Biochemistry of the Sphingolipids. XV. Structure of Phytosphingosine and Dehydrophytosphingosine*

H. E. CARTER AND H. S. HENDRICKSON†

From the Division of Biochemistry, Noyes Laboratory of Chemistry, University of Illinois, Urbana

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Mixtures of phytosphingosine and dehydrophytosphingosine have been prepared from phytoglycolipids of a number of seeds. Analysis of these mixtures gave the following ratios of saturated to unsaturated base: flax, 15:85; soybean, 20:80; peanut, 50:50; corn, 90:10. Oxidative cleavage of the double bond in dehydrophytosphingosine showed it to be in the C 8,9 position. Studies on the stereochemistry of phytosphingosine established the structure as D-*ribo*-1,3,4-trihydroxy-2-amino-octadecane. Dehydrophytosphingosine, therefore, has the structure D-*ribo*-1,3,4-trihydroxy-2-amino-8-*trans*-octadecene.

Cerebrin base was first isolated from mushrooms by Zellner (1911) and later from yeast by Reindel (Reindel, 1930; Reindel *et al.*, 1940). Carter *et al.* (1954) isolated a long-chain base from corn phosphatide and showed it to be identical to cerebrin base.[1] Its structure was established as 1,3,4-trihydroxy-2-amino-octadecane (I) and it was named phytosphingosine.

$$CH_3(CH_2)_{13}\underset{OH}{CH}-\underset{OH}{CH}-\underset{NH_2}{CH}-CH_2OH$$

I

Carbon 2 was shown to have the D configuration by the formation of *N*-benzoyl-L-serinal after periodate oxidation of the *N*-benzoyl base.

An unsaturated analog of phytosphingosine, dehydrophytosphingosine (II), was isolated from soybean and

$$CH_3(CH_2)_xCH{=}CH(CH_2)_{11-x}\underset{OH}{CH}-\underset{OH}{CH}-\underset{NH_2}{CH}-CH_2OH$$

II

flax phosphatides by Carter *et al.* (1958a, 1958b, 1962). This base was shown by hydrogenation and infrared studies to be identical to phytosphingosine except for the presence of a *trans* double bond.

During the acid hydrolysis of phytosphingolipids a small amount of anhydrophytosphingosine is formed in addition to free phytosphingosine (Reindel, 1930; Reindel *et al.*, 1940). Structural studies by Carter *et al.* (1954) and O'Connell and Tsein (1959) showed that anhydrophytosphingosine has a tetrahydrofuran structure (III).

$$CH_3(CH_2)_{13}\underset{O}{CH}-\underset{OH}{CH}-\underset{NH_2}{CH}-CH_2$$

III

The work described in this paper was undertaken to establish the position of the double bond in dehydrophytosphingosine and establish the configuration of carbons 3 and 4 of phytosphingosine.

In order to accomplish these objectives it was necessary to prepare moderate quantities of the two bases in relatively pure form. The acidic methanol hydrolysis of phytoglycolipid previously employed gives only moderate yields of long-chain base together with significant amounts of the anhydro base (III). There is also the possibility of isomerization occurring in the acidic hydrolysis. Therefore various alkaline hydrolysis procedures were investigated, but in each case the yield of long-chain base was too low to provide a satisfactory preparative method. These experiments did, however, indicate the absence of isomerization in the acidic hydrolysis, since the base fractions obtained by the two procedures showed closely similar properties (melting point, optical rotation) and gave identical derivatives.

The preparation of phytosphingosine and dehydrophytosphingosine, each uncontaminated by the analog, was facilitated by use of the analytical procedure devised by Sweeley and Moscatelli (1959) to characterize long-chain bases. The method involves vapor phase chromatographic identification of the aldehydes produced by periodate oxidation of the base. By this

$$RCH(OH)-CH(OH)-CH_2OH(NH_2) \xrightarrow{IO_4^-} RCHO + 2HCO_2H + H_2CO + NH_3$$

procedure, it was shown that corn phosphatide contains mainly phytosphingosine and flax phosphatide mainly dehydrophytosphingosine, with other sources giving intermediate values (ratio of saturated to unsaturated base: flax, 15:85; soybean, 20:80; peanut, 50:50; corn, 90:10). It was further established that phytosphingosine derivatives from corn and dehydrophytosphingosine derivatives, regardless of the source, could not be completely freed of the contaminating analog even after repeated crystallization from various solvents. Furthermore the two bases were not satisfactorily resolved by silicic acid chromatography in the systems tested.

