diffraction in order to determine the relative configuraion of the lactone rings. They established that aemonin is in the trans configuration and that the cyclobutane ring is not planar, but assumes a bent configuration with a dihedral angle of 152°.

25



A literature search on protanemonin, anemonin and ranunculin produced over 100 articles on these compounds. Baer, Holden and Seegal (42) showed that protoanemonin is an antibacterial agent from Anemone pulsatilla (buttercup), and that anemonin inhibited growth of E. coli at a dilution factor of 2000. These types of compounds (and others that contain the presence of an unsaturated lactone ring) are unique in that they are active against both Gram-positive and Gram-negative bacteria. Anemonin has been shown to have tuberculostatic action (43), produce a dermatis with blisters (44), cause kidney damage, paralysis of respiration and the beating of the heart (45).

The author proposes that the active ingredient in Clematis hirsutissima used by Native Americans to stimulate horses is protonanemonin **26**, which readily dimerizes to amenonin **25**.

2. Pharmacological Screens

The water soluble extracts of Clematis hirsutissima exhibited toxicity towards bacteria deficient in DNA repair activity. Because of this the water soluble extracts will be studied in the near future for potential anti-cancer compounds. Due to the small amount (5 mg) of aemonin isolated, antifungal screens were not carried out on this compound. Antifungal screens were carried out on 5 fractions (7-11) from the first LH-20 separation. The screens were all negative.

Summary. Anemonin **25**, was isolated and characterized from the

dichloromethane soluble extracts of the rhizomes of Clematis hirsutissima. Anemonin, known to be in other species of the Ranunculaceae is derived from the dimerization of protoanemonin **26**, which, in turn, is derived from the glucoside ranunculin **27** in the intact plant. Protoanemonin is presumed to be the active principal which is responsible for the stimulation of horses in usage by Native Americans.

EXPERIMENTAL

1. Extraction and Initial Separations - Clematis hirsutissima

Plant Material. Whole plant material was collected in July 1981 near the Fish Hatchery on Bridger Mountain Drive, 2 miles north of Bozeman, Montana. The collection site was located approximately ½ miles southwest of the Fish Hatchery on an open grassland slope.

Extraction. The rhizomes (156 g fresh weight) of Clematis hirsutissima were ground in a Waring blender and steeped for 24 hours in methanol (x2). The methanolic extracts were removed by suction filtration and evaporated, in vacuo, to a brown syrup. The ground rhizomes were then steeped in dichloromethane for 24 hours (x2). The reduced methanolic extracts were suspended in 100 ml water with 10 ml of concentrated ammonia and equilibrated with the dichloromethane extracts.

The dichloromethane extracts were then extracted with 0.1 M HCl (3 x 30 ml). The acidic layer was brought to pH ~10 by the addition of potassium carbonate. The basic aqueous phase was then extracted with diethyl ether (3 x 20 ml). The diethyl ether extract was then evaporated in vacuo, to a colorless oil, 5.2 mg (0.003% fresh weight). The dichloromethane extract was then washed with water (2 x 20 ml) and evaporated, in vacuo, to a brown syrup, 846 mg (0.54% fresh weight).

Initial Separation of the Dichloromethane Extract. The crude

dichloromethane (846 mg) extract was permeated through Sephadex LH-20 (195 x 2.5 cm column) using dichloromethane-methanol (1:1) as the eluent; thirteen fractions were collected.

2. Anemonin

Isolation. Fractions 10 (26 mg) and 11 (5 mg) from the LH-20 separation were combined and permeated once more through Sephadex LH-20 (2 x 120 cm column) using dichloromethane-methanol (1:1) as the eluent. Four fractions were collected; the fourth fraction, 21 mg, was permeated through Bio-Beads SX-8 (120 x 2 cm column) using dichloromethane-cyclohexane (3:2) as the eluent. Five fractions were collected; the fourth fraction was purified by HPLC with an Altex Ultrasphere-Cyano (10 mm x 25 cm column), using dichloromethane-hexane (95:5) as the eluent. The fourth fraction, 5.5 mg, afforded anemonin, **25**, a white solid. Recrystallization from ethanol-dichloromethane afforded colorless crystals, mp. 150-151°C.

An additional 1 mg of 25 was obtained from fraction 12 of the original Sephadex LH-20 separation by a similar sequence.

Characterization. IR: $\nu_{max}^{CHCl_3}$ 3570,2910,1790,1610,1110,1010, 980,910 cm^{-1}; UV: λ_{max}^{MeOH}: 221 nm (ε = 13,200); ^{1}H-NMR ($CDCl_3$): δ7.7(2H, d, J = 5.75); 6.1(2H, d, J = 5.75); 2.4(4H, complex m); ^{13}C-NMR ($CDCl_3$): 170.2(s), 153.2(d), 121.3(d), 90.4(s) 23.9(t); ms: m/z 192(M^+, 8.3%), 164(30), 96(100), 68(75).

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