Purification of hexadecanol-1-C□14 for solubility studies
by Clark Samuel Hoffman

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Chemistry
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Abstract:
Since the discovery that monomolecular films retard water evaporation, increased attention has been focused on the basic chemical and .physical properties of long chain alcohols which form such films. The present study is part of an effort to measure water solubility, a property of these compounds which has economic significance. Three samples of hexadecanol tagged with carbon-14 were purchased from three different companies and were used in the preliminary solubility measurements. Each- sample proved to be impure with significant amounts of radioactivity appearing in the impurities. : - , Column, preparative thin layer, thin layer, and .gas chromatographic techniques were used in attempts to purify the commercial samples-of hexadecanol-1-C14 . Preparative thin layer chromatography resulted in a 99.5% radiopurity for one sample of hexadecanol-1-C14; however, the alcohol still contained some lower molecular weight homologs and a small amount of water soluble tagged impurities. The failure of radiotracer experiments to produce a reasonable solubility for hexadecanol was attributed to the presence of these water soluble carbon-14 labeled impurities.

PURIFICATION OF HEXADECANOL-1-C^{14} FOR SOLUBILITY STUDIES

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ABSTRACT

Since the discovery that monomolecular films retard water evaporation, increased attention has been focused on the basic chemical and physical properties of long chain alcohols which form such films. The present study is part of an effort to measure water solubility, a property of these compounds which has economic significance. Three samples of hexadecanol tagged with carbon-14 were purchased from three different companies and were used in the preliminary solubility measurements. Each sample proved to be impure with significant amounts of radioactivity appearing in the impurities.

Column, preparative thin layer, thin layer, and gas chromatographic techniques were used in attempts to purify the commercial samples of hexadecanol-1-C^{14}. Preparative thin layer chromatography resulted in a 99.5% radiopurity for one sample of hexadecanol-1-C^{14}; however, the alcohol still contained some lower molecular weight homologs and a small amount of water soluble tagged impurities. The failure of radiotracer experiments to produce a reasonable solubility for hexadecanol was attributed to the presence of these water soluble carbon-14 labeled impurities.

INTRODUCTION

Modern civilization has placed an increasing burden on the natural resources of the United States. The future needs of our exploding population require efficient conservation of these resources. Water, a vital resource, must be available in abundant quantities; its shortage would cause a serious decline in our nation's economy. The water conservationist's task is complicated by an uneven seasonal distribution of precipitation coupled with varying regional climatic conditions. Storing spring runoff in large, man made reservoirs is one method used to insure an evenly distributed annual water supply. Stored water is then available during dry summers for crops, livestock, industry, and human consumption. Reservoirs in providing a solution to the seasonal problem have created several new ones, such as, water loss through seepage and evaporation.

In the Western United States evaporation accounts for an annual water loss of 25 million acre feet (1). According to Magin and Randall (2), "The evaporation loss is serious because it is usually greatest in dry areas where the supply is most valuable. For example, in the Great Basin and the Colorado River Basin, where the needs for water exceed the amounts available, evaporation dissipates almost one-sixth of the available water supply. Great economic gain would result in all arid areas if as much as one-fourth of the evaporation from stored water could be prevented."

Economical methods which retard evaporation are becoming more of a necessity each year. One method presently being studied uses long chain primary alcohols. The alcohol, or mixture of alcohols, when applied to the water surface forms a film one molecule thick (2,3). Under ideal laboratory conditions these monomolecular films or monolayers are effective evaporation

retardents (4); however, they are not as successful in retarding evaporation from large bodies of water (1,5). Wind, temperature, humidity, and various other natural phenomena reduce their effectiveness (2). Means by which alcohol may disappear from the monolayer include decomposition by ultraviolet radiation from the sun (6), bacterial attrition (7,8), evaporation(9), and solubilization (9). In the present work I am concerned with the last of these, specifically the solubility of hexadecanol. This alcohol was chosen as it is representative of the series of alcohols from C_{16} to C_{20} which presently seem to be the most useful in retarding water evaporation.

