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CHARACTERIZATION OF A MICROBIAL SULFOLIPID AS A 1,14-DOCOSANEDIOL-1,14-DISULFATE

BY

GEORGE LOUIS MAYERS

A dissertation submitted to the Graduate Faculty in Chemistry in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

1967

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This manuscript has been read and accepted for the University Committee in Chemistry in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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Dedicated to

Professor Francis E. Condon

with appreciation for his inspiration and guidance

during my academic career.

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ABSTRACT

This dissertation presents the isolation, determination of structure and synthesis of the sulfatide, 1,14-docosanediol-1,14-disulfate. The sulfatide was isolated from a protozoan algae, Ochromonas danica. The sulfate ester was cleaved by solvolysis in moist dioxane. Studies on the solvolysis of sulfate esters in moist dioxane showed that the reaction was both stereospecific and quantitative. Mass spectral studies on long chain alkanediols showed that the position of a secondary hydroxyl group could be determined accurately. The solvolysis products from the sulfolipid preparation were analyzed by gas chromatography. Quantitative data showed that 1,14-docosanediol-1,14-disulfate accounted for 62% of the sample. The rest of the sample was composed of at least four other compounds.

1,14-Docosanediol-1,14-disulfate dipotassium salt was synthesized as follows. 12-Bromolauric acid was reacted with sodio-diethyl malonate. The resulting diester was saponified and yielded 1,1,12-dodecanetricarboxylic acid. The tricarboxylic acid was esterfied with dihydropyran, converted to a sodio-malonic ester and reacted with nonanoyl chloride. The 14-ketotripyranyl-1,13,13-docosanetricarboxylate was hydrolyzed and decarboxylated. The 14-ketodocosanoic acid was reduced with lithium aluminum hydride to 1,14-docosanediol. The alkanediol was sulfated

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with chlorosulfonic acid. The properties of the synthesized compounds were compared to those of the isolated natural products.

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CHARACTERIZATION OF A MICROBIAL SULFOLIPID AS A 1,14-DOCOSANEDIOL-1,14-DISULFATE

INTRODUCTION

Lipids are all substances that can be extracted from biological materials with non-polar solvents such as chloroform. The polar lipids isolated from biological tissues to date have one structural characteristic in common. They consist of a long aliphatic chain of at least 14 methylene carbons with a polar functional group at one end of the molecule. The polar group is phosphate, glycerol, inositol, a glycoside, an amine, sulfate or a combination of these. To date no lipids present in significant quantity have been reported which have such polar groups at both ends of the molecule.

This work reports the isolation, determination of structure, and synthesis of a 1,14-docosanediol-1,14-disulfate (Fig. 1) which is a new sulfolipid* having two polar groups separated by 13 methylene carbons. This is the third sulfolipid to be characterized to date, and the

*The term "sulfolipid" applies to any lipid that contains sulfur. A sulfatide is a sulfolipid in which the sulfur is in the form of a sulfate ester; and a sulfonolipid is a sulfonic acid (1).

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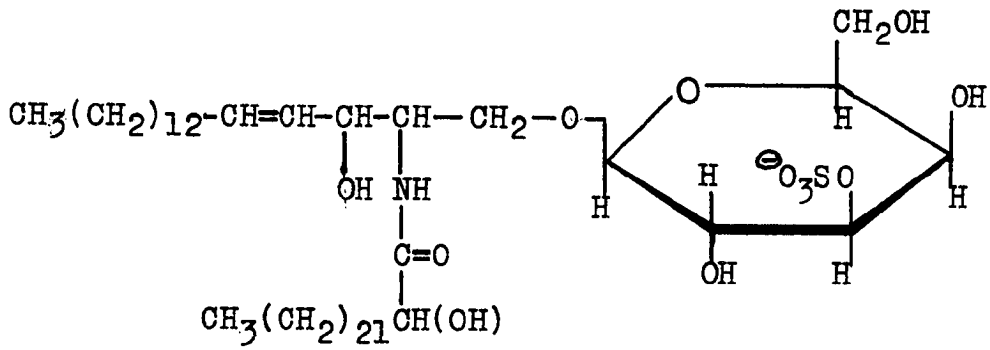
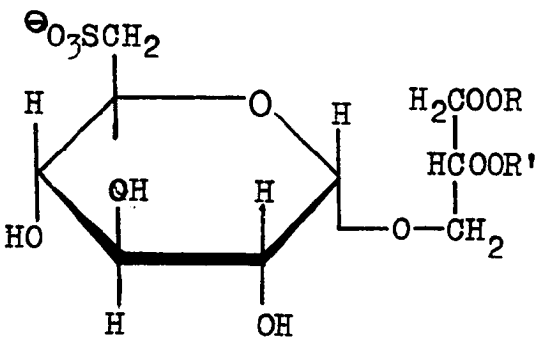
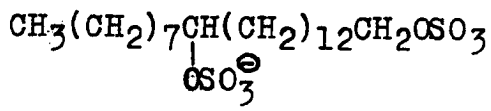


CEREBROSIDE SULFATE



6-SULFOQUINOVOSYL MONOGLYCERIDE



1,14-DOCOSANEDIOL-1,14-DISULFATE

FIGURE 1: The structure of three sulfolipids. Cerebroside sulfate was characterized by Blix in 1933 (3). 6-Sulfoquinovosyl monoglyceride was characterized by Benson et al. in 1962(6). 1,14-Docosanediol-1,14-disulfate is described in this thesis.

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first aliphatic sulfate.

Cerebroside sulfate (Fig. 1), first isolated by Koch in 1907 (2), was partially characterized by Blix in 1933 (3). The structure was not completely elucidated until 1962, when Yamakawa (4) established that the galactose was esterified with sulfuric acid in the 3 position rather than the 6 position (5). A chloroplast sulfonolipid. 6-sulfo-D-α-quinovopyranosyl-(1-1')-2',3'-di-O-acyl-D-glycerol (Fig. 1) was characterized by Benson, et al. (6,7). A sulfolipid has been found in a virulent strain of M. tuberculosis which has been identified as a sulfonic acid from its infrared spectrum (8).

Other sulfate esters have been found in biological material. Steroid sulfates, such as estrone sulfate, first characterized by Butenandt and Hofstetter in 1939 (9), have received considerable attention (10-16). Sulfated polysaccharides such as heparin and chondroitin sulfates have been found in many biological tissues. Tyrosine-O-sulfate, an amino acid residue, has been isolated from fibrinogen. None of these, however, have a structure similar to that of the docosanediol disulfate characterized here.

As one might suspect from its structure, the sulfatide described herein was a surface-active agent. Its aqueous solutions foamed readily. For many years the bulk of the synthetic surface-active agents (detergents) were

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alkylbenzenesulfonates. As it became apparent that some of these detergents were not biodegradable and were polluting the drinking water, detergent manufacturers sought a biodegradable synthetic detergent. Sewage degradative tests showed alkyl sulfates were biodegradable. Biodegradability depended on the cleavage of the sulfate. Many manufacturers turned to the use of alkyl sulfates such as sodium dodecyl sulfate for detergents.

Further evidence for biodegradability of alkyl sulfates has been reported by Payne et al. (17). They have isolated a primary alcohol sulfatase from a Pseudomonas species which clearly demonstrated that a biological system was capable of degrading alkyl sulfates. Payne has subsequently isolated a secondary alcohol sulfatase (18). The sulfolipid described herein is a long chain alkanediol disulfate. The presence of this structure in nature is further evidence for the biodegradability of alkyl sulfates, since it may be assumed that anything synthesized by nature can be degraded by nature.

The sulfolipid characterized in this thesis had been isolated by Haines as a crude proteolipid mixture from the protozoan algae, Ochromonas danica (1). Haines found that the sulfolipid contained sulfur in the form of a sulfate ester (19). Elemental analysis indicated the sulfatide was a 12 carbon hydroxyalkyl sulfate, a conclusion shown herein to be incorrect.

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In the work reported here, the sulfatide was further purified and obtained as a dipotassium salt of an alkanediol disulfate free of proteinaceous material by enzymatic hydrolysis. Its structure was elucidated by careful solvolysis to an alkanediol in dilute mineral acid and in moist dioxane; by synthesis of the alkanediol; and comparison of the physical properties, including spectra, of the natural and synthetic materials.

Solvolysis in moist dioxane offered several advantages over acid hydrolysis. Solvolysis of known sulfate esters in moist dioxane gave quantitative yields of both the alcohol and potassium hydrogen sulfate, under mild conditions. Furthermore, the solvolysis in moist dioxane is demonstrated to yield optically active (-)-2-octanol from the corresponding optically active sulfate.

The organic residue was analyzed by gas chromatography and purified by column chromatography. The carbon-containing moiety of the sulfolipid was identified as 1,14-docosanediol by elemental analysis, infrared spectrophotometry, mass spectroscopy and gas-liquid chromatography. The structure of the natural diol was corroborated by the synthesis of 1,14-docosanediol. The properties of the natural and synthetic alkanediol disulfate (Fig. 1) were also compared.

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ISOLATION AND CHARACTERIZATION OF THE SULFOLIPID

Isolation of the crude sulfolipid (proteolipid).

Ochromonas danica was grown on a synthetic medium (20) at 29°C under continuous light for a period of five days. The cells were harvested by continuous centrifugation and stored at -5°C to reduce degradation, until large quantities of the cells had been obtained.

The procedures for the isolation of the sulfolipid (Fig. 2) were chosen to maintain a minimum of decomposition of the sulfate ester. The isolation was carried out in the presence of potassium salts so that the sulfatide would be isolated as a potassium salt. In order to avoid sulfate ester hydrolysis the solutions were maintained at a pH greater than 7 except for a brief acidification to remove the fatty acids.

The cells were combined and extracted with chloroform:methanol::2:1 (v/v). The insoluble residue was removed by filtration and the chloroform and methanol were removed *in vacuo*. The residue, after evaporation of the chloroform and methanol, was saponified with a dilute solution of potassium hydroxide in methanol. The suspension was diluted with water and extracted with chloroform to remove the non-saponifiables. The suspension was acidified (pH 4.0) and extracted with chloroform to remove the fatty acids.

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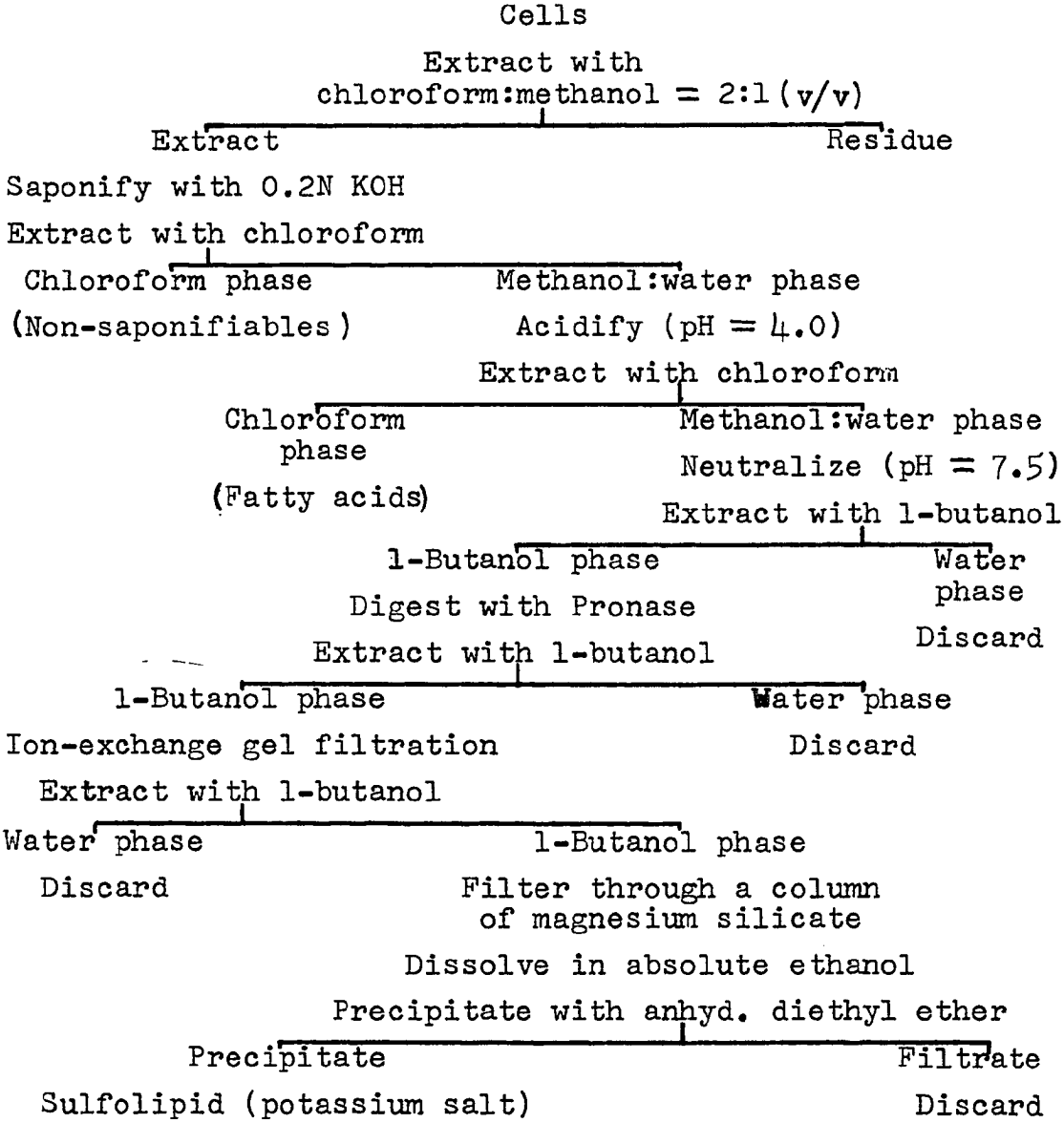

FIGURE 2: Procedure for the isolation of the sulfolipid from O. danica cells.

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The aqueous solution was neutralized (pH 7.5) with 1N potassium hydroxide and extracted with three 1000 ml portions of 1-butanol. 1-Butanol was chosen as it not only acted as an excellent solvent, but also helped to prevent emulsions and foaming and to remove water as an azeotrope. The 1-butanol was removed *in vacuo*.

It was very difficult to obtain truly anhydrous samples of the sulfolipid. In order to accomplish this, however, small amounts of absolute ethanol were added to the sulfolipid and then removed *in vacuo*. Unfortunately, this procedure was not completely effective. Samples dried for periods of time up to 48 hours *in vacuo* at 60°C still contained a small amount of water. These samples were regarded as a crude preparation of the sulfolipid as a potassium salt. This material ran as a single spot on thin layer chromatography. The material analyzed for 3.5% nitrogen and had infrared absorption at 1725 and 1550 cm^{-1}, which could be ascribed to the carbonyl absorption of a secondary amide. This suggested that the preparation was contaminated with a peptide.

The sulfatide appeared to be bound to protein in the cell (1). Isolation by solvent extraction as reported herein gave a sulfatide-protein complex that chromatographed as a single spot on TLC. It may be referred to as a proteolipid because of its solubility in organic solvents (21).

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Isolation of the protein-free sulfatide.

In order to establish the presence of protein, the sulfolipid preparation was hydrolyzed with aqueous acid and the resulting products were chromatographed in two dimensional paper chromatography. The paper chromatogram showed a spectrum of amino acids, but the basic amino acids seemed to be absent or present in very small quantity. The protein was removed by enzymatic hydrolysis with pronase, a proteolytic enzyme (22). These mild conditions were chosen to avoid decomposition of the sulfatide. The protein was hydrolyzed to amino acids and small peptides which were separated from the sulfolipid by filtration through sephadex and magnesium silicate columns.

The sulfatide that was isolated from these columns was a faint green. It was further purified by precipitation from absolute ethanol by addition of anhydrous ether. The sulfatide, obtained as an off-white powder, gave an elemental analysis which indicated that the carbon to sulfur ratio was 11:1.

Spectral studies on alkyl sulfates.

The infrared spectra of the salts of organic sulfate esters were studied in order to interpret the infrared spectrum of the sulfatide. A systematic study of the infrared absorption spectra of the salts of organic sulfate esters was reported by G. Chihara in 1960 (23,24). The salts of all the organic sulfate esters measured

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showed two very strong and characteristic absorptions at 1260-1240 and 1220-1210 cm^{-1} (Table 1). Long chain alkyl sulfates showed strong absorption in the region of 1081-1062 cm^{-1} and the position of the absorption tended to shift to a higher wave number with lengthening of the chain. The absorption of ν_{O-S} in aliphatic sulfates appeared in the region of 757-838 cm^{-1} and shifted to a higher wave number region with lengthening of the hydrocarbon chain. La Lau and Dahmen (25) showed that the infrared spectra of long chain alkyl sulfates were very similar if they were substituted in the same position, while there were many differences, particularly in the 800 cm^{-1} region, for an isomeric series in which the chain length was kept constant.

In 1961, Lloyd et al. (26) reported on a study of the infrared spectra of salts of alkyl sulfates and compared their spectra with those of the parent alcohols. They found strong absorption in the 1210 to 1260 cm^{-1} region; however, they reported a narrower range for ν_{O-S} of 770-810 cm^{-1} which shifted to higher frequency for secondary sulfates.

In 1962, Hummel (27) reviewed the subject for long chain alkyl sulfates in a book on the infrared absorption of surface-active compounds. He presented many spectra to corroborate the absorption ranges for primary and secondary sulfates. For primary sulfates he found

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TABLE 1: Position of Characteristic Infrared Absorptions in Organic Sulfate Esters.

Sulfate ester	Reference	Absorptions (in cm^{-1})			
		$\nu_{as\,S=O}$	$\nu_{s\,S=O}$	ν_{C-O}	ν_{O-S}
Potassium methyl sulfate	A	1252, 1215	1063	1020[B]	757
Potassium ethyl sulfate	A	1256, 1218	1062	1020[B]	773
Potassium n-propyl sulfate	A	1247, 1220	1067	1040[B]	777
Potassium n-butyl sulfate	A	1247, 1219	1069		819 (790[B])
Potassium n-pentyl sulfate	B	1240, 1210	1070	990	800
Potassium n-hexyl sulfate	B	1240, 1210	1070	990	800
Sodium n-octyl sulfate	A	1245, 1215	1079		830
Sodium n-decyl sulfate	A	1244, 1220	1078		834
Sodium n-dodecyl sulfate	A	1246, 1219	1081	1020[C], 990	836
Sodium n-tetradecyl sulfate	A	1252, 1211	1078		838
Potassium isopropyl sulfate	A	1246, 1216	1041	930[B]	761
Potassium cyclohexyl sulfate	A	1241, 1204	1075	960[B]	822
Potassium 2-octyl sulfate	C	1240-1220	1060	940	780
Sulfatide	C	1260-1200	1065	985, 935	815

A. Values reported by Chihara (23, 24).
B. Values obtained from spectra reported by Lloyd et al. (26).
C. Values obtained from spectra in Fig. 3.

absorption at 1267-1220 cm^{-1} (7.9-8.2 μ) (very strong, generally split), 1100-1075 cm^{-1} (9.1-9.3 μ) (medium), about 1000 cm^{-1} (10 μ) (medium, repeatedly split up) and at 840-834 cm^{-1} (11.9-12 μ) (medium, lower alkyl sulfates absorb in wider ranges). For secondary sulfates he found absorption at 1250 cm^{-1} (8.0 μ) and 1228 cm^{-1} (8.15 μ) (very strong, pair of bands) 1075-1063 cm^{-1} (9.3-9.4 μ) (strong) and 945-925 cm^{-1} (10.6-10.8 μ) (medium).

