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

Synthetic Studies on Sphingolipids. IV. The Synthesis of Sphingomyelin

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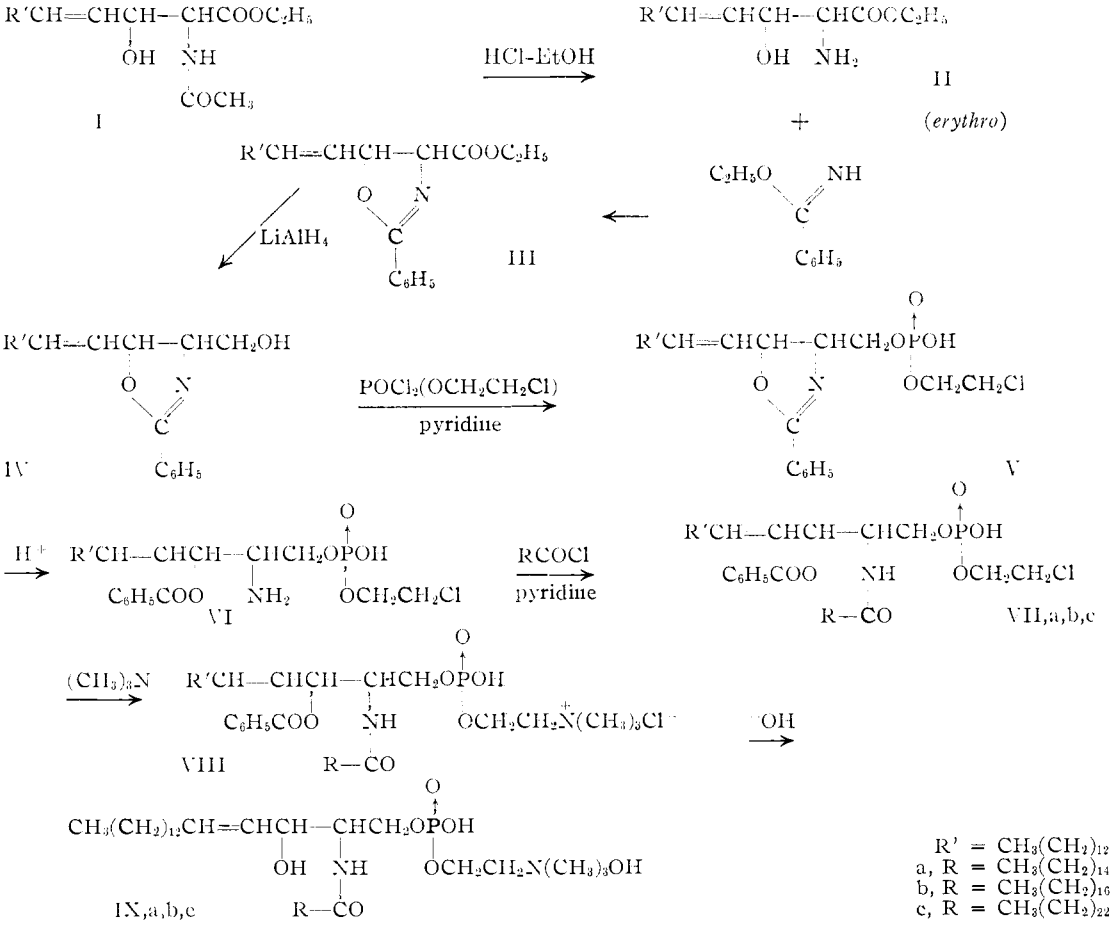
The synthesis of palmitoyl-, stearoyl- and lignoceroyl-sphingomyelin (IX,a,b,c) is described. *cis*-2-Phenyl-4-hydroxymethyl-5-(1-pentadecenyl)-2-oxazoline (IV) is phosphorylated with β-chloroethylphosphoryl dichloride. Hydrolysis of V and acylation of the amino ester VI lead to the amide VII. Quaternization of the latter with trimethylamine followed by treatment with alkali affords the sphingomyelins IX.

In a previous communication[1] we reported the synthesis of palmitoyl-, stearoyl- and, as a model compound, benzoyl-dihydrosphingomyelin. As key intermediate we employed the dihydro derivative of the *cis*-oxazoline III (CH_3 instead of C_2H_5) which we obtained by cyclization of methyl *threo*-α-benzamido-β-hydroxystearate with thionyl chloride. We now wish to describe the synthesis of the more common unsaturated lipids of this series by a similar procedure.

