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## Microbial Sulfolipids. III. The Disulfate of (+)-1,14-Docosanediol in *Ochromonas danica*\*

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**ABSTRACT:** A new sulfolipid, the disulfate of (+)-1,14-docosanediol, was isolated from the phytoflagellate, *Ochromonas danica*. It was characterized by synthesis of (DL)-1,14-docosanediol and its disulfate and by comparison of the synthetic materials with the natural materials. In order to determine the orientation of the natural secondary sulfate, it was necessary to explore the mechanism of solvolysis of sulfate esters in dioxane.

It was found that solvolysis of secondary sulfates to

alcohols in dioxane occurs with retention of configuration around the C-O bond of the alcohol. Comparison of the natural (+)-1,14-docosanediol rotation with the rotations of hydroxy acids of known absolute configuration suggest the natural sulfolipid is 1-(S)-14-docosanediol 1,14-disulfate. With one exception all other natural hydroxy fatty acids (where the hydroxyl is isolated and beyond the  $\beta$  position) have the *R* configuration. Chlorodocosanediols are obtained from the solvolyzed sulfatides.

Phospholipids have been found in all organisms in which they have been sought to date. Sulfolipids have been less sought than phospholipids but it now appears they are ubiquitous. The retarded development of sulfolipid research has been due to the poorer analytical methods for sulfate, whereas phosphate is conveniently assayed by molybdate. The recent availability of sulfur-35 has permitted the identification of a variety of sulfolipids including the sulfonolipid<sup>1</sup> of the chloroplast (Daniel *et al.*, 1961), the sulfatide of *Halobacterium cutirubrum* (Kates, 1967), and the sulfatides described in this series.

Perhaps the most surprising aspect of the sulfatide, herein described, is the fact that the polar groups, always charged because of the extremely low  $pK_a$  of sulfates, are essentially at both ends of the molecule. To our knowledge, no similar lipid has been found in living tissue although several compounds with polar groups at both ends of the molecule have been found to be excreted by yeasts (Stodola *et al.*, 1967; Gorin *et al.*, 1961).

In order to characterize the sulfatide, it was necessary to determine the configuration of the carbon at position 14. It was not possible to obtain the rotation of the secondary sulfate directly because of its poor solubility and its tenacity for

trace impurities throughout its isolation and purification. Acid hydrolysis was expected to produce some racemization and a poor yield of diol. Solvolysis in dioxane appeared to be a mild, rapid, and highly specific technique (McKenna and Norymberski, 1957; Burstein and Leiberman, 1958). Unfortunately the effect of solvolysis of secondary alkyl sulfates upon the configuration of the C-O bond was not known. Experiments with optically active 2-octanol 2-sulfate demonstrated that solvolysis occurred with retention of configuration.

This procedure, together with the unambiguous synthesis of 1,14-docosanediol 1,14-disulfate, has permitted a complete characterization of the sulfatide in *Ochromonas danica*. This structure had earlier been proposed for the sulfatide (Mayers and Haines, 1967).

### Experimental Procedure

**Materials.** All reagents were analytical grade. Solvents were distilled before use. Dioxane was distilled from lithium aluminum hydride and used immediately. 12-Bromolauric acid (Sapon Laboratories) was recrystallized from heptane and then from methylene chloride (mp 51.5–52.5°). Diethyl malonate, dihydropyran (City Chemical Co.), and nonanoic acid were redistilled before use and checked for purity by gas chromatography. (–)-2-Octanol (Aldrich Chemicals),  $[\alpha]_D^{23}$  –10.05° (c 8.6, ethanol), was checked for purity by gas chromatography.

**Isolation of the Sulfatide.** *O. danica* was cultured on defined medium as described earlier (Haines, 1965). The procedure for isolation of the sulfatide has already been described (Mayers and Haines, 1967; Haines, 1965). A brief summary of this procedure follows.

Cells were collected by centrifugation and extracted three

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<sup>1</sup> The term *sulfolipid* in this paper means a sulfur-containing lipid; *sulfatide* refers to a lipid sulfate ester; *sulfonolipid* is a sulfolipid in which the sulfur occurs as a sulfonic acid.

