ultraviolet absorption: λ_{max} 217 mμ (ϵ 22,000), 239 (33,000), 254 (28,000), 282 (8,200), 293 (8,800), 315sh. (4,300).

Anal. Calcd. for $C_{18}H_{15}O_3N$: C, 67.0; H, 6.4; N, 6.0. Found: C, 67.2; H, 6.3; N, 6.3.

O^4-Methylbalfourodinium Iodide (XIX).—A solution of balfourodine (XVIII) (300 mg., 1.04 mmoles) in methyl iodide (25 ml.) was allowed to stand for 3 days. The white precipitate which formed was collected, and addition of hexane to a solution of this material in absolute ethanol gave O^4-methylbalfourodinium iodide which was extremely hygroscopic; λ_{max} 217 mμ (ϵ 43,000), 254 (36,000), 301 (8,100), 324sh. (4,600).

Anal. Calcd. for $C_{17}H_{22}NO_4I \cdot H_2O$: C, 45.5; H, 5.4; I, 28.2. Found: C, 45.9; H, 5.5; I, 27.7.

Balfourolone (V) from O^4-Methylbalfourodinium Iodide (XIX).—A solution of O^4-methylbalfourodinium iodide (200 mg., 0.46 mmole) in water buffered at *p*H 11 was allowed to stand for 16 hours. The solution was acidified to *p*H 1.5 and extracted with methylene chloride (3 × 25 ml.). Evaporation of the combined organic phases gave an oily substance which after recrystallization from ether gave balfourolone, m.p. 98–99°. A quantitative study using ultraviolet spectral data showed that at least 84% of the starting O^4-methylbalfourodinium iodide was converted to balfourolone within 8 hours and that there was no further change after this time.

Balfourolone Precursor; O^4-Methylbalfourodinium Perchlorate (XIX).—Starting with 327 ml. of plant extract (1.5 kg. of plant) the usual extraction scheme was followed through extraction at *p*H 7 with ether. At this point the aqueous phase was made 4 *M* in sodium chloride and extracted with butanol (3 × 800 ml.). On evaporation of the butanol at reduced pressure, 19.4 g. of solid material was obtained. This material was taken up in 200 ml. of absolute ethanol (about 2 g. remained undissolved). A 25-ml. portion of the ethanolic solution was treated with 15 ml. of 1 *N* perchloric acid in ethanol followed by dilution with 200 ml. of ether. A light yellow solid precipitated (about 1 g.) which after recrystallization from water and then from ethanol–ether melted at 124–125°, $[\alpha]_D$ +9°; ultraviolet absorption: λ_{max} 215 mμ (ϵ 31,000), 254 (36,000), 301 (7,500), 324sh. (3,700).

Anal. Calcd. for $C_{17}H_{22}NO_3Cl$: C, 50.6; H, 5.5. Found: C, 50.3; H, 5.8.

Conversion of O^4-Methylbalfourodinium Perchlorate (XIX) to Balfourolone (V).—O^4-Methylbalfourodinium perchlorate (0.5 g., 1.2 mmoles) was dissolved in water buffered at *p*H 10.5 and allowed to stand for 3 days. Upon extraction with methylene chloride (3 × 50 ml.), an oil (300 mg., 0.95 mmole) was obtained which on crystallization from ether gave balfourolone, m.p. 97–98°.

BERKELEY, CALIF.

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

Synthetic Studies on Sphingolipids.[1] III.[2] The Synthesis of Dihydrosphingomyelin[3]

BY DAVID SHAPIRO, H. M. FLOWERS AND SARAH SPECTOR-SHEFER

RECEIVED JANUARY 16, 1959

The synthesis of benzoyl-, palmitoyl- and stearoyldihydrosphingomyelin (XXI) is described. *cis*-2-Phenyl-4-hydroxymethyl-5-pentadecyl-2-oxazoline (VII) is phosphorylated with β-chloroethylphosphoryl dichloride. The reaction product XVIb is hydrolyzed with diluted hydrochloric acid, and the resulting ester XIVb is acylated with the corresponding fatty acid chloride to give XIX. Quaternization with trimethylamine and removal of the benzoyl group by mild alkaline hydrolysis lead to the sphingomyelins XXIb,c. Conversion of XIVb to the barium salt XVIIa, followed by treatment with trimethylamine affords XXIa.

Sphingomyelin was discovered, in 1884, by Thudichum[4,5] who isolated it from an alcoholic extract of brain tissue. Subsequent workers later found that the main product of complete hydrolysis was the unsaturated base sphingosine (I), in addition to fatty acids, choline and phosphoric acid. The structural investigation of the sphingolipids which continued for about five decades until recent years was concentrated mainly on the chemistry of sphingosine.[6–10] Its structure was established by Carter and his collaborators in 1947.[11–12] Following this conclusion, the complete stereochemistry of sphingosine was soon determined by several investigators who were able to show that the carbons 2 and 3 have the *erythro* form,[13–18] and that the double bond has the *trans* configuration.[19–21] These results have been recently confirmed by synthesis.[2a,22,23]

The presence in sphingomyelin of phosphorylcholine as structural unit, an assumption which was based largely on analogy with the lecithins, has been recently substantiated.[24,25] The ester linkage of the phosphoric acid with the primary hydroxyl has been conclusively proved by Stotz and co-workers,[25,26] and the structure of sphingo-

(1) We adopted the term sphingolipid as proposed by Carter, *et al.*, *J. Biol. Chem.*, **169**, 77 (1947), to designate the group of lipids which incorporate sphingosine. They include in the main the sphingomyelins, the cerebrosides and the gangliosides.

(2) (a) D. Shapiro, H. Segal and H. M. Flowers, THIS JOURNAL, **80**, 1194 (1958), is considered as part I of this series; (b) part II, D. Shapiro, H. Segal and H. M. Flowers, *ibid.*, **80**, 2170 (1958).

(3) Presented in part before the XVI International Congress of Pure and Applied Chemistry, Paris, 1957.

(4) J. W. L. Thudichum, "A Treatise on the Chemical Constitution of Brain," Bailliere, Tindall and Cox, London, 1884.

(5) H. Thierfelder and E. Klenk, "Die Chemie der Cerebroside und Phosphatide," Verlag Julius Springer, Berlin, 1930, pp. 65 ff.

(6) P. A. Levene and W. A. Jacobs, *J. Biol. Chem.*, **11**, 547 (1912).

(7) P. A. Levene and C. J. West, *ibid.*, **16**, 549 (1913).

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(9) E. Klenk, *Z. physiol. Chem.*, **185**, 169 (1929).

(10) E. Klenk und W. Diebold, *ibid.*, **198**, 25 (1931).

