Partial Identity of Sphingosine Methyl Ethers*

BENJAMIN WEISS

WITH THE TECHNICAL ASSISTANCE OF RICHARD L. STILLER

From the Department of Biochemistry, New York State Psychiatric Institute, and the College of Physicians and Surgeons, Columbia University, New York

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Sphingosine, *O*-methylsphingosine I, and *O*-methylsphingosine II were converted to *N*-carbobenzoxy derivatives which were permethylated in dimethylformamide. It was concluded from infrared spectra and specific rotations that these three compounds differed from each other. Similar results were obtained when the corresponding dihydro compounds were subjected to the same sequence of reactions. Performylation of 1,3-*O*-diacetyl-*N*-carbobenzoxysphingosine and *O*,*N*-diacetyl-*O*-methylsphingosines I and II, followed by mild alkaline hydrolysis and periodate oxidation, vapor-phase chromatography of the long-chain aldehydes, and formation of their 2,4-dinitrophenylhydrazones, disclosed that the *O*-methyl ethers were a mixture. The concentration of 1-hydroxy-2-amino-5-methoxy-3-octadecene, formed by an allylic rearrangement, in bases I and II is 71 and 72%, respectively; the remainder of 29 and 28% constitutes the 3-*O*-methyl ethers. The specific rotations of the sodium salts of the acids obtained from oxidation of the corresponding 2-methoxypentadecanals with CrO_3 in glacial acetic acid established the configurations at carbon atom 5 of the 5-methoxy isomer in bases I and II as D and L, respectively.

Methyl ethers of sphingosine are formed during preparation of the base from sphingolipids by hydrolysis in methanolic sulfuric acid (Carter *et al.*, 1947). Isolation from the crude mixture of two crystalline ethers of sphingosine as the *p*-hydroxyazobenzene-*p'*-sulfonate and hydrochloride salts was achieved by Carter *et al.* (1951). They concluded both from periodate oxidation and hydrogenation studies in which hydrogenolysis of the methoxyl group occurred that the bases were methylated at the allylic hydroxyl group. In addition, they stated that the possibility of an allylic rearrangement had not been eliminated. It was postulated by these investigators that during hydrolysis a carbonium-ion intermediate was formed which gave rise to two diastereoisomers of opposite configuration at carbon atom 3 (Fig. 1a,b,d,e). Without assignment of configuration, these bases were arbitrarily designated I and II.

EXPERIMENTAL

To establish the identity of these bases, the hydrochlorides of *O*-methylsphingosine I and *O*-methylsphingosine II were isolated from hydrolysates of beef spinal cord lipids by the method of Carter *et al.* (1951); melting points of 113–117° and 142–145° for bases I and II, respectively, were in agreement with those reported (Carter *et al.*, 1951). Sphingosine and the *O*-methyl ethers of sphingosine were *N*-carbobenzoxylated (Weiss, 1957), and then permethylated in a single step by the procedure of Kuhn *et al.* (1955) with Ag_2O and CH_3I in dimethylformamide. The infrared spectra of the compounds derived from methylation of *N*-carbobenzoxy-*O*-methylsphingosines I and II were similar but different from the spectrum of the compound obtained from *N*-carbobenzoxysphingosine in the ether region of 1160–1095 cm^{-1}. Absence of the –NH– deformation band at 1512 cm^{-1} indicated methylation of the secondary amide group; this was confirmed by elementary analysis. All the compounds had different specific rotations in chloroform.

When the corresponding dihydro compounds were subjected to the same sequence of reactions, the infrared spectra of the derivatives from *O*-methyldihydrosphingosines I and II were different from the spectrum of the derivative from dihydrosphingosine. The same results were obtained when the corresponding unsaturated, permethylated, *N*-carbobenzoxy derivatives were hydrogenated over platinum in ethanol; the carbobenzoxy group is unaffected under these conditions. Ealing models of these compounds disclosed a very tightly packed structure with some restricted rotation of the $N\text{-}CH_3$ group. Removal of the carbobenzoxy group from the unsaturated or saturated derivatives by hydrogenation over platinum in glacial acetic acid or over palladium in ethanol still resulted in three different spectra with a strong single absorption band near 1100 cm^{-1} replacing the previous ether triplet; each compound gave a negative ninhydrin reaction.

When methylation was conducted under the same conditions but with benzene as solvent, no *N*-methylation occurred. The compound derived from *N*-carbobenzoxydihydrosphingosine differed in its infrared spectrum and physical properties from the one obtained from *N*-carbobenzoxy-*O*-methyldihydrosphingosine II. It was concluded from these methylation studies that the *O*-methyl ethers of sphingosine differed fundamentally from the parent compound. It was assumed that an allylic rearrangement had occurred and that the methoxyl group was now on carbon atom 5 of the base. This assumption would still be in agreement with the periodate and hydrogenation findings of Carter *et al.* (1951).

The validity of this assumption was examined by hydroxylating 1,3-*O*-diacetyl-*N*-carbobenzoxysphingosine, and *O*,*N*-diacetyl-*O*-methylsphingosines I and II with performic acid according to the procedure of Swern (1953). After mild alkaline hydrolysis, the products were characterized by reacetylation in pyridine. None of the compounds showed absorption at 980 cm^{-1} for the *trans* double bond. No attempt was made to separate the mixture of diastereoisomers in which the newly formed pair of hydroxyl groups are

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All analyses were done by the Schwartzkopf Microanalytical Laboratory. The low *N*-methyl values may have been due to the presence of other substituents on the nitrogen atom bearing the methyl group; the limits of this determination are plus or minus 5%.