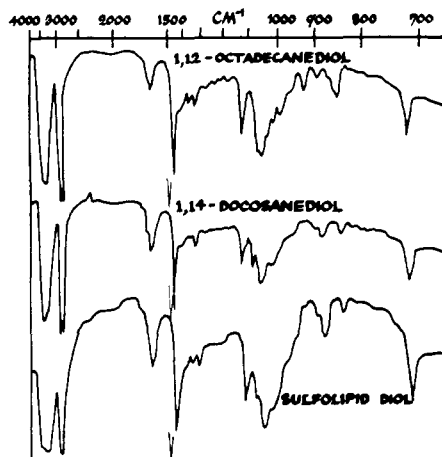


FIGURE 1: Infrared spectra of 1,12-octadecanediol, synthetic 1,14-docosanediol, and natural 1,14-docosanediol obtained by solvolysis of *O. danica* sulfatide in dioxane. The spectra were taken on potassium bromide wafers using an Infracord Model 137.

times with 20 volumes of chloroform-methanol (2:1, v/v). The solvent was removed *in vacuo* and the residue was saponified in 0.2 N potassium hydroxide in methanol for 45 min at 37°. The solution was diluted with an equal volume of water, exhaustively extracted with chloroform, acidified, and again extracted with chloroform. The solution was neutralized and the sulfatides were then extracted with 1-butanol. The 1-butanol was removed *in vacuo*.

**Solvolysis of Crude Sulfatide in Dioxane.** The crude sulfatide (2.6 g) was added to 195 ml of anhydrous dioxane. The suspension was refluxed for 1 hr, and two portions of 1.9 ml of deionized water were added at 15 and 30 min, respectively. After cooling, hexane (500 ml) was added to the suspension which was extracted twice with 125 ml of water. The clear hexane was dried over anhydrous sodium sulfate, filtered, and the solvent was removed *in vacuo*. The residue (935.3 mg) was examined by thin-layer chromatography and gas-liquid partition chromatography. In addition to the sulfatide products obtained by solvolysis of pure sulfolipid (Mayers and Haines, 1965) (Figure 1), the crude solvolysate contained some steroids (Gershengorn *et al.*, 1968) and fatty acids.

**Thin-Layer Chromatography.** Chromatograms consisting of thin layers (250  $\mu$ ) of silicic acid with 10% calcium sulfate binder (Supelcosil B from Supelco Laboratories, Bellefonte, Pa.) were *not* activated. The developing solvent was chloroform-methanol-water (65:35:5, v/v); plates were visualized by examination under ultraviolet light after spraying with 2',7'-dichlorofluorescein (0.2% w/v in methanol) or by slowly heating the plate over a hot plate after spraying with 25% (w/v) sodium bisulfate containing 1% (v/v) concentrated sulfuric acid.

**Chromatography on Silicic Acid.** A column (32  $\times$  3 cm) of silicic acid (Supelcosil B; 154 g) in hexane-diethyl ether (7:3, v/v) was charged with the crude desulfated sulfatide mixture. The products were eluted with the same solvent and collected in 5-ml fractions. The fractions were monitored with thin-layer chromatography and gas-liquid partition chromatography. Fractions containing pure diol on thin-layer chromatography were combined and the solvent was removed *in vacuo*. The product was recrystallized from hexane and then from carbon

tetrachloride. The crystals were fine white feathers (128 mg): mp 87–87.5°,  $[\alpha]_D^{25} + 0.40 \pm 0.01$  (c 2.75, dimethylacetamide).

**Gas-Liquid Partition Chromatography.** A Perkin-Elmer 881 gas chromatograph with a 6 ft  $\times$  1/8 in. stainless steel column packed with 3% JXR on 100–200 mesh Chromosorb Q (Applied Science Laboratories, Pa.) column at 230°. The procedure has been described elsewhere (Gershengorn *et al.*, 1968).