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

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

(13) H. E. Carter and C. G. Humiston, *ibid.*, **191**, 727 (1951).

(14) J. Kiss, G. Fodor and D. Banfi, *Helv. Chim. Acta*, **37**, 1471 (1954).

(15) E. Klenk and H. Faillard, *Z. physiol. Chem.*, **299**, 48 (1955).

(16) H. E. Carter, D. Shapiro and J. B. Harrison, THIS JOURNAL, **75**, 1007 (1953).

(17) H. E. Carter and D. Shapiro, *ibid.*, **75**, 5131 (1953).

(18) E. F. Jenny and C. A. Grob, *Helv. Chim. Acta*, **36**, 1936 (1953).

(19) K. Mislow, THIS JOURNAL, **74**, 5155 (1952).

(20) G. Marinetti and E. Stotz, *ibid.*, **76**, 1347 (1954).

(21) G. Fodor and J. Kiss, *Nature*, **171**, 651 (1953).

(22) D. Shapiro and H. Segal, THIS JOURNAL, **76**, 5894 (1954).

(23) C. A. Grob and F. Gadient, *Helv. Chim. Acta*, **40**, 1145 (1957).

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(25) G. Rouser, J. F. Berry, G. Marinetti and E. Stotz, THIS JOURNAL, **75**, 310 (1953).

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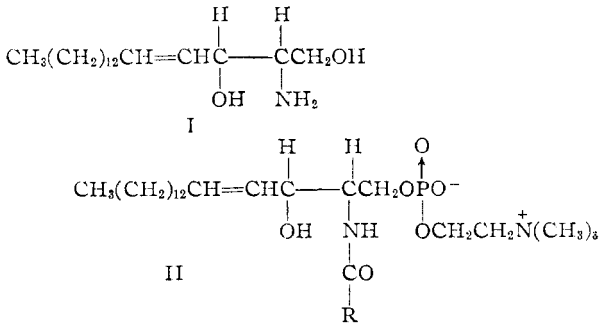
myelin may accordingly be expressed by formula II.



The pure natural product consists of several sphingomyelins, since it yields on hydrolysis a mixture of fatty acids, such as palmitic, stearic, nervonic and lignoceric acid. Carter and coworkers[27] isolated dihydrosphingosine from brain and spinal cord, and Thannhauser and Boncoddo[28] showed its presence in sphingomyelin together with sphingosine.

The allylic system present in sphingosine imparts to the molecule considerable chemical sensitivity. It seemed, therefore, advisable to explore the approach to the sphingomyelins by studying first the synthesis of a dihydro derivative. With this in mind, we set out to prepare benzoyl-dihydrosphingomyelin (XXIa) which was the simplest model that could be developed from the starting material employed. In a recent brief communication[29] we announced the synthesis of dihydrosphingomyelin. In the present paper we wish to report the results of this investigation in detail.

Dihydrosphingosine with its three functional groups is obviously not a suitable starting material for an unambiguous synthesis. An ideal key intermediate seemed to be the substituted oxazoline VII, a derivative of dihydrosphingosine in which both the amino group and the secondary hydroxyl are blocked. To prepare this compound, we initially employed as starting material the mixture of the diastereomeric methyl esters of α-benzamido-β-hydroxystearic acid[30] (IIIa) which we obtained by reduction of the corresponding ketoester with sodium borohydride. It is well known that epimeric pairs of type III react with thionyl chloride to give oxazolines with respective inversion of configuration.[31,32] Since the isolation of the pure *threo* epimer in a fairly good yield was rather difficult, we converted the original mixture into all-*threo* form following the method of Elliott,[31a] which is based on the observation that *cis*-oxazolines invert into the stable *trans*-oxazolines under the influence of alkali. The pure *threo* form of the acid IIIa thus obtained was esterified with diazomethane and converted to the *cis*-oxazoline VI. Careful treatment with lithium aluminum hydride afforded the desired key intermediate VII.

The method just described involves the use of 2-phenyl-4-(1-hydroxypalmitylidene)-oxazolone-5 and suffers from the difficulty of obtaining this product in consistent yields and good quality. We found it preferable to prepare the oxazoline VII *via* the acetamido acid IIIb whose ethyl ester may be conveniently obtained by a different route.[2b] The free amino ester V was prepared in good yield either by methanolysis of the ester IVb with 15% methanolic hydrochloric acid, or by refluxing the acid IIIb with gaseous methanolic hydrochloric acid. The latter method was, however, less convenient, since it required prolonged reaction time, and even so, a considerable part of the acid remained unesterified, thus making the separation of the ester difficult.

Preliminary phosphorylation experiments showed that the alcoholic group in VII had a rather low reactivity. Thus, it failed to react with dibenzyl chlorophosphate which was extensively used by Todd[33] in nucleotide chemistry. Applying the more reactive phenylphosphoryl dichloride XIa we were able to isolate, after hydrolysis, the phosphate esters XIVa and XVa in analytically pure form. However, attempts to bring about the reaction of the intermediate XIIa with choline chloride in a manner described by Baer[34] met with complete failure. We finally turned to β-chloroethylphosphoryl dichloride (XIb) which seemed preferable to other phosphorylating agents, since it has no blocking groups to be later removed by catalytic hydrogenation. This was borne in mind in view of the prospective synthesis of the unsaturated lipid.

Surprisingly we found that phosphorylation of VII with either XIa or XIb was accompanied by scission of the oxazoline ring. The rearrangement of oxazolines to β-chloroalkylamides at elevated temperature was reported earlier.[35-37] Thus, Gabriel and Heymann[38] obtained a high yield of β-chloroethylbenzamide by heating 2-phenyloxazoline with concentrated hydrochloric acid. The conversion of XII to XIII at 25–30° finds its parallel in the rearrangement at room temperature of the complex X, formed from the corresponding amidoalcohol and thionyl chloride, to IX.[39]

In general, substituted oxazolines may be opened in the following positions. By aqueous acids, rupture occurs between the carbons 2 and 3, while the dry hydrochloride opens on warming at the position 1-5. A reasonable explanation for this behavior was given by Goldberg and Kelly[37] by assuming the existence of an ammonium-oxonium hybrid which may be attacked at the positions 2 or 5.

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

(28) S. J. Thannhauser and N. F. Boncoddo, *ibid.*, **172**, 141 (1948).

(29) D. Shapiro, H. M. Flowers and S. Spector-Shefer, THIS JOURNAL, **80**, 2339 (1958).

(30) H. E. Carter, J. B. Harrison and David Shapiro, *ibid.*, **75**, 4705 (1953).

