by addition of wet tetrahydrofuran, and the precipitate separated by filtration through Super-cel. The filtrate was dried and evaporated to leave a gum which crystallized on trituration with acetone. Recrystallization from acetone yielded 2.8 g. (75.5%) of white prisms, m.p. 123–124°.

Anal. Calcd. for $C_8H_{15}NO_2$: C, 61.11; H, 9.61; N, 8.91. Found: C, 61.10; H, 9.76; N, 8.76.

The hydrochloride was prepared in ethanol and crystallized from ethanol–ether; m.p. 111–112.5°.

Anal. Calcd. for $C_8H_{15}NO_2 \cdot HCl$: C, 49.60; H, 8.33. Found: C, 49.63; H, 8.62.

The picrate prepared in ethanol and crystallized from ethanol–ether gave shining yellow prisms, m.p. 166–167°.

Anal. Calcd. for $C_8H_{15}NO_2 \cdot C_6H_3N_3O_7$: C, 43.52; H, 4.69; N, 14.50. Found: C, 43.50; H, 5.19; N, 13.98.

1-Chloromethyl-2-chloropyrrolizidine.—A mixture of 1.3 g. of 1-hydroxymethyl-2-hydroxypyrrolizidine in 5 ml. of chloroform and 3 ml. of thionyl chloride was heated under reflux for 4 hr. at the end of which time most of the solvent and excess of thionyl chloride were removed under diminished pressure, the residue taken up in chloroform, washed with 10% aqueous sodium hydroxide, the organic layer dried and the solvent evaporated. The resulting oil was converted into its picrate in ethanol, and recrystallized from ethanol; m.p. 168–169°.

Anal. Calcd. for $C_8H_{13}Cl_2N \cdot C_6H_3N_3O_7$: C, 39.72; H, 3.81; N, 13.24. Found: C, 39.62; H, 3.77; N, 13.56.

1-Hydroxymethyl-2-hydroxypyrrolizidine failed to react with thionyl chloride at 0°. At the end of 30 min. the starting material was recovered as the hydrochloride.

[CONTRIBUTION FROM THE DANIEL SIEFF RESEARCH INSTITUTE, THE WEIZMANN INSTITUTE OF SCIENCE, REHOVOTH, ISRAEL]

Synthetic Studies on Sphingolipids. VI. The Total Syntheses of Cerasine and Phrenosine

BY DAVID SHAPIRO AND H. M. FLOWERS

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The galactocerebrosides cerasine (VIIIa) and phrenosine (VIIIb) and the Gaucher spleen glucocerebroside have been synthesized and found identical with the natural products. The N-acyl-3-O-benzoylsphingosine bases (VI), were condensed with the corresponding aceto-bromo sugars in the presence of mercuric cyanide, and the resulting acylated glycosides (VII) were saponified to the glycosides VIII. The β-configuration has been assigned to the natural cerebrosides.

The cerebrosides, first isolated from brain tissue in 1874,[1] were found to be composed of sphingosine, D-galactose and a long-chain fatty acid. The amide linkage of the acidic component and the glycosidic nature involving one of the two hydroxylic groups have long been recognized by early investigators.[2–5] However, it was not until 1952 that structure II was established for the two cerebrosides by Carter and co-workers,[6] who showed that the galactosyl group is attached to C-1 of the sphingosine molecule. The structure of sphingosine as *trans*-D-*erythro*-1,3-dihydroxy-2-amino-4-octadecene (I) was proven both by degradation[7–8] and by synthesis.[9]

The term cerasine has been applied to the cerebroside IIa in which the acidic component is lignoceric acid, while phrenosine (IIb) contains cerebronic acid. Although these lipids certainly exist in nature as individual compounds, the fatty acids could not be obtained in a pure form by hydrolysis. Lignoceric (*n*-tetracosanoic) acid was found to contain small amounts of homologous acids.[10] The identity and purity of natural cerebronic acid has been the subject of considerable discussion. Klenk's assumption[11–12] that it is pure α-hydroxy-*n*-tetracosanoic acid was not substantiated by later investigators.[13–15] Indeed, Chibnall, *et al.*,[10] were able to show that the purified acid contained up to 15% of the C-26 homolog. Various melting-points and specific rotations have been reported for cerebronic acid. The optically active α-hydroxytetracosanoic acid synthesized in the present investigation closely corresponded to the physical data which characterize the purest cerebronic acid obtained from natural sources.[10]

In a preliminary communication[16] we reported a synthesis of dihydrocerebrosides which involved the Koenigs–Knorr reaction[17] of a DL-ceramide[18] of type VI with 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide in the presence of silver carbonate. The reaction proceeded sluggishly over a period of 48 hr., even with a large excess of the bromo-sugar. The products showed the expected infrared absorption spectra and gave good analytical values, including the percentage of galactose. The relatively low melting points of 125–130° were attributed to the presence of a conglomerate of D-galactosides instead of a racemic compound. Surprisingly, we later found that this reaction, when applied to the benzoyl-ceramides of D-sphingosine and D-dihydrosphingosine (VIa and

(1) J. L. W. Thudichum, "Researches on the Chemical Configuration of the Brain," Report of the Medical Officer of the Privy Council and Local Government Board 3, No. 5, 113 (1874).

(2) J. L. W. Thudichum, "Die Chemische Konstitution des Gehirns des Menchen und der Tiere," Pietzcker, Tuebingen, 1901.

(3) E. Klenk, *Z. physiol. Chem.*, **153**, 74 (1926).

(4) E. Klenk and R. Haerle, *ibid.*, **178**, 221 (1928).

(5) I. Pryde and R. W. Humphreys, *Biochem. J.*, **18**, 661 (1924).

(6) H. E. Carter and F. L. Greenwood, *J. Biol. Chem.*, **199**, 283 (1952).

(7) H. E. Carter, F. J. Glick, W. P. Norris and G. E. Phillips, *ibid.*, **170**, 285 (1947).