Hexadecanol solubility estimates to date have been dependent on extrapolation of data obtained for C_4 to C_{10} n-primary alcohols. In 1926, Hill and Walisoff (10) reported the solubility of butyl alcohol in water. These authors were able to measure the solubility of butanol over a wide temperature range using standard volumetric techniques. In 1933, Butler, Thomson, and Maclennan (11) determined the water solubilities of C_5 to C_8 n-primary alcohols by comparing the refractive indices of saturated solutions with those obtained for solutions of known concentration. The water solubilities of C_5 to C_{10} alcohols have also been determined (12,13,14) by surface tension measurements. In a typical determination, a plot of log concentration versus surface tension was made for unsaturated solutions of known concentration. The surface tension of the saturated solution was then measured, the linear plot was extrapolated to this point, and the solubility of the solution was graphically determined. Measurements of this type were made by Addison (12) for C_5 to C_8 alcohols and by Addison and Hutchinson (13) for decyl alcohol. These authors measured the surface tension using the "roughened plate"

method. Kinoshita, Ishikawa, and Shinoda (14) determined the solubilities of C_4 to C_{10} alcohols using the "drop weight" method to measure surface tensions.

Solubility measurements for alcohols with chain lengths greater than ten carbons have not been reported in the literature. However, several authors (13,14,15) have given equations from which one can estimate solubilities of long chain alcohols. All equations are based upon the linearity of log solubility versus carbon number plots. Table I contains a list of references, equations, and calculated solubilities of hexadecanol in water. The equation reported by Kinoshita, Ishikawa, and Shinoda (14) was not consistent with the solubility values they obtained for C_4 - C_{10} alcohols. I applied the method of least squares to their data and obtained the equation $\log C = -.602\ m + 2.40$. The solubility of hexadecanol obtained from this equation is listed in Table I.

J. H. Brooks and A. E. Alexander (9) have also estimated the solubility of hexadecanol. They used the plots of log concentration versus surface tension reported by Posner, Anderson, and Alexander (16) for C_4 to C_8 alcohols and by Addison and Hutchinson (13) for decyl alcohol. From these plots they observed that for a given lowering of surface tension (film pressure of 35 dynes/cm.) a plot of bulk concentration in the aqueous solution against chain length was linear. Through extrapolation the solubility of hexadecanol was obtained. Their result has been included in Table I. The present investigation is an attempt to measure the solubility of hexadecanol directly. There is no guarantee that the equations of Table I can be used outside the range for which they satisfactorily

TABLE I

Calculated Solubilities of Hexadecanol

Reference	Equation	Solubility (mol./l.)	Temp. (°C.)
Addison & Hutchinson (13)	$1 = 5.65 - 1.78 \log S$ (a)	6.2×10^{-8}	20
Erichsen (15)	$\log X = 3.4991 + (-0.043100) M$ (b)	6.1×10^{-8}	20
	$\log X = 3.5621 + (-0.045054) M$	2.4×10^{-8}	40
Kinoshita, Ishikawa & Shinoda (14)	$\log C = -1.39 m + 5.53$ (c)	2.1×10^{-17}	25
Least Squares Method	$\log C = -.602 m + 2.40$ (d)	5.9×10^{-8}	25
Brooks & Alexander (9)	None (e)	1.0×10^{-7}	40

(a) 1 = chain length, S = solubility (wt. %)
(b) M = molecular wt., X = solubility (mole %)
(c) See Least Squares Value
(d) C = solubility (mol./l.), m = chain length, Data from Ref. 14
(e) Film pressure 35 dynes/cm.

correlate actual solubility data.

Synthetic and analytic procedures for obtaining solubility values have been developed (17). The synthetic method involves changing the temperature and pressure of the system until all the solute present dissolves. The analytic method, in general, consists of obtaining equilibrium saturation and then analyzing the solution by either chemical or physical means. The analytic method does not require complete solubilization of the solute; equilibration may be obtained by intimately mixing the solute and solvent(18), percolation of the solvent through the solute (19), or by convection of the solvent through the solute (20). When the solute and solvent are intimately mixed, separation is best effected by filtering the excess solute from the saturated solution.