(31) (a) D. T. Elliott, *J. Chem. Soc.*, 589 (1949); (b) D. T. Elliott, *ibid.*, 62 (1950).

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(34) (a) E. Baer and M. Kates, THIS JOURNAL, **72**, 942 (1950); (b) E. Baer and J. Maurukas, *ibid.*, **74**, 158 (1952).

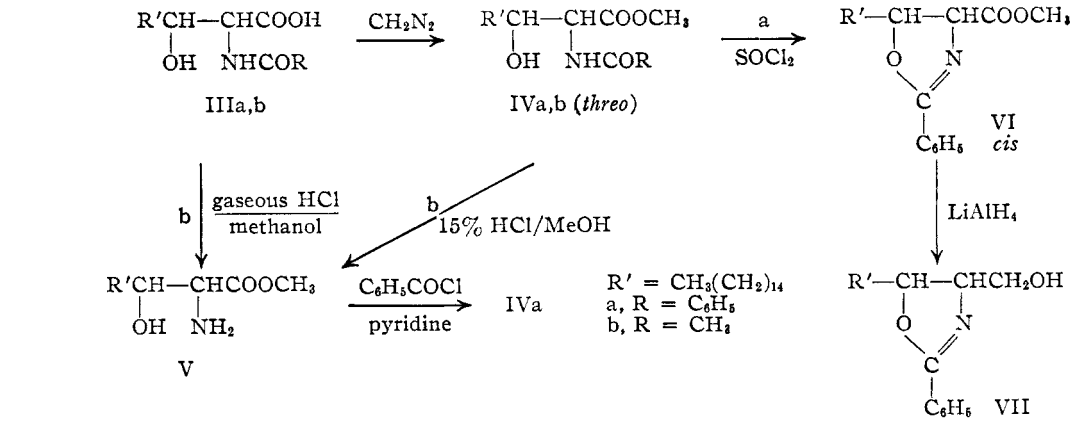
(35) W. Wislicenus and H. Körber, *Ber.*, **35**, 164 (1902).

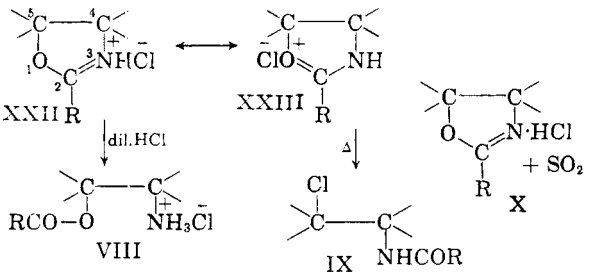
(36) H. Wenker, THIS JOURNAL, **60**, 2152 (1938).

(37) A. Goldberg and W. Kelly, *J. Chem. Soc.*, 1919 (1948).

(38) S. Gabriel and Th. Heymann, *Ber.*, **23**, 2493 (1890).

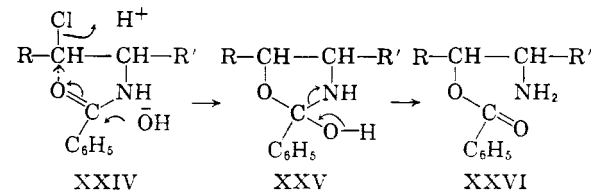
(39) E. M. Fry, *J. Org. Chem.*, **14**, 887 (1949).





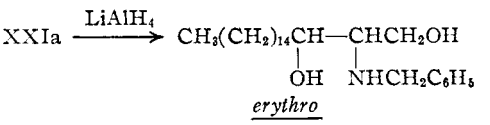
The ammonium ion leads to the ester hydrochloride VIII, while the oxonium ion yields the β-chloroamide IX.

When the oxazoline VII was treated at 25–30° with β-chloroethylphosphoryl dichloride (XIb) in the presence of pyridine, the reaction product was found to consist mainly of the β-chloroamide XIIIb. Subsequent treatment with warm water resulted in the formation of the amino ester XIVb. Reactions of the latter type have been reported earlier[38–40] and proceed probably *via* a cyclic intermediate. It may involve the following mechanism which is similar to that proposed by Welsh for the N→O acyl migration.[41–43]



Preparation of the barium salt of XIVb and its treatment with trimethylamine[44,45] effected O→N acyl migration and quaternization to give the substituted choline chloride XVIIIa. Removal of the chlorine by Amberlite IRA-400 afforded benzoyldihydrosphingomyelin (XXIa).

Extensive studies of reactions involving rupture of the oxazoline ring have been recently made by Fry[46] and by Sicher and co-workers.[47] The latter authors investigated oxazolines with diastereomeric carbon atoms and found that transformations of the type XII → XIII → XIV entail a double Walden inversion. This result made it necessary to prove that the carbon atoms 2 and 3 in benzoyldihydrosphingomyelin still had the *erythro* configuration. Treatment of XXIa with excess of lithium aluminum hydride effected both removal of the phosphorylcholine moiety and reduction of the benzoyl group. The product obtained in 70% yield was identical with *erythro*-N-benzyl-dihydrosphingosine which resulted from a similar reduction of *erythro*-N-benzoyldihydrosphingosine.



We now faced the problem of replacing the benzoyl group at some stage. A logical application of the above results would obviously be to start with the corresponding 2-alkyloxazoline (VII, alkyl instead of Ph). However, we encountered unexpected difficulties in preparing such compounds in pure form. The pure palmitamido-ester (IV, R = $C_{15}H_{31}$) was obtained in a fairly good yield, but the reduction of the corresponding oxazoline (VI, $C_{15}H_{31}$– instead of C_6H_5–) with lithium aluminum hydride gave a product with deviating carbon values, although the absorption spectrum showed correctly the characteristic bands of oxazoline and alcoholic OH. Indeed, attempts to phosphorylate this product did not lead to the isolation of any definite compound. Similar results were obtained with the 2-methyloxazoline (VII, CH_3 instead of C_6H_5) and its seems that, generally, 2-alkyloxazolines are more susceptible to attack by lithium aluminum hydride than the oxazoline VI in which the phenyl group may have a stabilizing effect.