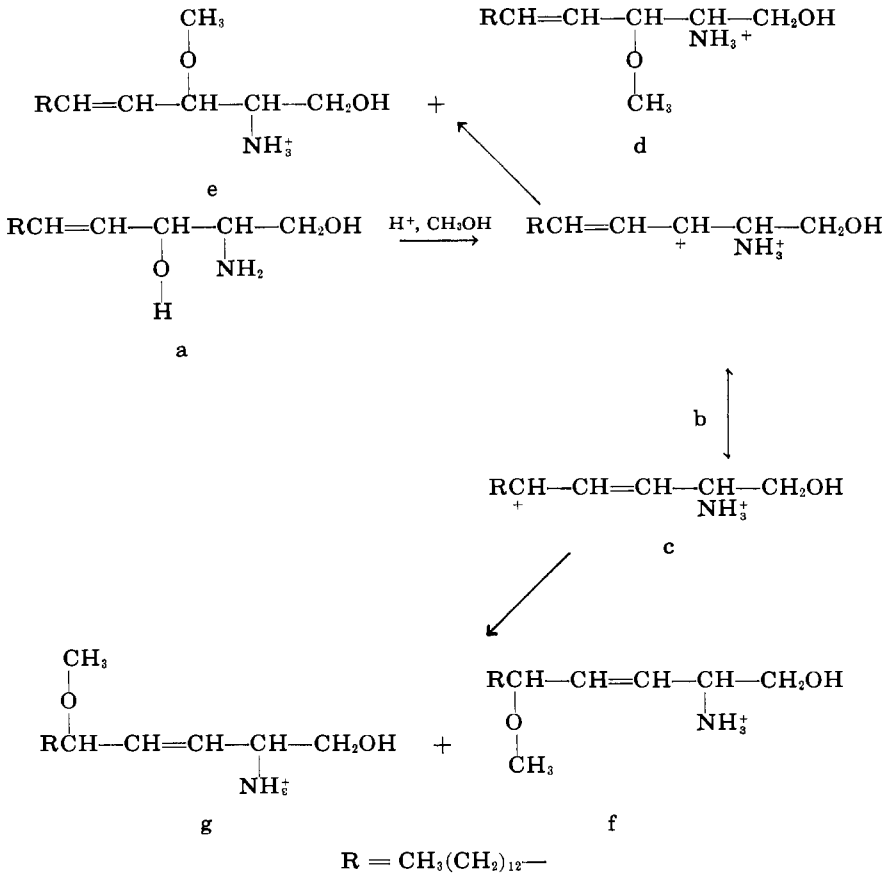


FIG. 1.—The formation of methyl ethers of sphingosine during preparation of the base from sphingolipids by hydrolysis in methanolic sulfuric acid may be accounted for by an allylic rearrangement. See text for details.

both *cis* and anti to the hydroxyl group on either carbon atom 3 or 5 owing to inversion upon opening of the intermediate epoxide (Swern, 1948). After characterization, the ester groups were cleaved, and the resulting *N*-acylated compounds were degraded with periodic acid. The 2,4-dinitrophenylhydrazones of the long-chain aldehydes from *O*-methyl derivatives I and II melted at 68–72° and 65–69°, respectively; the 2,4-dinitrophenylhydrazone of the aldehyde from the parent base agreed with that for the same derivative of tetradecanal, mp 103–104°. The specific rotations of the long-chain aldehydes from *O*-methyl compounds I and II were $[\alpha]_D^{23}$ +34.0 and $[\alpha]_D^{23}$ −36.3, respectively. Vapor-phase chromatography of aldehydes from the parent base disclosed a single peak with the retention time of tetradecanal; however, the aldehydes from *O*-methyl derivatives I and II gave two peaks, a minor one corresponding to tetradecanal and a major one to to 2-methoxypentadecanal. Both from a comparison of the peak areas in the gas chromatograms and the methoxyl contents of the 2,4-dinitrophenylhydrazones, the average concentration of 1-hydroxy-2-amino-5-methoxy-3-octadecene in bases I and II is 71 and 72%, respectively; the remainder of 29 and 28% represents the 3-*O*-methyl ethers. It is assumed that the methoxyl group in the compound from the allylic rearrangement is on carbon atom 5 and that no further migration of the methoxyl group has occurred. This assumption is supported by the infrared spectra of the 2-methoxypentadecanals which showed only an ether absorption band at 1110 cm^{-1}, whereas tetradecanal, which has a free methylene group, exhibited both an alcohol, 1125 cm^{-1}, and an ether doublet, 1075–1055 cm^{-1}, absorption band, probably, owing to an aldollike condensation in which a substituted 1,3-dioxane is formed from trimer (Fig. 2) (Royals, 1958; Bellamy, 1958).