REACTION SCHEME



One of the chief obstacles which we encountered in the application of the previous route to the synthesis of the sphingomyelins lay in obtaining the pure *threo* isomer of I necessary for the preparation of the *cis*-oxazoline III. Although, as was earlier shown, the pure *erythro* form of I may be obtained in a fairly good yield by fractional crystallization,[2] we have not been able to isolate an appreciable amount of its diastereomer from the mother liquors. Likewise, attempts to convert the mixture into the pure *threo* epimer *via* the oxazolines[1] were not successful, an indefinite product being obtained after repeated crystallization. An explanation for this result may lay in the behavior of the chlorosulfinate esters which presumably are formed as intermediates by action of thionyl chloride on I. It is well known that polar factors facilitate the decomposition of chlorosulfinates to alkyl chlorides in compounds having the ester group in the benzylic[3] or the allylic[4] position.

(1) D. Shapiro, H. M. Flowers and S. Spector-Shefer, THIS JOURNAL, **81**, 3743 (1959).

(2) D. Shapiro, H. Segal and H. M. Flowers, *ibid.*, **80**, 1194 (1958).

(3) C. K. Ingold in "Structure and Mechanism in Organic Chemistry," Bell and Sons, London, 1953, p. 392.

(4) J. Meisenheimer and J. Link, *Ann.*, **479**, 211 (1930).

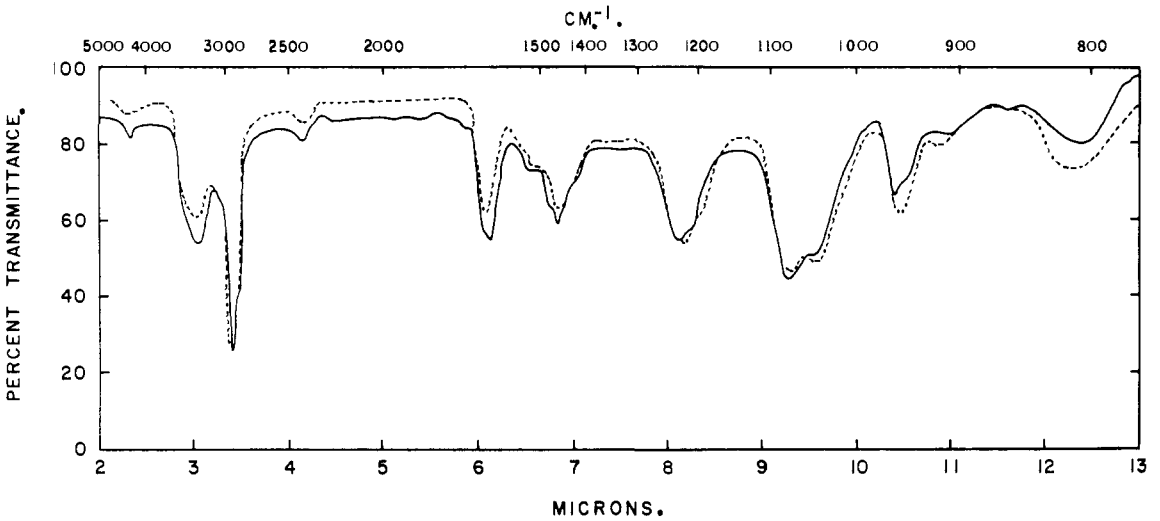


Fig. 1.—Infrared spectra of N-stearoyl-sphingomyelin (IXb) (dashed line) and dihydro-sphingomyelin (solid line) in a 5% chloroform solution; cell thickness 0.01 mm. The absorption peaks are: sphingomyelin, 3.01, 3.42, 3.51, 6.08, 6.46, 6.84, 8.16, 9.25, 9.48, 10.26, 10.88 and 12.22 μ; dihydrosphingomyelin, 3.01, 3.42, 3.51, 6.08, 6.45, 6.84, 8.08, 9.23, 9.46, 10.26, 10.88 and 12.28 μ.