A more promising alternative was the introduction of the fatty acid prior to the formation of the amide XIII. This implied that phosphorylation should take place with the oxazoline ring remaining intact. We, therefore, investigated this reaction more closely by means of infrared spectroscopy and succeeded in finding the conditions which

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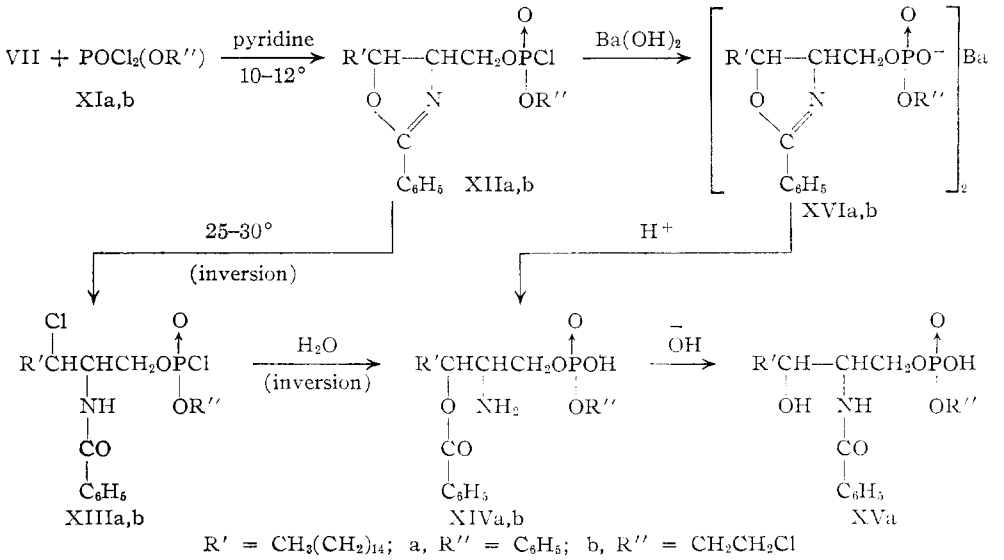
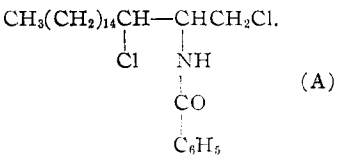
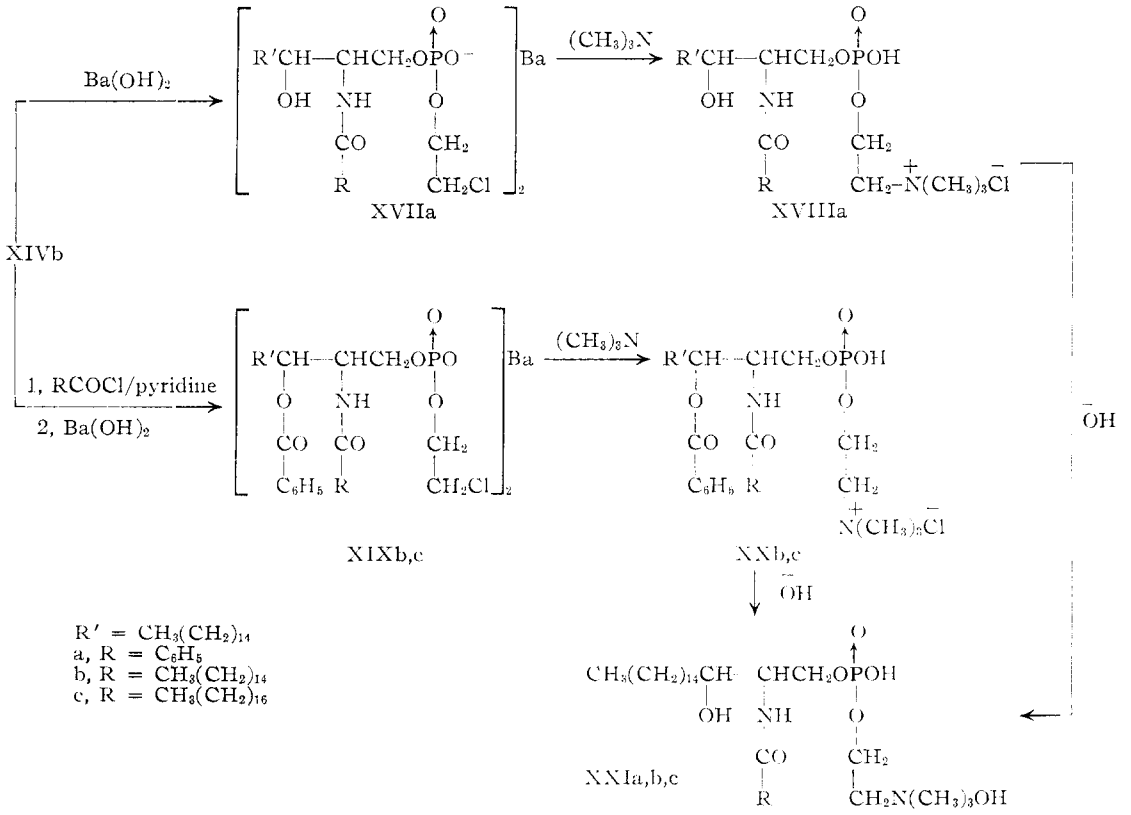
(41) M. Bergmann, E. Brand and F. Weinmann, *Z. physiol. Chem.*, **131**, 1 (1923).
(42) L. H. Welsh, THIS JOURNAL, **71**, 3500 (1949).
(43) A. P. Phillips and R. Baltzly, *ibid.*, **69**, 200 (1947).
(44) A. Gruen and F. Kade, *Ber.*, **45**, 3367 (1912).
(45) E. Baer, D. Buchnea and A. G. Newcombe, THIS JOURNAL, **78**, 232 (1956).
(46) E. Fry, *J. Org. Chem.*, **15**, 802 (1950).
(47) J. Sicher and M. Pankova, *Coll. Czech. Chem. Communs.*, **20**, 1409 (1955).





was demonstrated by the fact that keeping the mixture at +5° for 15 hours did not affect the oxazoline ring, while on stirring for 2 hours at 25–30° the product showed the presence of a strong band at 1560 cm.$^{-1}$ (–NH). This indicated that the formation of XIII in the previous method in-



prevented rupture of the heterocycle. Thus, we were able to isolate the barium salt of the phosphate ester XVIb in good yield when the oxazoline VII was allowed to react with excess of β-chloroethylphosphoryl dichloride (XIb) and pyridine at a temperature not exceeding 10–12°. The dependence of this reaction on the temperature

volved the transitory existence of XII and that the amide band observed there was not due to rearrangement of VII prior to phosphorylation.[48]

Acid hydrolysis of oxazolines is known to proceed without inversion of configuration, and as a result

(48) When the hydrochloride of VII was phosphorylated at 37°, a considerable amount of product was isolated which was recognized as (A).

we obtained the *erythro* form of the ester XIVb by refluxing XVIb with a slight excess of normal hydrochloric acid in aqueous dioxane.