(8) H. E. Carter, F. J. Glick, W. P. Norris and G. E. Phillips, *ibid.*, **142**, 449 (1942).

(9) (a) D. Shapiro and H. Segal, *J. Am. Chem. Soc.*, **76**, 5894 (1954); (b) D. Shapiro, H. Segal and H. M. Flowers, *ibid.*, **80**, 1194 (1958); (c) C. A. Grob and F. Gadient, *Helv. Chim. Acta*, **40**, 1145 (1957).

(10) A. Chibnall, S. H. Piper and E. F. Williams, *Biochem J.*, **30**, 100 (1936).

(11) E. Klenk, *Z. physiol. Chem.*, **174**, 241 (1928).

(12) E. Klenk and W. Diebold, *ibid.*, **215**, 79 (1933).

(13) F. A. Taylor and P. A. Levene, *J. Biol. Chem.*, **84**, 23 (1929).

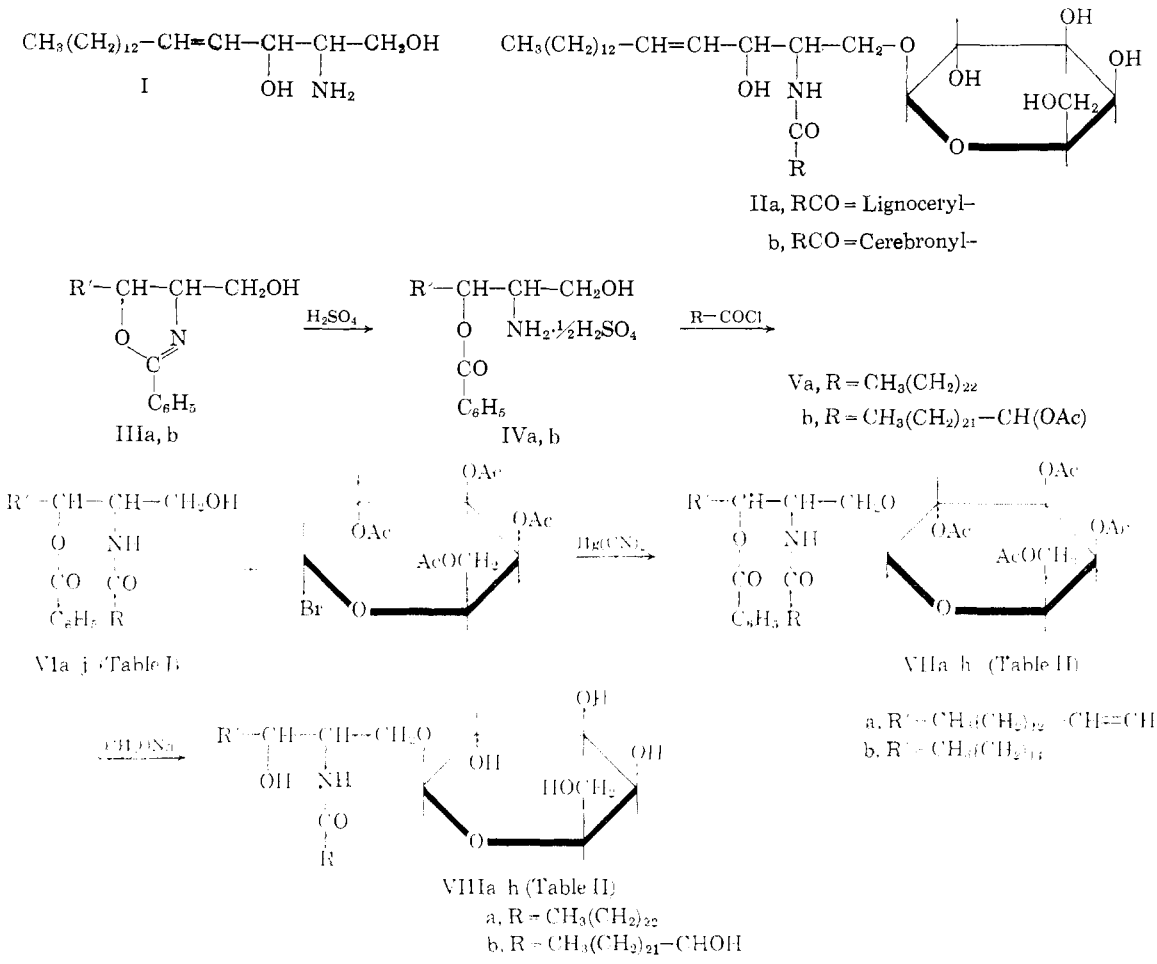
(14) D. M. Crowfoot, *J. Chem. Soc.*, 716 (1936).

(15) J. C. Smith, *ibid.*, 625 (1936).

(16) D. Shapiro and H. M. Flowers, *J. Am. Chem. Soc.*, **81**, 2023 (1959).

(17) W. Koenigs and E. Knorr, *Ber.*, **34**, 957 (1901).

(18) The fatty acid amides resulting from partial hydrolysis of the sphingolipids are referred to as ceramides.



VIh), respectively, yielded cerebrosides which deviated from the natural products by exhibiting considerably higher positive rotations. Furthermore, the L-ceranide (VIe) gave rise to a compound with a similar optical rotation. The latter result seemed to indicate that the difference may lie in the glycosidic linkage or in a rearrangement of the sugar moiety caused by the prolonged reaction time. The identity of these glycosides is being investigated further, bearing in mind the possibility that they are anomers of the natural lipids.

Many investigators have elucidated the factors which govern the Koenigs–Knorr reaction. It is now known that its course may be affected by the polarity of the solvent and, particularly, by the type and amount of the catalyst. Ineffective buffering by the latter may even result in the anomerization of a glycoside initially formed under certain conditions.[19] A number of variations and improvements of the original method have been described in the literature.[20–24] Recently, Helferich and Weis[25] achieved excellent results by employing mercuric cyanide as condensing agent and nitromethane as solvent. These authors obtained various glycosides in yields up to 90%. When this method was applied in the present investigation, the reaction proceeded smoothly in a relatively short time and gave rise to the glycosides (VIII) which were identical in every respect with the natural lipids, as will be seen below.