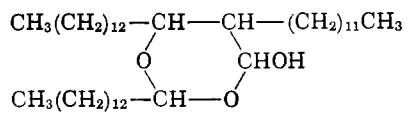
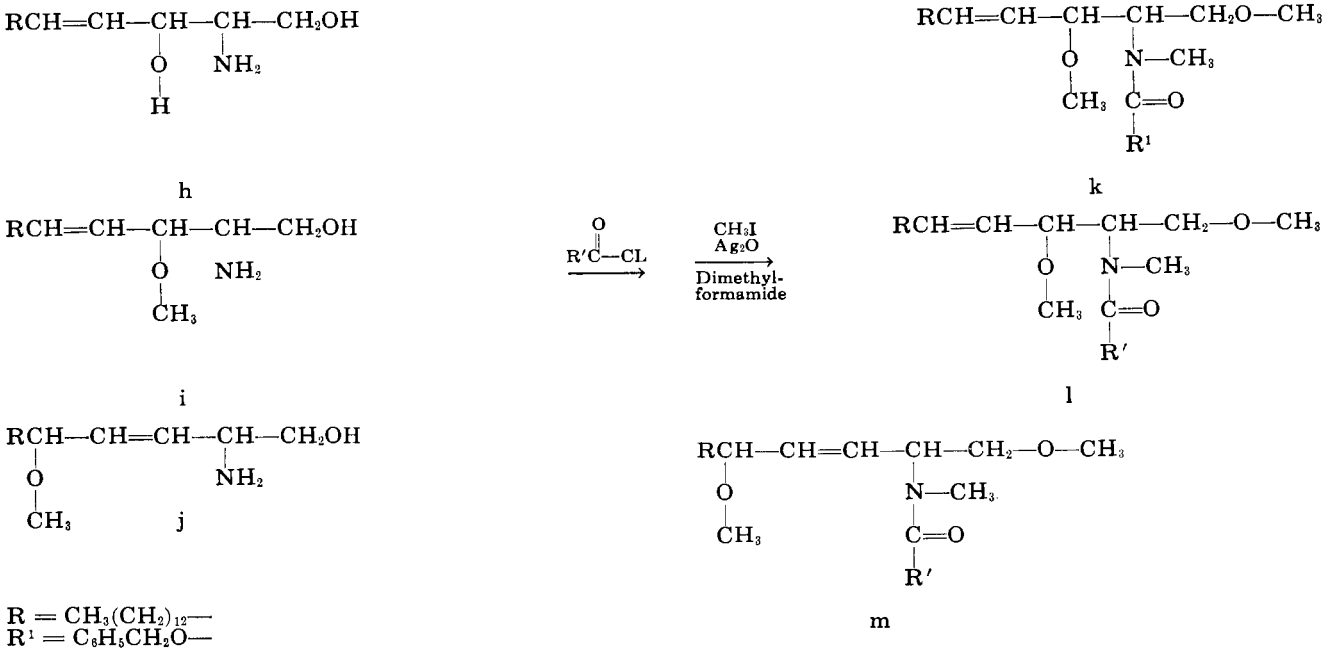


FIG. 2.—Postulated structure of trimer of tetradecanal.

The carbonium-ion intermediate (Fig. 1b) previously postulated by Carter *et al.* (1951) appears to be in equilibrium with the intermediate from the allylic rearrangement (Fig. 1c); each of these would produce its respective pair of diastereoisomers (Fig. 1d,e,f,g). The configuration at carbon atom 5 was established by oxidation with CrO_3 in glacial acetic acid of the aldehydes from the performylated *O*-methyl bases after periodate degradation. The sodium salts in 90% ethanol of the 2-methoxypentadecanoic acids from *O*-methyl derivatives I and II had specific rotations of $[\alpha]_D^{23}$ +20.7 and $[\alpha]_D^{23}$ −21.9, respectively. Since the specific rotation of the sodium salt of D-2-hydroxypentadecanoic acid is $[\alpha]_D$ +15 (*c*, 1% in 50% aqueous ethanol; Lemieux, 1953), the configurations at carbon atom 5 of 1-hydroxy-2-amino-5-methoxy-3-octadecenes I and II are D and L, respectively. The configurations at carbon atom 3 of 3-*O*-methylsphingosines I and II remain to be determined. Since the *O*-methyl bases I and II are mixtures which exhibit strong *trans* double-bond absorption, the presence of a *cis* double bond in any of the diastereoisomers, although unlikely, has not been excluded.

Permethylation in Dimethylformamide.—The *N*-carbobenzoxy derivatives of sphingosine, *O*-methylsphingosine I, mp 65–66°, or *O*-methylsphingosine II, 2.0 g, each prepared as previously described (Weiss, 1957), were dissolved in 25 ml of distilled dimethylformamide, followed by the addition of 4.5 ml of distilled CH_3I and



$R = CH_3(CH_2)_{12}$—
$R^1 = C_6H_5CH_2O$—

FIG. 3.—Sphingosine (h) and *O*-methyl ethers (i and j) were carried through the above reaction sequences and their products (k, l, m) were compared. The 3- and 5-*O*-methyl ethers (i and j) illustrate the reactions and do not necessarily represent the composition of the *O*-methylsphingosines studied. See text for details.

4.5 g of freshly prepared Ag_2O (Fig. 3). The reaction mixture was magnetically stirred in an amber-tinted bottle in the dark for 17 hours at room temperature. After centrifugation of the reaction mixture, the precipitate was washed three times with 30-ml portions of chloroform. The combined supernatant liquids, after addition of 250 ml of ether, were filtered and successively washed with 25 ml of 5% KCN, twice with 50-ml portions of water, with 25 ml of 5% $Na_2S_2O_3$, and, finally, with water. The organic layer was dried over Na_2SO_4 and concentrated. The residue was dried over P_2O_5 *in vacuo;* yield, 1.6 g. The products were faint yellow sirups.