O→N acyl migration usually occurs at *p*H 7.5–8, and according to Fry[46] it also takes place in an anhydrous medium in the presence of trimethylamine. In order to eliminate the possibility of migration,[49] we initially acylated XIVb in a buffer solution of sodium acetate and acetic acid. The yields, however, were rather poor and inconsistent. Subsequently, we found that fairly good yields of XIX could be obtained when XIVb was slowly added to an excess of the acylpyridinium chloride prepared from equal equivalents of acyl chloride and pyridine. After heating XIX with trimethylamine, the benzoyl group in XX was removed by mild alkaline hydrolysis. Treatment with an anion exchange resin led to the desired palmitoyl- and stearoyl-dihydrosphingomyelins (XXIb and XXIc).

All three dihydrosphingomyelins gave analytical carbon values which correspond closely to the "hydrated" forms XXI, and not to the zwitterionic structure as in II. This result, which is consistent with the behavior of the lecithins reported by Baer,[50] will be discussed in more detail in the following paper.

Experimental[51]

erythro- **and** *threo-***Methyl α-Benzamido-β-hydroxystearate.**—A solution of methyl α-benzamido-β-ketostearate (60 g.) in methanol (2,100 ml.) was reduced at 20° by the dropwise addition of a cold solution of sodium borohydride (3.0 g.) in methanol (120 ml.), stabilized with a few drops of *N* sodium hydroxide solution. After 30 minutes, ice and water were added and the mixture acidified with acetic acid. The bulky white precipitate was filtered to remove most of the solvent, and the wet mass remaining on the filter extracted with chloroform. After evaporation of the solvent, the residue was recrystallized from methanol and yielded 58 g. of m.p. 88–94°.

*threo-α-***Benzamido-β-hydroxystearic Acid (IIIa).**—A solution of the mixed esters (58 g.) in dry chloroform (145 ml.) was added dropwise, with stirring, to thionyl chloride (58 ml.) at 5–8°. The mixture was left overnight at 5° and then concentrated *in vacuo* below 35° to an oil. To remove completely the remaining traces of thionyl chloride a fresh portion of chloroform was added and evaporated.

A cold solution of sodium (33.5 g.) in absolute alcohol (1450 ml.) was added and the mixture left at room temperature for 10 minutes. After the addition of water (270 ml.), the suspension was boiled for 20 minutes and cooled in ice. Concentrated hydrochloric acid (97 ml.) was now added carefully and the mixture left at room temperature for 4 hours. A cooled solution of sodium methylate (40 g.) in methanol (400 ml.) was added, followed by 2 *N* sodium hydroxide until *p*H 10. After 10 minutes the solution was cooled and acidified with hydrochloric acid. On the addition of ice, a bulky white precipitate appeared which was filtered and extracted with ether. The thoroughly washed ethereal solution was concentrated to a white solid which was recrystallized from methanol, giving 48 g. of m.p. 82–86°.

*threo-***Methyl α-Benzamido-β-hydroxystearate (IVa).**—a. The acid (48 g.) was suspended in ether (480 ml.) and esterified by the addition of an ethereal solution of diazomethane. Evaporation of the solvent and recrystallization from methanol gave 41 g. of m.p. 80–84°.

Alternatively, the cooled ether solution gave a partial precipitation of the ester (29.5 g. of m.p. 87–88°, identical with an authentic specimen) and the filtrate, when evaporated and recrystallized from methanol, gave additional 14.5 g. of m.p. 81–83°.

b. To a stirred solution of V (23.1 g., see below) in dry chloroform (90 ml.) and pyridine (35 ml.) was added at 20°, over a period of 20 minutes, a solution of benzoyl chloride (8.8 ml.) in chloroform (25 ml.). The clear solution produced was stirred a further 3 hours at 20° and, after diluting with excess of ether, washed successively with cold hydrochloric acid, sodium bicarbonate solution and water. The dry organic layer was evaporated and the residue recrystallized from petroleum ether to give 28.0 g. (92%), m.p. 86–88°.

*cis-***2-Phenyl-4-carbomethoxy-5-pentadecyl-2-oxazoline (VI).**—The preceding dried ester (44 g.) was treated with thionyl chloride as described above for the epimeric mixture. After evaporation of the solvent the pasty residue was redissolved in a little chloroform and poured into a hand-stirred, cold, 10% solution of sodium carbonate (in excess). The separated solid was extracted with ether and the dry ether solution evaporated to an oil which was crystallized from methanol, giving 35 g. of a white solid of m.p. 42–44° (83%). A sample, recrystallized from methanol, melted at 43–45°.

Anal. Calcd. for $C_{26}H_{41}O_3N$: C, 75.13; H, 9.94; N, 3.37. Found: C, 75.38; H, 10.11; N, 3.36.

*cis-***2-Phenyl-4-hydroxymethyl-5-pentadecyl-2-oxazoline (VII).**—A stirred suspension of lithium aluminum hydride (2.6 g.) in dry ether (260 ml.) was boiled for 15 minutes and cooled in ice. To this was added, dropwise, over a period of 20 minutes, a solution of the ester VI (35 g.) in ether (220 ml.).

The mixture was stirred for a further 20 minutes at room temperature (20°), then boiled for 10 minutes. After cooling to 0°, ethyl acetate (10 ml.) was added dropwise, followed by the rapid addition of *N* hydrochloric acid (130 ml.) and 25% acetic acid (87.5 ml.). The bulky white precipitate was dissolved by adding more ether and a little chloroform and methanol to facilitate separation. The organic layer was washed successively with ice-water, sodium carbonate solution until *p*H 9, and again with water, and concentrated *in vacuo* to a white solid. Recrystallization from 10 parts of ethyl acetate and petroleum ether (1:1) gave 25 g. of m.p. 97–99°; infrared spectrum: 2.97 (OH) and 6.09 μ (oxazoline).

Anal. Calcd. for $C_{25}H_{41}O_2N$: C, 77.5; H, 10.7; N, 3.6. Found: C, 77.6; H, 10.3; N, 3.9.

*threo-α-***Acetamido-β-hydroxystearic Acid (IIIb).**—The crude acid was prepared from the mixture of the epimeric ethyl esters (70 g.), as described above for the benzamido derivative. It was recrystallized from methanol and ethyl acetate (1:4) and gave 52 g. (81%) of m.p. 132–134°. A second recrystallization gave a product of m.p. 135–136°.

Anal. Calcd. for $C_{20}H_{39}O_4N$: C, 67.19; H, 11.00; N, 3.92. Found: C, 67.00; H, 11.14; N, 4.14.