In recent publications,[26] we described the preparation of the substituted oxazolines IIIa and IIIb and pointed out their suitability as key intermediates in the development of unequivocal syntheses in the sphingomyelin series. However, attempts to prepare the glycosides of these compounds were wholly unsuccessful. When IIIb was allowed to react with the acetylated bromo-sugar in the presence of silver carbonate, 40–50% of the former was recovered unchanged in addition to an oily mixture of indefinite composition. On treatment of the oil with alkali to effect deacetylation, a further quantity of IIIb was isolated, the total recovery amounting to 80–90%. Since in many other experiments IIIb was recovered nearly quantitatively, it is reasonable to assume that glycosidation had, in part, taken place, but that the glycosidic linkage was ruptured during deacetyla-

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(20) (a) G. Zemplén, *Ber.*, **62**, 990 (1929); (b) G. Zemplén and Z. S. Nagy, *ibid.*, **63**, 368 (1930).

(21) B. Helferich and K. F. Wedemeyer, *Ann.*, **563**, 139 (1949).

(22) (a) B. Helferich and K. F. Wedemeyer, *Ber.*, **83**, 538 (1950); (b) K. F. Wedemeyer and W. Hans, *ibid.*, **83**, 541 (1950).

(23) C. McClosky, R. Pule and G. H. Coleman, *J. Am. Chem. Soc.*, **66**, 349 (1949).

(24) G. Zemplén, Z. Bruckner and A. Gerecs, *Ber.*, **64**, 744 (1931).

(25) B. Helferich and K. Weis, *ibid.*, **89**, 314 (1956).

(26) (a) D. Shapiro, H. M. Flowers and S. Spector-Shefer, *J. Am. Chem. Soc.*, **81**, 3743 (1959); **81**, 4360 (1959).

TABLE I

3-O-BENZOYL-N-ACYL DERIVATIVES OF SPHINGOSINE AND DIHYDROSPHINGOSINE BASES (VI)

VI	Base, 3-O-benzoyl	N-Acyl	M.p., °C.	$[\alpha]^{28}D$ (chlf.)	Formula	Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N	Yield, %
a	D-Sphingosine	Tetracosanoyl	86–88	+17.7°	$C_{49}H_{87}NO_4$	78.03	11.63	1.86	78.02	11.67	1.95	94
b	D-Sphingosine	D-2-Acetoxy-tetracosanoyl	56–58	+19.5°	$C_{51}H_{89}NO_6$	75.41	11.04	1.72	75.10	10.96	1.83	37
c	DL-Sphingosine	Tetracosanoyl	81–82		$C_{49}H_{87}NO_4$	78.03	11.63	1.86	77.98	11.51	2.08	83
d	DL-Sphingosine	DL-2-Acetoxy-tetracosanoyl	76–78		$C_{51}H_{89}NO_6$	75.41	11.04	1.72	75.25	11.01	1.55	56
e	L-Sphingosine	Tetracosanoyl	84–86	−10.7°	$C_{49}H_{87}NO_4$	78.03	11.63	1.86	77.81	11.86	1.85	87
f	DL-Dihydro-sphingosine	Tetracosanoyl	78–79		$C_{49}H_{89}NO_4$	77.81	11.86	1.85	77.95	11.84	2.01	80
g	DL-Dihydro-sphingosine	DL-2-Acetoxy-tetracosanoyl	57–59		$C_{51}H_{91}NO_6$	75.21	11.15	1.72	75.53	11.31	1.59	48
h	D-dihydro-sphingosine	Tetracosanoyl	80–81	+20.0°	$C_{49}H_{89}NO_4$	77.81	11.86	1.85	77.49	11.58	2.03	75
i	D-Sphingosine	Behenyl	88–90	+18.5°	$C_{47}H_{93}NO_4$	77.74	11.52	1.93	77.50	11.43	2.10	93
j	DL-Sphingosine	Behenyl	81–83		$C_{47}H_{93}NO_4$	77.74	11.52	1.93	77.81	11.58	2.27	92

tion under the influence of alkali. Alkali sensitivity of glycosides is generally considered as a function of the aglycon. It may withdraw electrons from the glycosidic bond[27] as a result of activation by substituents in the β-position. The grouping —N=C—Ph in III may be expected to exercise such an effect.

Substituted oxazolines are known to suffer ring scission at the position 2–3 under the influence of acids, with retention of configuration.[28] Thus, the *cis*-oxazolines IIIa,b, on treatment with dilute sulfuric acid, gave rise to the formation of *erythro*-3-O-benzoyl-sphingosine and -dihydrosphingosine (IVa,b), respectively. For acylation, the racemic sulfates were not isolated but were treated directly with the fatty acid chlorides in the presence of sodium acetate and afforded in most cases excellent yields of the benzoyl-ceramides VI (Table I). Attempts to bring about the optical resolution of III were not successful. It either gave unstable salts or it suffered ring rupture, as was the case when bromocamphorsulfonic acid was tried.

It was found that both IVa and IVb formed nicely crystalline salts with tartaric acid, and the resolution could be effected easily by alternate treatment of the pure sulfates with barium L- and D-tartrates. Samples of the separated optically active salts were converted either into the triacetyl- or the tribenzoyl-derivatives, in order to identify the antipode deriving from natural sphingosine. For further characterization or comparison with the natural products, some of the compounds VI were saponified by mild alkali to the ceramides, which are well defined fragments formed in the hydrolytic degradation of sphingolipids.