1,3-Dimethoxy-2-carbobenzoxamido-(*N*-methyl)-4-octadecene (A); $[\alpha]_D^{23} = -31.5$ (*c*, 1.00% in $CHCl_3$).

Anal. Calcd. for $C_{29}H_{49}O_4N$ (475.6): C, 73.17; H, 10.38; OCH_3, 13.04; NCH_3, 6.10. Found: C, 72.33; H, 10.32; OCH_3, 13.21; NCH_3, 5.10.

Mixture (B) of 1,3-dimethoxy-2-carbobenzoxamido-(*N*-methyl)-4-octadecene and 1,5-dimethoxy-2-carbobenzoxamido-(*N*-methyl)-3-octadecene from *O*-methylsphingosine I; $[\alpha]_D^{23} = +15.9$ (*c*, 0.44% in $CHCl_3$).

Anal. Calcd. for $C_{29}H_{49}O_4N$ (475.6): C, 73.17; H, 10.38; OCH_3, 13.04; NCH_3, 6.10. Found: C, 72.87; H, 10.28; OCH_3, 13.22; NCH_3, 6.08.

Mixture (C) from *O*-methylsphingosine II; $[\alpha]_D^{23} = -16.9$ (*c*, 1.08% in $CHCl_3$).

Anal. Calcd. for $C_{29}H_{49}O_4N$ (475.6): C, 73.17; H, 10.38; OCH_3, 13.04. Found: C, 72.72; H, 10.23; OCH_3, 13.23.

The infrared-absorption bands for compounds A, B, and C in chloroform are: 3500 $cm^{-1}(w)$, 3000 $cm^{-1}(s)$, 1770 $cm^{-1}(w)$, 1720 $cm^{-1}(s)$, 1470 $cm^{-1}(s)$, 1420 $cm^{-1}(m)$, 1360 $cm^{-1}(m)$, 1240 $cm^{-1}(w)$, 1195 $cm^{-1}(w)$, triplet 1160–1105 $cm^{-1}(s)$, 980 $cm^{-1}(m)$, and 915 $cm^{-1}(w)$. The peaks of the triplet ether bands are: compound A, 1160 $cm^{-1}(m)$, 1125 $cm^{-1}(w)$, and 1105 $cm^{-1}(s)$; compound B, 1160 $cm^{-1}(s)$, 1125 $cm^{-1}(m)$, and 1110 $cm^{-1}(m)$; compound C, 1160 $cm^{-1}(s)$, 1125 $cm^{-1}(m)$, and 1110 $cm^{-1}(m)$.

Permethylation of Dihydro Compounds.—The *N*-carbobenzoxy derivative of dihydrosphingosine, *O*-methyldihydrosphingosine I, or *O*-methyldihydrosphingosine II, 2.0 g., were treated in the same manner as in the preparation of compound A; the dihydro compounds were prepared by hydrogenation of the free base (Fig. 3h,i,j) over platinum in ethanol. The products were faint yellow sirups; yield, 1.7 g. The same results were obtained by reduction of the corresponding unsaturated compounds A, B, or C, over platinum in ethanol.

1,3-Dimethoxy-2-carbobenzoxamido-(*N*-methyl)octadecane (D); $[\alpha]_D^{23} = -4.8$ (*c*, 1.05% in $CHCl_3$).

Anal. Calcd. for $C_{29}H_{51}O_4N$ (477.6); C, 72.86; H, 10.76; OCH_3, 12.98. Found: C, 73.48; H, 10.95; OCH_3, 12.99.

Mixture (E) of 1,3-dimethoxy-2-carbobenzoxamido-(*N*-methyl)octadecane and 1,5-dimethoxy-2-carbobenzoxamido-(*N*-methyl)octadecane from *O*-methyldihydrosphingosine I; $[\alpha]_D^{23} = +10.2$ (*c*, 0.84% in $CHCl_3$).

Anal. Calcd. for $C_{29}H_{51}O_4N$ (477.6); C, 72.86; H, 10.76; OCH_3, 12.98. Found: C, 72.80; H, 10.66; OCH_3, 12.78.

Mixture (F) from *O*-methyldihydrosphingosine II; $[\alpha]_D^{23} = +12.6$ (*c*, 1.00% in $CHCl_3$).

Anal. Calcd. for $C_{29}H_{51}O_4N$ (477.6): C, 72.86; H, 10.76; OCH_3, 12.98. Found: C, 73.16; H, 10.86; OCH_3, 12.91.

The infrared-absorption bands for compounds D, E, and F in chloroform are: 3500 $cm^{-1}(w)$, 3000 $cm^{-1}(s)$, 1770 $cm^{-1}(w)$, 1720 $cm^{-1}(s)$, 1470 $cm^{-1}(s)$, 1420 $cm^{-1}(m)$, 1360 $cm^{-1}(m)$, 1240 $cm^{-1}(w)$ and triplet 1160–1095 $cm^{-1}(s)$. The peaks of the triplet ether bands are: compound D, 1160 $cm^{-1}(m)$, 1120 $cm^{-1}(w)$, and 1100 $cm^{-1}(s)$; compound E, 1160 $cm^{-1}(w)$, 1130 $cm^{-1}(m)$, and 1095 $cm^{-1}(s)$; compound F, 1160 $cm^{-1}(w)$, 1130 $cm^{-1}(s)$, and 1095 $cm^{-1}(m)$.