**Analyses and Spectra.** The elemental analyses were carried out by Schwartzkopf Microanalytical Laboratories, New York, N. Y. The infrared spectra were determined on potassium bromide wafers using an Infracord Model 137. Samples were prepared by drying at 0.5 mm at 60°. Mass spectra were obtained by Morgan Schaffer Corp. on an Hitachi RMU-6 mass spectrometer using the indirect inlet at 250°. The melting points are corrected. Optical rotations were determined on a Rudolph spectral polarimeter.

**Synthesis of 1,1,12-Dodecanetricarboxylic Acid (I).** Sodium (3.0 g, 0.13 mol) was dissolved in 75 ml of absolute ethanol. Diethyl malonate (24 g, 0.15 mol) was added to the sodium ethoxide solution over a period of 15 min. 12-Bromolauric acid (14.45 g, 0.052 mol) was dissolved in 100 ml of absolute ethanol and slowly added to the solution of sodiomalonic ester. The reaction mixture was refluxed for 2 hr. Water was added until the solution clarified. Sodium hydroxide pellets (20 g) were slowly added while the solution was refluxing so that the final solution was approximately 0.2 N in base. The solution was allowed to stand overnight. The sodium salt of the resulting triacid was filtered and washed with ethanol. The sodium salt of I was dissolved in 250 ml of water. The viscous solution was acidified to pH 1 with 3 N HCl and the product was filtered. Compound I was washed with deionized water and dried in a desiccator over phosphorous pentoxide overnight. The yield was 15.5 g (99%), mp 94.5–96°. The substance was recrystallized from methylene chloride and then from water (mp 99–100°). The sample, in the melting point capillary, was heated in the melting point apparatus at 160–180° for 10 min. After decarboxylation of I to 1,12-dodecanedicarboxylic acid the melting point was 121.5–122.5° (lit. (Sisido *et al.*, 1959) mp 124.5–125.0°). Compound I was titrated with standardized NaOH. The observed equivalent weight was 103.9 g/equiv (Calcd: 100.7 g/equiv). *Anal.* Calcd for  $C_{18}H_{36}O_6$  (302): C, 59.60; H, 8.61. Found: C, 59.50; H, 8.92.

**Synthesis of 14-Ketodocosanoic Acid (II).** Compound I (9.1 g, 0.052 mol), suspended in 25 ml of benzene, was added to a solution of dihydropyran (15 g, 0.179 mol) in 50 ml of benzene containing 1 drop of sulfuric acid. The solution was stirred with a magnetic stirrer and maintained below 30° with a cold water bath. The solution clarified after 2 hr. Potassium hydroxide (5 g) was added and the suspension was stirred for 5 min. The solution was decanted from the solid and the solvent was removed *in vacuo* below 30°. The residual ester in 100 ml of benzene was added to a suspension of sodium hydride (2.8 g, 0.12 mol; washed with benzene) in benzene (100 ml) and stirred for 2.5 hr. Nonanoyl chloride (15 g, 0.085 mol) was added and the reaction mixture was stirred for 30 min. Water (10 ml) was added to destroy unreacted nonanoyl chloride and the reaction mixture was extracted with 100 ml of 1 N potassium hydroxide. The organic layer was dried with sodium sulfate and the benzene was removed *in vacuo*. The residue was dissolved in acetic acid (20 ml) and acetic anhydride (20 ml). The solution was refluxed at 150° for 3 hr;

1 l. of 1 N potassium hydroxide was added and the solution was extracted with diethyl ether. The aqueous solution was acidified to pH 1 and extracted with diethyl ether. The ether solution was dried over sodium sulfate and the solvent was removed *in vacuo*. The residue was recrystallized from hexane and 95% (v/v) aqueous ethanol yielding 4.5 g (24%) of II (mp 89.5–90.0, lit. (Bowman, 1950a) mp 85°).