Figure 3 gives the infrared spectra of a primary and a secondary alkyl sulfate. The spectrum of sodium dodecyl sulfate (a pure sample contributed by Dr. H. L. Rosano) shows the characteristic bands for a primary sulfate at 1250-1220, 1080, 1020, 1000, and 835 cm^{-1}. The alkyl chain is evident in the CH_2 rocking at 720 cm^{-1}. The spectrum of 2-octyl sulfate (Fig. 3) shows bands characteristic of a secondary alkyl sulfate at 1240-1220, 1060, 1030, and 938 cm^{-1}. This spectrum compares well with other 2-alkyl sulfates reported by la Lau and Dahmen (25).

Figure 3 also shows the spectrum of the sulfolipid isolated from Ochromonas danica. The extremely strong sulfate absorption is noted in the region of 1200-1250 cm^{-1}. The spectrum is nearly identical to that of sodium dodecyl sulfate with two important differences. The first is a shoulder at 926-945 cm^{-1} which, as noted earlier, is characteristic of secondary sulfates and appears in the spectrum of 2-octyl sulfate. A second difference between

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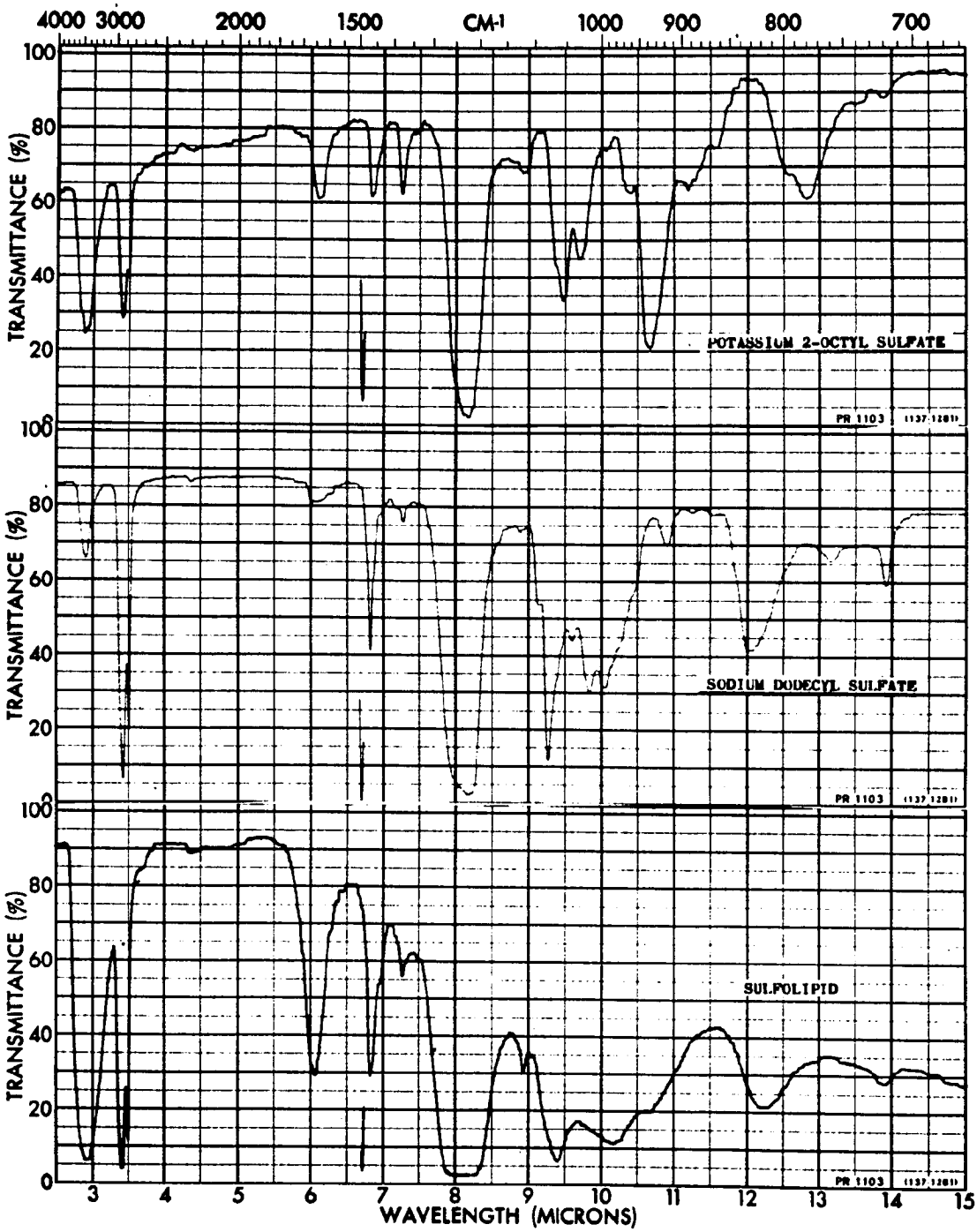

FIGURE 3: INFRARED SPECTRA OF THE POTASSIUM SALT OF THE SULFATIDE, OF POTASSIUM 2-OCTYL SULFATE AND OF SODIUM DODECYL SULFATE. EACH SPECTRUM WAS TAKEN OF A POTASSIUM BROMIDE WAFER WITH AN INFRACORD MODEL 137.

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the sulfatide and the dodecyl sulfate infrared spectra is the presence of intense water peaks at 3500 cm^{-1} and 1650 cm^{-1}. These peaks justify the inclusion of at least two molecules of water in the calculated elemental analysis. Rigorous attempts to remove the water were unsuccessful.

The nmr spectrum of the sulfatide (Fig. 4) in deuterium oxide showed a sharp peak at 8.7 τ typical of aliphatic methylene (28). A triplet at 9.1 τ was typical of a terminal methyl on a long chain. A triplet at 6.5 τ was identified with the methylene group of the primary sulfate ester. Integration indicated that the triplet contained the proton on the carbon with the secondary sulfate. It was not possible, however, to determine either the splitting pattern or the precise chemical shift. The sharpness of the methylene peak and the lack of any other peaks which could be ascribed to another methyl group excluded the possibility of branching. Thus the sulfatide appeared to be a long chain alkyl sulfate.

A long chain alkyl sulfate has not previously been reported in nature. Such compounds have been synthesized commercially for use as synthetic detergents for many years. It has been found (1) that the substance characterized here is synthesized by O. malhamensis, Chlorella pyrenoidosa (Van Neil), Tetrahymena pyriformis, Chlamydomonas, Pseudomonas (sea water bacterium) and Streptomyces griseus. Thus it appears that the new

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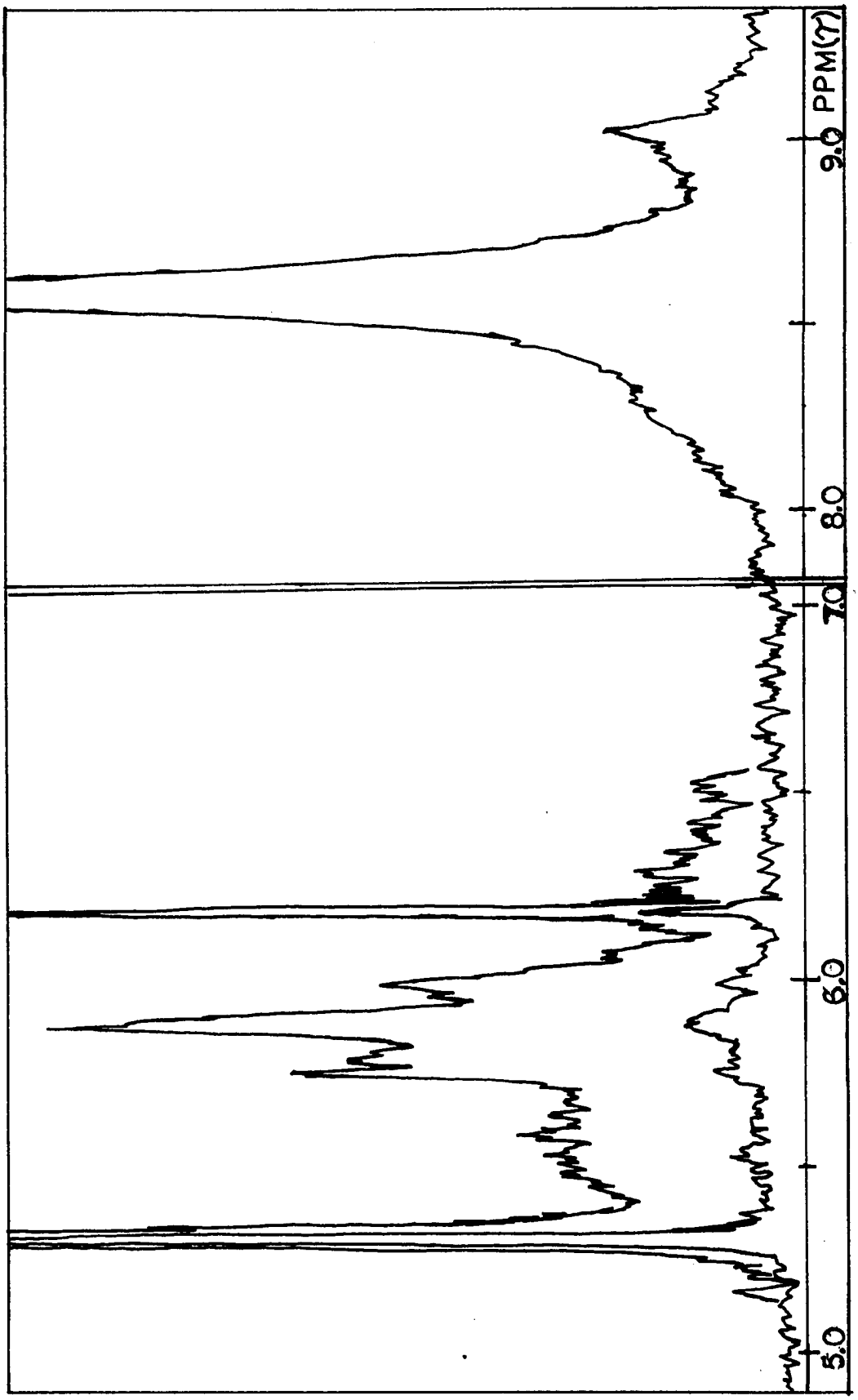

FIGURE 4: Proton resonance spectrum of the sulfatide (potassium salt), determined in deuterium oxide solution.

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sulfolipid is ubiquitous in micro-organisms.

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DETERMINATION OF STRUCTURE

Isolation of the sulfolipid diol.

The sulfolipid appeared to be an alkyl sulfate, but further purification proved extremely difficult, undoubtedly because of its surface-active properties. It was thought that if the sulfate ester were hydrolyzed, the resulting compound with a hydroxyl group would be easier to isolate. Two methods of hydrolysis were used, aqueous mineral acid (15,29-35) and moist dioxane (9,12,14,16,36). The hydrolysis products were isolated and investigated so as to elucidate the structure of the sulfolipid.

The aqueous hydrolysis of alkyl hydrogen sulfates has been investigated several times (15,29-35). Acid hydrolysis has been shown to proceed by sulfur-oxygen fission (35). Lieberman (15) explained the retention of configuration in the acid hydrolysis of steroid sulfates in a similar manner. Burwell (29) also found that the acid hydrolysis of sodium s-butyl sulfate yielded 7 to 48% iso-butylene. Therefore, several products were expected from the hydrolysis of the sulfolipid, and several were obtained.

The thin layer chromatogram of the products from the aqueous acid hydrolysis of the sulfatide showed six spots, the major one of which had a R_f identical with that of 1,12-octadecanediol (R_f 0.12). Compounds with

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small variations in chain length or isomers were not separable in this thin layer chromatography system. Chromatography of the hydrolysis products on magnesium silicate separated the major product from the other components. The analysis indicated an empirical formula, $C_{10.2}H_{21.6}O$, but the melting point (87.0-87.5°C) and its chromatographic behavior were more indicative of an alkanediol. The alkanediol was assumed to be eicosanediol ($C_{20}H_{42}O_2$), heneicosanediol ($C_{21}H_{44}O_2$) or docosanediol ($C_{22}H_{46}O_2$).

The infrared spectrum of the alkanediol isolated from the sulfatide is shown in Fig. 5. The infrared spectra of two other long chain alkanediols, 1,12-octadecanediol and 1,4-eicosanediol, are shown for comparison. The spectra show many similarities, especially in the bands that correspond to the C-O stretch for primary (1060 cm^{-1}) and secondary (1120 cm^{-1}) hydroxyls.

Mass spectral studies on alcohols.

The mass spectra of secondary alcohols were first studied by Friedel, Shultz and Sharkey (37). The position of a secondary hydroxyl on an aliphatic chain was determined by the presence of fragments in the spectrum derived from cleavage on either side of the secondary carbon. The fragment containing the hydroxyl was especially stable. In addition, fragments of low intensity consistently appeared in these spectra that showed

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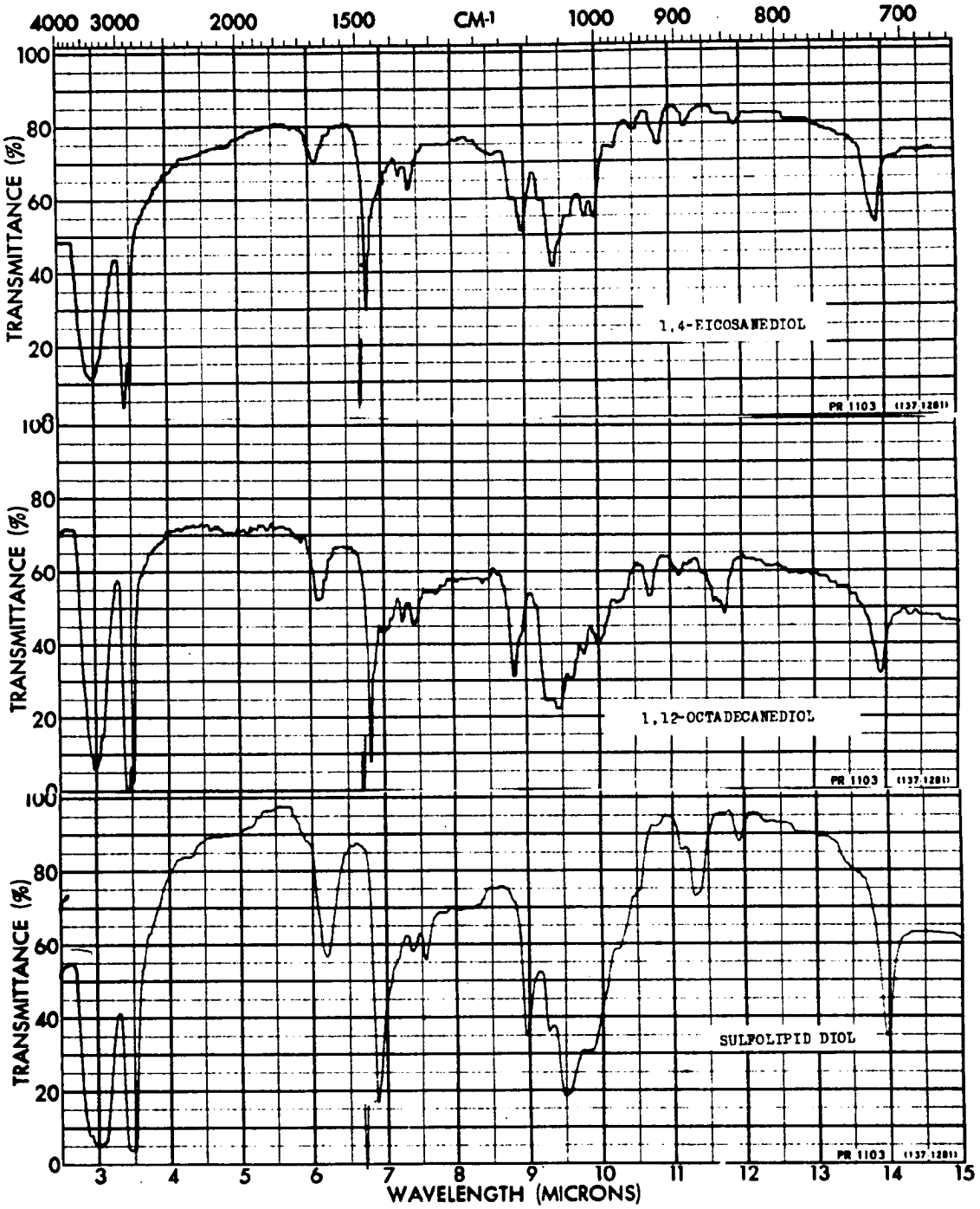

FIGURE 5: INFRARED SPECTRUM OF THE SULFOLIPID DIOL. THE SPECTRA OF 1,4-EICOSANEDIOL AND 1,12-OCTADECANEDIOL ARE SHOWN FOR COMPARISON. THE SPECTRA WERE TAKEN OF POTASSIUM BROMIDE WAFERS WITH AN INFRACORD MODEL 137.

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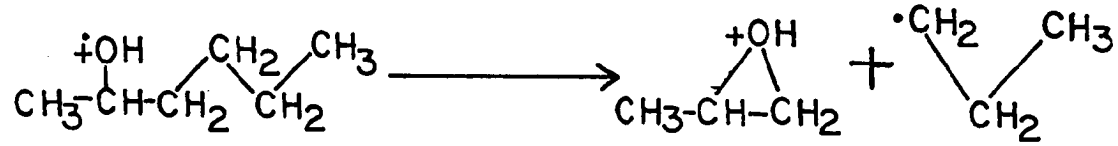
cleavage in the β position and in which the hydroxyl was retained. There were two peaks whose mass numbers differed by one hydrogen that could be ascribed to β-cleavage. Both peaks were present in the spectra of Friedel et al. (37), but the relative intensities varied from compound to compound. In the case of 2-hexanol (M.W. 102), the peak at m/e 58 ($CH_3-\overset{+OH}{\overset{\|}{C}}-CH_2^{\cdot}$) was 4 times as intense as the peak at m/e 59 ($CH_3\overset{+OH}{\overset{/\ \backslash}{C}}H-CH_2$). The peak at m/e 59 could result from β-cleavage of the molecular ion with formation of an oxonium ion:



The peak at m/e 58 was probably the result of β-cleavage of the (M - 1) ion, $CH_3\overset{+OH}{\overset{\|}{C}}-(CH_2)_3CH_3$. In 3-hexanol (M.W. 116), the peak at m/e 73 was 2½ times the peak at m/e 72; while in 3-octanol the results were similar to those for 2-hexanol. Thus it appeared that the peaks for β-cleavage of a secondary alcohol could result from cleavage of the molecular ion and the molecular ion that had lost a hydrogen.