*threo-***Methyl α-Acetamido-β-hydroxystearate (IVb).**—A solution of IIIb (34.5 g.) in ether was esterified with diazomethane. The residue, after evaporation of the ether, was recrystallized from ethyl acetate, giving 33.8 g. of m.p. 88–90°. A sample for analysis, prepared by a second recrystallization from ethyl acetate, melted at 89–91°.

Anal. Calcd. for $C_{21}H_{41}O_4N$: C, 67.88; H, 11.12; N, 3.77. Found: C, 67.94; H, 10.80; N, 4.02

*threo-***Methyl α-Amino-β-hydroxystearate (V). Method A.**—Into a boiling solution of the acid IIIb (60 g.) in dry methanol (600 ml.) was passed a steady stream of dry hydrogen chloride for 4–6 hours. The resulting solution was concentrated *in vacuo* to a paste, and excess of cold sodium carbonate solution added. The precipitated amino-ester was extracted with ether and separated from the insoluble sodium *threo-α*-amino-β-hydroxystearate. The ether layer was washed and dried and the solvent evaporated. Recrystallization of the residue from petroleum ether gave 40 g. (73%) of m.p. 76–78° (identical with an authentic specimen[30]).

Method B.—The acetamidoester IVb was hydrolyzed by boiling under reflux for 2 hours with a 15% solution of hydrogen chloride in methanol. The acid was removed by evaporation and the residue treated with 10% sodium carbonate solution and extracted with ether. The residue obtained after evaporation of the dried solvent was recrystallized from petroleum ether to give a 90% yield of V.

(49) It was later found that acyl migration proceeds rather sluggishly in this series. A more detailed discussion of this observation will be included in the subsequent paper IV.

(50) E. Baer, THIS JOURNAL, **75**, 621 (1953).

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

threo-Methyl α-Hexadecanamido-β-hydroxystearate.—To a stirred solution of the amino-ester V (10 g.) in dry tetrahydrofuran (60 ml.) and pyridine (60 ml.), warmed to 40–45°, was added dropwise a solution of palmitoyl chloride (9 ml.) in tetrahydrofuran (25 ml.). The solution was maintained at 45–50° for 1 hour and then poured into hydrochloric acid (60 ml.) and crushed ice.

The white precipitate was extracted with ether, the ethereal layer washed with cold sodium carbonate solution and water, and dried. The residue, after evaporation of the ether, was crystallized from alcohol, giving 14.5 g. (84%) of m.p. 70–75°. Recrystallization from methanol gave 11.0 g., m.p. 75–77°.

Anal. Calcd. for $C_{35}H_{69}O_4N$: C, 74.02; H, 12.25; N, 2.47. Found: C, 74.19; H, 12.29; N, 2.7.

cis-2,5-Dipentadecyl-4-hydroxymethyl-2-oxazoline.—A solution of the palmitamido ester (17 g.) in dry chloroform (50 ml.) was added dropwise to thionyl chloride (60 ml.), stirred at 5°. The ice-bath was removed, and stirring was continued for 45 minutes. The temperature of the mixture was then slowly raised during 30 minutes and maintained for two hours at 38°. Evaporation of the solvent left a low-melting solid which was treated with excess of cold sodium carbonate solution and extracted with ether. The dry ethereal solution was evaporated to an oil (17 g.), presumably crude *cis*-2,5-dipentadecyl-4-hydroxymethyl-2-oxazoline.

This oil was dissolved in dry ether (170 ml.) and added dropwise over a period of 25 minutes to a cooled stirred suspension of lithium aluminum hydride (2.0 g.) in ether (150 ml.). After cooling for 1 hour further at 5°, ethyl acetate (5 ml.) was added dropwise, followed by cold water (50 ml.) and 0.5 *N* sodium hydroxide (50 ml.). The ethereal layer was washed and evaporated and the residue recrystallized from acetone, giving 11.2 g., m.p. 61–63°. Two more recrystallizations from acetone raised the m.p. to 63–64°; infrared spectrum: 2.9 (OH) and 6.01 μ (oxazoline).

Anal. Calcd. for $C_{34}H_{67}O_2N$: C, 78.3; H, 12.9; N, 2.7. Found: C, 77.3; H, 12.96; N, 2.98.

3-O-Benzoxy-1-phenylphosphoryldihydrosphingosine (XIVa).—To a stirred mixture of phenylphosphoryl dichloride (1 ml., 0.0066 mole), pyridine (0.6 ml., 0.0066 mole) and dry chloroform[52] (10 ml.) which had been prepared in the cold, was added over a period of 15 minutes at 5–8° a solution of the oxazoline VII (2.6 g., 0.0066 mole) in chloroform (20 ml.). The temperature was allowed to rise slowly to 25–30°, and stirring was continued at this temperature for 3 hours. The solvent was then evaporated *in vacuo*, dry ether (200 ml.) was added, and the solution was cooled for two hours in an ice-bath. The separated pyridine hydrochloride together with a small amount of unchanged starting material was filtered off. A sample of the filtrate was evaporated, and the remaining oil showed a strong band at 1558 cm.$^{-1}$ (–NH). The rest of the filtrate was poured into cold water (100 ml.) and stirred for one hour at room temperature and then for two hours with reflux of the ether. The ethereal layer was washed and evaporated to dryness. The oil (2.95 g.) contained benzoate and amide in an approximate ratio of 7:3. To assure complete hydrolysis of the chloroamide, the oil was dissolved in a mixture of dioxane (15 ml.), 2 *N* hydrochloric acid (2 ml.) and water (4 ml.), and the solution refluxed for one hour. After cooling overnight in the refrigerator, a sticky solid (0.9 g.) was collected and crystallized from 90% ethanol; m.p. 145–147°; infrared spectrum: 5.82 μ.

Anal. Calcd. for $C_{31}H_{48}NO_6P$: C, 66.22; H, 8.6; N, 2.5; P, 5.5. Found: C, 66.20; H, 8.45; N, 2.55; P, 5.57.

N-Benzoyl-1-phenylphosphoryldihydrosphingosine (XVa).—The oil obtained in the preceding experiment after evaporation of the ether was stirred with 0.1 *N* sodium hydroxide (15–20 parts) at 50–55° until it dissolved (1–2 hours). After acidification of the cooled solution, the precipitate was taken up with ether. The solvent was evaporated and the semi-solid which remained was crystallized 2–3 times from methanol, m.p. 93–95°; infrared spectrum: 6.09 and 6.46 μ (amide).

Anal. Calcd. for $C_{31}H_{48}NO_6P$: C, 66.22; H, 8.6; N, 2.5. Found: C, 66.62; H, 9.16; N, 2.96.