Methylation in Benzene.—The *N*-carbobenzoxy derivatives of dihydrosphingosine or *O*-methyldihydrosphingosine II, 2.0 g, were methylated in the same manner as in the preparation of compound A, except that the solvent was benzene instead of dimethylformamide; a small amount of ether was added to the reaction mixture containing *N*-carbobenzoxydihydrosphingosine to effect solution. The products were white amorphous solids; yield, 1.4 g.

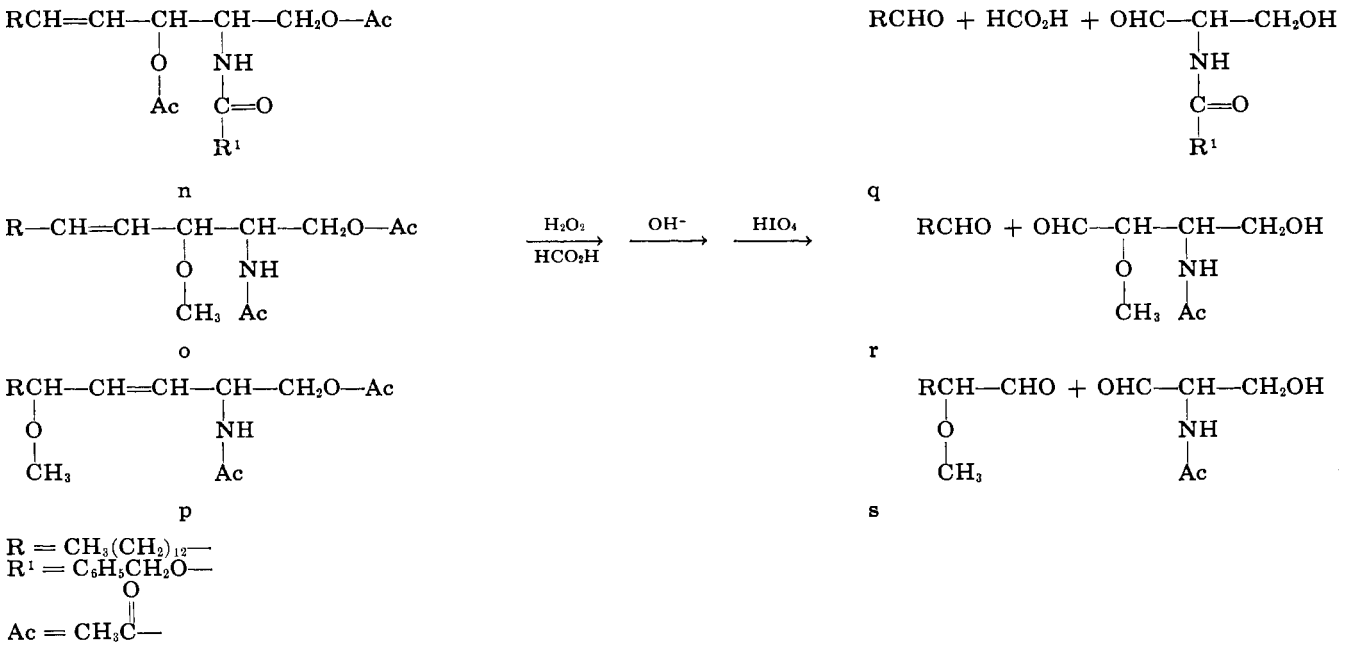


FIG. 4.—Performylation followed by mild alkaline hydrolysis and periodate oxidation disclosed that the *O*-methyl ethers are a mixture. See text for details.

1,3-Dimethoxy-2-carbobenzoxamidooctadecane (G); mp, 74–86°.

Anal. Calcd. for $C_{28}H_{49}O_4N$ (463.6): C, 72.48; H, 10.65; OCH_3, 13.38; NCH_3, 0. Found: C, 72.18; H, 10.58; OCH_3, 13.07; NCH_3, 0.

Mixture (H) of 1,3-dimethoxy-2-carbobenzoxamidooctadecane and 1,5-dimethoxy-2-carbobenzoxamidooctadecane from *O*-methyldihydrosphingosine II, mp, 32–42°.

Anal. Calcd. for $C_{28}H_{49}O_4N$ (463.6): C, 72.48; H, 10.65; OCH_3, 13.38; NCH_3, 0. Found: C, 72.67; H, 10.79; OCH_3, 13.20; NCH_3, 0.

The infrared-absorption bands for compound G in chloroform are: 3500 $cm^{-1}(m)$, 3000 $cm^{-1}(s)$, 1730 $cm^{-2}(s)$, 1512 $cm^{-1}(s)$, 1470 $cm^{-1}(m)$, 1410 $cm^{-1}(w)$, 1380 $cm^{-1}(w)$, 1315 $cm^{-1}(w)$, 1240 $cm^{-1}(s)$, 1110 $cm^{-1}(s)$, and 1030 $cm^{-1}(w)$.

The infrared-absorption bands for compound H in chloroform are: 3500 $cm^{-1}(m)$, 3000 $cm^{-1}(s)$, 1730 $cm^{-1}(s)$, 1512 $cm^{-1}(s)$, 1470 $cm^{-1}(m)$, 1410 $cm^{-1}(w)$, 1380 $cm^{-1}(w)$, 1315 $cm^{-1}(m)$, 1240 $cm^{-1}(s)$, 1110 $cm^{-1}(s)$, and 1030 $cm^{-1}(w)$.