In a study of the mass spectra of long chain hydroxyalkanoates Ryhage and Stenhagen (38) have obtained similar data. Their data also showed that β-cleavage occurred to give two peaks whose mass numbers differ by

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1 unit. Thus for methyl 7DL-hydroxyoctadeconoate (M.W. 314), their data showed m/e 187 (M - $(CH_2)_9CH_3$), 1.1%; m/e 186 (M - 1 - $(CH_2)_9CH_3$), 4.4%; m/e 165 (M - $(CH_2)_4COOMe$), 1.3%; and m/e 164 (M - 1 - $(CH_2)_4COOMe$), 0.4%.

Ryhage, et al. (39) also studied the spectrum of 2,4-eicosanediol as a model in determining the structure of phthiocerol. In addition to fragments resulting from cleavage on either side of carbon four they found an unusally intense peak at C_{15} indicative of β-cleavage.

The mass spectrum of 1,4-eicosanediol is shown in Fig. 6. A complete tabulation of the peaks is given in Table 4. The assignment of peaks can be seen in Table 2. Major peaks at masses 89 and 255 arise through initial cleavage of the molecular ion at the bonds adjacent to the secondary hydroxyl group. As was the case with the other diols, the alkyl ion peaks did not appear in the spectrum. The peak at mass 268 is an example of β-cleavage of the (M-1) ion. The molecular ion peak had low intensity in all three spectra but the peak for M - $2H_2O$ was present in high concentration and together with M - H_2O forms a good basis for the identification as an alkanediol.

The results from the mass spectrum of 1,12-octadecanediol obtained by the Morgan-Schaffer Corporation (Montreal, Quebec, Canada) are fully tabulated in Table 4 and summarized in Table 2. Major peaks at masses 115 and

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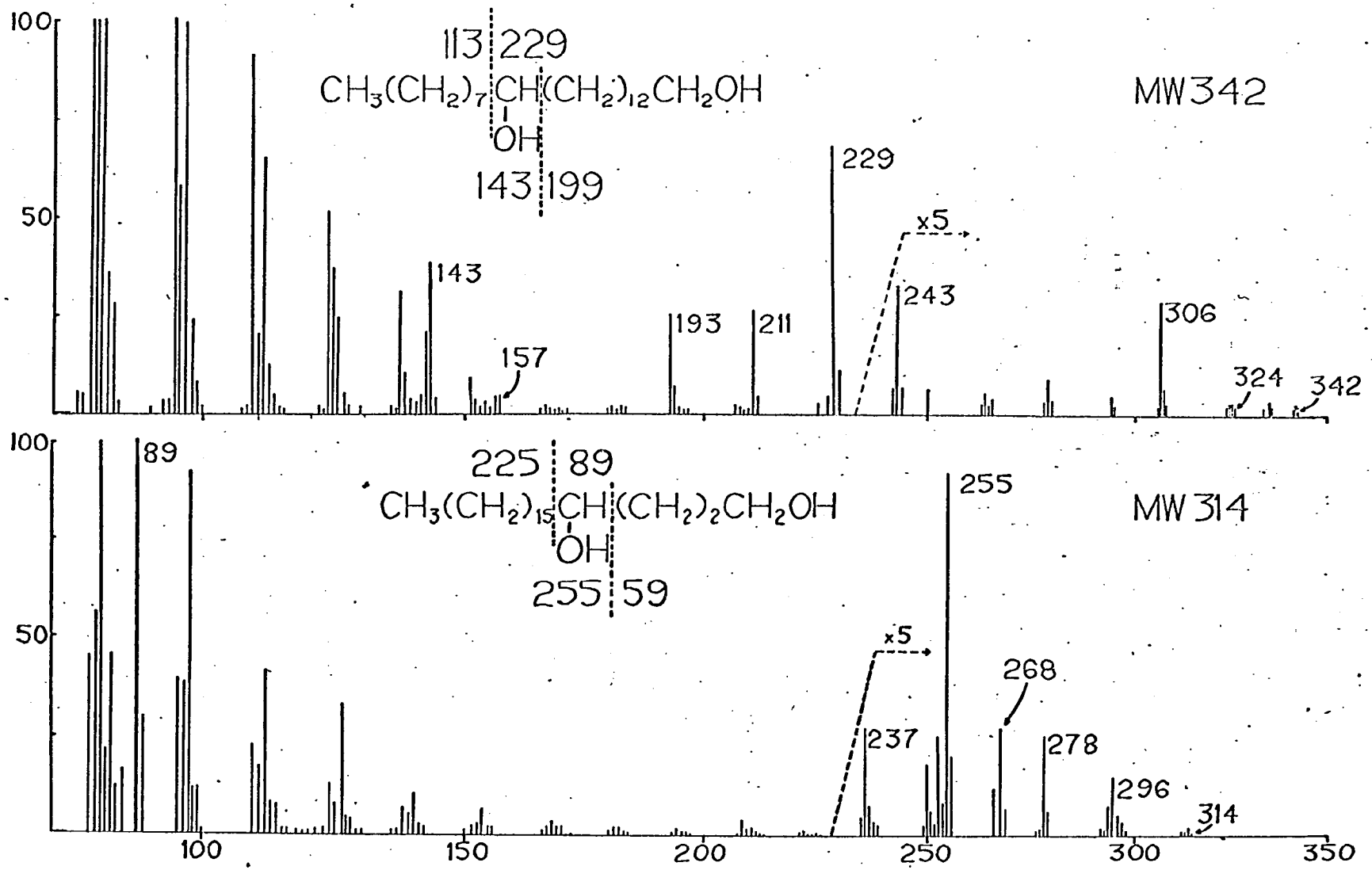

FIGURE 6: Mass spectra of the sulfolipid diol and 1,4-eicosanediol.

TABLE 2: Mass spectra of alkanediols.

1,12-Octadecanediol	1,4-Eicosanediol	Sulfolipid diol	Peak Designation	Ion
286	314	342	M[a]	$CH_3(CH_2)_x\overset{+\cdot OH}{C}H(CH_2)_yOH$
285	313	341	M - 1	$CH_3(CH_2)_x\overset{+OH}{\overset{\|}{C}}-(CH_2)_yOH$
268	296	324	M - H_2O	
250	278	306	M - $2H_2O$	
201	89	229	A[b]	$HOCH_2(CH_2)_y\overset{+}{C}HOH$
215	103	243	A + CH_2	$HOCH_2(CH_2)_y\overset{+OH}{\overset{/}{C}}H-\overset{\diagdown}{C}H_2$
183	71	211	A - H_2O	
165		193	A - $2H_2O$	
214	102	242	B[c]	$HOCH_2(CH_2)_y\overset{+OH}{\overset{\|}{C}}-CH_2\cdot$
115	255	143	C[d]	$CH_3(CH_2)_x\overset{+}{C}HOH$
129		157	C + CH_2	$CH_3(CH_2)_x\overset{+OH}{\overset{/}{C}}H-\overset{\diagdown}{C}H_2$
128	268	156	D[c]	$CH_3(CH_2)_x\overset{+OH}{\overset{\|}{C}}-CH_2\cdot$

[a]M = molecular ion. The molecular ion contains X methylene groups between the terminal methyl group and the secondary hydroxyl group and Y methylene groups between the secondary hydroxyl group and the primary hydroxyl group. X and Y are determined from the structure of the diol. [b]A = fragment resulting from cleavage of the C-C bond adjacent to the secondary hydroxyl in the direction of the terminal methyl group. [c]B and D = fragments resulting from β-cleavage of the (M - 1) ion. [d]C = fragment resulting from cleavage of the C-C bond adjacent to the secondary hydroxyl and in the direction of the primary hydroxyl group.

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201 arise from initial cleavage of the molecular ion α to the secondary hydroxyl group. The peaks at masses 214 and 128 are the result of β-cleavage of the (M-1) ion. The peak at m/e 129 is due to β-cleavage of the molecular ion.

Comparisons of the mass spectra of 1,12-octadecanediol and 1,12-octadecanediol-1,12-diacetate were obtained from the spectra taken by Ralph T. Holmann, (Hormel Institute, Austin, Minnesota). The results are tabulated in Table 5. It is important to note that in these spectra it was not possible to determine the position of the secondary acetate in the case of the diacetate, but the cleavage is clear in the case of the diol.

The mass spectrum of the sulfatide diol is shown in Fig. 6. The data are tabulated in Table 4 and summarized in Table 2. The peak at mass 342 represents the peak of highest mass and is the molecular ion peak. Peaks at masses 324 (M - H_2O) and 306 (M - $2H_2O$) justify the diol assignment and confirm the molecular weight as 342. The major peaks of masses 143 and 229 indicate that the secondary hydroxyl is at C_{14}. The peaks at masses 211 and 193 show that the fragment of mass 229 contains two hydroxyl groups. Peaks at masses 243 and 157 apparently result from β-cleavage of the molecular ion previously described. Peaks at 242 and 156 probably result from β-cleavage of the (M-1) ion. The absence of

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peaks (other than those mentioned) which interrupt the normal alkane pattern of fragmentation indicates that the methylene chain is unbranched. The absence of major peaks separated by 14 or 28 mass units indicate that the substance is a single compound rather than a mixture of diols of varying chain length. Thus, the mass spectrum of the diol obtained by acid hydrolysis of the sulfatide shows unequivocally that the diol is 1,14-docosanediol.

The elemental analysis of the sulfatide indicates that there are two sulfate groups to 22 carbons. The infrared spectrum (Fig. 3) of the sulfolipid shows that one is secondary (926-945 cm^{-1}) and that the other is primary (830 cm^{-1}). It is therefore concluded that the sulfatide is a 1,14-docosanediol-1,14-disulfate.

Dioxane Solvolysis.

Since the aqueous acid hydrolysis yielded several products, there was a possibility that it might be obscuring some other initial products from the sulfolipid. Solvolysis in moist dioxane offered a milder method by which the sulfolipid could be hydrolyzed than by use of aqueous acids. As a model, 1,12-octadecanediol-1,12-disulfate dipotassium salt was synthesized and then solvolyzed in moist dioxane. This reaction yielded 1,12-octadecanediol as the sole ether-soluble product.

Since the solvolysis of steroid sulfates in moist dioxane (12-14,16) was reported to give quantitative

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yields of both the free sterol and potassium hydrogen sulfate, the solvolysis procedure was expected to be quantitative for alkyl sulfates as well. Potassium dodecyl sulfate was solvolyzed in moist dioxane. The solvolysis resulted in quantitative yields of lauryl alcohol and potassium hydrogen sulfate, which were identified by their infrared spectra.

The sulfatide was solvolyzed in moist dioxane. The yields of organic and inorganic material were close to those expected for 1,14-docosanediol-1,14-disulfate dipotassium salt, but the recovered organic material was at least 10% greater than expected and the inorganic material indicated it was primarily potassium sulfate. Thus the solvolysis hinted that the sulfolipid was not pure 1,14-docosanediol-1,14-disulfate dipotassium salt. Thin layer chromatography of the organic products showed five spots, but only two seemed to be major. One had the same R_f as 1,12-octadecanediol (R_f 0.12) and was therefore assumed to be docosanediol. The mixture was analyzed by gas chromatography, but this technique failed to give a clean separation of the compounds (Fig. 7).

The solvolysis products were acetylated and analyzed by gas chromatography as this permitted separation of the components (Fig. 8). Acetylation of 1,12-octadecanediol was followed by gas chromatography, which demonstrated that the reaction was essentially complete after $3\frac{1}{2}$ hrs.

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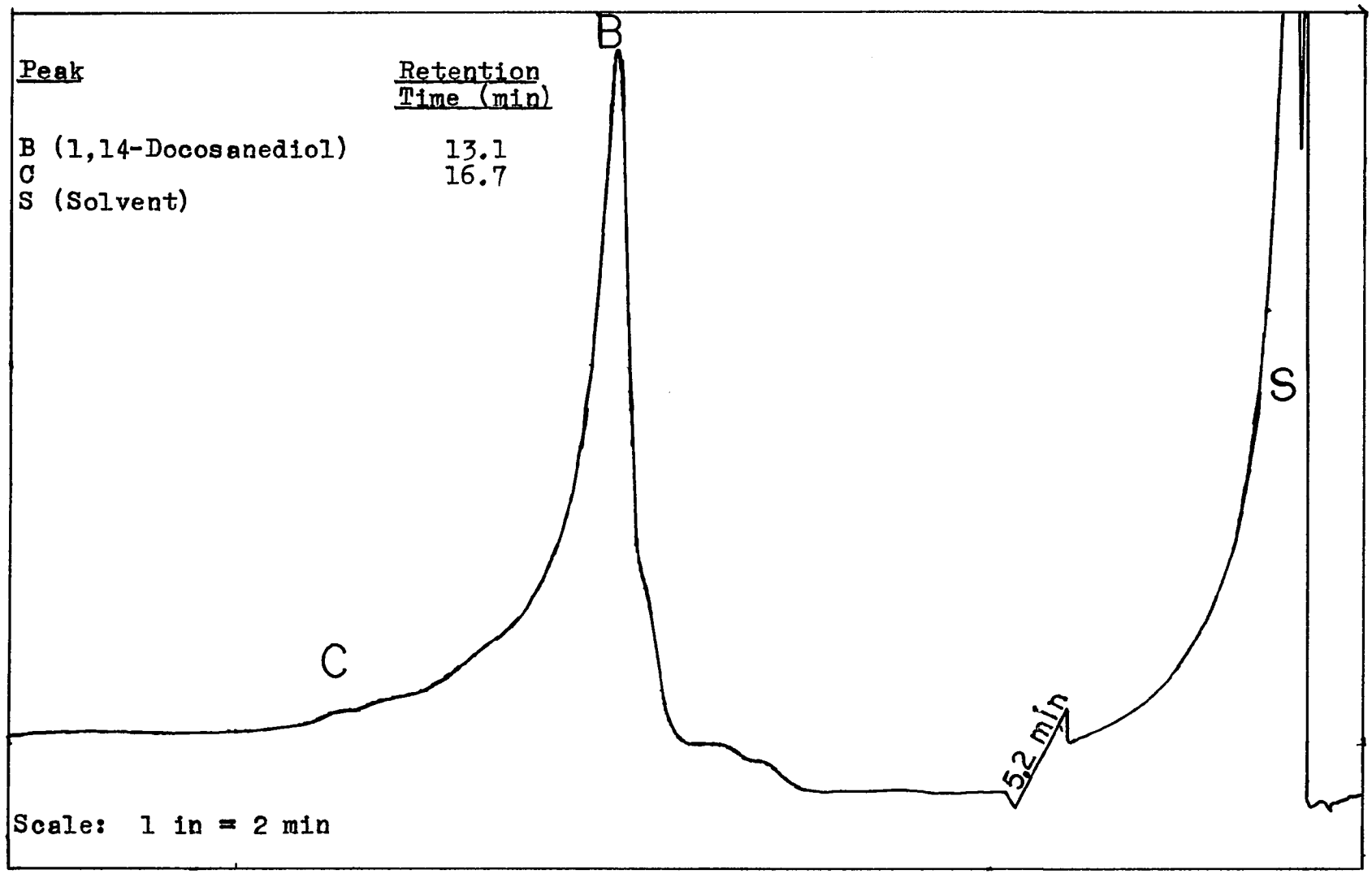

FIGURE 7: Gas Chromatogram of the products of the solvolysis of the sulfatide in moist dioxane. Column: 3% JXR on Chromosorb Q. Programed: 200 to 250°C.

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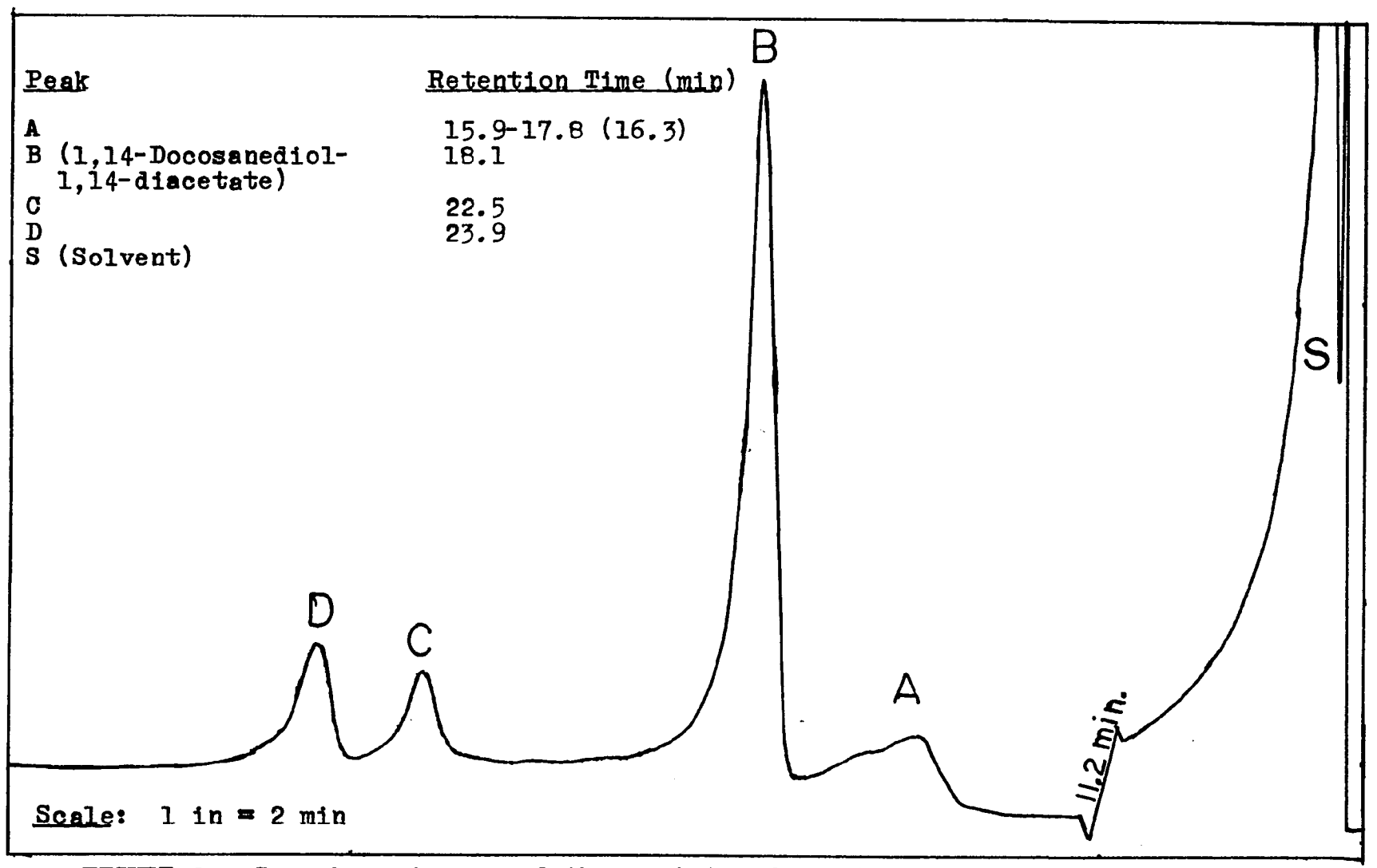

FIGURE 8: Gas chromatogram of the acetates from the solvolysis of the sulfolipid in moist dioxane. Column: 3% JXR on Chromosorb Q. Programed: 200 to 250°C.