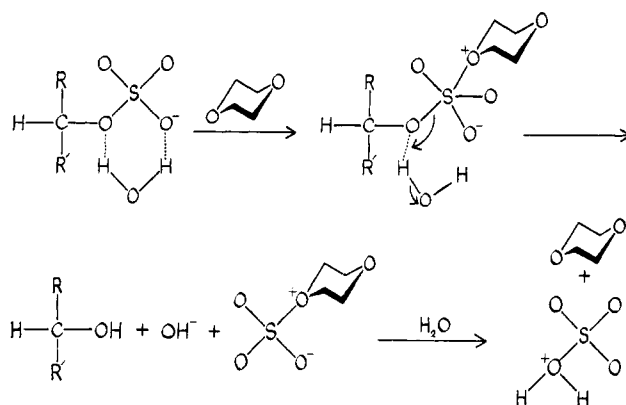
**Synthesis of 1,14-Docosanediol (III).** Compound II (1.26 g, 3.7 mmol) was dissolved in diethyl ether (200 ml). Lithium aluminum hydride (1.0 g, 0.029 mol) was added to the diethyl ether solution and the suspension was stirred for 10 hr. Water (100 ml) was slowly added to decompose the unreacted lithium aluminum hydride. Hydrochloric acid (3 N) was added to dissolve the salts and the organic layer was separated. The aqueous layer was extracted twice with 150 ml of diethyl ether. The combined ether fractions were washed with water (25 ml) and dried over anhydrous potassium carbonate. The diethyl ether was removed *in vacuo*. The residual III (1.1 g, 87%) was recrystallized from hexane and then from methylene chloride (mp 78–78.5°). The infrared spectrum of III is shown in Figure 1. *Anal.* Calcd for  $C_{22}H_{46}O_2$  (342): C, 77.19; H, 13.45. Found: C, 76.67; H, 13.21.

**1,14-Docosanediol 1,14-Disulfate Dipotassium Salt (IV).** Chlorosulfonic acid (0.5 ml, 3.7 mmol) was dissolved in 10 ml of dioxane (distilled from lithium aluminum hydride) at 4°; III (0.5 g, 1.4 mmol), dissolved in dioxane (25 ml), was added slowly over the period of 1 hr with stirring at 4°. The reaction was maintained at 4° for an additional hour and then was permitted to come to room temperature. The mixture was poured into a solution of potassium hydroxide so that the final concentration was 0.5 N in base. The solution was extracted four times with 100 ml of 1-butanol. The combined 1-butanol extracts were washed twice with water (50 ml). The solvent was removed *in vacuo* and the residue (700 mg) was recrystallized from ethanol. The infrared spectrum is shown in Figure 3. *Anal.* Calcd for  $C_{22}H_{44}K_2O_8S_2 \cdot H_2O$ : C, 44.30; H, 7.72; S, 10.74. Found: C, 44.70; H, 7.73; S, 10.85.

**Potassium (+)-2-Octyl Sulfate.** Chlorosulfonic acid (3.16 ml, 0.048 mol) was added to 80 ml of dioxane and the mixture was cooled to 4°. (–)-2-Octanol (6.34 ml, 0.04 mol), dissolved in 100 ml of dioxane, was slowly added over a period of 40 min. The reaction mixture was permitted to come to room temperature (approximately 2 hr) and then poured into 100 ml of cold 1.8 N potassium hydroxide. Water (400 ml) was added to clarify the solution. The clear solution was extracted three times with 250 ml of petroleum ether (bp 30–60°). The petroleum ether was dried over sodium sulfate and the solvent was removed *in vacuo* to yield a residue of 102 mg of unreacted (–)-2-octanol. The water fraction was then extracted three times with 250 ml of 1-butanol. The 1-butanol extracts were combined, washed three times with water (100 ml), and the solvent was removed *in vacuo*. The residual potassium (+)-2-octyl sulfate (10.5 g) was recrystallized from ethanol: mp 187–191° dec,  $[\alpha]_D^{25} +1.00 \pm 0.05^\circ$  (c 7.3, water–ethanol, 1:4, v/v). *Anal.* Calcd for  $C_8H_{17}KO_4S$  (248): C, 38.71; H, 6.85; S, 12.90. Found: C, 38.64; H, 6.81; S, 12.81.