A suitable analytic technique must be available for accurately measuring the amount of soluble solute per unit volume of solution. Since the estimates of the solubility of hexadecanol are quite small (Table I), the common procedures of Reilly and Raye (18), Fox (19), and Gibson (20), which are relatively insensitive, are unsatisfactory. Jordan (21), Caddock and Davies (22), and Jones and Monk (23) have proposed radioisotope procedures for determining the solubility of a slightly soluble material. Since hexadecanol-1-C^{14} is readily available, it was decided to attempt the measurement of the solubility of hexadecanol by tracer methods.

EXPERIMENTAL

The experimental section can best be separated into two parts: 1) Purification of Hexadecanol-1-C^{14} and 2) Solubility Measurements.

Appendix I contains a listing of chemicals and materials used in the work. The listing includes manufacturer and, when available, purity specifications.

Purification of Hexadecanol-1-C^{14}

Hexadecanol-1-C^{14} samples were purchased from Tracerlab Inc., Volk Radiochemical Company, and Nichem Inc. Preliminary experimental work (described later) indicated that the samples of hexadecanol-1-C^{14} were contaminated with water soluble carbon-14 labeled impurities. Before accurate solubility measurements could be attempted, these impurities had to be removed. Purification techniques were limited to column and thin layer chromatography. Gas chromatography was used only to check on the efficiency of thin layer separations. The solvents used in chromatographic techniques were redistilled and stored in glass containers.

Column chromatography was carried out using solid supports of Unisil (silicic acid), Woelm Neutral Alumina, and 20% Silver Nitrate-Unisil. Ungefug (24) has reported the basic packing, loading, and eluting techniques used with these columns. The columns were of glass construction with an inside diameter of 1.2 cm. Columns containing 4.5 g. Unisil and 5.0 g. 20% $AgNO_3$-Unisil were ca. 8.5 cm. high. The 20% $AgNO_3$-Unisil columns were prepared by dissolving silver nitrate (20% by weight) in distilled water and then mixing the solution with the Unisil. The water was removed by vacuum rotatory evaporation, and the support was reactivated at 110°C. Woelm Neutral Alumina columns were deactivated with 1.5% distilled water. The alumina and water were placed in a one liter round bottom flask and rotated to obtain even adsorption of the water by the alumina. These 7.5 g.

columns were ca. 6.8 cm. high.

Thin layer chromatographic techniques and the preparation of plates have been reported by Mangold (25) and by Ungefug (24). These techniques were employed with four adsorbents: Silica Gel G, Silica Gel H, Adsorbosil I, and Adsorbosil II. Silica Gel G and Adsorbosil I contained 13 and 10 percent $CaSO_4$ binder, respectively.

When preparative thin layer chromatography was required, the techniques reported by Ungefug (24) were modified in the following way. Thin layer plates (250 microns thick) were prepared in the usual manner. One μl. spots of hexadecanol-1-C^{14} in benzene were placed on the plate side by side, 1 cm. from the bottom, so that they overlapped to form a band. The plates were placed in a solvent tank until the eluent reached the 15 cm. mark. Plates were then removed from the tank, air dried, and about one-half centimeter of the right and left hand edges were sprayed with Rhodamine 6 G. The alcohol band was located by examination of the sprayed portion under long wave ultraviolet light. The alcohol fluoresced a yellowish color and was visible at both sides of the plate. The leading and tailing edges of the band were marked with a sharp dissecting needle. A plastic template was then placed over the plate and lines were drawn through the adsorbent connecting the marks. In this way the location of the entire band was determined, and the sprayed portion of the adsorbent was scraped from the plate and discarded. The unsprayed portion of the alcohol band was scraped from the plate and extracted with ether. The adsorbent was separated from the alcohol by filtration through a medium pore sintered glass filter. The filter had previously been cleaned with chromic acid and

then washed with distilled water, acetone, and ether. The alcohol was concentrated by vacuum rotatory evaporation and the remaining solvent (ca. 2 cc.) was removed by a nitrogen stream. The alcohol purity was then determined by normal thin layer tests and gas chromatography. Band spotting hexadecanol-1-C^{14} enables one to purify approximately 10 mg./plate.