The components of the mixture were quantitated by the areas of the peaks. A sample of 1,14-docosanediol (previously isolated from the products of acid hydrolysis) was acetylated and then was found to co-chromatograph with the major peak in the acetates from the solvolysis mixture. The 1,14-docosanediol-1,14-diacetate thus identified accounted for 62% of the mixture. This percentage was in good agreement with the amount of alkanediol isolated from a silica gel column. The group of peaks in front of the 1,14-docosanediol-1,14-diacetate (Fig. 8) accounted for 18% of the volatile compounds while peaks C and D (Fig. 8) accounted for 8% and 12% respectively.

Stereochemistry of the solvolysis of sulfate esters in moist dioxane.

Since the sulfatide had an asymmetric center at the carbon with the secondary sulfate group and had been isolated from natural material, it was expected to exhibit optical activity. It was very difficult to purify the sulfatide. Consequently, it was not possible to interpret the optical rotation of the sulfolipid preparation.

Previous work had indicated that solvolysis in moist dioxane should leave the stereochemistry of the secondary hydroxyl group intact. 1,14-Docosanediol could be isolated as a pure substance by column chromatography on silica gel. It was hoped therefore that the optical rotation could be determined on the 1,14-docosanediol

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obtained from solvolysis in moist dioxane.

The stereochemistry of solvolysis in moist dioxane was determined on (+)-2-octyl sulfate. Optically active 2-octanol was sulfated with chlorosulfonic acid and yielded 2-octyl sulfate with retention of configuration. This alkyl sulfate was then solvolyzed in moist dioxane and found to yield 2-octanol with 97.5% retention of configuration. Solvolysis of the sulfolipid in moist dioxane therefore should yield 1,14-docosanediol with retention of configuration for the secondary hydroxyl group.

The solvolysis products were chromatographed on a column of magnesium silicate and the 1,14-docosanediol was isolated. The 1,14-docosanediol collected from the column was gas chromatographed and found to contain two minor contaminants (Fig. 9). The 1,14-docosanediol accounts for more than 95% of the sample. The two contaminants seem to have a polarity similar to that of 1,14-docosanediol, judging from their behavior in column chromotography and solubility in hexane and other non-polar solvents. One of the contaminants had a retention time in gas chromatography which approximated that of 1,4-eicosanediol. It was possible, therefore, that the two minor contaminants were an eicosanediol and a tetracosanediol.

Sufficient 1,14-docosanediol was isolated to indicate that the specific rotation (sodium lamp) was less than 1°. This result was in accordance with data reported

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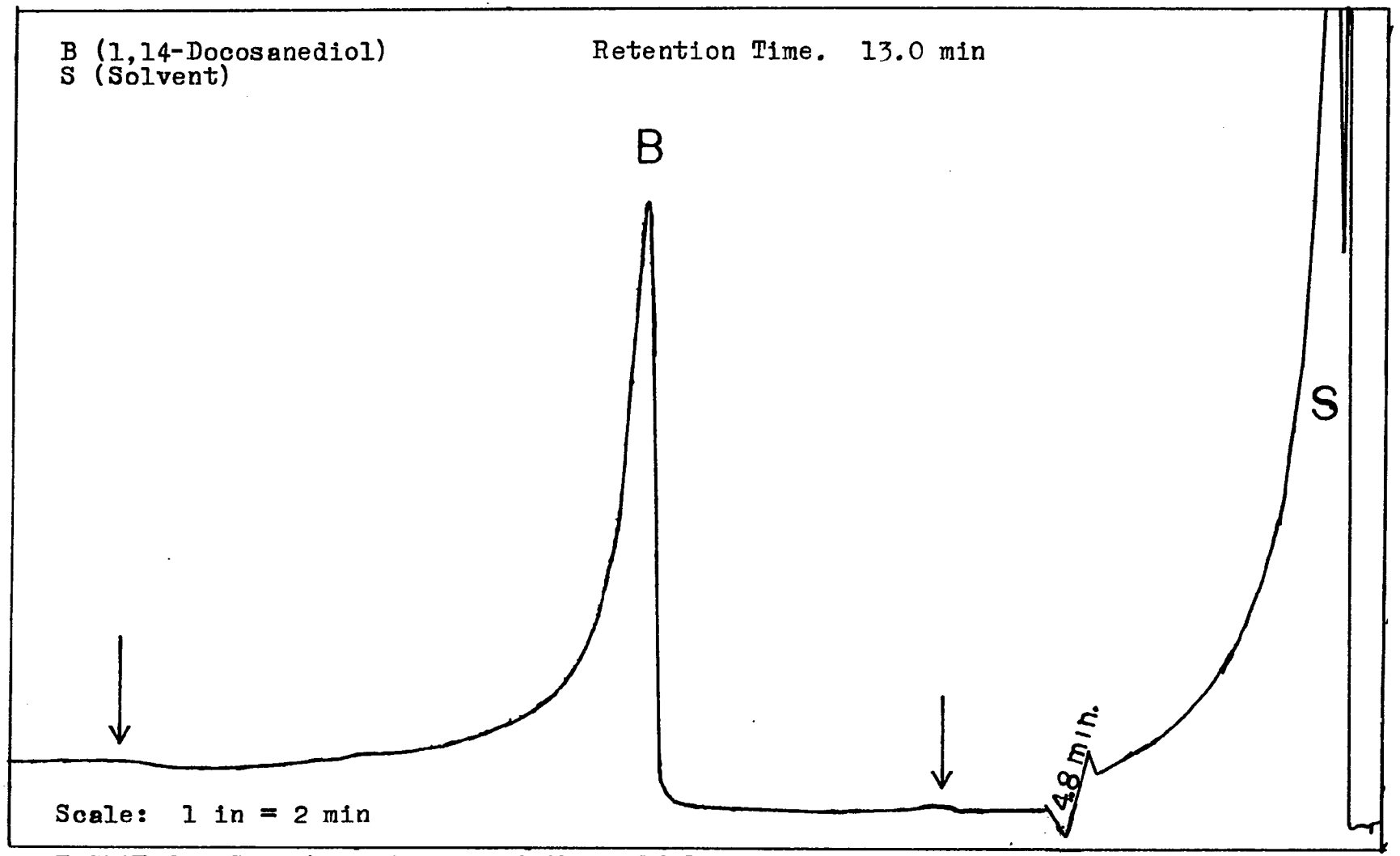

FIGURE 9: Gas chromatogram of the sulfolipid diol isolated from the solvolysis of the sulfolipid. Column: 3% JXR on Chromsorb Q. Programed: 200 to 250°C.

by Schroepfer and Block (40) for methyl 9D-hydroxyoctadecanoate which had a specific rotation of - 0.17° ± 0.05° and for methyl (-)-10-hydroxyoctadecanoate which had a specific rotation of - 0.15° ± 0.05°.

Other compounds in the sulfolipid preparation.

Since dioxane solvolysis indicated that the sulfolipid contained other components, attempts were made to characterize them. As noted earlier, the components all seemed to form acetates. When the alkanediol was removed from the sample, the next major component appeared to be peak "C". When peak "C" was acetylated and gas chromatographed the change in retention time appeared to be commensurate with the formation of a diacetate.

Compound "C" was collected from the gas chromatograph in capillary tubes. The capillary tubes were placed in the splitter and acted as air condensers with gradient cooling. When the collected peak was reinjected, it separated into two peaks, compound "C" and compound "X" (Fig. 10). The infrared spectrum of compound "C" was characterized by an absence of peaks for most functional groups (Fig. 11). Compound "C" does not show carbonyl absorption in its infrared spectrum, but compound "X" (Fig. 11) absorbed at 1720 cm^{-1}. The infrared spectra of these samples were very weak because they were taken as thin films on approximately 1-2 mg of material.

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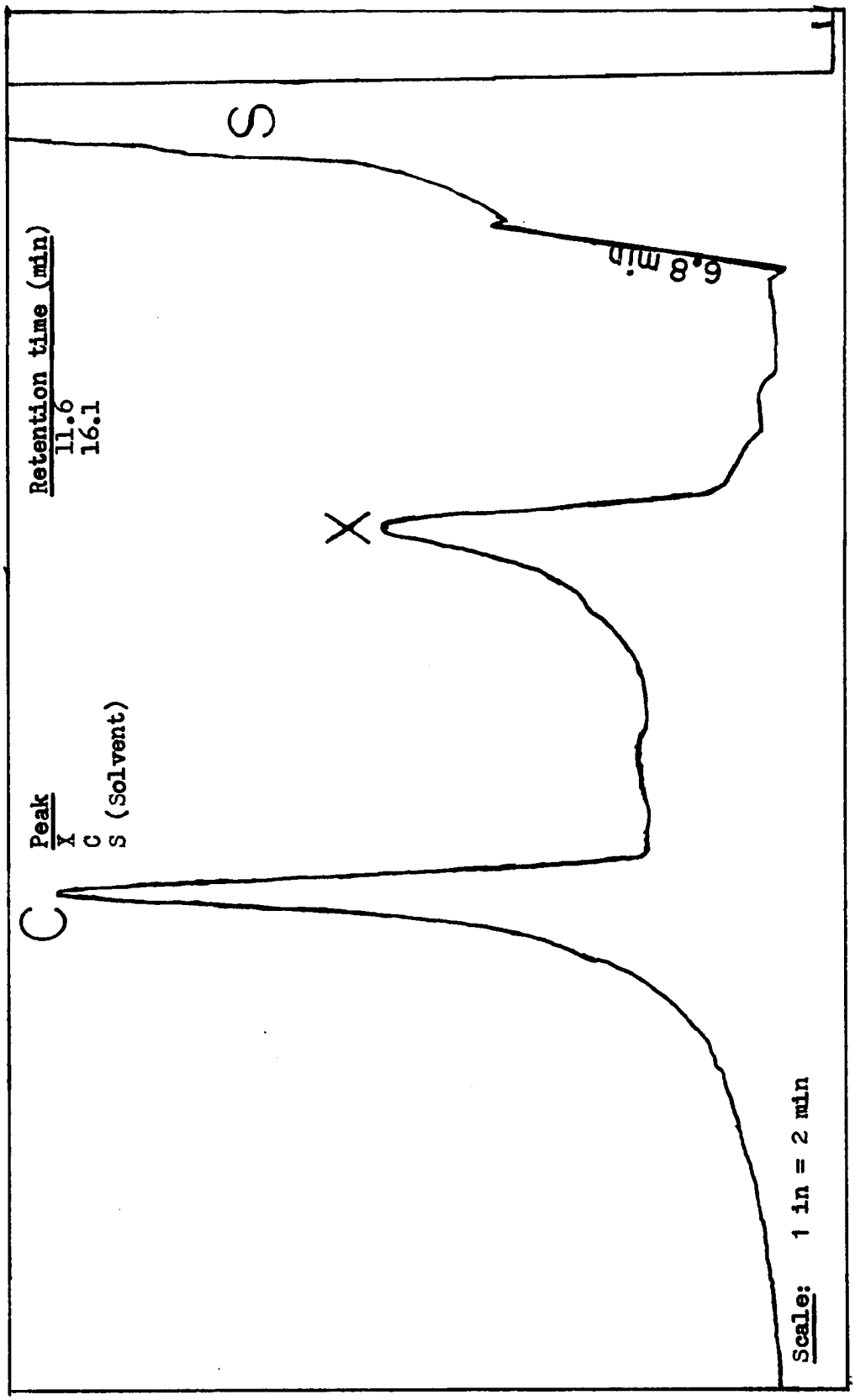

Figure 10: Gas chromatogram of Peak "C" collected from the gas chromatograph.
Column: 3% JXR on Chromsorb Q. Programed: 200 to 250°C.

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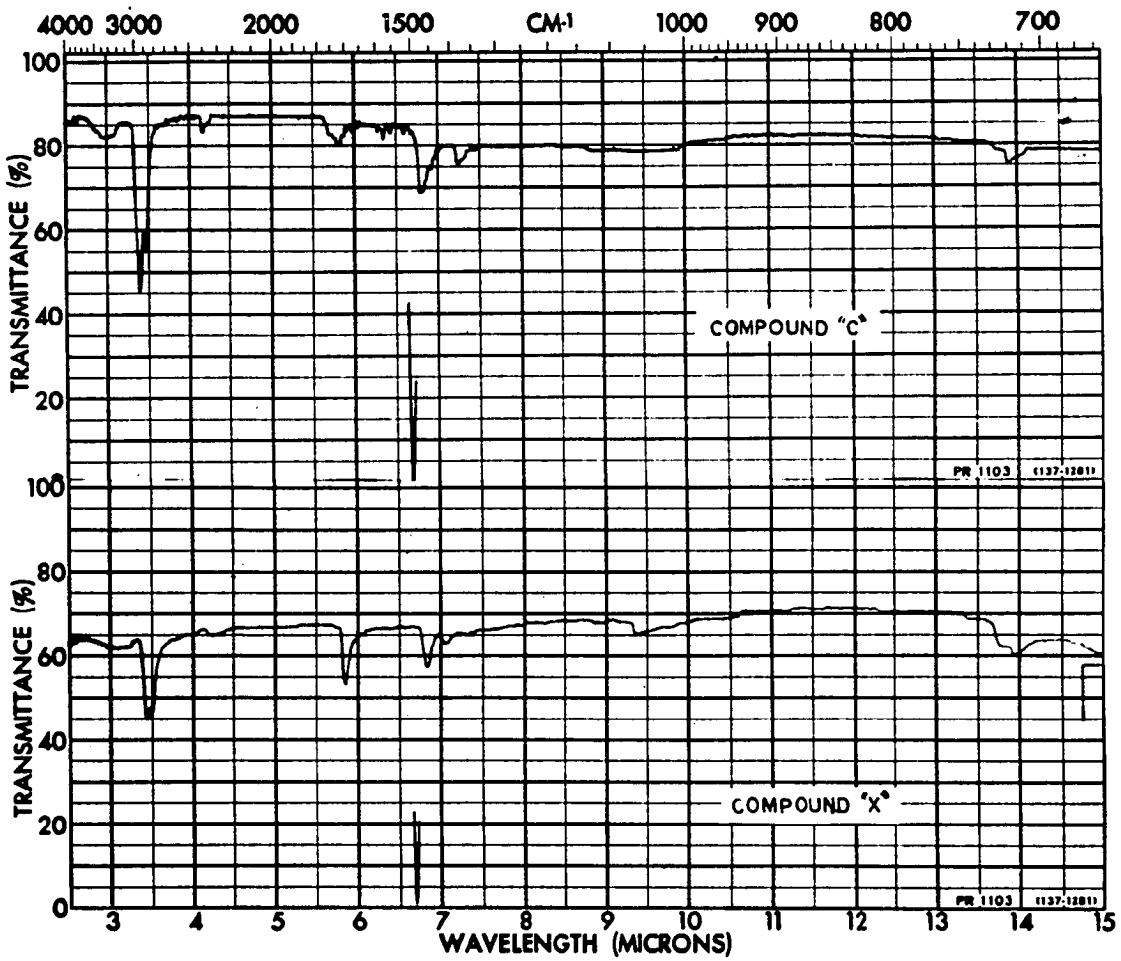

FIGURE 11: INFRARED SPECTRA OF COMPOUND "C" AND COMPOUND "X". THE SPECTRA ARE TAKEN AS A AS A THIN FILM BETWEEN SODIUM CHLORIDE PLATES.

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Since it was observed that compound "C" was converted to compound "X" in the gas chromatograph at 250°C, we collected all of compound "C" as compound "X". Compound "X" showed carbonyl absorption in the infrared at 1720 cm^{-1} (Fig. 11). Compound "X" was reduced with lithium aluminum hydride. The product of the reduction co-chromatographed with 1,14-docosanediol. Since reduction of compound "X" resulted in its conversion to a compound which co-chromatographed with 1,14-docosanediol, compound "C" appears to be closely related to the alkanediol.

The mass spectrum of compound "C" was that of 14-ketodocosanol (M.W. 340). There is a peak at m/e 322 for loss of one molecule of water. Peaks at m/e 227 and 141 are those for cleavage α-to the keto-group. The peaks at m/e 242 and 156 are attributable to the McLafferty rearrangement (41-43) observed with β-cleavage in ketones. However the infrared spectrum suggested that this structure was more suitable for compound "X". It is likely that the heat in the inlet port of the mass spectrograph was responsible for conversion of compound "C" to compound "X". The mass spectrum definitely excludes the possibility of compound "C" being an epoxide, since the epoxide would yield a mixture of ketones on heating and this mixture would be observed in the mass spectrum. The structure of compound "C" remained undetermined.

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SYNTHESIS OF
1,14-DOCOSANEDIOL-1,14-DISULFATE
DIPOTASSIUM SALT

The properties of the major solvolysis product in the various chromatographic procedures indicated that the sulfolipid was the disulfate of a long chain alkanediol. The analysis suggested that the diol was a docosanediol. The mass spectrum demonstrated that its structure was that of 1,14-docosanediol. This compound and its disulfate dipotassium salt were synthesized in order to compare the chemical and spectroscopic properties of the natural and synthetic materials.

The syntheses were accomplished as follows and as shown in Fig. 12. 14-Ketodocosanoic acid was synthesized in a manner similar to that described by Bowman for aliphatic long chain ketones (44-46). 12-Bromolauric acid was added to a solution of sodio-diethyl malonate in absolute ethanol and the product was saponified with dilute sodium hydroxide. 1,1,12-Dodecanetricarboxylic acid was isolated and characterized.