**Solvolysis of Potassium (+)-2-Octyl Sulfate.** Potassium (+)-2-octyl sulfate (2.21 g, 8.9 mmol) was dissolved in 99% (v/v) aqueous dioxane (50 ml). The solution was refluxed for 15 min, cooled, and diluted with water (200 ml). The water was extracted three times with 100 ml of diethyl ether. The ether extracts were combined and washed twice with water

SCHEME I



(50 ml), dried over anhydrous potassium carbonate, filtered, and the solvent was removed *in vacuo*. The residual (–)-2-octanol (1.02 g, 87%) was analyzed by gas-liquid partition chromatography and thin-layer chromatography. The infrared spectrum was identical with that of 2-octanol. The specific rotation (absolute ethanol) was  $[\alpha]_D^{25} -9.86^\circ$  (c 8.3, ethanol) identical with that of (–)-2-octanol.

## Results and Discussion

**Solvolysis of Sulfates in Dioxane.** The cleavage of sulfate esters is unique in mechanistic organic chemistry. In base, a Walden inversion occurs with a negatively charged group attacking a carbon to which a negative group (sulfate) is bonded (Green and Kenyon, 1950). In acid, attack is on the sulfur as hydrolysis occurs with retention of configuration (Burwell, 1952) although some racemization occurs as well as  $\beta$  elimination. Cyclic sulfates are also cleaved in base *via* attack upon the sulfur (Kaiser and Zaborsky, 1968). Solvolysis of steroid sulfates, first discovered<sup>2</sup> by Grant and Beall (1950), also occurs with retention of configuration when it is acid catalyzed (Burstein and Lieberman, 1958). Generally speaking, the variety of reactions that sulfate esters undergo in acid, elimination, racemization, etc., are autocatalyzed by the production of acid during the reaction (Burwell, 1952). It was deemed desirable therefore to explore the mechanism of solvolysis of secondary alkyl sulfate ester salts in dioxane and its effect on the configuration of the C–O bond.

(–)-2-Octanol was converted into (+)-2-octanol 2-sulfate by reaction with chlorosulfonic acid. The solvolysis of the potassium salt of the latter compound yielded (–)-2-octanol with retention of configuration in 15 min with essentially a quantitative yield. The 87% yield of material is undoubtedly due to losses during the isolation. The same procedure with long-chain sulfates and disulfates gives better than 97% yield. The unusual stability of the sulfur trioxide–dioxane complex, first described by Suter *et al.* (1938) as a white solid and a very effective sulfating reagent, demonstrates the affinity of dioxane with this Lewis acid. A mechanism for the solvolysis of sulfate esters in dioxane is proposed in Scheme I. A large number of

<sup>2</sup> As early as 1939, Butenandt and Hofstetter observed that estrone sodium sulfate decomposed in hot organic solvents.