Condensation of VI with 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide by Helferich's method led to the acylated galactosides VII which were obtained in fairly good yield. Most of these compounds gave somewhat high carbon values, owing to the difficulty of removing completely the unchanged benzoyl-ceramides by crystallization. However, fully acetylated derivatives, when prepared from the pure glycosides VIII, gave excellent elemental analyses. Outstanding examples are the pentaacetate of VIIIa (pentaacetyl-cerasine) and the hexaacetate of VIIIb (hexaacetylphrenosine). The acylated glycosides VII were saponified by means of catalytic amounts of sodium methoxide dissolved in anhydrous methanol, following the procedure of Zemplén.[29]

The physical properties of VIIIa and VIIIb are in good agreement with those of natural cerasine and phrenosine, respectively, and are listed in Table II. The close similarity of the infrared spectrum of VIIIb to that of the natural product is shown in Figs. 1 and 2. It is noteworthy that the typical change in specific rotation from a negative value

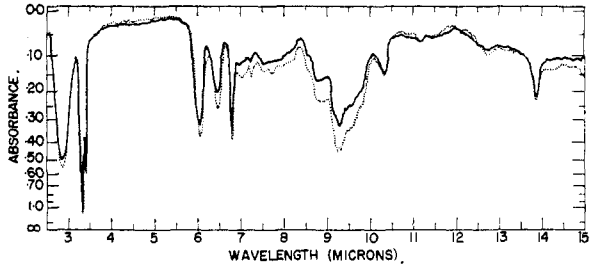

Fig. 1.—Infrared spectra of synthetic phrenosine (VIIIb) (solid line) and natural phrenosine (dotted line), pressed in KBr.

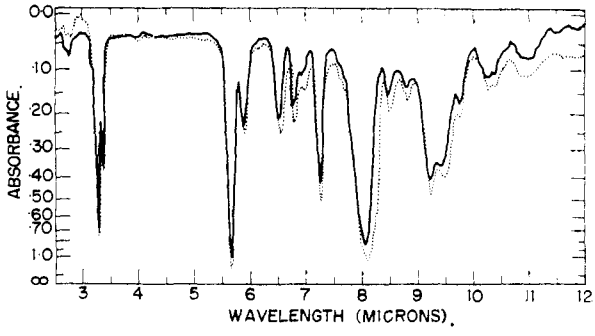

Fig. 2.—Infrared spectra of synthetic hexaacetylphrenosine (solid line) and natural hexaacetylphrenosine (dotted line), pressed in KBr.

(27) C. E. Ballou, *Advances in Carbohydrate Chem.*, **9**, 63 (1954).

(28) J. Sicher and M. Pankowa, *Coll. Czech. Chem. Communs.*, **20**, 1409 (1955).

(29) G. Zemplén, *J. Am. Chem. Soc.*, **52**, 2568 (1930).

TABLE II

THE KOENIGS–KNORR REACTION OF 3-O-BENZOYL-CERAMIDES (VI) WITH 2,3,4,6-TETRA-O-ACETYL-α-D-GALACTOPYRANOSYL BROMIDE: VI → VII → VIII

	VII		VIII			Calcd.			Found		
VI	M.p., °C.	$[\alpha]^{26}D$ (chlf.)	M.p., °C.	$[\alpha]^{26}D$ (pyridine)	Formula	C	H	N	C	H	N
a	48–50	+8.2°	182	−3.4°	$C_{48}H_{93}NO_8$[a]	70.98	11.55	1.72	71.13	11.80	1.51
b	41–44		195	+4.4°	$C_{48}H_{95}NO_7$[b]	69.65	11.24	1.69	69.86	11.50	1.87
c	42–44	−5.2°	178–180	−0.5°	$C_{48}H_{93}NO_8$	70.98	11.55	1.72	71.38	11.50	1.69
d	42–44		178–179	+2.0°	$C_{48}H_{93}NO_9$	69.65	11.24	1.69	69.55	11.33	1.67
e	Not isolated		182–183	+2.0°	$C_{48}H_{93}NO_8$	70.98	11.55	1.72	70.44	11.62	1.57
f	46–48	0°	183–185	+4.9°	$C_{48}H_{95}NO_8$	70.80	11.80	1.72	70.91	12.24	1.72
g	37–39		182	+2.8°	$C_{48}H_{95}NO_9$	69.46	11.52	1.69	69.55	11.50	1.91
h	44–47	+1.6°	180	+5.5°	$C_{48}H_{95}NO_8$	70.80	11.80	1.72	70.84	12.10	1.59

[a] % galactose (anthrone): calcd. 22.2; found, 21.8. [b] % galactose (anthrone): calcd. 21.7; found, 21.3.

for natural cerasine (−3.71°)[30] to a positive value for dihydrocerasine (+5.31°)[31] was demonstrated by catalytic hydrogenation of VIIIa to VIIIh. The considerable increase in levorotation exhibited by the natural cerebrosides on conversion into their fully acetylated derivatives also has been observed with the synthetic products.

The galactosides listed in Table II include dihydro-derivatives and differ either by the optical configuration of the sphingosine moiety or by the fatty acid residue. In addition, we synthesized for further comparison a natural cerebroside[32] (and its racemic form), in which the carbohydrate component is glucose, namely, N-docosanoyl-1-D-sphingosyl-β-D-glucopyranoside. The "abnormal" lipid is deposited in the spleen in Gaucher's disease. Its structure as behenyl-β-glucocerebroside has been confirmed recently by Rosenberg and Chargaff,[33] who were able to prepare a purified crystalline material. It was found that the physical properties of the synthetic product were virtually identical with those of the natural lipid.