The 1,1,12-dodecanetricarboxylic acid was esterified below 30°C with dihydropyran in benzene in the presence of a catalytic amount of concentrated sulfuric acid. The resulting tritetrahydropyranyl-1,1,12-dodecanetricarboxylate was converted to a substituted sodio-malonic

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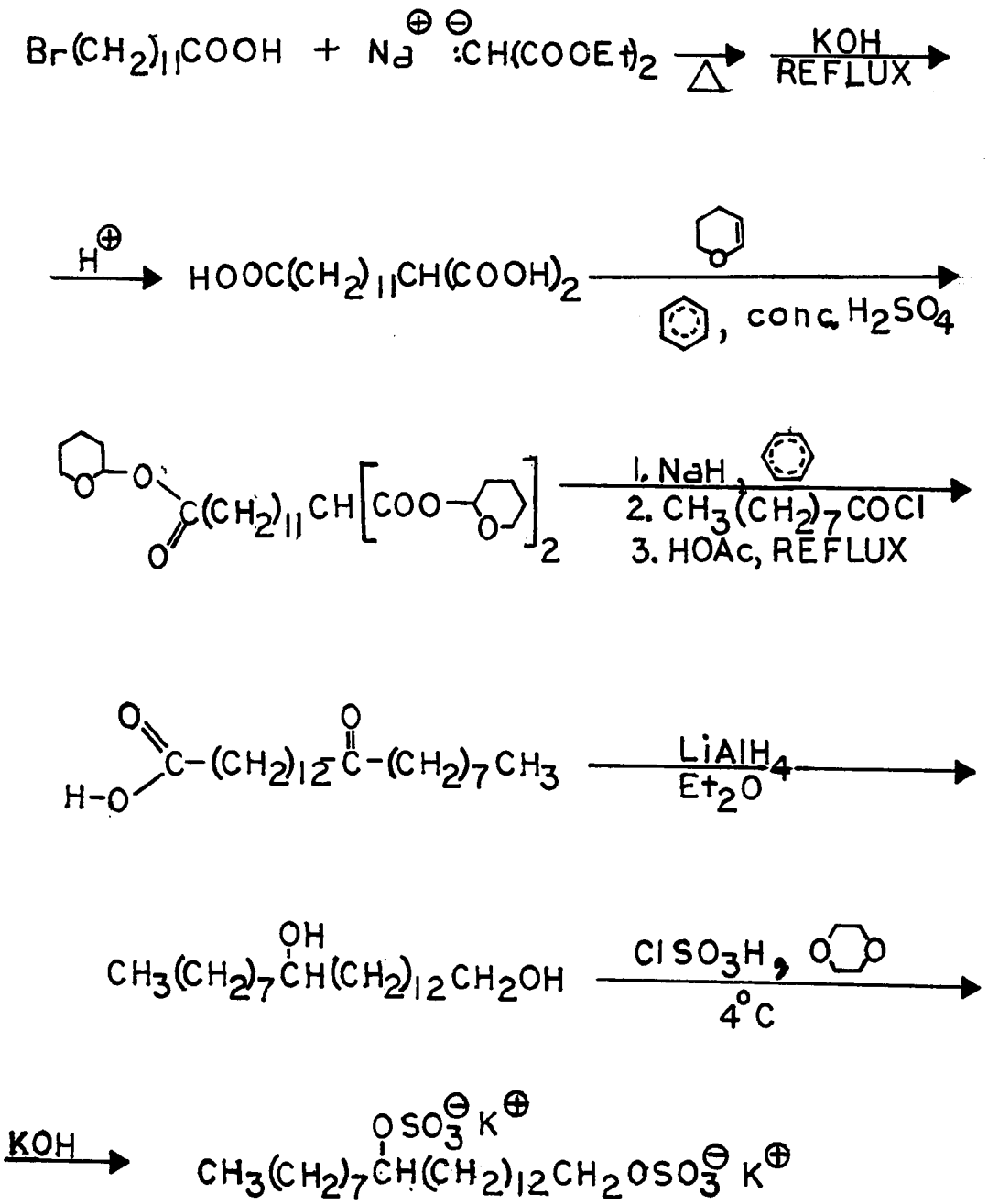

FIGURE 12: Scheme for the synthesis of 1,14-docosanediol-1,14-disulfate dipotassium salt.

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ester. The anhydrous benzene solution was refluxed under acidic conditions to decarboxylate the β-keto-ester. 14-Ketodocosanoic acid was isolated from the reaction mixture.

The 1,14-docosanediol was obtained by reduction of 14-ketodocosanoic acid with lithium aluminum hydride. In 1961, Nikishin et al. (47) reported that they had obtained an 80% yield of 1,14-docosanediol (mp 80-80.5°C) from the lithium aluminum hydride reduction of 13,14-epoxydocosanoic acid. Their basis of identification was the melting point of a derivative, 14-ketodocosanoic acid (mp 83.5-85°C) which they compared to the ketodocosanoic acid (mp 83.5-85.°C) obtained from treating behenolic acid (13-docosynoic acid) with concentrated sulfuric acid, a reaction which is expected to give approximately equal yields of the 13-keto- and 14-ketodocosanoic acids (48). Therefore, Nikishin et al. apparently had obtained a mixture of 1,13-docosanediol and 1,14-docosanediol. Julietti et al. (49) and Pigulerskii and Sokolova (50) have reported that the reduction of 13,14-epoxydocosanoic acid with lithium aluminum hydride gave a mixture of 1,13- and 1,14-docosanediols. Pigulerskii and Sokolova found that the docosanediol mixture melted at 80.0-80.5°C and the mixture of 13-(and 14-) oxodocosanoic acid melted at 84-86°C

The properties of the synthesized 1,14-docosanediol were compared with those of the alkanediol isolated

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from the sulfolipid. The infrared spectra of the two diols appears in Fig. 13. These spectra were identical in all respects, and particularly the 1150-800 cm^{-1} region, where the spectra of 1,12-octadecanediol and 1,4-eicosanediol (Fig. 5) differ markedly. The synthesized and natural diol were examined by gas chromatography and found to co-chromatograph both as the free diols and as the diacetates.

The mass spectra of the synthetic and natural alkanediol were compared in Table 6. The same fragmentation pattern was found in both spectra. There were differences in the intensities of many of the peaks, but the two spectra were unquestionably of the same compound. As can be seen from the table both spectra had a peak at mass 306 ($M - 2H_2O$) that confirmed the alkanediol structure and indicated that the peak at m/e 342 was the molecular ion. The spectrum of the synthesized 1,14-docosanediol showed peaks for α- and β-cleavage that were described previously for the sulfolipid diol.

The melting points of the synthetic and natural diol differed. The natural compound melted at 87.0-87.5°C, and the synthesized melted at 82.5-83.0°C. The difference in the melting points may be indicative of the fact that the natural compound is an optical isomer. Baker and Gunstone (51) have found similar differences for the melting points of 9DL-hydroxyoctadecanoic acid (mp 74-77°C) and 9D-hydroxyoctadecanoic acid (mp 82.5-83.5°C).

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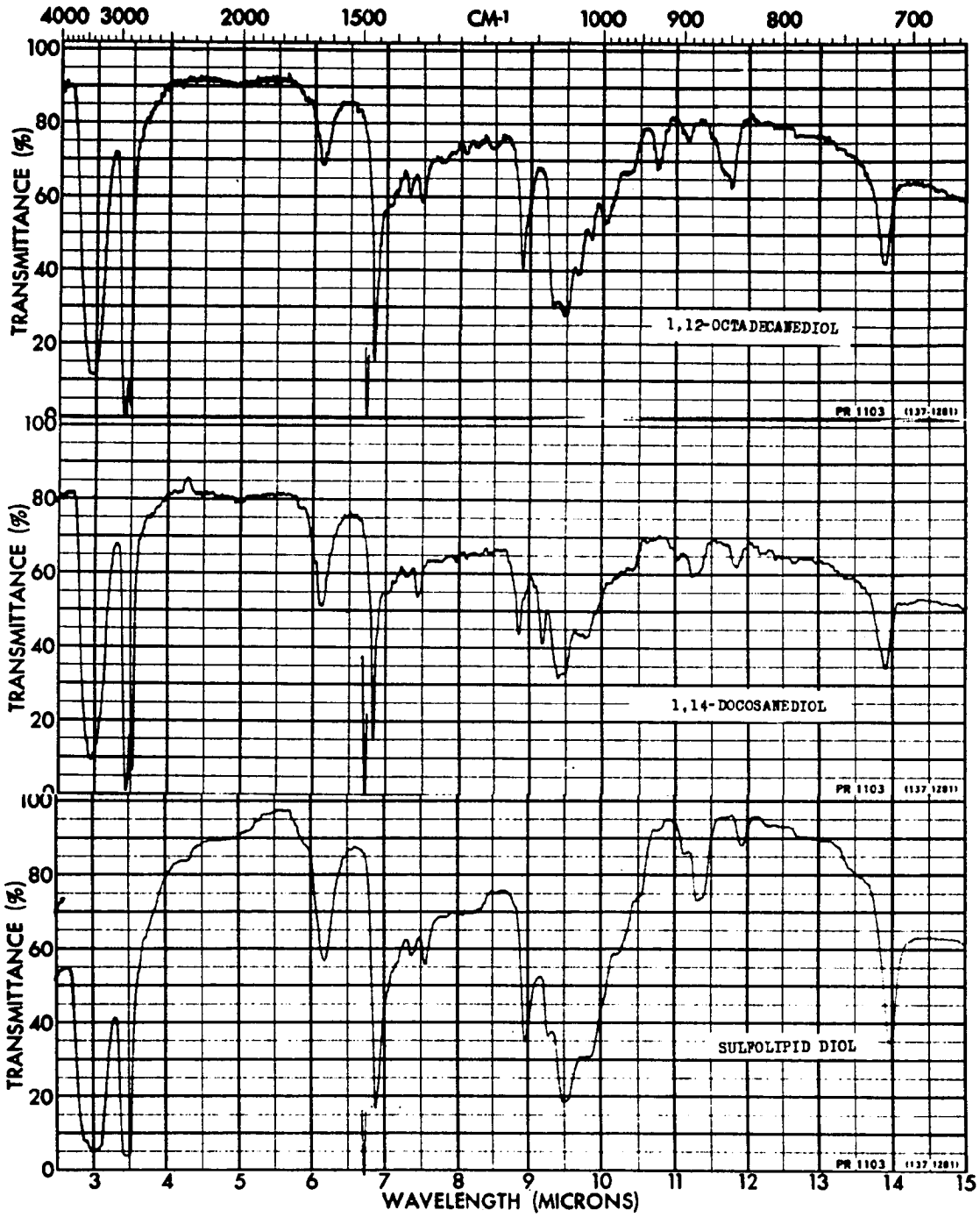

FIGURE 13: INFRARED SPECTRA OF 1,14-DOCOSANEDIOL, THE SULFOLIPID DIOL, AND 1,12-OCTADECANEDIOL. THE SPECTRA WERE TAKEN OF POTASSIUM BROMIDE WAFERS WITH AN INFRACORD MODEL 137.

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1,14-Docosanediol was converted to 1,14-docosanediol-1,14-bis(hydrogen sulfate) with chlorosulfonic acid (52). The sulfuric acid ester was neutralized with potassium hydroxide and the 1,14-docosanediol-1,14-disulfate dipotassium salt was isolated. The properties of 1,14-docosanediol-1,14-disulfate dipotassium salt were compared to those of the sulfolipid.

The infrared spectra of the synthetic salt and the sulfolipid are shown in Fig. 14. The similarity is especially remarkable because the solvolysis of the sulfolipid indicated only 62% of the sulfolipid was 1,14-docosanediol-1,14-disulfate dipotassium salt. It is important to note that the intensity of the secondary sulfate absorption at 940 cm^{-1} is almost equal to that of the primary at 1000 cm^{-1} in the synthetic material, but in the sulfolipid the secondary sulfate absorption is weaker than the primary. The differences in intensities of ν_{C-O} indicates that the ratio of secondary to primary sulfate in the natural product is less than 1.

The synthetic disulfate salt was solvolyzed with moist dioxane. This was done to compare the products with those obtained from the corresponding solvolysis of the sulfatide. The solvolysis yielded 1,14-docosanediol as the sole ether-soluble product, according to analysis by gas chromatography.

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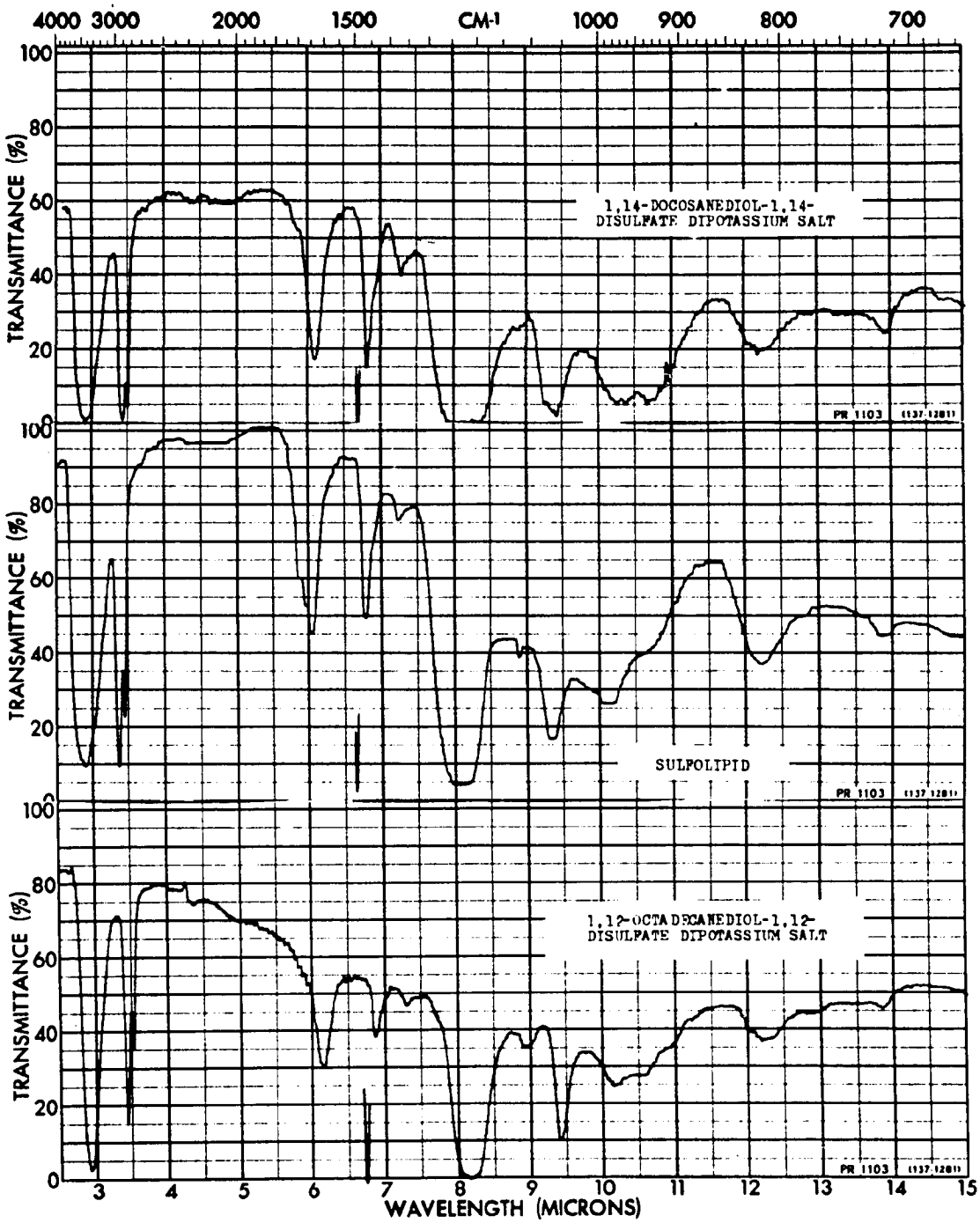

FIGURE 14: INFRARED SPECTRA OF 1,14-DOCOSANEDIOL-1,14-DISULFATE DIPOTASSIUM SALT, THE SULFOLIPID, AND 1,12-OCTADECANEDIOL-1,12-DISULFATE DIPOTASSIUM SALT. THE SPECTRA WERE TAKEN OF POTASSIUM BROMIDE WAFERS WITH AN INFRACORD MODEL 137.

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CONCLUSION

It has been established that a 1,14-docosanediol-1,14-disulfate is present in Ochromonas danica. This is based on the infrared spectrum of the sulfatide which showed absorption for both a primary and a secondary sulfate and on the structure of the carbon moiety obtained from solvolysis in moist dioxane. The properties of the sulfatide and the synthetic disulfate are compared in Table 3.

It was apparent from the results of solvolysis in moist dioxane that, although the sulfolipid behaved as a homogeneous material in thin layer chromatography, the preparation was a mixture of several components. The analysis and the solvolysis both quantitated for one sulfate to eleven carbons. The alkanediol isolated from the sulfolipid analyzed for one oxygen to eleven carbons. Gas chromatographic analysis indicated that the major component of the solvolysis products was 1,14-docosanediol. It was therefore concluded that the sample was a mixture of sulfolipids of similar structure, and that the main constituent was a 1,14-docosanediol-1,14-disulfate. It was not known in what salt form the natural disulfate exists in the cells.

The structure of the alkanediol, that accounted for 62% of the organic moiety, was established by spectral methods and was verified by synthesis to be 1,14-docosane-

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TABLE 3: Comparison of the properties of the sulfatide and the synthetic 1,14-docosanediol-1,14-disulfate dipotassium salt.

	Sulfatide	1,14-Docosanediol-1,14-disulfate
IR Spectrum	$\nu_{S=O}$ 1250-1210 cm^{-1}, 1060 cm^{-1} ν_{C-O} (primary) 980 cm^{-1} ν_{C-O} (secondary) 935 cm^{-1} ν_{S-O} 810 cm^{-1} The intensity of the primary absorption was greater than that of the secondary.	$\nu_{S=O}$ 1250-1210 cm^{-1}. 1060 cm^{-1} ν_{C-O} (primary) 980 cm^{-1} ν_{C-O} (secondary) 935 cm^{-1} ν_{S-O} 810 cm^{-1} The intensities of the secondary and primary absorptions are about equal.
Analysis	Carbon 42.56 Hydrogen 7.90 Sulfur 10.51	Carbon 44.30 Hydrogen 7.73 Sulfur 10.85
TLC	R_f 0.9	R_f 0.9
Solvolysis in moist dioxane	The organic moiety was primarily 1,14-docosanediol, but there were several other products.	The organic moiety was only 1,14-docosanediol.

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TABLE 4: Comparison of the properties of the natural and synthetic 1,14-docosanediol.

	Sulfolipid diol		1,14-Docosanediol	
IR Spectrum	C-O (primary) 1060 cm^{-1} C-O (secondary) 1120 cm^{-1} The infrared spectrum was identical to the synthetic material.		C-O (primary) 1060 cm^{-1} C-O (secondary) 1120 cm^{-1}	
Analysis	Carbon Hydrogen	76.46 13.59	Carbon Hydrogen	76.67 13.21
TLC	R_f	0.12	R_f	0.12
GLC	The natural compound and the synthetic 1,14-docosanediol co-chromatographed both as the alkanediols and the alkanediol diacetates.			
Melting point*	87.0-87.5°C		82.5-83.0°C	
Mass Spectrum	Important peaks at 342 (M), 324 (M - H_2O), 306 (M - $2H_2O$), 229 (M - $(CH_2)_7CH_3$), 211 (229 - H_2O), 193 (229 - $2H_2O$) and 143 (M - $(CH_2)_{12}CH_2OH$).		Important peaks at 342 (M), 324 (M - H_2O), 306 (M - $2H_2O$), 229 (M - $(CH_2)_7CH_3$). 211 (229 - (H_2O), 193 (229 - $2H_2O$) and 143 (M - $(CH_2)_{12}CH_2OH$).	