Gas chromatograms were obtained using an F&M Biomedical Gas Chromatograph, Model No. 400. The output from the chromatograph was fed to a Honeywell one millivolt strip chart recorder equipped with a Disc integrator. Flame ionization detection was used with a 3.8% SE-30 on 80/100 mesh diataport S column. Instrument settings were: temperature, 160°C.; carrier (helium) flow rate, 65 ml./min.; and chart speed, 1/2 in./min.

Solubility Measurements

Hexadecanol-1-C^{14} samples were weighed into 1 cc. beakers by means of a Mettler Micro Balance M5. The weights were accurate to $\pm$ 2 μg. The samples and beakers were then placed in 125 ml. screw top Erlenmeyer flasks. The flasks and beakers had previously been soaked in warm chromic acid, washed in phosphoric acid to remove adsorbed chromium, and rinsed extensively in tap water. They were then washed with distilled water until a negative acid test was obtained from blue litmus and finally, were dried in an oven at 110°C. (26). The teflon lined caps were wiped thoroughly with lens tissue to remove dust, soaked extensively in distilled water to remove soluble impurities, and dried at 110°C. before use.

Upon completion of the weighings for a series of samples, either 50 cc. of water, distilled over alkaline $KMnO_4$, or 50 cc. of dilute $AgNO_3$ (50 parts

silver nitrate per billion parts distilled water) solution were added to each flask. The flasks were capped, placed in a thermostat at 30°C. ± 0.1°C., and shaken vigorously by a Burrel Wrist-Action Shaker, Model No. 00. At approximately twenty-four hour intervals an aliquot of each solution was withdrawn by a hypodermic syringe equipped with a Leur-Lok tip containing a 5/8 in. 25 gauge needle. These aliquots (ca. 1 cc.) were then filtered through either type AA or HA, white, 13 mm. diameter, Millipore filters. A Swinny Hypodermic Adapter (Millipore Filter Corp., Bedford, Mass.) allowed direct filtration from the syringe into a 5 dram counting vial. The volume of filtrate was determined by weighing the counting vial before and after addition of the solution.

Fifteen cc. of a naphthalene-dioxane counting solution (27) was then added to each vial. The counting solution consisted of 60 g. naphthalene, 4 g. PPO (2,5-diphenyloxazole), 0.2 g. POPOP (1,4-bis-2-(5-phenyloxazolyl)-benzene), 20 ml. ethylene glycol, 100 ml. methanol (absolute), and redistilled 1,4 dioxane to one liter. Samples were counted in a Packard Tri-Carb Liquid Scintillation Spectrometer, Series 314A. Counting conditions were: freezer temperature, 0°C.; high voltage, 900 or 1,000 volts; discriminator settings, 10 v. - 50 v. - 100 v.; and analysis mode No. 3 with the C discriminator on.

RESULTS AND DISCUSSION

Preliminary solubility experiments were conducted as previously described with samples of hexadecanol-1-C^{14} as received from Tracerlab and Nichem. The alcohol concentration is proportional to the count rate per cc.

of a filtered aliquot. In these experiments the count rate increased when the amount of labeled alcohol used to make up a mixture was increased. This is illustrated in Figure 1, where the weight of hexadecanol-1-C^{14} used is plotted against the dpm./ml. for 1 cc. aliquots at a counting efficiency of 40.5%. After the mixtures had been prepared and placed in the shaker, aliquots were withdrawn daily, filtered, and counted. The count rates for a given mixture increased with time at the start and then leveled off. The points shown in Figure 1 represent the maximum values obtained.