The discovery that the *N*-benzoyl-triacetyl derivatives of the two bases could be separated by thin layer chromatography on siliconized silica Gel G with an acetonitrile-containing solvent system led to a study of countercurrent distribution methods. A satisfactory separation of the *N*-benzoyl-triacetyl derivatives was finally achieved by an extended (800-transfer) distribution in an acetonitrile-hexane system, thus providing pure dehydrophytosphingosine for characterization studies. It seems probable that this same procedure will prove useful in the separation of sphingosine analogs.

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† Predoctoral Research Fellow (GF 17,382), United States Public Health Service. Present address, Department of Biochemistry, University of California, Berkeley. This work is taken from the thesis submitted by H. S. Hendrickson to the Graduate College of the University of Illinois in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry.

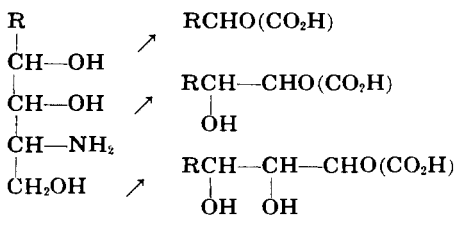
[1] It was subsequently shown by Prostenik and Stanačev (1958) that the yeast base consists mainly of the C_{20} homolog of phytosphingosine.

The position of the double bond in dehydrophytosphingosine was determined by two different degradations. In one experiment, the *N*-benzoyl-triacetyl derivative was hydroxylated with performic acid. Cleavage of this product with periodate yielded a long-chain aldehyde which was oxidized to the corresponding fatty acid with silver oxide. In a second experiment, oxidative ozonolysis of dehydrophytosphingosine gave a fatty acid identical to that obtained in the first experiment and which was identified as decanoic acid by vapor phase chromatographic analysis of its methyl ester. Thus, the double bond in dehydrophytosphingosine is established as being in the C 8,9 position (formula IV).

$$CH_3(CH_2)_8CH{=}CH{-}(CH_2)_3\underset{OH}{CH}{-}\underset{OH}{CH}{-}\underset{NH_2}{CH}{-}CH_2OH$$

IV

It was hoped to establish the configuration of carbon atoms 3 and 4 of phytosphingosine by partial periodate degradation and characterization of the mono and dihydroxy acid fractions. Possible oxidation products are shown below:



R = $CH_3(CH_2)_{13}$

The main product of the periodate–silver oxide oxidation was pentadecanoic acid. However, sufficient α-hydroxypalmitic acid was produced (characterized by vapor phase chromatography) to determine its specific rotation and, thus, establish its configuration as D-α-hydroxypalmitic acid. Thus, carbon 4 of phytosphingosine has the D-configuration as related to carbon 1. Unfortunately, insufficient amounts of dihydroxy acid were obtained to permit characterization. However, evidence concerning the configuration of carbon 3 was obtained by a study of the behavior of *N*-benzoyl-anhydrophytosphingosine. In this derivative an $N \rightarrow O$ shift of the benzoyl group under relatively mild conditions would be expected only if the adjacent hydroxyl group were *cis* to the amide. For instance, in 1 N acid at 25°, *cis*-2-acetamidocyclohexanol was found to undergo migration about six times as rapidly as the *trans* form (McCasland, 1951). Migration studies on *cis*- and *trans*-2-benzamidocyclohexanol showed an even greater difference (Fodor and Kiss, 1949). Similarly, studies on the all *trans* *N,N'*-dibenzoylstreptamine showed no migration even after several weeks in 0.7 N ethanolic HCl at room temperature (Dyer, 1954). In a five-membered ring an even greater difference in the rates of migration of the *cis* and *trans* isomers would be expected.