*The difference in melting points probably indicates that the natural material is one enantiomer and the synthetic compound is a racemic mixture.

diol. The properties of the synthetic and the natural diol were compared in Table 4. This work demonstrated that the structure of a long chain alkanediol could be established from its mass spectrum. The infrared spectrum, analysis, and chromatographic properties of the sulfolipid diol supported the structure. The difference in the melting points was attributed to the fact that the natural product was only one enantiomer, but the synthetic 1,14-docosanediol was a racemic mixture.

The stereospecific solvolysis of alkyl sulfates in moist dioxane was demonstrated with (+)-2-octyl sulfate. The reaction resulted in hydrolysis of the alkyl sulfate yielding 2-octanol with retention of configuration. Solvolysis of alkyl sulfates in moist dioxane were also shown to yield quantitative amounts of the alcohol and potassium hydrogen sulfate.

These data left little doubt that the major portion of the sulfolipid preparation was the sulfatide, 1,14-docosanediol-1,14-disulfate.

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EXPERIMENTAL PROCEDURES

Materials. All reagents were analytical grade. Solvents were distilled before use. 1,12-Octadecanediol (K and K Laboratories, Plainview, New York) was recrystallized first from hexane and then from methylene chloride before use (mp 72-72.5°C; lit. mp 71.2°C (54)). (-)-2-Octanol was purchased from Aldrich Chem. Co., Milwaukee, Wisc. The specific rotation, $[\alpha]_D^{23}$, in absolute ethanol was -10.05° (lit. $[\alpha]_D^{17}$ -9.9° neat (55,56)).

Methods. The gas liquid chromatography column used in this work was a 1/8 in. x 6 ft. stainless steel tube packed with 3% JXR on 100-120 mesh Chromosorb Q (Applied Science Laboratories, State College, Pa.). A Perkin-Elmer Gas Chromatograph Model 881 was used.

The infrared spectra were taken on a Perkin-Elmer Infracord 137 Spectrophotometer. The spectra were calibrated with a polystyrene reference at 1495 cm^{-1} (6.692 μ).

Mass spectra were obtained by Morgan-Schaffer Corporation (Montreal, Quebec, Canada) on an Hitachi Mass Spectrometer using the indirect inlet at 250°C at voltages 700, 1750, and 2000. Some of the spectra were obtained by Dr. Ralph T. Holmann at the Hormel Institute (Austin, Minn.) on a Hitachi Mass Spectrometer.

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The nuclear magnetic resonance (nmr) spectrum was determined with a Varian A-60 spectrometer (Varian Assoc., Paulo Alto, Calif.) on a deuterium oxide solution.

Optical rotations were measured on a Rudolph Polarimeter (O.C. Rudolph & Sons, Inc., Caldwell, N. J.) using a sodium lamp.

The elemental analyses were carried out by Schwartzkopf Microanalytical Laboratories, New York.

All melting points are corrected.

Growth of the Cells. The cells were grown in twelve 2-liter Fernbach flasks on a synthetic medium (20). This medium is given in Table 5. Each flask was inoculated with 10 ml of a 4- to 5-day old culture in the logarithmic phase of growth. The twelve flasks were incubated at 29°C under continuous light for a period of five days. The cells were harvested by continuous centrifugation at 9000 g on a Servall refrigerated centrifuge at 4°C. The cells were washed twice by resuspension in 200 ml of distilled water and centrifugation. The pellet weighed a little less than 100 g (wet wt.). It was stored at -5°C until four batches were obtained.

Isolation of the proteolipid. Four batches (about 400 g, wet wt.) were combined and extracted three times with 20 volumes (8 liters) of chloroform:methanol:: 2:1 (v/v). The chloroform and methanol were removed on a rotary evaporator. The residue was suspended in 1200 ml

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TABLE 5: Defined media for the growth of Ochromonas danica.

NH_4Cl	0.05 g
KH_2PO_4	0.03 g
$MgSO_4 \cdot 7H_2O$	0.1 g
$MgCO_3$ (basic)	0.04 g
(Ethylenedinitrilo)-tetraacetic acid	0.02 g
$CaCO_3$	0.005 g
L-Glutamic acid	0.3 g
Thiamine·HCl	0.1 mg
Glucose	1.0 g
L-Histidine·HCl	0.04 g
L-Arginine·HCl	0.04 g
Metals*	1.0 mg
Biotin	1.0 µg
pH	5.0
H_2O to 100 ml	
Temperature incub. 29°C	

*The mixture of metals consists of the following metals as salts: Fe (as $Fe(NH_4)_2SO_4 \cdot 6H_2O$), 0.2 mg; Zn (as $ZnSO_4 \cdot 7H_2O$), 0.1 mg; Mn (as $MnSO_4 \cdot H_2O$), 0.05 mg; Cu (as $CuSO_4 \cdot 5H_2O$), 0.008 mg; Co (as $CoSO_4 \cdot 7H_2O$), 0.01 mg; B (as H_3BO_3), 0.01mg; Mo (as $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$), 0.005 mg; and V (as $Na_3VO_4 \cdot 16H_2O$), 0.001 mg.

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of 0.2N potassium hydroxide in methanol and maintained at 37°C for 45 min. The suspension was diluted with 1200 ml of water and extracted with a liter of chloroform. The extraction was repeated twice until the extract was a clear pale green. The aqueous fraction was acidified (pH 4.0) with 3N hydrochloric acid with vigorous stirring and extracted 5 times with 500 ml of chloroform, to remove the fatty acids. The aqueous solution was neutralized (pH 7.5) with 1N potassium hydroxide and extracted with three 1000 ml portions of 1-butanol. The 1-butanol extracts were combined and the solvent removed *in vacuo*. The residue was washed with 25 ml of acetone. Small volumes of absolute ethanol were added several times and removed *in vacuo* to remove the 1-butanol. The residue (4.5 g) was regarded as a crude preparation of the sulfolipid as a potassium salt. The material ran as a single spot on thin layer chromatography but further examination verified it was not a single substance.

Analysis: C, 49.07; H, 7.88: S, 10.33; N, 3.50.

The infrared spectrum (Fig. 15) had bands at 3400 (strong, broad), 2950, 2875, 1725 (medium, shoulder), 1650 (strong, broad), 1550 (shoulder, broad), 1460, 1380, 1240 (extremely strong, broad), 1060, 975 (medium, broad), 810 (broad) and 720 cm^{-1}.

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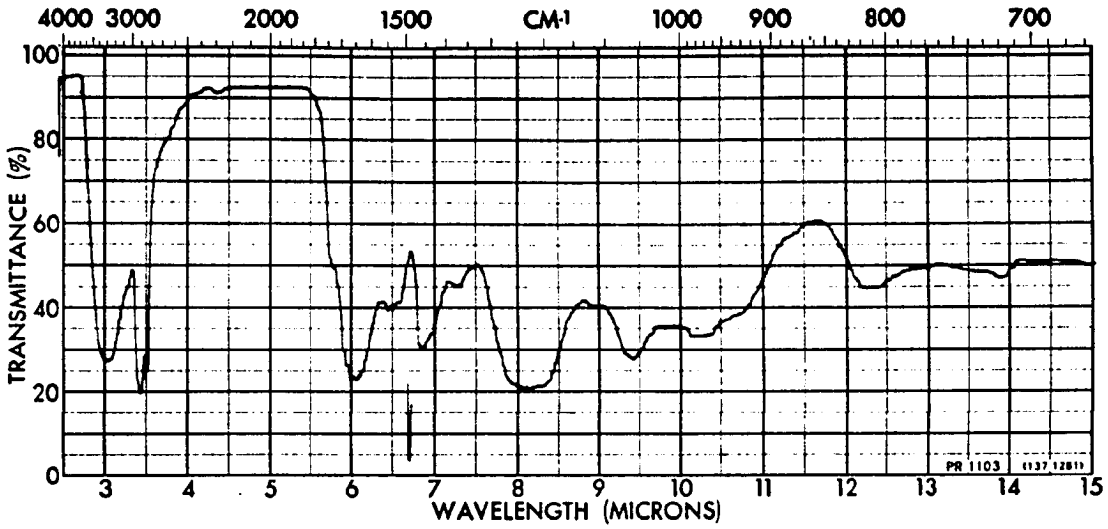

FIGURE 15: INFRARED SPECTRUM OF THE SULFATIDE. THE SPECTRUM WAS TAKEN OF A POTASSIUM BROMIDE WAFER WITH AN INFRACORD MODEL 137.

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Acid hydrolysis and chromatography of the sulfolipid preparation. A portion of the crude sulfolipid preparation (20 mg) was refluxed with 5 ml of 6N hydrochloric acid overnight. The reaction mixture was evaporated to dryness. Distilled water was added and removed by evaporation several times until the pH was 4.0. The residue was paper chromatographed against standard amino acids. The chromatography was conducted in two dimensions on 20 cm squares of No. 3MM Whatman Filter Paper. The chromatograms were developed in one direction with TBF (t-butyl alcohol:88% formic acid:water::14:3:3 (v/v/v)) and in a second direction with Solvent 1 (95% aqueous ethanol:iso-butyl alcohol:28.9% ammonia:water::60:20:5:15). The chromatograms were visualized by spraying with 0.2% (w/v) ninhydrin in acetone followed by heating in an oven at 110°C for 10 min.

Enzymatic hydrolysis of the protein in the sulfolipid preparation. The protein was removed from the proteolipid by digestion with pronase, a proteolytic enzyme excreted by Streptomyces griseus (22) and obtained from Enzyme Development Corporation, New York, New York. In accordance with a procedure described by Haines (1), 4.5 g of the proteolipid, was digested in 400 ml of a solution which contained 100 mg of pronase, 8 ml of 2N potassium carbonate and 40 ml of ethanol. The mixture was maintained at 37°C for 48 h, and then extracted three times

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with 250 ml of 1-butanol. The 1-butanol fractions were combined and washed twice with 25 ml of water. The 1-butanol was removed under vacuum.

Ion-Exchange gel filtration of the sulfolipid. A column (6 cm x 2 cm) of Sephadex SE-25 (3 g) (Pharmacia Fine Chemicals, Piscataway, N. J.) suspended in 0.16M potassium phosphate buffer (pH 7.5) was prepared. The residue obtained from pronase digestion (1.75 g) in 7 ml of water was placed on the gel. The material was washed onto the column successively with 3 ml of water and 6 ml of potassium phosphate buffer (pH 7.5). The sulfolipid was eluted with further addition of phosphate buffer (pH 7.5). Fractions were monitored with thin layer chromatography. Those containing the sulfatide were combined. The sulfolipid was extracted with 1-butanol. The 1-butanol fraction was washed with small volumes of water and the solvent was removed in vacuo. The product, (2.5 g), was contaminated with sodium phosphate.

Thin layer chromatography (TLC). Chromatograms, consisting of thin layers (250 μ) of magnesium silicate with 10% calcium sulfate binder (Adsorbosil M-1, Applied Science Laboratories, State College, Pa.) were not activated, i.e., the plates were not heated at 110°C for one hour prior to use, so as not to increase the adsorptivity of the magnesium silicate. The developing solvent for the

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sulfolipid was chloroform:methanol:water::65:33:2 (v/v/v). Plates were run for 45 min (7 cm) and visualized by spraying with 2,7-dichlorofluorescein (0.2% in methanol). Before pronase digestion the sulfolipid chromatographed as a single spot (R_f 0.95). After digestion with pronase the R_f was 0.9.

Filtration of the sulfatide through magnesium silicate. A column (13.5 cm x 2 cm) of magnesium silicate (15 g) (Adsorbosil M-2, Applied Science Laboratories, State College, Pa.) in chloroform:methanol::2:1 (v/v) was charged with the sulfatide and phosphate isolated from the Sephadex column. The sulfatide was eluted with the same solvent and collected in 5 ml fractions. The fractions were monitored with TLC. Fractions containing "pure" sulfatide were combined and the solvent removed *in vacuo*. The material was dried *in vacuo* at 60°C. The substance was a faint green and weighed 594 mg. The green contaminant was removed by dissolving the sulfatide in a minimum volume of absolute ethanol and then precipitating it by addition of 20 volumes of anhydrous diethyl ether. This precipitation was repeated twice. The product, an off-white fine powder, was dried *in vacuo* at 60°C (wt. 176 mg). The isolation procedure is summarized in Figure 2.

Analysis of the sulfolipid. Calculated for $C_{22}H_{44}S_2O_8K_2 \cdot 2H_2O$: C, 42.94; H, 7.80; S, 10.40. Found: C, 42.56; H, 7.90; S, 10.51; N, 0.63.

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The infrared spectrum had bands at 3400 (strong) (broad), 2950, 2875, 1640, 1460, 1250-1210 (extremely strong, broad), 1060, 980, 935 (shoulder, broad), 810 (medium, broad), and 720 cm^{-1}. The nuclear magnetic resonance spectrum (Fig. 4) in deuterium oxide showed a sharp peak at 8.7τ, for the methylene protons, a triplet at 9.1τ, for the methyl, and a triplet at 6.5τ for the protons on the carbons to which the sulfate groups are attached.

Aqueous Acid Hydrolysis of the Sulfolipid. The sulfatide was placed in 25 ml of 1N hydrochloric acid and the mixture was refluxed for 2 h. The solution was cooled and made basic with 6N potassium hydroxide. The basic reaction mixture was extracted with diethyl ether. The diethyl ether solution was washed with water until neutral, dried over anhydrous sodium sulfate and evaporated *in vacuo*. The residue was chromatographed on TLC using diethyl ether:petroleum ether (bp 30-60°C)::3:7 (v/v) as the developing solvent. The aqueous solution was acidified and extracted with diethyl ether. The diethyl ether solution was washed with water until neutral, dried over anhydrous sodium sulfate and evaporated *in vacuo*. The residue was negligible.

Chromatography of the sulfolipid diol on magnesium silicate. A column (18 cm x 2 cm) of magnesium

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silicate (Adsorbosil M-2) (15 g) in diethyl ether:petroleum ether (bp 30-60°C)::3:7 (v/v) was charged with crude diol (150 mg) from the sulfatide hydrolysis. The column was eluted with the same solvent. The eluent was collected in 3 ml fractions which were monitored with TLC. Fractions containing pure diol were combined and evaporated *in vacuo*. The product was recrystallized from hexane and then from carbon tetrachloride. The crystals were fine white feathers (25 mg); mp 87-87.5°C.

Analysis: Calculated for $C_{22}H_{46}O_2$: C, 77.19; H, 13.45. Found: C, 76.46; H, 13.59.

The infrared spectrum (Fig. 5) showed peaks at 3350 (very strong), 2920, 2850, 1470, 1360, 1120, 1060, and 718 cm^{-1}.

The peaks from the mass spectrum (Fig. 6) with their relative intensities appear in Table 4. Important peaks appear at 342 (M), 324 (M - H_2O), 306 (M - $2H_2O$), 229 (M - $(CH_2)_7CH_3$), 211 (229 - H_2O), 193 (229 - $2H_2O$), and 143 (M - $(CH_2)_{12}CH_2OH$).

Synthesis of 1,12-octadecanediol-1,12-disulfate dipotassium salt. 1,12-Octadecanediol (286 mg, 1 mmol) was dissolved in 25 ml of dioxane (distilled from lithium aluminum hydride). The solution was slowly added to a solution of chlorosulfonic acid (0.2 ml, 3 mmol) in dioxane (10 ml) at 4°C. The mixture was stirred in an ice bath for one hour. The ice bath was removed and the solution

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Solvolysis of potassium dodecyl sulfate. Potassium dodecyl sulfate (74.2 mg, 0.24 mmol) was dissolved in 10 ml of hot dry dioxane (distilled from lithium aluminum hydride) and refluxed for 30 min. Petroleum ether (bp 30-60°C) (50 ml) was added and the suspension centrifuged. The precipitate was washed with an additional 50 ml of petroleum ether (bp 30-60°C). The petroleum ether fractions were combined and the solvent was removed *in vacuo*. The residue, 31.2 mg, was a colorless oil. The precipitate isolated from the above suspension was dissolved in water and the water removed *in vacuo*. The residue from the aqueous solution weighed 22.5 mg.

Synthesis of potassium (+)-2-octyl sulfate from (-)-2-octanol. Chlorosulfonic acid, 3.16 ml (0.048 mol), was added to 80 ml of dioxane (distilled from lithium aluminum hydride) and the mixture was cooled to 4°C with an ice bath. (-)-2-Octanol, 6.340 ml (0.04 mol), dissolved in 100 ml of dioxane, was slowly added over a period of 40 min with stirring. The reaction mixture was permitted to come to room temperature (approximately 2 h) and then poured into 100 ml of cold 1.8N potassium hydroxide. Water (400 ml) was added to clarify the solution. The clear solution was extracted 3 times with 200 ml of petroleum ether (bp 30-60°C) to remove unreacted alcohol. The solvent was removed *in vacuo*. A small residue of unreacted (-)-2-octanol (102 mg) was obtained. The water

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solution was then extracted 3 times with 250 ml of 1-butanol. The 1-butanol extracts were combined, washed 3 times with water and evaporated to dryness on a rotary evaporator. The residue weighed 10.5 g. The potassium (+)-2-octyl sulfate was recrystallized from ethanol (mp dec 187-191°C).

Analysis: Calculated for $C_8H_{17}O_4SK$: C, 38.71; H, 6.85; S, 12.90. Found: C, 38.64; H, 6.81; S, 12.81.

The infrared spectrum (Fig. 3) showed bands at 3350, 2930, 2875, 1640, 1470, 1380, 1230 (strong, broad), 1060, 1035, 940, 780, and 720 cm^{-1}.

The nmr spectrum shows a broad peak at 6.0τ for the proton on the carbon with the secondary sulfate. The peaks between 8.5 and 9.5τ for the two methyls and the rest of the methylene chain were not separated.

Solvolysis of potassium (+)-2-octyl sulfate. Potassium (+)-2-octyl sulfate, 2.2079 g (8.9 mmol), was added to 50 ml of 99% aqueous dioxane. The solution was refluxed for 15 min, cooled and diluted with 200 ml of water. The water solution was extracted 3 times with 100 ml of diethyl ether. The diethyl ether extracts were combined and washed twice with 50 ml of water. The diethyl ether solution was dried with anhydrous potassium carbonate. The solution was filtered and the solvent was removed *in vacuo*. The residue was added to water and extracted with

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diethyl ether again. The diethyl ether solution was dried and the solvent evaporated *in vacuo*. The residue, 1.0117 g (7.9 mmol, 87%), was gas chromatographed. The infrared spectrum was identical with the spectrum of (-)-2-octanol. The optical rotation of the (-)-2-octanol recovered from the solvolysis reaction was taken in absolute ethanol. The specific rotation, $[\alpha]_D^{23}$, was -9.86° (97.5% optically pure).