The Anomeric Configuration.—As to the nature of the glycosidic linkage in the cerebrosides, it would appear that the β-configuration derives from the method of synthesis. The glycosides obtained by Helferich[25] with mercuric cyanide as catalyst were shown to be β-anomers, irrespective of the nature of the aglycon or the sugar portion. In the present investigation, no trace of another anomer could be detected in the mother liquors of the crude reaction products which were obtained in high yields and differed in specific rotation only slightly from the purified cerebrosides. This finding is of significance in view of the low optical rotations, which are typical of β-galactosides. Thus, methyl β-galactoside has a rotation of +2.6°, while the value for the α-form is +178.8°. Helferich[34] has also shown that phychosine[35] is split by almond emulsin and attributed the fact that phrenosine is not attacked by this enzyme to its insolubility in aqueous medium. According to a more recent report,[36] the cerebrosides can be split by β-galactosidase.

The complete identity of the synthetic glucocerebroside with the Gaucher spleen lipid may be considered as strong support for the specificity of the synthesis in yielding β-anomers. The β-configuration of the natural lipid was recognized earlier,[37] since it could be split by almond emulsin which is known to be free of α-glycosidase. Additional evidence was obtained by Rosenberg and Chargaff[33] from the spectrum which showed a characteristic maximum at 11.22 μ. Kuhn[38] investigated a large number of sugars and their derivatives and showed that the anomeric forms readily may be distinguished by their infrared spectra. Tipson and Isbell[39] reported that the β-forms of both galactosides and glycosides are characterized by a band at 11.22 μ which is absent in the α-anomers and that a band at 11.44 μ is typical of the galactopyranose ring.[40] As can be seen in Fig. 3, both the galactoside and the glucoside show the characteristic absorption at 11.22 μ. The former lipid also shows the expected band at 11.44 μ.

On the basis of the evidence now accumulated, it seems justified to assign the β-configuration to the natural cerebrosides.

In a recent brief communication, Kiss[31] reported the condensation of N-tetracosanoyl-dihydrosphingosine of m.p. 88° with acetobromogalactose and arrived at the conclusion that natural cerasine has the α-configuration. For lack of experimental details this result cannot be commented upon. It may be pointed out, however, that the melting point of 88° is not consistent with that reported in the literature (99–100°).[41] A melting point of 102° was observed in this Laboratory for the synthetic compound.

Synthetic Lignoceric Acid.—Natural lignoceric acid, isolated from hydrolysates of plant oils, has been recognized as a gross mixture from which the pure tetracosanoic acid can be obtained in low yields only after prolonged purification.[42]

Levene and Taylor[43] described a tedious synthesis, starting from stearic acid and adding one carbon atom at a time. A five-step synthesis was devised by Robinson, *et al.*,[44–45] which involved

(30) O. Rosenheim, *Biochem. J.*, **10**, 142 (1916).
(31) J. Kiss, *Chimia*, **13**, 115 (1959).
(32) E. Klenk, *Z. physiol. Chem.*, **267**, 128 (1940).
(33) A. Rosenberg and E. Chargaff, *J. Biol. Chem.*, **233**, 1323 (1958).
(34) B. Helferich, H. Appel and R. Gootz, *Z. physiol. Chem.*, **215**, 277 (1933).
(35) A term applied to galactosidosphingosine obtained by alkaline hydrolysis of cerebrosides.
(36) Y. Fujino and T. Negishi, *C. A.*, **51**, 6745 (1957).
(37) N. Halliday, H. J. Deuel, L. J. Tragerman and W. E. Ward, *J. Biol. Chem.*, **132**, 171 (1940).
(38) L. P. Kuhn, *Anal. Chem.*, **22**, 276 (1950).
(39) R. S. Tipson and H. S. Isbell, *J. Research Natl. Bur. Standards*, **64A**, 239 (1960).
(40) W. B. Neely, *Advances in Carbohydrate Chem.*, **12**, 13 (1957).
(41) G. Marinetti and E. Stotz, *J. Am. Chem. Soc.*, **76**, 1347 (1954).
(42) F. A. Taylor, *J. Biol. Chem.*, **91**, 541 (1931).
(43) P. A. Levene and F. A. Taylor, *ibid.*, **59**, 905 (1924).

the condensation of ethyl 11-bromoundecanoate and lauryl chloride with acetoacetic ester and a Clemmensen reduction of 13-oxotetracosanoic acid. We found it more convenient to synthesize larger quantities of pure tetracosanoic acid, following a method described by Jones for other fatty acids.[46] Thus, condensation of ω-carbethoxypentanoyl chloride with octadecyl-zinc chloride gave 80% of 6-oxo-tetracosanoic acid. Similar yields of the 8-oxo- and 10-oxo-derivatives were obtained, starting with the chlorides of the ω-substituted heptanoic and nonanoic acids, respectively. A Wolf–Kishner reduction[47] of these oxo-acids afforded pure tetracosanoic acid in 90% yield.

DL-α-Hydroxy-*n*-tetracosanoic acid, obtained from α-bromo-*n*-tetracosanoic acid, according to Robinson[44] was resolved[48] *via* the brucine salt, and the D-isomer was converted into the acetoxy derivative as described by Klenk.[49]

Experimental[50,51]

Ethyl *erythro*-2-Amino-3-hydroxyoctadecanoate.—The crude mixture of diastereomers (73 g.) resulting from the reduction of ethyl 2-acetamido-3-ketoöctadecanoate with sodium borohydride[52] was treated with hot ethanolic hydrogen chloride following the procedure described earlier for the unsaturated series.[26b] Recrystallization of the crude product from a mixture of ethyl acetate and dioxane gave the ester hydrochloride (46 g.) of m.p. 125–127°. The amino ester, liberated by treatment with sodium carbonate, was obtained in pure form after two crystallizations from petroleum ether; m.p. 56–58°; yield 32.6 g.

Anal. Calcd. for ($C_{26}H_{41}NO_3$): C, 69.92; H, 12.03; N, 4.08. Found: C, 70.00; H, 12.06; N, 4.35.