The behavior of *N*-benzoyl-anhydrophytosphingosine in 0.7 N ethanolic HCl at room temperature was investigated. Migration of the benzoyl groups occurred to the extent of about 50% in 91 hours and was complete in 15 days. These results establish that the hydroxyl and amino groups in anhydrosphingosine bear a *cis* relationship to each other and that the same relationship exists in phytosphingosine, provided only that no epimerization occurs at carbon 3 during the formation of anhydrophytosphingosine. The fact that the anhydro base is obtained readily as a single isomer (*cis*) argues strongly against this possibility as does also the stability toward acid hydrolysis of carbon 3 in dihydrosphingosine ceramides. Since the amino carbon of phytosphingosine has the D configuration, carbon atom 3 also may be assigned the D structure. Thus the structure for phytosphingosine is established as D-*ribo*-1,3,4-trihydroxy-2-amino-octadecane and that of dehydrophytosphingosine as D-*ribo*-1,3,4-trihydroxy-2-amino-8-*trans*-octadecene. Sphingosine and dihydrosphingosine also have the D configuration at carbons 2 and 3 (D-*erythro* structure).

Experimental

Materials.—Phytoglycolipid was prepared from crude flax phosphatides as described by Carter *et al.* (1962).

Vapor Phase Chromatographic Analysis of Long-Chain Bases.—An Aerograph A-350 gas chromatographic apparatus[2] equipped with a thermal conductivity detector was used in this work. A Disc integrator[2] was used to integrate the peaks. Long-chain aldehydes were analyzed with use of a 0.25-inch column containing glutaric acid–diethylene glycol polyester on siliconized Celite prepared according to Sweeley and Moscatelli (1959).

Long-chain bases were analyzed by the procedure of Sweeley and Moscatelli (1959) with slight modifications. The lipid sample was hydrolyzed with methanolic sulfuric acid, and the crude base fraction, prepared as described for phytosphingosine from phytoglycolipid (Carter *et al.*, 1954), was applied to a small silicic acid column and eluted with chloroform followed by chloroform-methanol (1:4). The chloroform-methanol (1:4) fraction was evaporated and treated with periodate according to the procedure of Sweeley and Moscatelli. The reaction mixture was diluted with water and extracted twice with methylene chloride. The methylene chloride extracts were filtered and evaporated to dryness under a stream of nitrogen. The residue was dissolved in benzene and evaporated under nitrogen to a 1% solution (based on the original weight of long-chain base). This solution was then analyzed by vapor phase chromatography with the polyester column at a temperature of 180° and a flow rate of 75 ml per minute of helium.

Isolation and Purification of Dehydrophytosphingosine. (a) Alkaline Hydrolysis—Flax phytoglycolipid (10 g) was refluxed with 1 liter of saturated Ba(OH) solution for 6 hours. The hot hydrolysate was filtered through a medium sinter filter and the residue was washed with water and air dried (yield: 7.65 g; N, 1.23%; P, 1.74%). This material (7.5 g) was extracted with ether in a Soxlet apparatus for 6 hours. The ether extract was evaporated to give 772 mg of a mixture of ceramide and long-chain base. This residue was dissolved in 200 ml of boiling methanol. The solution was cooled overnight and filtered to remove most of the ceramide. The filtrate was treated with decolorizing charcoal and evaporated to dryness. The residue was recrystallized repeatedly from ether, giving 73 mg of crystalline base melting at 92–94°; $[\alpha]_D^{25} = +8.5°$ (*c*, 1.2% in ethanol).

Anal. Calcd. for $C_{18}H_{37}NO_3$: C, 68.36; H, 11.79; N, 4.43. Found: C, 67.04; H, 11.48; N, 4.23.

Infrared analysis showed strong absorption at 970 cm^{-1} (*trans* double bond).

The free base was recrystallized from acetone, giving an acetone compound melting at 114–116°; $[\alpha]_D^{30} = +15.9°$ (*c*, 3.4% in chloroform).

Anal. Calcd. for $C_{21}H_{41}NO_3$: C, 70.99; H, 11.63; N, 3.94. Found: C, 70.67; H, 11.41; N, 3.97.

[2] Wilken's Instrument and Research, Inc., P. O. Box 313, Walnut Creek, Calif.

(b) ACID HYDROLYSIS.—One gram of flax phytoglycolipid was hydrolyzed with methanolic sulfuric acid as described by Carter *et al.* (1954). The crude base (145 mg) was dissolved in 5 ml of boiling acetone, and the solution was decolorized with decolorizing charcoal, filtered, and cooled to 4°. After several hours the nicely crystalline precipitate which formed was filtered, giving 55 mg of acetone compound melting at 111–117°. Two recrystallizations from acetone gave 35 mg of pure acetone compound melting at 116–118°; $[\alpha]_D^{28}$ = +20.1° (*c*, 1.4% in chloroform). Periodate oxidation followed by vapor phase chromatographic analysis of the long-chain aldehydes showed 15% saturation and 85% unsaturation.