Thus, Sicher[5] was able to show that the *p*-nitrobenzamido derivative of nor-ψ-ephedrine reacts with thionyl chloride to give a mixture of *erythro*- and *threo*-1-phenyl-1-chloro-2-*p*-nitrobenzamidopropane rather than the corresponding oxazoline. The reaction is being investigated further.

In a recent paper[2] we described the hydrolysis of the pure *erythro* epimer of I to the hydrochloride of II by a rather tedious procedure. We have now found that the latter could be more advantageously prepared by alcoholysis of the original crude mixture of diastereomers resulting from the reduction of the corresponding keto-ester. The reaction proceeds rather fast, and it would appear that it does not involve an appreciable N→O acyl migration[6-8] since the *erythro* form of II was obtained in a comparatively high yield. Furthermore, neither of the epimeric acetamido esters was recovered from the mother liquor when treated with alkali. Similar results with even higher yields were achieved with the corresponding saturated esters, which we prepared earlier by a different procedure.

Condensation of II with ethyl iminobenzoate followed by careful treatment with lithium aluminum hydride afforded a 60% yield of the required 4-hydroxymethyl-oxazoline IV.

The course of the phosphorylation of IV with β-chloroethylphosphoryl dichloride in the presence of pyridine was followed in various experiments by infrared spectroscopic measurements, and it was found that 5–6 hours at $-5°$ was sufficient to ensure complete reaction. It was also found that the addition of ether used in the previous method to separate the pyridine hydrochloride reduced the yield considerably. Instead, the reaction product was immediately treated with a cold solution of barium hydroxide, and the phosphate ester V was isolated as barium salt in consistent yields of 40–50%.

In the course of the investigation we observed a difference in stability between V and the corresponding saturated phosphate. Whereas the barium salt of the latter, when treated with cold diluted hydrochloric acid, quickly suffered scission of the oxazoline ring, under the same conditions V precipitated in excellent yield and hydrolyzed to VI after prolonged reaction. The presence of the strongly electrophilic alkylphosphate group at position 4 might affect the stability of the oxazoline ring, and, in fact, IV is quite stable to cold hydrochloric acid.[9] However, the different relative rate of hydrolysis of V and its saturated counterpart must be attributed to the allylic system, although it is not evident whether it is due to a polar or steric effect. We utilized this observation as a welcome tool for separation of small amounts of saturated phosphate occasionally present as impurity. It arose from the dihydro derivative of IV probably produced during the reduction of III with lithium aluminum hydride. It is noteworthy that V suffers a similar ring opening by mere warming with organic solvents, such as dioxane or ethyl acetate.

As indicated in part III of this series, acyl migration in compounds of type VI takes place rather slowly. In various experiments it was observed that even after prolonged treatment with barium hydroxide at *p*H 9, a considerable quantity of ester remains unchanged. It was, therefore, not surprising that blank experiments with chloroform solutions of VI and pyridine, having a *p*H of approximately 7.3, did not show any migration after 10–15 hours. This result enabled us to acylate VI in good yields in the presence of an excess of pyridine. Under these conditions, however, a by-product always appeared which was recognized

(5) I. Sicher and M. Pánková, *Collect. Czechoslov. Chem. Communs.*, **20**, 1409 (1955).

(6) L. H. Welsh, THIS JOURNAL, **69**, 128 (1947).

(7) E. Van Tamelen, *ibid.*, **73**, 5773 (1951).

(8) G. Fodor and J. Kiss, *J. Chem. Soc.*, 1589 (1952).

(9) A scission of the oxazoline ring under "physiological" conditions in the presence of diisopropyl phosphofluoridate has been recently reported by Rydon, *et al.*, *Nature*, **182**, 927 (1958).