(52) The chloroform used throughout this investigation was distilled over phosphorus pentoxide and stored in a dark bottle

Benzoyldihydrosphingomyelin (XXIa).—Phosphorylation was carried out as above, except that two moles of β-chloroethylphosphoryl dichloride (2.6 g.) were used for one mole of the oxazoline (2.6 g.). Here, too, the infrared spectrum showed that the reaction product was predominantly an amide. After treatment with water, the isolated oil gave occasionally too high chlorine values, indicating that the amide XIIIb had not been completely converted to the ester XIVb. To achieve this, the oil was dissolved in dioxane (90 ml.) and *N* hydrochloric acid (10 ml.), and the solution warmed for one hour at 60°. The ethereal solution of the ester was added to an excess of 0.3 *N* barium hydroxide solution, and the mixture stirred for one hour. The ethereal layer which contained the barium salt was washed until neutral and evaporated, leaving a thick yellowish oil (2.7 g.). Its spectrum still showed the presence of a considerable amount of ester.[49] The completion of the acyl migration was effected during the subsequent treatment with trimethylamine. A mixture of the barium salt (2.3 g.), benzene (6 ml.) and trimethylamine (5 ml.) was warmed in a sealed tube for 4 days at 60°. The solvent and excess of trimethylamine were distilled off *in vacuo*, the semi-solid residue dissolved in methanol (35 ml.), and the solution allowed to stand overnight at room temperature. The clear solution was decanted from a sticky solid and concentrated *in vacuo* to dryness. The residue was refluxed for one hour with dioxane (10 ml.), water (5 ml.) and *N* hydrochloric acid (3 ml.). The solid which separated on cooling was crystallized from 55% dioxane and gave 0.6 g. of a hygroscopic solid. It was dissolved in 95% methanol (100 ml.) and the solution was passed first over a column of Amberlite IRC-50 and then over IRA-400. After evaporation of the effluent, the residue was crystallized from acetone and a few drops of methanol; m.p. 210–213° (sintered at 80° and turned glassy at 140°); infrared spectrum (chloroform): 3.05, 3.42, 6.09, 6.35, 6.81, 7.49, 8.08, 9.20, 9.46, 10.34 and 10.88 μ.

Anal. Calcd. for $C_{30}H_{57}N_2O_7P$: C, 61.2; H, 9.76; N, 4.7; P, 5.27. Found: C, 60.84; H, 9.99; N, 5.03; P, 4.93.

Confirmation of the *erythro* Configuration of Benzoyldihydrosphingomyelin (XXIa).—A solution of XXIa (0.6 g.) in dry tetrahydrofuran (15 ml.) was added over a period of 15 minutes to a suspension of lithium aluminum hydride (0.6 g.) in dry ether (20 ml.) and the mixture refluxed for 90 minutes. After cooling, the excess of the lithium compound was decomposed by ethyl acetate (5 ml.), and ice-water (5 ml.) was added, followed by 5% sodium hydroxide solution (50 ml.). The suspension was extracted with ether, the ether solution washed, dried and evaporated. The residue (0.55 g.) was crystallized from petroleum ether and yielded 0.275 g. of a product melting at 61–63°. A mixed melting point with an authentic sample of *erythro*-N-benzyl-dihydrosphingosine was not depressed.

cis-2-Phenyl-4-(β-chloroethylphosphorylmethyl)-5-pentadecyl-2-oxazoline (Barium Salt, XVIb).—A solution of the oxazoline VII (10.4 g., 0.0264 mole) in dry chloroform (80 ml.) was added in a thin stream to a mixture of β-chloroethylphosphoryl dichloride[53] (15.6 g., 0.08 mole) in chloroform (45 ml.) and pyridine (3.6 ml., 0.04 mole), stirred at −10°. The temperature was then allowed to rise slowly to 10–12°, and stirring was continued at this temperature for 5–6 hours. After leaving the clear solution for 12 hours in the refrigerator, cold dry ether (600 ml.) was added slowly with stirring, while care was taken that the temperature did not rise above 10°. The mixture was cooled for two hours to complete the separation of pyridine hydrochloride. The latter was filtered off and the filtrate was transferred into a dropping funnel. These operations were carried out under exclusion of moisture to prevent partial hydrolysis of the oxazoline ring. The ether–chloroform solution was then added to an excess of 0.3 *N* barium hydroxide solution (420 ml.) and the mixture stirred for 60–90 minutes at room temperature. The upper layer was separated as completely as possible, washed with water until neutral and cooled in the refrigerator for 24 hours. The precipitate was filtered and dried over phosphorus pentoxide. The barium salt (4.6 g.) was recrystallized from ethyl acetate and melted at 143–45°; infrared spectrum: 6.08 μ (oxazoline).

(53) R. R. Renshaw and C. Y. Hopkins, THIS JOURNAL, **51**, 953 (1929).

Anal. Calcd. for $C_{54}H_{88}Cl_2N_2O_{10}P_2Ba$: C, 54.2; H, 7.4; Cl, 5.94; N, 2.4; P, 5.2; Ba, 11.5. Found: C, 53.95; H, 7.41; Cl, 6.07; N, 2.58; P, 5.1; Ba, 11.6.

3-O-Benzoyl-1-β-chloroethylphosphoryldihydrosphingosine (XIVb).—The above barium salt (1.2 g.) was dissolved in dioxane (20 ml.) and *N* hydrochloric acid (3.9 ml.) was added, followed by water (1 ml.) to dissolve the precipitated barium chloride. The solution was refluxed for 4 hours, cooled and poured into water. The ether extract was washed to *p*H 4 and evaporated *in vacuo* to dryness. The residue was taken up twice with chloroform to remove azeotropically the last traces of moisture. The remaining sticky sirup (1 g.) was used for the subsequent acylations (Cl calcd. 6.4, found 6.35); infrared spectrum: 5.80 μ (ester) and a weak band at 6.12 μ.

Acylation of XIVb.—a. To a cold solution of freshly distilled acid chloride (1.2 ml.) in dry chloroform (8 ml.) was added a mixture of pyridine (0.36 ml.) and chloroform (2 ml.). After stirring for 15 minutes, a solution of the benzoxy ester (1 g.) in chloroform (10 ml.) was added at +10°, and the mixture was allowed to stand in the refrigerator (+5–8°) for 15 hours. The clear solution was poured into cold water, shaken twice with cold 2 *N* hydrochloric acid, and the lower layer washed with water to *p*H 5. To the cooled chloroform solution, ether (100 ml.) was added, and the mixture stirred for 30 minutes with a slight excess of barium hydroxide solution. The heavy white precipitate (1.9 g.) was collected, dried and extracted with hot ethyl acetate. The clear filtrate was evaporated *in vacuo* to a volume of about 5–6 ml., and the barium salt crystallized almost immediately after the addition of methanol (10 ml.). Occasionally, the barium salt failed to precipitate completely from the ether–chloroform layer. The latter was then washed with water, evaporated to dryness, and the remaining semi-solid was recrystallized from ethyl acetate and methanol (1:1). For the preparation of the free phosphoric acid, the barium salt was dissolved in ether and a little chloroform, the solution shaken several times with cold *N* hydrochloric acid until no more barium ions were present, washed to neutral and evaporated *in vacuo*. Yields of 65–75% were obtained.