Anal. Calcd. for $C_{21}H_{41}NO_3$: C, 70.99; H, 11.63; N, 3.94. Found: C, 70.04; H, 11.44; N, 4.03.

(c) SILICIC ACID CHROMATOGRAPHY.—Crude flax base (4.2 g) obtained by acid hydrolysis was applied to a 300-g column of silicic acid which was developed with chloroform-methanol solvents of increasing methanol concentration. Fractions were collected and characterized by thin layer chromatography on Silica Gel G with chloroform-methanol (4:1) as a solvent (R_F of dehydrophytosphingosine, 0.13; R_F of anhydro base, 0.8). Dehydrophytosphingosine was eluted from the silicic acid column with 20% methanol in chloroform. About 2 g of white powder was obtained which, after repeated recrystallizations from ethyl acetate, gave a microcrystalline material melting at 91–93°. Periodate oxidation and vapor phase chromatographic analysis of the resultant long-chain aldehydes showed 15% saturation and 85% unsaturation. There was no difference in composition between the earlier and later fractions eluted from the column.

(d) *N*-BENZOYL- and *N*-BENZOYL-TRIACETYLDEHYDROPHYTOSPHINGOSINE. — Dehydrophytosphingosine (250 mg) was converted to the *N*-benzoyl derivative by the Schotten-Baumann procedure (Carter *et al.*, 1954), giving 92.3 mg of derivative melting at 125–127°. The *N*-benzoyl derivative was acetylated with acetic anhydride in pyridine (Carter *et al.*, 1954), giving 110 mg of *N*-benzoyl-triacetyl derivative melting at 72–75°; $[\alpha]_D^{25}$ = +10° (*c*, 0.76% in chloroform). This material (100 mg) was recrystallized twice from hexane to give 80 mg of white crystalline product melting at 77–78°.

Anal. Calcd. for $C_{31}H_{48}NO_7$: C, 68.01; H, 8.85; N, 2.56. Found: C, 68.12; H, 8.77; N, 2.71.

A 10-mg sample of this substance was saponified in 1 N methanolic KOH at room temperature overnight. The solution was diluted with water and extracted several times with ether. The ether extract was treated with periodate. Analysis of the long-chain aldehydes showed 15% saturation and 85% unsaturation.

(e) THIN LAYER CHROMATOGRAPHY.—A Silica Gel G thin layer plate was immersed in a 5% solution of Dow Corning Silicone 200 Fluid in ether (Malins and Mangold, 1960). After the ether had evaporated the plate was spotted with the *N*-benzoyl-triacetyl derivatives of corn and flax base and developed in an acetonitrile–acetic acid–water (70:10:25) solvent system. The plate was exposed to iodine vapors to detect the unsaturated derivatives. The saturated derivatives were detected by spraying with a 1% solution of α-cyclodextrin in 30% ethanol followed by exposure to iodine vapors giving a white spot on a purple background. The R_F values are shown in Table I.

(f) COUNTERCURRENT DISTRIBUTION.—Countercurrent distribution was carried out in a 200-tube (10 ml per phase) Craig automatic apparatus. *N*-Benzoyl-triacetyl flax base (1.05 g) was added to the first four tubes and an 800-transfer distribution was made with the recycling technique with an acetonitrile-hexane (2:3) solvent system. The weight distribution is shown in Figure 1. The partition coefficients for the peaks at tubes 72 and 101 were calculated as K = 0.099 and K = 0.144 respectively.