Decarbobenzoxylation.—Compounds D, E, or F, 500 mg, were each hydrogenated over 100 mg of palladium in 25 ml of ethanol at room temperature for 12 hours with magnetic stirring. The reaction mixture was filtered and concentrated. The dried products were colorless sirups which gave negative ninhydrin reactions. When reduction was effected with 100 mg of platinum in 20 ml of glacial acetic acid, the reaction was made alkaline, and the mixture was treated with ether; the ether extract was washed, dried, and concentrated.

1,3-Dimethoxy-2-methylaminooctadecane (J) from compound D.

Anal. Calcd. for $C_{21}H_{45}O_2N$ (343.4): C, 73.39; H, 13.21; OCH_3, 18.06; NCH_3, 8.45. Found: C, 72.97; H, 13.14; OCH_3, 17.64; NCH_3, 7.09.

Mixture (K) of 1,3-dimethoxy-2-methylaminooctadecane and 1,5-dimethoxy-2-methylaminooctadecane from compound E.

Anal. Calcd. for $C_{21}H_{45}O_2N$ (343.4): C, 73.39; H, 13.21; OCH_3, 18.06; NCH_3, 8.45. Found: C, 73.04; H, 13.19; OCH_3, 17.83; NCH_3, 6.99.

Mixture (L) from compound F.

Anal. Calcd. for $C_{21}H_{45}O_2N$ (343.4): C, 73.39; H, 13.21; OCH_3, 18.06; NCH_3, 8.45. Found: C, 73.70; H, 13.00; OCH_3, 17.65; NCH_3, 7.45.

The infrared-absorption bands for compound J in chloroform are: 3500 $cm^{-1}(w)$, 3000 $cm^{-1}(s)$, 1770 $cm^{-1}(m)$, 1700 $cm^{-1}(w)$, 1470 $cm^{-1}(s)$, 1410 $cm^{-1}(w)$, 1370 $cm^{-1}(w)$, and 1110 $cm^{-1}(s)$.

The infrared-absorption bands for compounds K and L in chloroform are: 3500 $cm^{-1}(w)$, 3000 $cm^{-1}(s)$, 1770 $cm^{-1}(m)$, 1700 $cm^{-1}(w)$, 1470 $cm^{-1}(s)$, 1370 $cm^{-1}(m)$, and 1095 $cm^{-1}(s)$.

Performylation.—1,3-*O*-Diacetyl-*N*-carbobenzoxysphingosine, mp, 77–79°, or *O*,*N*-diacetyl-*O*-methylsphingosines I, mp, 67–68°, or II, mp, 73–74°, 2.0 g. in 40 ml of distilled formic acid and 1.5 ml of 30% H_2O_2 were each incubated at 45° (Fig. 4). The addition of 1.5-ml portions of H_2O_2 at 2-hour intervals was repeated two additional times. After standing overnight at 45°, the reaction mixture was lyophilized. The residue was dissolved in 45 ml of methanol and 5 ml of 10 N KOH. After the solution had remained 10 hours at room temperature, 150 ml of water was added, and the reaction mixture was treated with several 100-ml portions of ether. The washed ether layer was concentrated and the dried residue was dissolved in 25 ml of pyridine followed by the addition of 5 ml of acetic anhydride. After 1 hour the reaction mixture was poured into 200 ml of ice water and the product was removed with ether. The products from the washed and dried ether solutions after concentration were faint yellow sirups; yield, 1.5 g.

1,3,4,5-Tetraacetoxy-2-carbobenzoxamidooctadecane (M).

Anal. Calcd. for $C_{34}H_{53}O_{10}N$ (635.4): C, 64.21; H, 8.41; N, 2.20. Found: C, 64.65; H, 8.40; N, 2.12.

Mixture (N) of 1,4,5-triacetoxy-2-acetamido-3-methoxyoctadecane, and 1,3,4-triacetoxy-2-acetamido-5-methoxyoctadecane from *O*-methylsphingosine I.

Anal. Calcd. for $C_{27}H_{49}O_8N$ (515.4): C, 62.86; H, 9.58; OCH_3, 6.02. Found: C, 62.48; H, 9.51; OCH_3, 5.94.

Mixture (O) from *O*-methylsphingosine II.

Anal. Calcd. for $C_{27}H_{49}O_8N$ (515.4): C, 62.86; H, 9.58; OCH_3, 6.02. Found: C, 62.51; H, 9.40; OCH_3 6.10.

The infrared-absorption bands for compound M in chloroform are: 3500 $cm^{-1}(m)$, 3000 $cm^{-1}(s)$, 1770 $cm^{-1}(s)$, 1510 $cm^{-1}(w)$, 1470 $cm^{-1}(w)$, 1375 $cm^{-1}(m)$, 1245 $cm^{-1}(s)$, and 1050 $cm^{-1}(s)$.

The infrared-absorption bands for compounds N and O in chloroform are: 3500 $cm^{-1}(m)$, 3000 $cm^{-1}(s)$, 1770 $cm^{-1}(s)$, 1690 $cm^{-1}(s)$, 1525 $cm^{-1}(m)$, 1470 $cm^{-1}(m)$, 1375 $cm^{-1}(s)$, 1250 $cm^{-1}(s)$, and doublet 1100–1055 cm^{-1} (m).