N-Palmitoyl-3-O-benzoxy-1,-β-chloroethylphosphoryldihydrosphingosine (XIXb, free acid) crystallized from methanol or hexane; m.p. 98–99°; infrared spectrum: 5.8 (ester) and 6.0 μ (aliphatic amide).

Anal. Calcd. for $C_{43}H_{77}ClNO_7P$: C, 65.6; H, 9.86; Cl, 4.5; N, 1.78; P, 3.94. Found: C, 65.75; H, 9.8; Cl, 4.58; N, 1.93; P, 3.6.

N-Stearoyl-3-O-benzoxy-1-β-chloroethylphosphoryldihydrosphingosine (XIXc, free acid) crystallized from 4 parts of ethyl acetate and melted sharply at 96–97.5°.

Anal. Calcd. for $C_{45}H_{81}ClNO_7P$: C, 66.33; H, 10.03; Cl, 4.3; N, 1.7; P, 3.8. Found: C, 66.38; H, 10.45; Cl, 4.0; N, 1.82; P, 4.04.

b. Alternatively the acylation was carried out as follows: To the acid dioxane solution which resulted from hydrolysis of the barium salt (1.2 g.), sodium acetate (2.5 g.) was added, followed by palmitoyl chloride (0.5 ml.). After stirring for one hour, another portion of the acid chloride (0.5 ml.) was added and stirring was continued at room temperature for two hours. The suspension was allowed to stand overnight, extracted with ether, the ether solution washed and stirred with an excess of barium hydroxide solution. After working up the barium salt as above, only 0.35 g. of the free acid of m.p. 98–99° was obtained.

Preparation of the Barium Salts XIX from the Pure Acids.—The acids were dissolved in chloroform (20 volumes), methanol (5 volumes) added, and the solution stirred for 30 minutes with an excess of 0.3 *N* barium hydroxide. After addition of water, the chloroform layer was washed, dried and evaporated *in vacuo*. For further purification the barium salt was dissolved in ethyl acetate (4 volumes) and to the warm solution (35–40°), methanol (5 volumes) was added. Crystallization occurred in a few minutes.

N-Palmitoyl-3-O-benzoxydihydrosphingosinephosphorylcholine Chloride (XXb).—Quaternization with trimethylamine was carried out as described for XVII. The viscous benzene solution was separated from a small heavy precipitate by centrifugation, and the supernatant evaporated *in vacuo* at 30–35°. The residue was dissolved in methanol (12 vol.) with slight warming, and to the cooled solution 2 *N* methanolic hydrochloric acid (2 vol.) was added. The mixture was poured into cold *N* aqueous hydrochloric acid (20 vol.), extracted with ether and the upper layer washed once with a little water, then with 50% methanol to *p*H 4–5. Evaporation of the solvent *in vacuo* was carried out at the lowest possible temperature, and the residue was repeatedly distilled with small portions of isopropyl alcohol to remove the remaining water. After crystallization from acetone and a little methanol the chloride was obtained in a 70% yield, m.p. 170–175°. To remove completely the inorganic cations, a sample was dissolved in methanol, methanolic hydrochloric acid added, followed by water, and finally by acetone. The flocculent precipitate which separated on cooling was filtered and dried over phosphorus pentoxide.

Anal. Calcd. for $C_{46}H_{86}ClN_2O_7P$: C, 65.3; H, 10.3; N, 3.3; P, 3.67. Found: C, 65.39; H, 10.43; N, 3.14; P, 3.77.

N-Stearoyl-3-O-benzoxydihydrosphingosinephosphorylcholine Chloride (XXc) was obtained similarly in an 80% yield as heavy hygroscopic crystals from acetone–methanol (9:1), m.p. 170–172°.

Anal. Calcd. for $C_{48}H_{90}ClN_2O_7P$: N, 3.2; P, 3.55. Found: N, 3.35; P, 3.77.

Palmitoyldihydrosphingomyelin (XXIb).—The amidoester XXb (1 g.) was dissolved in methanol (22 ml.), *N* sodium hydroxide solution (2.2 ml.) was added, and the mixture allowed to stand at 30° for four hours. The gelatinous suspension was cooled, and 2 *N* methanolic hydrochloric acid (6 ml.) added followed by acetone (100 ml.). After cooling for 2 hours the precipitate was filtered and dissolved in methanol (20 ml.). To the clear filtrate was added *N* methanolic hydrochloric acid (3 ml.) and then acetone (40 ml.). The precipitate was collected and weighed 0.8 g. The product was dissolved in methanol (100 ml.), the solution filtered over a little silicic acid and, after addition of water (5 ml.), passed over a column of Amberlite IRA-45. The solvent was distilled off *in vacuo*, and the gelatinous precipitate which appeared during the evaporation was dissolved by repeated addition of acetone. The dry product was recrystallized from acetone and a little methanol and yielded 0.6 g. of a hygroscopic powder. After recrystallization from butyl acetate it melted sharply at 210–212° (previous sintering at 110–115°, transparent at 200–205°); infrared spectrum (Nujol): 2.95, 3.12, 3.45, 3.51, 6.09, 6.41, 6.73, 6.95, 8.13, 9.43, 10.31, 10.88, 11.17, 12.05 and 13.83 μ.

Anal. Calcd. for $C_{39}H_{83}N_2O_7P$: C, 64.7; H, 11.58; N, 3.87; P, 4.29. Found: C, 64.1; H, 11.65; N, 3.83; P, 4.17.

Stearoyldihydrosphingomyelin, prepared by the same procedure in similar yields, was recrystallized from butyl acetate and melted at 213–215° (with previous sintering at 190°); infrared spectrum: 2.94, 3.12, 3.43, 3.51, 6.10, 6.41, 6.73, 6.95, 8.13, 9.08, 9.43, 10.28, 10.77, 11.41, 12.02 and 13.94 μ.

Anal. Calcd. for $C_{41}H_{87}N_2O_7P$: C, 65.55; H, 11.67; N, 3.72; P, 4.12. Found: C, 65.97; H, 12.05; N, 3.89; P, 4.18.

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