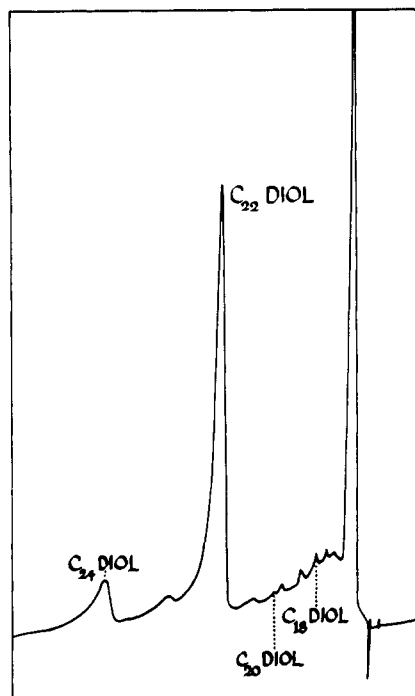


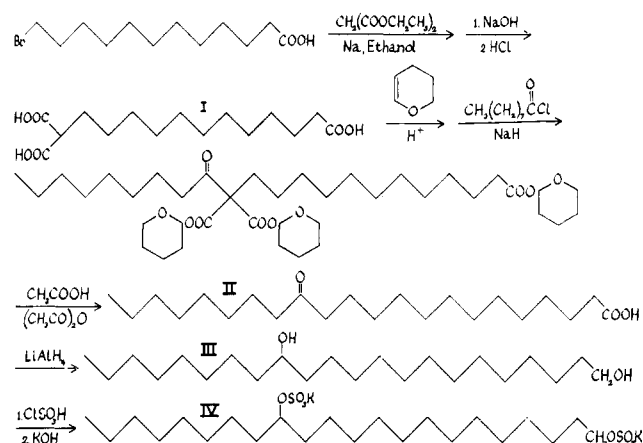
FIGURE 2: Gas chromatogram of natural 1,14-docosanediol obtained by solvolysis of the sulfatide in dioxane. The positions of the hydroxyls in the  $C_{18}$ ,  $C_{20}$ , and  $C_{24}$  diols are not known. The column, consisting of 3% JXR on Chromosorb Q, was maintained at  $230^{\circ}$ .

solvents have been used for solvolysis (McKenna and Norymberski, 1957; Burstein and Lieberman, 1958). Dioxane and tetrahydrofuran have the highest rate (McKenna and Norymberski, 1957). All the solvents used successfully have a pair of electrons to serve as a substitute for the attacking pair in dioxane.

The following points support the proposal. (1) Solvolysis of a 2-octyl sulfate occurs with complete retention of configuration. (2) An aryl pyridinium sulfate was solvolyzed much more rapidly than the corresponding derivatives of cholesterol and cholestan- $3\beta$ -ol. Thus displacement on the sulfur is facilitated by the attachment of the oxygen to an aromatic nucleus (McKenna and Norymberski, 1957). (3) It was shown that the solvolysis of steroid sulfates occurs with the following order of decreasing activity: dioxane and tetrahydrofuran > diethyl ether > diisopropyl ether > anisole (McKenna and Norymberski, 1957). This result can be interpreted as due to either decreasing availability of the pair of electrons (Brown and Horowitz, 1955) or due to increased steric hindrance in their attack on the central sulfur atom. (4) Dioxane exhibits a high affinity for sulfur trioxide; the complex is remarkably stable (Suter *et al.*, 1938). (5) The interatomic distances of the sulfate oxygens are such that a water molecule bonded between any two oxygens of the tetrahedron would be very stable.<sup>3</sup> (6) It has been observed (Cohen and Oneson, 1953)

<sup>3</sup> This undoubtedly accounts for the hygroscopic nature of sulfates as well as sulfuric acid. Although greater stability would be obtained by a water molecule hydrogen bonded between two negatively charged oxygens, this would not lead to the desired products. It is postulated therefore, that the heat required for the reaction is necessary to provide the initial reacting species in Scheme I.

## SCHEME II



and confirmed in our laboratory that water in excess of 2% inhibits the reaction dramatically. Presumably the entire sulfate group is hydrated under these circumstances and the dioxane does not penetrate through the shielding water molecules. It appears that a water molecule cannot attack the sulfur as does dioxane as it is preferentially hydrogen bonded to sulfur oxygens. (7) Solvolysis of cholestan- $3\beta$ -yl potassium sulfate in ethanol produced ethyl sulfate (McKenna and Norymberski, 1957), implying attack on the central sulfur atom by the free pair of electrons on the ethanol.

Besides the above observations Burstein and Lieberman (1958) observed that during the solvolysis of steroid hydrogen sulfates in ethyl acetate containing  $H_2^{18}O$ , no  $^{18}O$  was incorporated into the liberated sterol. It is likely that under these conditions the oxygen between the sulfur and the carbon is protonated as in acid hydrolysis in aqueous media<sup>4</sup> (Burwell, 1952).

The reaction provides a highly selective means for removing sulfate esters under mild conditions in the presence of glycosides, esters, and phosphate esters. It opens the possibility of using sulfate as a stereospecific blocking group in organic synthesis.