Periodic Acid Oxidation.—Compounds M, N, or O, 2.0 g, were each hydrolyzed at room temperature for 8 hours in 45 ml of methanol and 5 ml of 10 N KOH (Fig. 4). The *N*-acylated products were isolated in the same manner as described in the performylation procedure. Appropriate amounts of the dried bases were dissolved in 22 ml of methanol followed by the addition of 3 mmoles of periodic acid in 3 ml of 50% aqueous methanol; compound O was diluted to 97 ml before addition of periodate because of precipitate formation in the smaller volume. Portions of the reaction mixture were removed after 2 hours and titrated as previously described (Weiss, 1957). Compound M base, 0.88 mmole, 1.50 mmoles of compound N base, and 1.79 mmoles of compound O base required 1.79 mmoles, 2.15 mmoles, and 2.18 mmoles of periodate, respectively; the periodate base ratios, therefore, are 2.04, 1.44, and 1.21, respectively.

The long-chain aldehydes were removed from the reaction mixture with several portions of *n*-heptane as previously described (Weiss, 1963). Portions of the dried aldehyde were taken for preparation of the 2,4-dinitrophenylhydrazone, determination of specific rotation and infrared spectrum, vapor-phase chromatography, and oxidation to the acid with CrO_3.

2,4-Dinitrophenylhydrazone of tetradecanal from compound M; mp, 103–104°; the 2,4-dinitrophenylhydrazone of a known sample of tetradecanal melted at 103–104°.

Anal. Calcd. for $C_{20}H_{32}O_4N_4$ (392.3): C, 61.18; H, 8.22; OCH_3, 0. Found: C, 61.55; H, 8.43; OCH_3, 0.

2,4-Dinitrophenylhydrazone of 2-methoxypentadecanal from compound N; mp, 68–72°.

Anal. Calcd. for $C_{22}H_{36}O_5N_4$ (436.3): C, 60.51; H, 8.32; OCH_3, 7.11. Found: C, 61.54; H, 8.28; OCH_3, 5.29.

2,4-Dinitrophenylhydrazone of 2-methoxypentadecanal from compound O; mp, 65–69°.

Anal. Calcd. for $C_{22}H_{36}O_5N_4$ (436.3): C, 60.51; H, 8.32; OCH_3, 7.11. Found: C, 60.84; H, 8.60; OCH_3, 5.33.

The specific rotations of the aldehydes from compounds N and O are $[\alpha]_D^{23}$ +34.0 (*c*, 1.11% in $CHCl_3$) and $[\alpha]_D^{23}$ −36.3 (*c*, 0.96% in $CHCl_3$), respectively. These values have been corrected for the presence of tetradecanal whose concentration was determined by vapor-phase chromatography (*vide infra*). It is assumed that no effect is exerted by tetradecanal on the optical rotation.

The infrared-absorption bands for tetradecanal from compound M in chloroform are: 3500 $cm^{-1}(w)$, 3000 $cm^{-1}(s)$, 1775 $cm^{-1}(m)$, 1480 $cm^{-1}(m)$, 1390 $cm^{-1}(w)$, 1225 $cm^{-1}(m)$, 1125 $cm^{-1}(m)$, and doublet 1075–1055 $cm^{-1}(m)$.

The infrared-absorption bands for 2-methoxypentadecanal from compounds N and O in chloroform are: 3500 $cm^{-1}(w)$, 3000 $cm^{-1}(s)$, 1775 $cm^{-1}(m)$, 1480 cm^{-1} (m), 1390 $cm^{-1}(w)$, 1225 $cm^{-1}(m)$, and 1110 cm^{-1} (m).

Vapor-phase chromatography of a sample derived from compound M revealed only one peak, retention time 6.0 minutes, on diethylene glycol adipate polyester (15% on Chromosorb W, 60/80 mesh); the column temperature was 190° and the argon flow rate was 65 ml/min. This agreed with a known sample of tetradecanal. Samples from compounds N and O showed two peaks, the first corresponding to tetradecanal and the second, presumably, to 2-methoxypentadecanal, retention time 12.8 minutes. The concentration of 2-methoxypentadecanal, determined from the ratio of its area over the sum of the areas of 2-methoxypentadecanal and tetradecanal, is 68 and 69% in compounds N and O, respectively. The concentration of the same aldehyde, calculated from the methoxyl content of the 2,4-dinitrophenylhydrazones, is 74 and 75% in compounds N and O, respectively.

Approximately 200 mg of the 2-methoxyaldehydes from compounds N and O were oxidized with 75 mg of CrO_3 in 10 ml of glacial acetic acid at 45–50° for 15 minutes as previously described (Weiss, 1964). To the isolated fatty acids, containing traces of chromium salts, was added 6 ml of methanol and 0.1 N NH_4OH to *p*H 7.0. The solution was centrifuged and the turbid upper layer, removed from the green lower layer, was diluted with an equal volume of water. After acidification the solution was treated with several small portions of chloroform. Concentration of the washed chloroform extracts yielded 151 and 116 mg of fatty acids representing compounds N and O, respectively. Portions of the product were taken for vapor-phase chromatography and determination of specific rotations.