The plot strongly indicates the presence of tagged impurities in the commercial hexadecanol-1-C^{14} samples. If the alcohol had been free of water soluble tagged impurities, the line AOC would have coincided with the line AXE. The count rate would then have remained constant upon the addition of more alcohol. The figure shows that this situation did not exist. The fact that the activity is directly proportional to the amount of alcohol introduced into the system and does not level off indicates that water soluble impurities account for a large percentage of aliquot activity. If the points in Figure 1 were accurately known, the plot could be used to estimate the solubility of hexadecanol in the presence of impurities. An extrapolation of the OC line to zero concentration provides one with a method of obtaining the ratio of the activity from hexadecanol-1-C^{14} (interval GO, Fig. 1) to the activity from water soluble impurities (interval GF, Fig. 1). Once this ratio is determined, the solubility of hexadecanol can be calculated. My data were not consistent enough, however, to permit an accurate location of the true activity curve, and an estimate

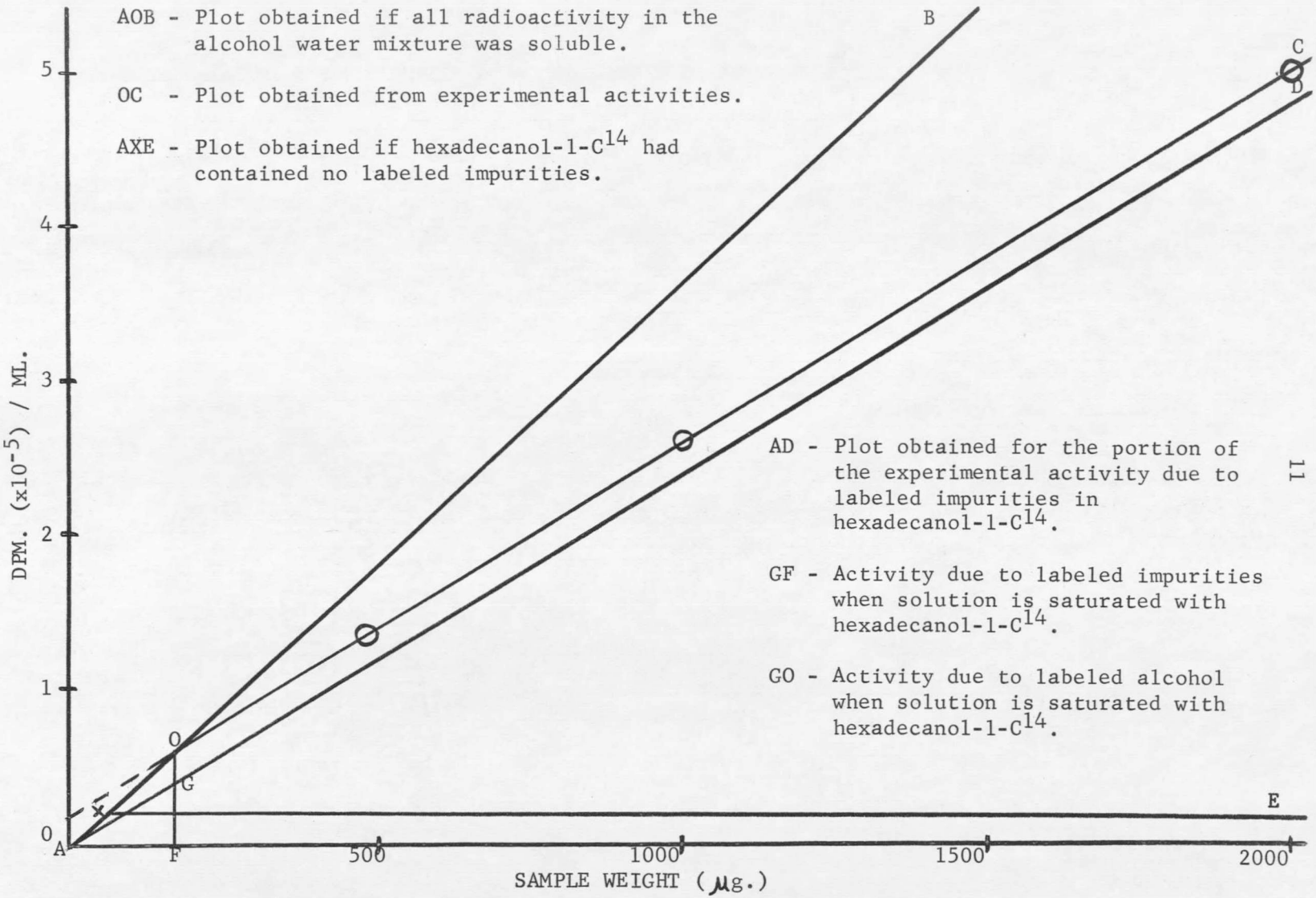

Figure 1. Initial Solubility Results

of the solubility was therefore not possible.

Dr. Shih Lu Chang (7) has pointed out that hexadecanol promotes the growth of bacteria, and that common bacteria, e.g. pseudomonas, are continually present in air and water. He has recommended the use of a 50 ppb. $AgNO_3$ solution as an effective deterrent to bacterial growth (28). Experiments were conducted to compare hexadecanol-1-C^{14}-distilled water mixtures with hexadecanol-1-C^{14}-50 ppb. $AgNO_3$ mixtures. Filtered aliquots of mixtures of hexadecanol and water containing $AgNO_3$ had count rates ca. 5×10^4 times higher than those from mixtures having the same amount of hexadecanol but no $AgNO_3$. No attempt was made to establish whether or not bacteria were responsible for this phenomenon, but it was decided to use the 50 ppb. $AgNO_3$ solution in the remaining experiments.

Hexadecanol-1-C^{14} Impurities