Solvolysis of the sulfolipid in dioxane. The sulfolipid, 207 mg, was refluxed in 15 ml of anhydrous dioxane (distilled from lithium aluminum hydride) for 15 min, at which time 0.15 ml of water was added. The reaction mixture was refluxed an additional 15 min, 0.15 ml of water was added once more, and the refluxing continued for a final 15 min. The reaction mixture was cooled and diluted with 50 ml of petroleum ether (bp 30-60°C). The precipitate was removed by filtration. The precipitate was washed with an additional 50 ml of petroleum ether. The filtrates were combined and washed twice with 20 ml of water. The petroleum ether solution was dried over anhydrous sodium sulfate and the solvent was removed on a rotary evaporator. The residue weighed 106.5 mg. The residue was monitored with thin layer chromatography. The mixture was analyzed by gas chromatography (Fig. 7).

Column chromatography of the natural diol on silica gel. A column (2 cm x 15 cm) was prepared from 20 g of silica gel (J. T. Baker Chemical Co., Philipsburg, N.J.)

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in petroleum ether (bp 30-60°C):diethyl ether::1:1. The solvolysis products from the sulfolipid (106.5 mg) were suspended in 2 ml of the same solvent and placed on the column. The material was rinsed into the column with three 2 ml aliquots of solvent. The compounds were then eluted with 120 ml of the same solvent followed by 100 ml of diethyl ether. The fractions were monitored with TLC. Those containing only the diol were combined and the solvent was removed on a rotary evaporator. The residue weighed 45.7 mg (mp 86.3-86.8°C). The sample was analyzed by gas chromatography (Fig. 9). The specific rotation, $[\alpha]_D^{24}$, in absolute ethanol was less than 1°.

Acetylation of 1,12-octadecanediol. 1,12-octadecanediol, 186 mg (0.65 mmol) was added to 5 ml of acetic anhydride. The solution was placed in a boiling water bath. Samples were removed every half hour for 4 h and analyzed by gas chromatography to follow the progress of the reaction. The reaction mixture was allowed to stand overnight and come to room temperature. A sample was also removed after 20 h and analyzed by gas chromatography. The acetic acid and excess acetic anhydride was evaporated *in vacuo*. The last traces were removed by placing the sample in a vacuum desiccator containing potassium hydroxide pellets. The 1,12-octadecanediol-1,12-diacetate was analyzed by gas chromatography. The yield was 238.8 mg (0.645 mmol, 99.3%).

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The mass spectrum showed important peaks at 355 (M - CH_3), 281 (M - CH_3, CH_3COOH), 250 (M - 2 CH_3COOH). A complete tabulation of the peaks appears in Table 5.

Acetylation of the solvolysis mixture. The solvolysis product (1.5 mg) was added to 1.0 ml of acetic anhydride. The solution was placed in a boiling water bath for 3½ h. The excess acetic anhydride and acetic acid were blown off with a stream of nitrogen. The acetates were dissolved in carbon disulfide for assay by gas chromatography. The areas were computed by use of a planimeter.

Acetylation of 1,14-docosanediol. 1,14-Docosanediol (5 mg) was added to 1.0 ml of acetic anhydride. The solution was placed in a boiling water bath for 3½ h. The acetic acid and excess acetic anhydride were blown off with a stream of nitrogen. The residual 1,14-docosanediol-1,14-diacetate was dissolved in carbon disulfide for analysis by gas chromatography.

Collection of compound "C" by gas chromatography. The remaining components of the hydrolysate of the sulfatide, after removal of 1,14-docosanediol, were separated on the gas chromatograph. The fractions corresponding to the peaks on the recorder were collected from the splitter in capillary tubes. The major fraction, compound "C", was reinjected to determine its purity. An infrared spectrum

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of compound "C" showed weak absorption at 3400, 1730, 1470, 1380 and 720 cm^{-1} as well as relatively strong absorption for CH at 2950 and 2875 cm^{-1}.

The mass spectrum was taken on a sample of compound "C" (less than one mg on the inside of a capillary tube as collected from the gas chromatograph). The mass spectrum showed important peaks at 340, 322 (340 - H_2O), 242 (340 - $CH_3(CH_2)_4CH{=}CH_2$), 227 (340 - $CH_3(CH_2)_7$), 209 (227 - H_2O), 156 (340 - $CH_2{=}CH(CH_2)_9CH_2OH$), 141 (340 - $(CH_2)_{12}CH\ OH$).

Collection of compound "X" by gas chromatography. Compound "C" was reinjected into the gas chromatograph and compound "X" was collected from the splitter on the gas chromatograph in capillary tubes. Compound "X" was found to be unchanged by reinjection into the gas chromatograph.

The infrared spectrum showed bands at 2950, 2875, 1720, 1470, 1060 and 720 cm^{-1}.

Reduction of compound "X" with lithium aluminum hydride. Compound "X" (about 1-2 mg collected from the gas chromatograph), dissolved in 3 ml of diethyl ether was added to 100 mg of lithium aluminum hydride. The reaction was left at room temperature for 6 h. Excess lithium aluminum hydride was hydrolyzed with 10 ml of water. The salts were dissolved with 10 ml of 3N hydrochloric acid. The acid solution was extracted twice with 20 ml of diethyl

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ether. The diethyl ether solution was dried with anhydrous sodium sulfate and the solvent was removed in vacuo. The product, dissolved in carbon disulfide and analyzed by gas chromatography, was found to co-chromatograph with 1,14-docosanediol.

Synthesis of 1,1,12-dodecanetricarboxylic acid. Absolute ethanol (75 ml) previously refluxed and distilled from calcium hydride was placed in a three necked flask fitted with a paddle stirrer, a reflux condenser, and an addition funnel. Sodium (2.3 g, 0.1 mol) was added and permitted to react until it had dissolved in refluxing ethanol. Diethyl malonate (27 ml, 0.17 mol) was dissolved in 35 ml of freshly distilled ethanol and the mixture was added to the sodium ethoxide solution with stirring over a period of 30 min at reflux. 12-Bromolauric acid (10.2 g, 0.037 mol) (Sapon Laboratories, L. I.; recrystallized from heptane and methylene chloride; mp 51.5-52.5°C; lit. mp 51.5°C (53), 52-53°C (54)) dissolved in 100 ml of freshly distilled ethanol was added over the period of 30 min. The mixture was maintained at a gently reflux for an additional hour. The solution clarified on addition of 25 ml of water. Aliquots of sodium hydroxide (4.0 g) were added at 30 min intervals until the solution was approximately 0.2M in base. Refluxing was continued for an hour after the addition of base. The reaction mixture was cooled, the sodium salt

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filtered and washed with cold ethanol. The tri-sodium salt was dissolved in 150 ml of water. The viscous solution was acidified with 3N hydrochloric acid in the cold and the 1,1,12-dodecanetricarboxylic acid was filtered and washed. The solid was dried under vacuum for 24 h, and weighed 11.9 g. This material was twice recrystallized from water and yielded 10.3 g (0.034 mol, 92%) of 1,1,12-dodecanetricarboxylic acid; mp 96-98°C. A portion of the material was further recrystallized and yielded tricarboxylic acid; mp 99-100°C. The capillary tube was heated in the melting point apparatus at 160-180°C for 10 min. 1,14-Dodecanedicarboxylic acid was formed (mp 121.5-122.5°C; lit. mp 124.5-125.6°C (55)). 1,1,12-dodecanetricarboxylic acid was titrated with standardized sodium hydroxide. The observed equivalent weight was 103.9 g/eq (Calculated: 100.7 g/eq).

Analysis: Calculated for $C_{15}H_{26}O_6$. C, 59.60; H, 8.61. Found: C, 59.50; H, 8.92.

The infrared spectrum (Fig. 16) showed carbonyl absorption at 1680 cm^{-1} (strong) with a shoulder at 1725 cm^{-1} several bands 1350-1180 cm^{-1} for the CH_2 vibrations, and a broad band at 885 cm^{-1}. The CH_2 rocking band at 720 cm^{-1} was also present.

Synthesis of 14-ketodocosanoic acid. 1,1,12-Dodecanetricarboxylic acid (9.1 g, 0.052 mol) suspended in 25 ml benzene, was added to a solution of dihydropyran

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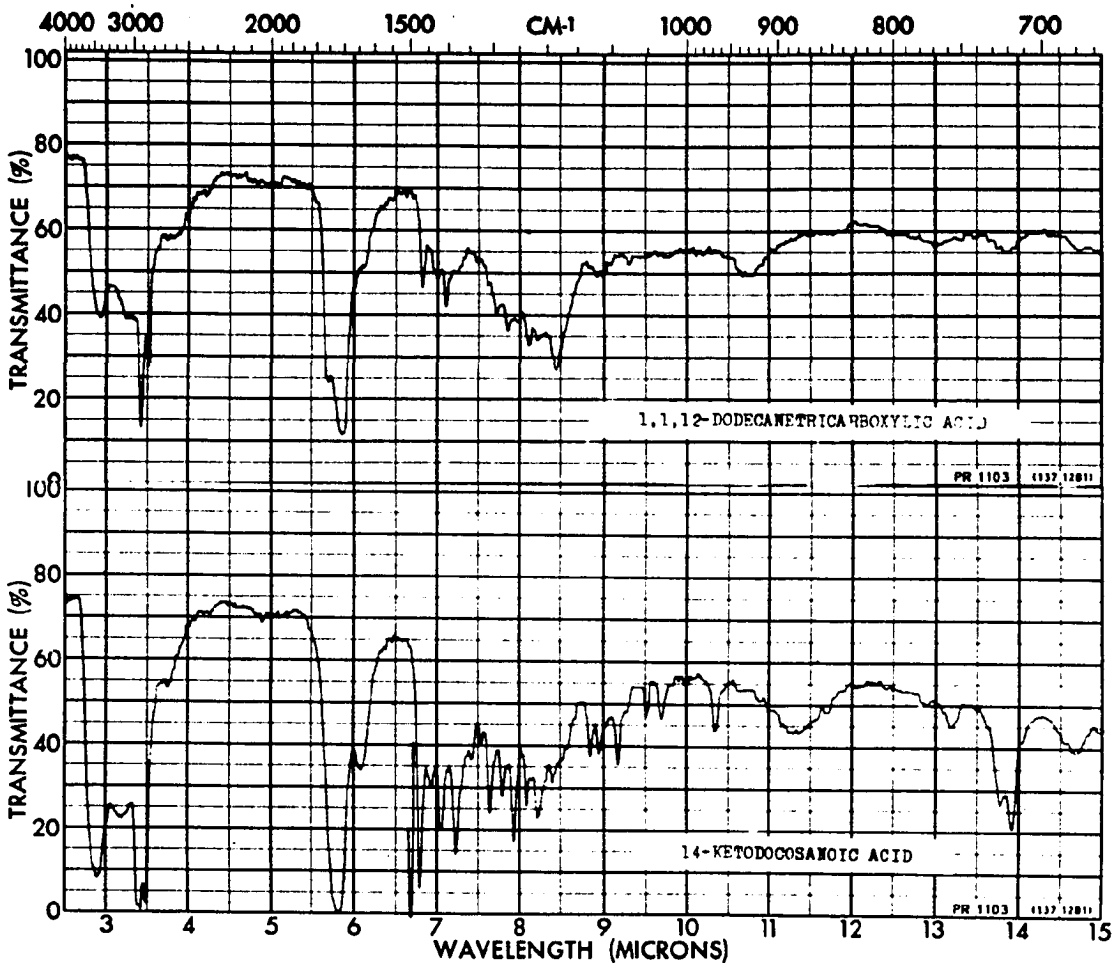

FIGURE 16: INFRARED SPECTRA OF 14-KETODOCOSANOIC ACID AND 1,1,12-DODECANETRICARBOXYLIC ACID. THE SPECTRA WERE TAKEN OF POTASSIUM BROMIDE WAFERS WITH AN INFRACORD MODEL 137.

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(12.6 g, 0.15 mol) in 50 ml of benzene containing 1 drop of concentrated sulfuric acid. The solution was stirred with a magnetic stirrer and kept below 30°C with a cold water bath. The solution became clear in 2 h and was stirred for an additional 30 min, at which time 4 g of solid potassium hydroxide and 2 g anhydrous potassium carbonate were added. The suspension was stirred for 1 h. The solution was decanted through a bed of potassium carbonate and the excess dihydropyran and benzene were removed *in vacuo* keeping the bath temperature below 30°C. The residual ester in 100 ml of benzene was added to sodium hydride (1.05 g, 0.044 mol) (previously washed with benzene) suspended in 100 ml of benzene. The mixture was stirred for 2½ h at which time nonanoyl chloride (10 g, 0.0567 mol) was added and the stirring was continued for 30 min. Water (10 ml) was added to destroy any remaining acyl chloride and the reaction mixture was extracted with 100 ml of 1M KOH. The organic layer was dried with sodium sulfate. After removal of drying agent, 25 ml of acetic acid and 10 ml of acetic anhydride were added to the reaction mixture and the solution was refluxed. There was vigorous evolution of carbon dioxide which ceased after 2½ h. The solution was refluxed for an additional 30 min. The benzene solution was extracted with 2000 ml of 1M KOH. The basic solution was acidified and extracted with ether. This gave 6.5 g of

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crude 14-ketodocosanoic acid which was extracted with petroleum ether (bp 30-60). The residual white solid (2.0 g) was recrystallized from 95% ethanol giving 1.26 g (6%) of 14-ketodocosanoic acid (mp 89.5-90.0°C; lit. mp 85°C (56)).

The infrared spectrum (Fig. 16) showed carbonyl absorption at 1720 cm^{-1} with shoulder at 1725 cm^{-1} several bands 1350-1180 cm^{-1} (weak) and the CH_2-methylene rocking band at 718 cm^{-1} with a shoulder at 722 cm^{-1}.

Reduction of 14-ketodocosanoic acid to 1,14-docosanediol. 14-Ketodocosanoic acid (1.26 g, 3.7 mmol) was dissolved in 200 ml of diethyl ether. Lithium aluminum hydride (1.0 g, 0.029 mol) was added to the diethyl ether solution and the mixture was stirred for 10 h. Then water was slowly added to decompose unreacted lithium aluminum hydride. Hydrochloric acid (3N) was added to dissolve salts. The diethyl ether fraction was separated and the water solution was extracted twice with 150 ml of diethyl ether. The combined diethyl ether fractions (500 ml) were washed with 25 ml of water and dried over potassium carbonate. The diethyl ether was removed in vacuo. The residual 1,14-docosanediol (1.1 g, 3.2 mmol, 87%) was recrystallized from hexane and then methylene chloride; mp 82.5-83.0°C.

Analysis: Calculated for $C_{22}H_{46}O_2$, C, 77.19; H, 13.45. Found: Carbon, 76.67; H, 13.21.

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aqueous dioxane (10 ml) and refluxed for 15 min. The reaction mixture was cooled and diluted with 40 ml of petroleum ether (bp 30-60°C) and filtered. The precipitate was washed with an additional 50 ml of petroleum ether (bp 30-60°C). The filtrates were combined and the solvent removed *in vacuo*. The residue weighed 75 mg (0.22 mmol, 60%) and was analyzed by gas chromatography.

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TABLE 6: Tabulation of mass spectral data on long chain alkanediols.

	1,12-Octadecanediol	1,4-Eicosanediol	Sulfolipid diol		1,12-Octadecanediol	1,4-Eicosanediol	Sulfolipid diol
M	A	B	C	M	A	B	C
m/e	286	314	342	m/e	286	314	342
				30	1.5	1.2	1.1
				31	19.6	8.5	15.8
12	...	...	...	32	...	2.3	1.5
13	...	...	...	33	...	...	...
14	...	0.6	0.4	34	...	...	...
15	...	0.5	0.6	35	...	...	...
16	...	0.5	0.4	36	...	...	...
17	...	1.3	1.3	37	...	...	...
18	4.6	6.3	4.1	38	...	...	...
19	...	3.0	0.6	39	6.2	6.2	4.1
20	...	...	...	40	2.0	1.4	1.3
21	...	...	...	41	54.5	46.2	55.2
22	...	...	...	42	14.6	33.8	13.3
23	...	...	...	43	51.5	100.0	59.7
24	...	...	...	44	6.1	8.5	6.9
25	...	...	...	45	4.8	1.9	3.5
26	...	...	...	46	...	0.2	...
27	11.3	10.0	7.6	47	...	0.7	...
28	3.2	13.3	8.5	48	...	...	...
29	28.3	25.8	24.9	49	...	...	...