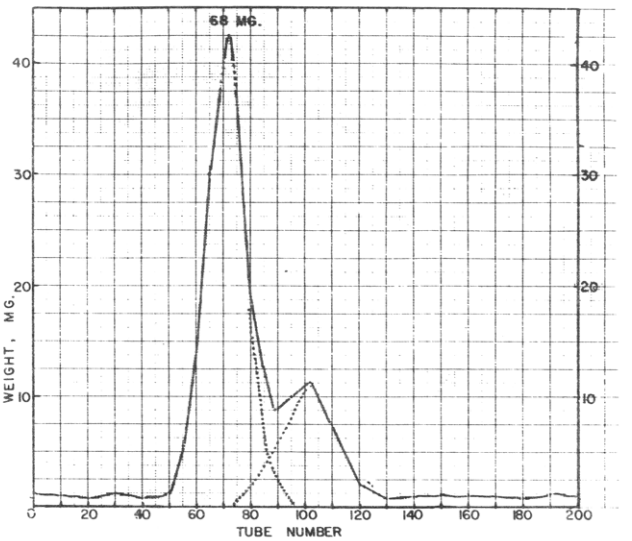


FIG. 1.—Countercurrent distribution of *N*-benzoyl-triacetyl flax base (1.05 g) in an acetonitrile-hexane (2:3) system; 800 transfers, recycling technique. Peaks at tubes 72 and 101 represent respectively dehydrophytosphingosine and phytosphingosine derivatives.

TABLE I
CHROMATOGRAPHY OF PHYTOSPHINGOSINE DERIVATIVES

Compound	R_F Values
N-Benzoyl-triacetyl corn base	0.21 (α-cyclodextrin-I_2)
N-Benzoyl-triacetyl flax base	0.21 (α-cyclodextrin-I_2) trace 0.23 (I_2) main spot

Tubes 50–70 were combined and the residue was crystallized from hexane, giving 332 mg of crystalline material melting at 77–79°.

Anal. Calcd. for $C_{31}H_{48}NO_7$: C, 68.01; H, 8.85; N, 2.56. Found: C, 68.12; H, 9.16; N, 2.64.

A 10-mg sample of this material was saponified in 1 N methanolic NaOH at room temperature overnight. Periodate oxidation and vapor phase chromatography of the long-chain aldehyde showed that this material was 100% dehydrophytosphingosine derivative.

Tubes 102–125 were combined and crystallized from hexane, giving 100 mg of crystals melting at 79.5–81°.

Anal. Calcd. for $C_{31}H_{50}NO_7$: C, 67.85; H, 9.18; N, 2.55. Found: C, 68.27; H, 9.08; N, 2.79.

Similar analysis of this material showed it to be 100% phytosphingosine derivative.

Base Composition of Phytoglycolipids from Various Sources.—One-gram samples of flax, soybean, peanut, and corn phytoglycolipids were analyzed by a modification of the Sweeley and Moscatelli procedure. The results of these analyses are shown in Table II.

The retention times of authentic dodecanal, tetradecanal, and octadecanal were 2.9, 4.6, and 11.9 minutes respectively. The calculated retention time for pentadecanal is 5.7 minutes (calculated from a plot of log retention time versus chain length).

Position of the Double Bond in Dehydrophytosphingosine.

(a) HYDROXYLATION OF *N*-BENZOYL-TRIACETYLDEHY-

TABLE II
ANALYSES OF BASES FROM VARIOUS PHYTOGLYCOLIPIDS

Source	Retention Times of Aldehydes (min.)		Phytosphingosine (%)	Dehydrophytosphingosine (%)
Crude flax base	5.7	6.2	15	85
Crude soybean base	5.7	6.2	20	80
Crude peanut base	5.7	6.2	50	50
Crude corn base	5.7	6.2	90	10

DROPHYTOSPHINGOSINE.—To a solution of 30 mg of *N*-benzoyl-triacetyl-phytosphingosine in 2 ml of redistilled formic acid, 0.05 ml of 30% H_2O_2 was added. The reaction mixture was shaken at 37° overnight and then lyophilized. The residue was dissolved in 15 ml of methanol and the solution was titrated to *p*H 10 with methanolic KOH and allowed to stand at room temperature for several hours to saponify any formate esters. The solution was neutralized with perchloric acid and filtered to remove the insoluble $KClO_4$. No attempt was made to purify the hydroxylated derivative at this point.

(b) PERIODATE OXIDATION OF HYDROXYLATED BASE.—One milliliter of 0.2 M $NaIO_4$ was added to the methanolic solution of hydroxylated base and the mixture was allowed to stand at room temperature in the dark. After one hour, the solution was diluted with water and extracted twice with hexane. The combined hexane extracts were dried over anhydrous sodium sulfate.