Condensation with benzimino ethyl ether and then reduction with lithium aluminum hydride yielded 61% of the oxazoline III.[26a]

3-O-Benzoyl-ceramides (VI).—A solution of the substituted oxazoline III (10 g.) in tetrahydrofuran (150 ml.) and 3 *N* sulfuric acid (30 ml.) was allowed to stand at room temperature for 15–18 hr. To the rapidly-stirred mixture were added simultaneously, during 30–40 minutes, 50% sodium acetate solution (200 ml.) and an equivalent amount of the acid chloride dissolved in 5–10 volumes of dry ether. After stirring for 2–3 hours, the ethereal extract was treated with sodium bicarbonate, washed, dried and concentrated *in vacuo*. The residue was recrystallized from methanol and, if necessary, a second time from *n*-hexane. The optically active derivatives were prepared similarly. The tartrates were dissolved in tetrahydrofuran (15 vol.) and treated with the acyl chlorides and sodium acetate, after addition of 1 *N* acetic acid (5 vol.). The infrared spectra showed significant bands at 2.9, 5.8, 6.0, 6.6, 7.9, 8.2 μ (acetate in acetoxy-lignoceroyl) and 10.4 μ (in the unsaturated compounds).

3-O-Benzoyl-DL-sphingosine Sulfate (IVa).—The tetrahydrofuran solution resulting from the hydrolysis of the oxazoline IIIa (10 g.) with sulfuric acid was diluted with ice-water. On cooling the mixture for a short time, the sulfate separated quantitatively. It was washed thoroughly with water and cold dilute methanol, dried and recrystallized from 70 parts of 90% methanol; yield 10.3 g.; m.p. 148–149°.

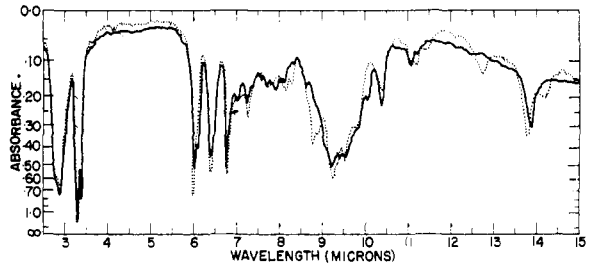

Fig. 3.—Infrared spectra of synthetic behenyl glucocerebroside (solid line) and synthetic cerasine (VIIIa) (dotted line), pressed in KBr.

Anal. Calcd. for $C_{25}H_{41}NO_3 \cdot {}^1/_2H_2SO_4$: C, 66.34; H, 9.43; N, 3.09; S, 3.53. Found: C, 66.23; H, 9.36; N, 2.79; S, 3.70.

3-O-Benzoyl-DL-dihydrosphingosine sulfate (IVb) was prepared similarly; m.p. 138–140°.

Anal. Calcd. for $C_{25}H_{43}NO_3 \cdot {}^1/_2H_2SO_4$: C, 66.06; H, 9.76; N, 3.10; S, 3.52. Found: C, 66.20; H, 9.65; N, 3.33; S, 3.83.

Resolution of 3-O-Benzoylsphingosine.—A warm solution of the DL-sulfate IVa (7.5 g.) in 50% tetrahydrofuran (160 ml.) was added with light swirling to a solution prepared from L-tartaric acid (2.5 g.), distilled water (95 ml.) and 0.38 *N* barium hydroxide solution (45 ml.). The mixture was warmed to boiling, and hot ethanol (800 ml.) was added. The warm suspension was filtered immediately by gravity and the filtrate was evaporated *in vacuo*. The wet product was taken up several times with small portions of isopropyl alcohol which was distilled off to remove the excess water. The dry salt thus obtained was recrystallized 2–3 times from ethanol (280 ml.) at room temperature and yielded 2 g. of m.p. 121–122° and $[\alpha]^{26}D$ −14° (*c* 1.2, in methanol). Melting point and specific rotation remained constant on further crystallization. Benzoylation of this tartrate with benzoyl chloride in the presence of pyridine gave a tribenzoyl derivative of m.p. 122–123°, and $[\alpha]^{26}D$ +8° (*c* 1, in chloroform), as compared with $[\alpha]^{27}D$ −11.2° reported[53] for the natural enantiomorph.

To obtain the D-isomer, 3 *N* sulfuric acid (10 ml.) was added to the filtrate, and the sulfate which precipitated by addition of ice-water was filtered and washed thoroughly with water. The dry product was converted to the D-tartrate as described above, and the salt was recrystallized 2–3 times from ethanol (200–240 ml. depending on the room temperature); yield 1.6 g. of m.p. 125–127°, $[\alpha]^{25}D$ +16.9° (*c* 1.3, in methanol). This product was converted into the triacetyl derivative and identified as the natural isomer as follows. The tartrate (0.25 g.) was dissolved in tetrahydrofuran (10 ml.), and 1 *N* acetic acid (5 ml.) and water (2 ml.) were added. The rapidly stirred mixture was treated with 50% sodium acetate solution (5 ml.) and acetic anhydride (2 ml.), which were added in 3–4 portions. After stirring for 3–4 hr., water and saturated sodium chloride were added and the ethereal extract was washed, dried and evaporated. The oily residue was saponified with 0.1 *N* sodium hydroxide solution. The raw N-acetyl derivative was treated with acetic anhydride in the presence of pyridine. D-Triacetylsphingosine, recrystallized from acetone, melted at 100–103° and had a specific rotation of −10.8°. (Reported −11.7° for the natural[53] and −12.8° for the synthetic product.[9b])

Resolution of 3-O-Benzoyldihydrosphingosine.—The L-tartrate of the natural antipode was found in this case to be the less soluble one, and a product of m.p. 149–150° and $[\alpha]^{27}D$ +20.8° crystallized from alcohol in a 45% yield. The tribenzoyl derivative melted at 144–145° and had a specific rotation of −26° (reported[53] −31°).

Glycosidation.—This reaction was based on a method described by Helferich and Weis.[25] To a stirred solution of the benzoyl-ceramide VI (0.002 mol.) in dry benzene (30 ml.) and dry nitromethane (30 ml.) were added tetraacetyl-

(44) R. Ashton, R. Robinson and J. C. Smith, *J. Chem. Soc.*, 283 (1936).