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TABLE 6: (continued)

m/e	A	B	C	m/e	A	B	C
50	...	...	...	90	...	6.7	...
51	...	0.3	...	91	1.0	1.5	...
52	...	0.2	...	92	...	0.3	0.2
53	4.4	3.3	2.4	93	1.6	0.7	1.0
54	11.2	6.2	9.1	94	1.4	0.7	0.9
55	100.0	42.1	79.8	95	36.4	9.4	43.6
56	19.4	11.8	18.5	96	22.9	9.1	16.6
57	22.4	54.6	48.8	97	48.5	25.1	34.0
58	5.8	8.5	4.1	98	7.5	3.2	6.7
59	3.1	3.3	2.1	99	2.3	3.2	2.4
60	...	1.8	...	100	0.9	0.7	0.6
61	...	1.3	...	101	1.3	0.2	0.2
62	...	1.3	...	102	...	0.6	...
63	...	0.5	...	103	...	0.1	...
64	...	...	...	104	...	0.2	...
65	1.1	0.6	0.4	105	...	0.2	...
66	2.1	1.5	3.3	106	...	...	...
67	24.6	13.7	24.3	107	1.5	0.2	0.6
68	15.9	13.3	16.9	108	1.1	0.3	0.7
69	42.5	32.6	100.0	109	29.4	4.8	26.0
70	11.7	8.5	16.7	110	6.3	3.2	6.3
71	9.6	63.7	17.1	111	3.3	11.6	18.6
72	11.7	10.9	1.5	112	2.2	1.5	2.8
73	1.1	1.0	0.7	113	3.7	1.2	1.1
74	...	...	...	114	15.8	0.3	0.6
75	...	...	...	115	14.3	0.3	0.4
76	...	...	...	116	1.9	...	...
77	1.1	0.5	...	117	...	0.2	...
78	...	...	...	118	...	0.2	...
79	2.7	1.5	1.9	119	...	0.2	...
80	1.7	1.0	1.7	120	...	...	...
81	27.2	11.6	29.8	121	1.5	0.3	0.7
82	18.2	13.7	29.3	122	0.9	0.3	0.4
83	35.9	25.6	68.2	123	8.8	2.5	13.9
84	9.5	5.5	10.4	124	3.0	1.7	10.6
85	6.9	11.5	8.1	125	2.1	5.0	6.9
86	2.5	2.8	1.3	126	2.5	0.9	1.6
87	1.0	3.8	...	127	0.9	0.7	0.6
88	...	1.2	...	128	2.3	...	0.3
89	...	32.6	...	129	1.5	...	0.7

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TABLE 6: (continued)

m/e	A	B	C	m/e	A	B	C
130	...	...	...	170	...	0.1	0.3
131	...	...	...	171	...	0.1	0.2
132	...	...	...	172	...	...	...
133	...	...	...	173	...	...	...
134	...	...	...	174	...	...	...
135	0.9	0.2	...	175	...	...	...
136	0.9	0.2	...	176	...	...	...
137	1.6	1.2	8.7	177	...	...	...
138	1.8	1.0	0.3	178	...	...	...
139	0.9	2.0	0.1	179	...	0.2	0.2
140	...	0.5	0.1	180	0.8	0.4	0.7
141	1.3	0.4	0.1	181	0.9	0.4	0.2
142	...	0.1	6.3	182	...	0.2	0.4
143	...	0.1	10.8	183	15.3	0.1	0.3
144	...	...	0.1	184	1.4	...	...
145	...	...	...	185	...	...	...
146	...	...	...	186	...	...	...
147	...	...	...	187	...	...	...
148	...	...	...	188	...	...	...
149	...	0.3	0.3	189	...	...	...
150	...	...	0.2	190	...	...	...
151	0.9	0.4	2.8	191	...	...	0.3
152	1.4	0.6	1.2	192	...	...	0.2
153	...	1.2	0.6	193	...	0.2	7.4
154	1.5	0.4	1.2	194	...	0.3	1.9
155	0.8	0.4	0.6	195	...	0.2	0.5
156	...	0.1	1.5	196	...	0.1	0.2
157	...	0.1	1.7	197	...	0.1	0.6
158	...	...	0.2	198	...	...	...
159	...	...	...	199	1.7	...	...
160	...	...	...	200	0.7	...	...
161	...	...	...	201	12.6	...	...
162	...	...	...	202	2.2	...	...
163	0.9	...	...	203	...	...	...
164	...	...	...	204	...	...	...
165	2.1	0.2	...	205	...	...	...
166	...	0.4	...	206	...	...	...
167	...	0.6	0.3	207	...	0.1	0.7
168	...	0.4	0.2	208	...	0.8	0.4
169	...	0.3	0.4	209	...	0.2	0.3

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TABLE 6: (continued)

m/e	A	B	C	m/e	A	B	C
210	...	0.3	0.2	250	1.8	0.7	2.6
211	...	0.2	7.5	251	0.5	0.2	...
212	...	...	1.3	252	...	0.1	...
213	...	...	...	253	...	1.0	0.2
214	1.5	...	...	254	...	0.3	...
215	0.3	...	0.3	255	...	3.6	...
216	...	...	...	256	...	0.7	0.1
217	...	...	...	257	0.2	...	0.1
218	...	...	...	258	...	...	...
219	...	...	...	259	...	...	...
220	...	...	...	260	...	...	...
221	0.2	0.2	...	261	...	...	...
222	0.9	0.1	0.3	262	...	...	...
223	0.2	...	0.2	263	...	...	...
224	0.2	0.1	0.3	264	...	...	0.2
225	0.3	...	0.9	265	...	...	...
226	0.4	...	0.3	266	0.1	0.4	...
227	1.1	...	1.3	267	0.3	0.1	...
228	0.3	...	0.4	268	0.2	1.0	...
229	0.2	...	19.4	269	0.2	0.2	...
230	...	...	3.4	270	0.1	...	...
231	...	...	0.4	271	...	...	...
232	...	...	...	272	...	...	...
233	...	...	...	273	...	...	...
234	...	...	...	274	...	...	...
235	...	0.1	0.1	275	...	...	...
236	...	1.1	0.2	276	...	...	...
237	...	0.3	0.1	277	...	...	...
238	0.4	0.1	...	278	...	1.0	0.3
239	0.2	...	0.2	279	...	0.2	...
240	...	...	...	280	...	...	...
241	...	...	0.2	281	...	...	...
242	...	...	0.4	282	...	...	...
243	...	...	2.1	283	...	...	...
244	...	...	0.4	284	0.2	...	...
245	...	...	...	285	0.2	...	...
246	...	...	...	286	0.1	...	...
247	...	...	...	287		...	...
248	...	...	...	288		...	...
249	...	...	0.1	289		...	...

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TABLE 6: (continued)

m/e	A	B	C	m/e	A	B	C
290		...	...	320			...
291		...	...	321			...
292		...	...	322			...
293		...	...	323			0.1
294		...	0.2	324			0.1
295		...	...	325			...
296		0.2	...	326			...
297		0.6	...	327			...
298		0.2	...	328			...
299		0.1	...	329			...
300		...	...	330			...
301		...	...	331			...
302		...	...	332			...
303		...	...	333			...
304		...	...	334			0.1
305		...	...	335			...
306		...	1.0	336			...
307		...	0.3	337			...
308		...	...	338			...
309		...	...	339			...
310		...	...	340			...
311		...	...	341			0.1
312		...	...	342			0.1
313		0.1	...				
314		0.1	...				
315			...				
316			...				
317			...				
318			...				
319			...				

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TABLE 7: Tabulation of mass spectral data on 1,12-octadecanediol and 1,12-octadecanediol-1,12-diacetate.

M	A	B	M	A	B
	1,12-Octadecanediol	1,12-Octadecanediol-1,12-diacetate		1,12-Octadecanediol	1,12-Octadecanediol-1,12-diacetate
m/e	286	370	m/e	286	370
			30	...	1.2
			31	...	2.0
12	...	1.0	32	...	6.5
13	...	1.1	33	...	...
14	...	3.1	34	...	...
15	...	11.1	35	...	...
16	...	4.1	36	...	2.1
17	...	24.1	37	...	...
18	0.6	95.5	38	...	0.9
19	...	...	39	...	13.7
20	...	...	40	...	3.1
21	...	...	41	0.3	71.1
22	...	...	42	0.7	18.5
23	...	...	43	1.6	33.2
24	...	...	44	0.4	2.1
25	...	...	45	0.3	6.9
26	...	...	46	...	0.5
27	...	13.3	47	...	...
28	0.5	54.8	48	...	...
29	...	28.5	49	...	...

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TABLE 7: (continued)

m/e	A	B	m/e	A	B
50	...	0.7	90	...	...
51	...	1.6	91	...	3.7
52	...	1.0	92	...	1.7
53	...	7.7	93	2.1	5.3
54	1.1	51.1	94	1.3	3.4
55	12.2	100.0	95	66.8	47.7
56	7.8	25.9	96	57.8	69.2
57	8.4	33.3	97	93.5	27.8
58	1.7	3.0	98	15.2	5.5
59	1.2	1.7	99	3.9	1.6
60	...	14.1	100	0.7	...
61	...	17.8	101	2.5	0.6
62	...	0.5	102	...	...
63	...	0.5	103	...	0.5
64	...	...	104	...	0.5
65	...	2.3	105	...	3.4
66	1.8	4.6	106	...	0.7
67	12.4	57.0	107	0.3	1.6
68	17.1	43.7	108	6.4	1.7
69	17.1	64.4	109	62.8	27.6
70	14.1	18.2	110	14.3	32.0
71	8.9	14.1	111	12.2	11.8
72	3.0	1.2	112	6.5	3.4
73	0.9	19.3	113	4.0	1.3
74	...	2.0	114	41.1	0.4
75	...	1.2	115	54.1	1.0
76	...	...	116	4.8	0.5
77	...	...	117	0.3	0.8
78	...	3.4	118	...	0.3
79	0.5	1.3	119	...	2.3
80	1.2	7.1	120	...	1.1
81	27.7	6.2	121	0.8	1.5
82	33.0	55.5	122	0.8	1.4
83	53.0	79.6	123	22.5	13.7
84	15.2	45.2	124	7.4	17.8
85	9.2	9.4	125	5.1	5.9
86	4.6	6.6	126	6.9	1.5
87	1.3	1.0	127	1.1	0.7
88	...	...	128	2.4	0.9
89	...	...	129	1.5	0.7

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TABLE 7: (continued)

m/e	A	B	m/e	A	B
130	...	...	170	0.2	0.5
131	...	...	171	0.4	...
132	...	...	172	0.1	...
133	...	1.0	173	0.4	...
134	...	0.7	174	...	...
135	1.2	1.8	175	...	...
136	2.1	1.4	176	...	...
137	3.7	8.3	177	...	...
138	4.0	11.1	178	...	...
139	1.3	2.1	179	0.9	1.6
140	0.8	1.3	180	1.4	2.9
141	1.2	5.9	181	0.8	0.6
142	0.3	1.3	182	1.3	...
143	0.2	...	183	31.5	...
144	...	...	184	4.4	...
145	...	...	185	0.5	...
146	...	...	186	...	...
147	...	1.6	187	...	...
148	...	0.4	188	...	...
149	...	0.8	189	...	...
150	...	0.7	190	...	...
151	1.4	4.1	191	...	0.7
152	2.7	7.6	192	...	...
153	0.8	1.4	193	7.3	1.6
154	3.3	1.0	194	1.3	2.4
155	1.3	2.4	195	0.3	0.5
156	0.2	0.7	196	0.3	...
157	0.3	...	197	1.2	...
158	...	...	198	0.3	...
159	...	...	199	4.1	...
160	...	...	200	0.7	...
161	...	...	201	100.0	...
162	...	...	202	15.0	...
163	0.6	...	203	1.3	...
164	0.7	...	204	...	...
165	43.3	2.7	205	...	...
166	7.4	4.4	206	...	...
167	2.5	1.1	207	0.4	1.6
168	1.0	0.7	208	0.9	1.8
169	1.0	0.9	209	0.3	0.4

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TABLE 7: (continued)

m/e	A	B	m/e	A	B
210	0.3	...	250	5.7	22.0
211	0.7	...	251	1.2	4.6
212	0.2	...	252	0.1	...
213	0.1	...	253	...	...
214	0.9	...	254	...	...
215	0.2	...	255	...	...
216	...	...	256	...	...
217	...	...	257	...	...
218	...	...	258	...	...
219	...	...	259	...	...
220	...	...	260	...	...
221	0.4	0.9	261	...	...
222	2.1	2.7	262	...	...
223	0.4	0.5	263	...	...
224	0.2	...	264	0.1	...
225	0.5	...	265	...	...
226	0.3	...	266	0.1	...
227	0.8	...	267	0.6	4.0
228	0.1	...	268	0.5	0.9
229	0.6	...	269	0.2	0.5
230	0.1	...	270	...	...
231	...	...	271	...	...
232	...	...	272	...	...
233	...	...	273	...	...
234	...	...	274	...	...
235	...	...	275	...	...
236	...	...	276	...	...
237	...	...	277	...	...
238	1.4	...	278	...	...
239	0.5	...	279	...	...
240	...	...	280	...	...
241	...	...	281	...	6.6
242	...	...	282	...	2.0
243	...	...	283	...	1.2
244	...	...	284	0.1	...
245	...	...	285	0.3	...
246	...	...	286	0.1	...
247	...	...	287		...
248	...	...	288		...
249	0.2	...	289		...

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TABLE 7: (continued)

m/e	A	B	m/e	A	B
290		...	330		...
291		...	331		...
292		...	332		...
293		...	333		...
294		...	334		...
295		...	335		...
296		...	336		...
297		...	337		...
298		...	338		...
299		...	339		...
300		...	340		...
301		...	341		3.4
302		...	342		1.2
303		...	343		0.6
304		...	344		...
305		...	345		...
306		...	346		...
307		...	347		...
308		0.2	348		...
309		0.2	349		...
310		0.4	350		...
311		...	351		...
312		...	352		1.0
313		...	353		0.3
314		...	354		...
315		...	355		6.2
316		...	356		2.4
317		...	357		1.4
318		...	358		0.4
319		...	359		...
320		...	360		...
321		...	361		...
322		...	362		...
323		...	363		...
324		0.3	364		...
325		0.9	365		...
326		0.3	366		...
327		0.3	367		...
328		...	368		...
329		...	369		...
			370		...

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TABLE 8: Tabulation of mass spectral data on the synthetic and natural 1,14-docosanediol

M	A	B	M	A	B
m/e	Sulfolipid diol 342	1,14-Docosanediol 342	m/e	Sulfolipid diol 342	1,14-Docosanediol 342
			30	1.1	2.1
			31	15.8	31.6
12	...	...	32	1.5	4.5
13	...	...	33	...	...
14	0.4	1.3	34	...	...
15	0.6	0.8	35	...	...
16	0.4	...	36	...	...
17	1.3	3.4	37	...	...
18	4.1	14.0	38	...	...
19	0.6	1.6	39	4.1	11.8
20	...	...	40	1.3	3.4
21	...	...	41	55.2	42.1
22	...	...	42	13.3	35.8
23	...	...	43	59.7	40.6
24	...	...	44	6.9	18.7
25	...	...	45	3.5	8.9
26	0.7	...	46	...	...
27	7.6	18.4	47	...	...
28	8.5	27.1	48	...	...
29	24.9	58.2	49	...	...

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TABLE 8: (continued)

m/e	A	B	m/e	A	B
50	...	...	90	...	...
51	...	...	91	...	1.3
52	...	...	92	0.2	...
53	2.4	7.6	93	1.0	3.7
54	9.1	32.4	94	0.9	3.7
55	79.8	60.7	95	43.6	38.5
56	18.5	62.6	96	16.6	59.0
57	48.8	42.2	97	34.0	100.0
58	4.1	18.9	98	6.7	22.1
59	2.1	8.2	99	2.4	9.0
60	...	...	100	0.6	2.2
61	...	...	101	0.2	0.9
62	...	...	102	...	...
63	...	...	103	...	...
64	...	...	104	...	...
65	0.4	1.8	105	...	...
66	3.3	11.1	106	...	...
67	24.3	67.6	107	0.6	2.2
68	16.9	58.2	108	0.7	2.1
69	100.0	80.0	109	26.0	83.5
70	16.7	56.6	110	6.3	22.6
71	17.1	59.0	111	18.6	61.6
72	1.5	5.5	112	2.8	11.6
73	0.7	2.6	113	1.1	4.3
74	...	...	114	0.6	2.4
75	...	...	115	0.4	1.3
76	...	...	116	...	...
77	...	1.8	117	...	...
78	...	...	118	...	...
79	1.9	7.4	119	...	...
80	1.7	5.8	120	...	...
81	29.8	98.2	121	0.7	3.2
82	29.3	98.2	122	0.4	1.6
83	68.2	46.1	123	13.9	51.4
84	10.4	32.6	124	10.6	36.6
85	8.1	27.4	125	6.9	24.8
86	1.3	4.3	126	1.6	6.1
87	...	1.2	127	0.6	2.6
88	...	...	128	0.3	1.3
89	...	...	129	0.7	3.3

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TABLE 8: (continued)

m/e	A	B	m/e	A	B
130	...	...	170	0.3	0.4
131	...	...	171	0.2	0.3
132	...	...	172	...	...
133	...	...	173	...	...
134	...	...	174	...	...
135	...	2.6	175	...	...
136	...	1.1	176	...	...
137	8.7	31.6	177	...	...
138	0.3	9.5	178	...	...
139	0.1	3.8	179	0.2	1.3
140	0.1	2.8	180	0.7	2.0
141	0.1	5.7	181	0.2	0.8
142	6.3	16.6	182	0.4	1.6
143	10.8	33.2	183	0.3	1.1
144	0.1	3.7	184	...	...
145	...	...	185	...	...
146	...	...	186	...	...
147	...	...	187	...	...
148	...	...	188	...	...
149	0.3	1.3	189	...	...
150	0.2	...	190	...	...
151	2.8	9.5	191	0.3	0.5
152	1.2	4.3	192	0.2	0.8
153	0.6	1.8	193	7.4	24.8
154	1.2	3.3	194	1.9	8.7
155	0.6	1.1	195	0.5	0.5
156	1.5	4.0	196	0.2	1.6
157	1.7	3.4	197	0.6	0.9
158	0.2	...	198	...	...
159	...	...	199	...	...
160	...	...	200	...	...
161	...	...	201	...	...
162	...	...	202	...	...
163	...	...	203	...	...
164	...	...	204	...	...
165	...	1.6	205	...	...
166	...	2.8	206	...	...
167	0.3	1.1	207	0.7	0.8
168	0.2	0.8	208	0.4	1.2
169	0.4	2.1	209	0.3	1.2

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TABLE 8: (continued)

m/e	A	B	m/e	A	B
210	0.2	0.8	250	2.6	0.9
211	7.5	25.2	251	...	...
212	1.3	4.3	252	...	...
213	...	0.8	253	0.2	0.5
214	...	...	254	...	...
215	0.3	...	255	...	...
216	...	...	256	0.1	...
217	...	...	257	0.1	...
218	...	...	258	...	...
219	...	...	259	...	...
220	...	...	260	...	...
221	...	...	261	...	...
222	0.3	1.1	262	...	...
223	0.2	...	263	...	...
224	0.3	0.9	264	0.2	0.66
225	0.9	0.9	265	...	...
226	0.3	...	266	...	...
227	1.3	5.8	267	...	...
228	0.4	1.3	268	...	...
229	19.4	51.5	269	...	...
230	3.4	8.4	270	...	...
231	0.4	0.9	271	...	...
232	...	...	272	...	...
233	...	...	273	...	...
234	...	...	274	...	...
235	0.1	0.3	275	...	...
236	0.2	0.9	276	...	...
237	0.1	0.4	277	...	...
238	...	...	278	0.3	1.5
239	0.2	0.5	279	...	...
240	...	...	280	...	...
241	0.2	...	281	...	...
242	0.4	2.2	282	...	...
243	2.1	0.5	283	...	...
244	0.4	...	284	...	...
245	...	...	285	...	...
246	...	...	286	...	...
247	...	...	287	...	...
248	...	...	288	...	...
249	0.1	0.4	289	...	...

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TABLE 8: (continued)

m/e	A	B	m/e	A	B
290	...	...	320	...	...
291	...	...	321	...	...
292	...	...	322	...	0.15
293	...	...	323	0.1	0.29
294	0.2	0.45	324	0.1	0.26
295	...	0.18	325	...	0.42
296	...	...	326	...	0.11
297	...	...	327	...	...
298	...	...	328	...	...
299	...	...	329	...	...
300	...	...	330	...	...
301	...	...	331	...	...
302	...	...	332	...	...
303	...	...	333	...	...
304	...	...	334	0.1	...
305	...	0.21	335	...	...
306	1.0	3.7	336	...	...
307	0.3	0.95	337	...	...
308	...	...	338	...	...
309	...	...	339	...	0.1
310	...	...	340	...	0.1
311	...	...	341	0.1	0.2
312	...	...	342	0.1	0.1
313	...	...			
314	...	...			
315	...	...			
316	...	...			
317	...	...			
318	...	...			
319	...	...			

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