(c) SILVER OXIDE OXIDATION.—The hexane solution from the periodate reaction was evaporated to dryness and the residue was dissolved in 10 ml of methanol. Silver nitrate (34 mg) was dissolved in the solution and 0.6 ml of 0.5 N NaOH was added dropwise with stirring. After stirring for 1.5 hours, the solution was filtered and the filtrate was acidified to *p*H 1 with dilute HCl. The solution was diluted with an equal volume of water and extracted with two equal volumes of hexane. The combined hexane extracts were dried over anhydrous sodium sulfate and evaporated to dryness, leaving 12.6 mg of crude acid.

(d) OZONOLYSIS OF DEHYDROPHYTOSPHINGOSINE.—Ozonolysis reactions were carried out in a homemade ozonizer similar to that described by Henne and Perilstein (1943). With use of an output of 18,500 volts from a high-voltage transformer (Jefferson Electric Co., primary voltage 136 volts), 0.78% ozone was produced at a rate of 0.091 moles of ozone per minute. Analysis of ozone was carried out as described by Smith *et al.* (1955).

Dehydrophytosphingosine (30 mg) was dissolved in a mixture of 2 ml of glacial acetic acid and 3 ml of methyl acetate and ozonized at −13°. Three milliliters of glacial acetic acid and 3 ml of 30% H_2O_2 were added to the solution and the mixture incubated at 37° for 3 days. The mixture was heated to boiling briefly and the solvents evaporated *in vacuo*. The residue (a viscous liquid) was diluted with 10 ml of water, acidified with dilute HCl to *p*H 1, and extracted with two equal volumes of hexane. The combined hexane extracts were dried over anhydrous sodium sulfate and evaporated to dryness, leaving 15 mg of crude acid.

In later experiments, the base was dissolved in absolute methanol (3 mg per ml) and ozonized at −60°. The solvent was removed *in vacuo*. Redistilled formic acid (0.1 ml per mg) and 30% H_2O_2 (0.05 ml per mg) were added and the mixture incubated at 37° for 3 days. The reaction mixture was processed exactly as described above. Similar results were obtained in both procedures, although side-reactions are less likely to occur in the latter.

(e) IDENTIFICATION OF FATTY ACIDS FROM THE DEGRADATIONS OF DEHYDROPHYTOSPHINGOSINE.—The fatty acids were converted to their methyl esters with diazomethane by the method of Schlenk and Gellerman (1960). The methyl esters were analyzed by vapor phase chromatography with the glutaric acid-diethylene glycol polyester column. The results are shown in Table III.

TABLE III
VAPOR PHASE CHROMATOGRAPHY OF FATTY ACID ESTERS

Methyl Ester	Retention Time (min.)	Column Temp. (°C)
From ozonolysis	5.4	180
Methyl decanoate	5.4	180
From Ag_2O oxidation	5.8	174
Methyl decanoate	5.8	174
Methyl dodecanoate	9.8	174
Methyl octanoate	3.7	174

N → O Acyl Migration of N-Benzoyl Anhydrophytosphingosine.—Twenty milligrams of *N*-benzoyl anhydrophytosphingosine (Carter *et al.*; 1954) was dissolved in 9.4 ml of absolute ethanol and 0.6 ml of concentrated HCl. The reaction mixture was incubated at room temperature. Aliquots (0.5 ml) were removed, evaporated to dryness under a stream of nitrogen, dissolved in methanol, and evaporated again to remove traces of HCl. The residue was then dissolved in 0.1 ml of chloroform and the infrared spectrum was taken with a 1-mm micro cell. After 91 hours the amide band (1650 cm^{-1}) was half its original intensity and an ester band (1730 cm^{-1}) of equal intensity had appeared. After 15 days the amide band had disappeared and the ester band was quite intense.

After 3 weeks the reaction solution was diluted with cold 1 N KOH and extracted with ether. The ether-soluble residue was crystallized from ether, giving a material melting at 130–139°. The infrared spectrum of this material was consistent with that of *O*-benzoyl anhydrophytosphingosine.

Stereochemistry of Phytosphingosine at Carbon 4.