**Synthesis of 1,14-Docosanediol 1,14-Disulfate Dipotassium Salt.** The synthesis of racemic sulfatide is essentially the synthesis of 14-ketodocosanoic acid following the general procedure of Bowman (1950b, Bowman and Fordham, 1952) with subsequent reduction and sulfation (Scheme II). Comparison of the infrared spectra (Figure 2) and the mass spectra (Mayers and Haines, 1967) of the natural and synthetic diols show the spectra to be essentially identical with minor differences in intensity of peaks. A marked difference is noted in the melting points. The natural diol melts at  $87-87.5^{\circ}$  while the synthetic material melts at  $78-78.5^{\circ}$ . The difference is presumably due to the racemic mixture obtained by synthesis.

Purified diol obtained from the natural sulfatide was examined by gas-liquid partition chromatography (Figure 1). The substance was obtained from a silicic acid column which does not separate aliphatic diols of varying chain length. The

<sup>4</sup> It should also be noted, however, that no effect on the rate of this hydrolysis could be observed by large variations in the hydrogen ion concentration (Burstein and Lieberman, 1958).

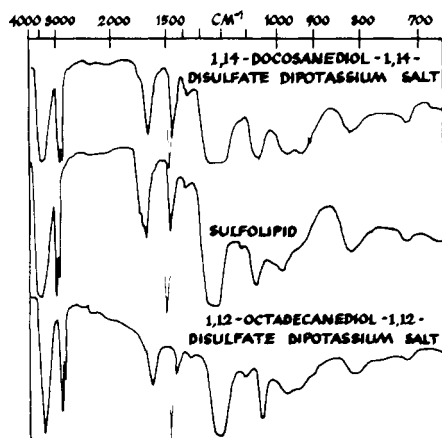


FIGURE 3: Infrared spectra of synthetic 1,14-docosanediol 1,14-disulfate, natural sulfolipid, and synthetic 1,12-octadecanediol 1,12-disulfate. The spectra were taken on potassium bromide wafers using an Infracord Model 137.

gas-liquid partition chromatography, however, showed that a series of diols were present with the 1,14-docosanediol representing approximately 94% of the total sample. Other diols present were  $C_{18}$ ,  $C_{20}$ , and  $C_{24}$  diols with only  $C_{24}$  present in greater than trace amounts.

The infrared spectrum of the sulfatide is shown in Figure 3 where it is compared with that of synthetic 1,14-docosanediol 1,14-disulfate and 1,12-octadecanediol 1,12-disulfate. The three spectra are hardly distinguishable as they are dominated by the absorption of water ( $3400$  and  $1640\text{ cm}^{-1}$ ), alkane ( $2950$ ,  $2875$ ,  $1460$ , and  $720\text{ cm}^{-1}$ ), and sulfate ( $1250$ – $1210$ ,  $1070$ ,  $980$ ,  $935$ , and  $810\text{ cm}^{-1}$ ). It is therefore difficult to use these spectra to distinguish between the disulfates. The significance of the spectra rests in the two bands at  $935$  and  $980\text{ cm}^{-1}$ . As shown in Figure 4 these two bands, which are due to the C–O bond of the sulfate ester (Lloyd *et al.*, 1961), distinguish between primary and secondary sulfates. Both bands are present in the natural material.

**Configuration of Carbon-14 of the Sulfatide.** The application of dioxane solvolysis to the sulfatide permitted isolation of a diol with the configuration of carbon-14 intact. The rotation of the natural 1,14-docosanediol was  $[\alpha]_D^{22.5} +0.40 \pm 0.01^\circ$  in dimethylacetamide.