(45) G. M. Robinson, *ibid.*, 745 (1930).

(46) R. G. Jones, *J. Am. Chem. Soc.*, **69**, 2350 (1947).

(47) Huang-Minlon, *ibid.*, **68**, 2487 (1946).

(48) A. Mueller and I. Binzer, *Ber.*, **72B**, 615 (1939), reported an unsuccessful resolution with strychnine. The natural isomer was obtained in "enriched form" with a rotation of +0.09°.

(49) E. Klenk and L. Clarenz, *Z. physiol. Chem.*, **257**, 268 (1939).

(50) Analyses were carried out in the Institute's microanalytical laboratory under the direction of Mr. Erich Meier.

(51) The acid chlorides were prepared *in situ* by boiling a solution of the acid in 3 vol. of petroleum ether with an excess of thionyl chloride and removing the latter by distillation of two freshly added portions of the solvent under diminished pressure.

(52) D. Shapiro, H. Segal and H. M. Flowers, *J. Am. Chem. Soc.*, **80**, 2170 (1958).

(53) H. E. Carter, W. P. Norris, F. J. Glick, G. E. Phillips and R. Harris, *J. Biol. Chem.*, **170**, 269 (1947).

α-D-galactosyl (or glucosyl) bromide (0.002 mole) and mercuric cyanide (0.002 mole), and the mixture was stirred at 35–40° for 8–10 hr. The filtered solution was evaporated *in vacuo* to dryness and the residue was dissolved in hot dry petroleum ether. After cooling the solution in ice for a short time, the small precipitate was removed by filtration and the filtrate was concentrated to an oil, which was dissolved in 30 parts of dry methanol. The methanolic solution was allowed to stand for several hours at about 25° to remove the unchanged ceramide-ester which may amount to 10–15% of the oily product. In all cases, the glycosidic esters separated, on cooling the filtrates, in yields ranging from 50–70%. The infrared spectra showed bands at 6.0 and 6.65 μ (amide), at 5.7 and 8.2 μ (acetate) and at 5.8 and 7.9 μ (benzoate). In the unsaturated compounds a band at 10.4 μ indicated the presence of the *trans*-ethylenic linkage.

For saponification, the acylated glycoside was suspended in 30 parts of dry methanol in which a catalytic amount[29] of sodium metal had been dissolved. The suspension was swirled for a few moments in a bath at 35–40°, and the clear solution was kept overnight at room temperature. Addition of cold water precipitated the glycoside, which was washed with cold dilute methanol, dried and recrystallized from methanol, giving a product melting at about 150°. Further purification was achieved by recrystallization from 10 parts of an acetic acid–chloroform mixture (2:1) and, if necessary, from pyridine–acetone–water (2:90:10).

3-O-Benzoyl-N-docosanoyl-1-DL-sphingosyl-2',3',4',6'-tetra-O-acetyl-β-D-glucopyranoside: m.p. 53–56°, $[\alpha]^{25}D$ −3.0° (chlf.)

Anal. Calcd. for $C_{61}H_{101}NO_{13}$: C, 69.38; H, 9.64; N, 1.33. Found: C, 69.42; H, 9.91; N, 1.21.

The corresponding D-sphingosine derivative was deacylated directly without being isolated.

N-Docosanoyl-1-DL-sphingosyl-β-D-glucopyranoside: m.p. 165°; $[\alpha]^{25}D$ −4.0° (pyridine).

Anal. Calcd. for $C_{46}H_{89}NO_8$: C, 70.45; H, 11.44; N, 1.79. Found: C, 70.06; H, 11.32; N, 1.74.

N-Docosanoyl-1-D-sphingosyl-β-D-glucopyranoside: m.p. 182–183° (reported[33] 183–185°); $[\alpha]^{25}D$ −7.6° (pyridine); (reported[33] −7.4°).

Anal. Found: C, 70.10; H, 11.28; N, 1.70.

Reduction of Cerasine to Dihydrocerasine (VIIIa → VIIIh).—A sample of synthetic D-cerasine (130 mg.) was dissolved in absolute alcohol (80 ml.) and hydrogenated during 2 hr. at 60 p.s.i. in the presence of platinum oxide. The residue obtained on evaporation of the filtrate was recrystallized from alcohol and gave 120 mg. of m.p. 182° and $[\alpha]^{23}D$ +5.5° (identical with VIIIh). The band at 10.4 μ indicative of the *trans*-ethylenic bond had completely disappeared.

Pentaacetyl-cerasine was prepared from VIIIa following the method of Carter[6] and was recrystallized from methanol; m.p. 57–58°; $[\alpha]^{25}D$ −18° (*c* 1, in methanol–chloroform 1:1). (Levene and West[54] observed m.p. 54–56° and $[\alpha]^{30}D$ −16.46°).

Anal. Calcd. for $C_{58}H_{103}NO_{13}$: C, 68.10; H, 10.12; N, 1.39. Found: C, 68.28; H, 10.19; N, 1.35.

Hexaacetylphrenosine, prepared similarly from VIIIb, melted at 40–41°, $[\alpha]^{25}D$ −10° (*c* 1.1 in chloroform–methanol 1:1) (reported[54] m.p. 40°; $[\alpha]^{20}D$ −11°.

Anal. Calcd. for $C_{60}H_{105}NO_{15}$: C, 66.71; H, 9.79; N, 1.29. Found: C, 66.70; H, 9.72; N, 1.49.

N-Tetracosanoyl-DL-dihydrosphingosine.—N-Tetracosanoyl-3-O-benzoyldihydrosphingosine (1 g.) was dissolved in warm methanol (90 ml.), and 1 *N* sodium hydroxide solution (10 ml.) was added. The clear solution was kept overnight at room temperature, and the precipitate formed on cooling was washed with cold dilute methanol. The dry product was recrystallized from ethyl acetate and gave 0.8 g. of m.p. 101–102°.