(a) PERIODATE OXIDATION OF PHYTOSPHINGOSINE.—Phytosphingosine (300 mg, 0.945 mmoles) was dissolved in 23 ml of methanol, and 2 ml of 0.442 M $NaIO_4$ was added. The mixture was incubated in the dark at room temperature. A 0.1-ml aliquot was removed after 10 minutes. Two milliliters of saturated $NaHCO_3$, 0.8 ml of 0.0102 M NaH_2AsO_3, and 0.2 ml of 20% KI in saturated $NaHCO_3$ were added to the aliquot in that order. Starch indicator was added and the mixture was titrated with 0.00996 M I_2. The I_2 required was 0.835 ml, representing an uptake of 0.884 mmoles of periodate.

After 30 minutes, the reaction mixture was diluted with 25 ml of water and extracted twice with ether. The ether extracts were combined, washed with water, and dried over Na_2SO_4. The ether was evaporated and the residue dissolved in 50 ml of methanol.

(b) SILVER OXIDE OXIDATION.—To the methanolic solution from the periodate reaction, 306 mg of $AgNO_3$ (dissolved in a few milliliters of water) was added and 5.4 ml of 0.5 N NaOH added dropwise with stirring. Stirring was continued for 1.5 hours in the dark. The reaction mixture was filtered through a fine sinter.

The filtrate was acidified to *p*H 1 with dilute HCl and diluted with 50 ml of water. This mixture was extracted three times with ether. The ether extracts were combined, washed with water, and dried over Na_2SO_4. The solvent was evaporated, leaving 143.4 mg of crude acid.

(c) SILICIC ACID CHROMATOGRAPHY.—Partition chromatography was carried out as described by Frankel *et al.* (1962). Fifty grams of silicic acid was slurried in 150 to 200 ml of benzene, and 40 ml of 20% methanol in benzene was added slowly with shaking. The slurry was applied to a column and allowed to settle. It was packed to a constant volume under nitrogen pressure. A 200-mg mixture of palmitic acid and α-hydroxypalmitic acid was applied to the column and elution begun with 2% methanol in benzene. Palmitic acid was eluted after 120 ml and α-hydroxypalmitic acid after 220 ml. The peaks were sharp and well resolved.

The mixture of crude acids (143 mg) from the silver oxide oxidation was applied to a similar column and elution begun with 2% methanol in benzene. Fifty-milliliter fractions were collected.

The fraction at 100–150 ml had an infrared spectrum consistent with a C_{15} saturated acid and had the same R_F as palmitic acid on thin layer chromatography (Silica Gel G, hexane–ether–acetic acid 60:40:1). The fraction at 200–250 ml (fraction A) showed two spots with dichlorofluorescein on thin layer chromatography in the same solvent system: a major spot at R_F 0.17, corresponding to α-hydroxypalmitic acid, and a minor spot at the origin.

(d) α-HYDROXYPALMITIC ACID.—Fraction A was dissolved in 75% aqueous ethanol and cooled, giving a few milligrams of precipitate (A-I). The filtrate was diluted with an equal volume of water and cooled, giving 9 mg of precipitate (A-II). A-I showed only a faint spot corresponding to α-hydroxypalmitic acid and a minor spot at the origin. A-II, which was amorphous, melted and crystallized at 80–82°. The crystals then melted at 85–91°. Two melting points are reported for α-hydroxypalmitic acid: 86–87° (Kuwata, 1938) and 93.3–93.6° (Horn and Pretorius, 1954).

One milligram of A-II was esterified with diazomethane according to the procedure of Schlenk and Gellerman (1960). Vapor phase chromatographic analysis (20% QF-1 on Chromosorb W, 196°, 85 ml per minute helium) showed two peaks: a major peak at 7.5 minutes corresponding to methyl α-hydroxypalmitate, and a minor peak (less than 5% of the total) at 3.3 minutes corresponding to methyl pentadecanoate.

A 7.7-mg sample of acid A-II (0.028 mM) was dissolved in 0.5 ml of methanol, 0.28 ml of 0.1 N NaOH, and 0.22 ml of water. The optical rotation of this solution was measured in a 1-dm cell (+0.081°) and the specific rotation was calculated to be $[\alpha]_D^{25} = +10.5°$ (*c*, 0.77% in 50% aqueous ethanol). The sodium salt of D-α-hydroxypalmitic acid has a specific rotation $[\alpha]_D^{22} = +16°$ (*c*, 1.49% in 50% aqueous ethanol) (Horn and Pretorius, 1954).

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