Thin layer chromatography was utilized to investigate the nature of the impurities present in commercial samples of hexadecanol-1-C^{14}. Silica Gel G plates spotted with the alcohol were developed with either benzene-ethyl acetate (9:1) or hexane-ether-acetic acid (90:10:1). Rhodamine 6G (mixed with the adsorbent before the plates were spread) served as the indicator. All plates were placed in a tank containing iodine vapor to facilitate the identification of any unsaturates present as impurities. When a plate is viewed under long-wave ultraviolet light, hexadecanol fluoresces yellow and impurities fluoresce either yellow (saturated) or blue (unsaturated) against a green background.

In determining the percentage activity of impurities versus alcohol,

the spots were individually scraped from the plate into separate 5 dram counting vials and 15 cc. of counting solution was added to each vial. The insoluble adsorbent settled to the bottom of the vial and the alcohol or impurity was dispersed throughout the counting solution. The solutions were then counted in the liquid scintillation counter.

Figures 2A and 2B are thin layer chromatograms of Tracerlab, Volk, and Nichem hexadecanol-1-C^{14}. The first plate (Fig. 2A) was developed with benzene-ethyl acetate (9:1) and the second (Fig. 2B) with hexane-ether-acetic acid (90:10:1). Both chromatograms show that impurities were present in each commercial sample. Approximate percentage impurities were determined from ratios of impurity activities and total activities. The results are: Tracerlab, 65%; Volk, 16%; and Nichem, 25%. It is obvious the use of commercial hexadecanol-1-C^{14} without purification would result in meaningless solubilities.

Column Chromatography

Because of the expense and limited amounts of hexadecanol-1-C^{14} on hand, it was decided to work out purification techniques using untagged alcohol (Lachat Chemical Company, 95% pure) containing impurities similar to those present in the tagged samples (compare Figs. 2A & 3A). The untagged samples of hexadecanol (50 mg. in 1 ml. of hexane) were placed on columns and eluted with various solvent combinations. Unisil columns were eluted successively with 60 ml. 18% benzene in hexane, 85 ml. 60% benzene in hexane, and 60 ml. benzene. Thin layer chromatography, benzene-ethyl acetate (9:1), of the concentrated fractions (Fig. 3B) showed that fraction