Anal. Calcd. for $C_{42}H_{85}NO_3$: C, 77.28; H, 13.14; N, 2.15. Found: C, 77.08; H, 13.08; N, 2.32.

N-Tetracosanoyl-DL-sphingosine, prepared similarly, melted at 91–92°. *Anal.* Calcd. for $C_{42}H_{83}NO_3$: C, 77.60; H, 12.87; N, 2.15. Found: C, 77.50; H, 12.77; N, 2.32.

N-Tetracosanoyl-D-dihydrosphingosine: m.p. 102–103°, $[\alpha]^{26}D$ 0° (pyridine).

Anal. Calcd. for $C_{42}H_{85}NO_3$: C, 77.28; H, 13.14; N, 2.15. Found: C, 77.26; H, 12.75; N, 2.21.

(54) P. A. Levene and C. J. West, *J. Biol. Chem.*, **31**, 635 (1917).

N-Tetracosanoyl-D-sphingosine: m.p. 93–95°, $[\alpha]^{25}D$ −2.0° (chlf.); −3.4° (pyridine); reported[55] $[\alpha]^{30}D$ 1.88° (chlf.)

Anal. Found: C, 77.39; H, 13.14; N, 2.35.

6-Oxo-tetracosanoic Acid.—A Grignard reagent was prepared from octadecyl bromide[56] (226 g.), dry ether (970 ml.) and magnesium turnings (31 g.). On completion of the reaction, the supernatant (910 ml.) was drawn by suction into stirred suspension of anhydrous zinc chloride (80.4 g.) in dry ether (200 ml.) which had been placed in a 3-necked 2-liter flask fitted with a reflux condenser and a calcium chloride tube. Ether was then distilled off during a period of 2 hr. until a volume of about 560 ml. remained, and a solution of ω-carbethoxy-pentanoyl chloride (prepared from 88 g. of ethyl hydrogen adipate[46,57]) in dry benzene (165 ml.) was added dropwise to the cooled, vigorously stirred, viscous solution. After boiling the mixture for 3 hr. with continued stirring, hydrochloric acid (2 *N*, 500 ml.) was added in a fairly rapid stream with partial cooling, and the hot mixture was transferred to a separatory funnel with the addition of hot benzene (600 ml.). The organic layer was washed once with hot 2 *N* hydrochloric acid (400 ml.), twice with hot water and concentrated *in vacuo* to about 150 ml. After digesting the solution on the steam-bath for 2 hr. with a solution of caustic soda (17 g.) in 80% alcohol (100 ml.), the resulting white solid was filtered, washed successively with warm benzene and water and suspended in hot hydrochloric acid (800 ml.). Hot benzene (about 500 ml.) was added and the mixture shaken for several minutes until complete solution was achieved. The benzene layer was separated, washed with hot water and allowed to cool to room temperature, giving 150 g. of m.p. 97–99°. Recrystallization from acetone did not affect the melting point.

Anal. Calcd. for ($C_{24}H_{46}O_3$): C, 75.34; H, 12.12. Found: C, 75.20; H, 11.85.

8-Oxo-tetracosanoic acid: m.p. 96–97°; Found: C, 75.62; H, 12.18.

10-Oxo-tetracosanoic acid: m.p. 96–98°; Found: C, 75.51; H, 11.82.

Tetracosanoic Acid.—A solution of 6-oxo-tetracosanoic acid (30 g.) and potassium hydroxide (18 g.) in digol[58] (220 ml.) and hydrazine (22 ml. of 64% aqueous solution) was refluxed for 90 minutes. The internal temperature was then raised and maintained at 195–200° for 5 hr. After cooling to about 50°, excess of concentrated hydrochloric acid was added at such a rate as to keep the liberated tetracosanoic acid in solution. Addition of cold water precipitated a white crystalline solid which was recrystallized from petroleum ether (500 ml.); yield 26 g. (90%), m.p. 84–86°. Recrystallization from alcohol gave 24 g. of m.p. 84–85°.

Anal. Calcd. for $C_{24}H_{48}O_2$: C, 78.19; H, 13.13. Found: C, 78.07; H, 13.10.

Resolution of 2-Hydroxytetracosanoic Acid.[44]—To a solution of the acid (3.1 g.) in warm chloroform (25 l.) was added a warm solution of brucine tetrahydrate (3.7 g.) in chloroform (15 ml.). The mixture was filtered, evaporated *in vacuo* to dryness, and the residue was recrystallized from alcohol. Two crystallizations sufficed to obtain a constant melting point of 93–95° and a constant rotation of $[\alpha]^{25}D$ 0° (in chloroform). The salt was decomposed by shaking with a mixture of diluted hydrochloric acid and ether, and the ether solution was evaporated. Recrystallization of the residue from acetone gave 1.5 g. of m.p. 100–101°, $[\alpha]^{25}D$ +3.9°; (*c* 1.34, in pyridine). Chibnall, *et al.*,[10] reported +3.3° (pyridine).

DL-2-Acetoxytetracosanoic Acid.—Acetylation of 2-hydroxytetracosanoic acid with acetyl chloride according to Klenk's method[49] gave over 80% yield of a product melting at 64–66°.

Anal. Calcd. for $C_{26}H_{50}O_4$: C, 73.19; H, 11.81. Found: C, 73.34; H, 11.63.

D-2-Acetoxytetracosanoic acid, prepared similarly, melted at 64–66°; $[\alpha]^{25}D$ 42° (*c* 1, in chloroform). Klenk reported a m.p. of 65–66° for the natural product.

(55) C. Tropp and V. Wiedersheim, *Z. physiol. Chem.*, **222**, 39 (1933).

(56) "Organic Syntheses," Coll. Vol. I, p. 29.

(57) Ref. 56, Vol. II, p. 276.

(58) Commercial name for β,β'-dihydroxyethyl ether (B. D. H.).