The absolute configuration of ricinoleic acid was established as (*R*)-12-hydroxy-9-*cis*-octadecenoic acid by synthesis of the enantiomer, (*S*)-12-hydroxyoctadecanoic acid, and by comparison with hydrogenated ricinoleic acid (Serck-Hanssen, 1958; Serck-Hanssen and Stenhagen, 1955). This work, together with that of Lemieux and Giguere (1951), has permitted the assignment of absolute configurations to aliphatic hydroxy acids (Baker and Gunstone, 1963; Applewhite *et al.*, 1967a,b; Badami and Morris, 1965; Chisholm and Hopkins, 1960; Powell *et al.*, 1967; Schroepfer and Bloch, 1963; Tallent *et al.*, 1966; Serck-Hanssen, 1967; Tulloch *et al.*, 1968; Gorin *et al.*, 1961). All of the natural hydroxy acids described in these papers are of the *R* configuration except for those excreted by yeasts (Tulloch *et al.*, 1968; Gorin *et al.*, 1961). These (*R*)-hydroxy acids are all found in higher plants or in one case in a bacterium (Schroepfer and Bloch, 1963; Wallen *et al.*, 1962). The *R* and *S* designations are those of Cahn *et al.* (1956). The *D*-hydroxy acids are of the

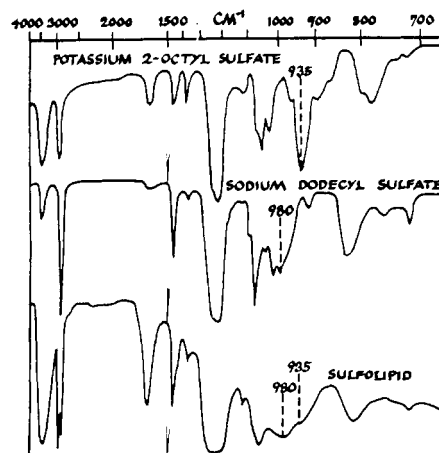


FIGURE 4: Infrared spectra of potassium 2-octanol sulfate, sodium dodecanol sulfate, and potassium sulfolipid. The spectra were taken on potassium bromide wafers using an Infracord Model 137.

*R* configuration despite statements to the contrary by Baker and Gunstone (1963). This is in agreement with Morris and Wharry (1966). The (*S*)-hydroxy acids excreted by yeasts (Tulloch *et al.*, 1968; Gorin *et al.*, 1961) are the only natural fatty acids of the *S* configuration that have been reported. In each case the magnitude of rotation of these hydroxy fatty acids have been less than  $0.5$  with the magnitude increasing with decreasing wavelength except when another functional group has been in the vicinity of the hydroxyl including for example a double bond (*e.g.*, Applewhite *et al.*, 1967a,b; Schroepfer and Bloch, 1963). Where a hydroxy acid is of the *R* configuration, the rotation is negative. In the one case where it has been tested, reduction of the hydroxy acid to a diol has had a negligible effect on the rotation (Applewhite *et al.*, 1967a,b). We therefore tentatively conclude that the sulfolipid is 1-(*S*)-14-docosanediol 1,14-disulfate.

**Solvolysis Products.** In a previous paper (Mayers and Haines, 1967) we reported that acid hydrolysis of the purified sulfolipid produced several ether-soluble products among which the major product was the 1,14-docosanediol described herein. Because of the side reactions that occur in the acid hydrolysis of 2-butyl sulfate (Burwell, 1952), we expected a variety of artifacts to be produced (along with some racemization of the isolated diol). Solvolysis of natural and synthetic sulfolipid in dioxane, however, showed that the natural sulfolipid was a mixture of disulfates, one of which is 1,14-docosanediol 1,14-disulfate and disulfates of related diols. Thin-layer chromatography of the solvolysis products (diols) showed seven spots. The gas-liquid partition chromatograph in Figure 1 was obtained from the most polar spot (lowest  $R_F$ ) and shows mostly 1,14-docosanediol with a small amount of tetracosanediol and traces of octadecanediol, etc. The contaminating series is *threo*-(*R*)-13-chloro-1-(*R*)-14-docosanediol and a series of polychlorodiols, di-, tri-, tetra-, penta-, and hexachlorodocosanediols (Haines *et al.*, 1969). The 1,14-docosanediol and the monochlorodocosanediol constitute 44% of the sulfolipid diols. Another spot which analyzes for hexachlorodocosanediol constitutes 34% and the remaining spots are each less than 10%.

These substances are currently under active investigation.

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