Vapor-phase chromatography on the same column used for the aldehydes of the acids derived from compounds N and O, after esterification with diazomethane (Tenny *et al.*, 1963), disclosed two peaks in each case; the first corresponded to methyl tetradecanoate, retention time 7.0 minutes, and the second corresponded presumably, to methyl 2-methoxypentadecanoate, retention time 20 minutes. The concentration of methyl tetradecanoate in each sample was 34%. Tetradecanal from compound M, after oxidation and esterification, gave a single peak with retention time of 7.0 minutes.

The specific rotations of the sodium salts of the 2-methoxypentanoic acids derived from compounds N and O are $[\alpha]_D^{23}$ +20.7 (*c*, 0.81% in 90% aqueous ethanol) and $[\alpha]_D^{23}$ −21.9 (*c*, 0.66% in 90% aqueous ethanol), respectively. The sodium salt was obtained by neutralization of the acid with the required amount of 0.36 N NaOH in the necessary volume of aqueous ethanol. Turbidity developed when the ethanol concentration was reduced to 50%. Fractionation of the fatty acids from various concentrations of aqueous ethanol produced only a slight enrichment in the methoxy acid content. The specific rotation values were corrected for the presence of tetradecanoic acid; it is assumed that no effect is exerted by its presence on the optical rotation.

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The Paraffin Hydrocarbons of Wool Wax. Normal, Iso, Anteiso, and Other Branched Isomers

JAMES D. MOLD, RICHARD E. MEANS, ROBERT K. STEVENS, AND JOHN M. RUTH

From the Research Department, Liggett and Myers Tobacco Company, Durham, N. C.

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The relative abundance of the homologs of the series of normal, iso (2-methyl), and anteiso (3-methyl) paraffin hydrocarbons has been determined for a sample of wool wax. The occurrence of pristane (2,6,10,14-tetramethylpentadecane) has been established for the first time in a wax not of marine origin. The presence of homologous cycloparaffins and at least two other homologous series of branched paraffins has been noted in wool wax.

Our discovery of the natural occurrence of the anteiso (3-methyl) series of paraffin hydrocarbons in a plant material, tobacco leaf wax (Mold *et al.*, 1963a) prompted an interest in a report of the presence of this class of compounds in wool wax (Downing *et al.*, 1960). A preliminary study of the hydrocarbon fraction of wool wax, utilizing the techniques which had been applied to tobacco leaf wax, indicated to us that the branched paraffins of wool wax did not consist solely of homologous series of iso and anteiso compounds but included other series of branched homologs. Furthermore, since we had observed that the iso paraffins of tobacco wax were predominantly homologs of odd numbers of carbon atoms and the anteiso paraffins were predominantly of even numbers of carbon atoms, it was of interest to establish whether a similar relationship existed for an animal wax. Downing *et al.* (1960) had reported that the reverse order of predominant homologs was true for wool wax, albeit on rather meager evidence.

Results

The paraffin hydrocarbons of wool wax, representing about 0.5% of the total wax, were separated into several groups of compounds by means of urea-adduct formation and treatment with a molecular sieve (5A, Linde). The normal compounds, which were complexed by the molecular sieve, were further separated by gas-liquid chromatography (Fig. 1) into the individual homologs. These compounds satisfied the requirement for a homologous series in that the logarithms of their retention times from isothermal chromatography gave a straight-line relationship when plotted against the number of carbon atoms. The series of normal hydrocarbons represented 16–20% of the total wool-wax paraffins. Small amounts of isomeric paraffins were carried along with the normal homologs into the sieve. This was corrected for in estimating the total amounts present. The relative amounts of the normal paraffins are given in Table I.

An interesting quantitative distribution of the normal homologs was observed. These compounds were grouped in two families. One of these included homologs in the range of about 13–25 carbon atoms. Each carbon number was represented in increasing amount up to the 20-carbon compound. The amounts then decreased regularly for the higher members. The second included homologs in the range of about 23–35 carbon atoms. These consisted predominantly of homologs of odd numbers of carbon atoms with the 29-carbon compound present in the largest amount.

TABLE I
AMOUNTS OF HOMOLOGOUS NORMAL PARAFFINS IN WOOL WAX[a]

Paraffin Carbon Number	Per Cent of Total Paraffins	Paraffin Carbon Number	Per Cent of Total Paraffins
12	Trace	24	0.34
13	0.08	25	0.50
14	0.11	26	0.17
15	0.14	27	1.04
16	0.29	28	0.43
17	0.65	29	2.45
18	1.05	30	Trace
19	1.55	31	1.55
20	1.55	32	Trace
21	1.18	33	0.88
22	0.76	34	Trace
23	0.71	35	Trace

[a] These values were calculated from the areas under the gas-liquid chromatograph. A correction was applied for low recovery from the molecular sieve but no correction was made for other losses in the isolation.

The branched paraffins, which formed urea adducts but were not removed from an isooctane solution by the molecular sieve, gave a gas-liquid chromatogram (Fig. 2) which indicated the presence of at least three homologous series with carbon contents ranging from 17 to 43 atoms per molecule. This group of compounds was present to the extent of about one-half that of the normal paraffins. The relative amounts of these components are given in Figure 3.

Individual homologs of these branched hydrocarbons were collected from gas-liquid chromatograms and identified by mass spectrometry. Examination of the infrared spectra for the total sample prior to gas-liquid chromatography and for several of the individual fractions collected from the chromatography indicated the absence of any olefinic impurities. The homologs of series III in the range from 17 to about 35 carbon atoms consisted largely of mixtures of 2-methyl and 3-methyl isomers. The relative intensities of the ions