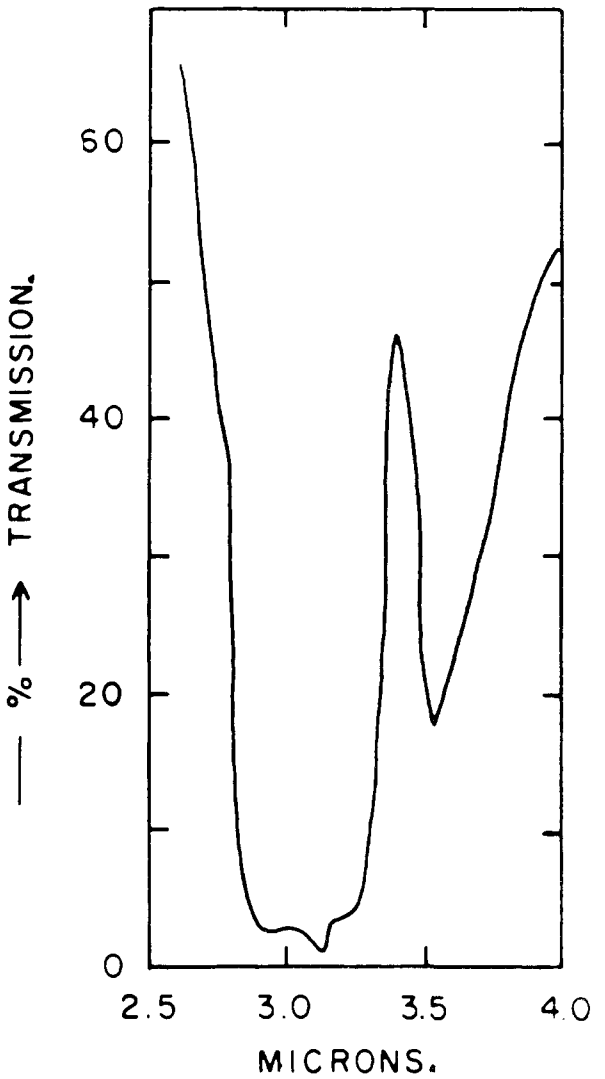


Fig. 2.—N-Stearoyl-sphingomyelin in Nujol. The absorption peaks are: 2.94, 3.10, 3.3 and 3.5 μ.

as the respective aliphatic acid anhydride whose formation is due to the acylating properties of the excess of acyl pyridinium chloride.[10] Their separation was facilitated by the extreme insolubility in methanol to which they are remarkably stable. To verify the course of the synthesis, the palmitoyl derivative VIIa was reduced catalytically in the presence of 5% palladium–charcoal at 10–15°, and the product obtained in good yield proved to be identical with the corresponding saturated amide.

The continuation of the synthesis proceeded essentially as described for dihydrosphingomyelin, except for the difficulty in isolating the choline chlorides VIII in a pure form. The allylic ester was extremely readily saponified by treatment with 0.05 *N* sodium hydroxide.

The sphingomyelins (IX) thus prepared form high melting, somewhat hygroscopic products whose infrared spectra are essentially identical with those of the natural lipids.[11] In accordance with the findings of Baer[12] in the field of lecithins the analytical values agree with the "hydrated" rather than with the "anhydrous" zwitterionic structure. Support for this view came from a closer examination of the infrared spectra.[13] Mulled in Nujol, all three sphingomyelins showed bands at about 2.94 and 3.1 μ. While the former was clearly due to alcoholic, hydrogen-bonded OH, the latter was assumed to be due to an ionic, hydrogen-bonded OH group. For comparison, the spectra of choline and trimethylbenzylammonium hydroxide were also measured and their OH absorptions were found to be at 3.12 and 3.13 μ, respectively, thus indicating the presence of an ionic OH group in the sphingomyelins under these conditions. The relatively long wave length of this OH group indicates very strong hydrogen bonds probably resulting from the free charge on the oxygen atom of the hydroxyl which makes it a good acceptor for the proton. A shoulder, at 3.3 μ, assigned to POH (acidic absorption) would seem further to confirm the hydrated form. In contrast, the chloroform solutions of the sphingomyelins (and dihydrosphingomyelins) show only one strong band at 3.0μ. The absence of the band at 3.1μ in solution seems to show that here the zwitterionic structure is dominant; the absorption at 3.0μ is probably due to the molecule of water librated during its formation.[14]

Experimental[15]

Ethyl *erythro*-2-Amino-3-hydroxy-4-octadecenoate (II).—A solution of I (38 g.) in 15% absolute alcoholic hydrochloric acid (300 ml.) was boiled under reflux for 1.5 hours. After evaporation of the solvent, excess of dry ether was added to the cooled paste, the precipitate filtered and washed thoroughly with dry ether. A single recrystallization from ethyl acetate gave 20.1 g. of m.p. 110–112°, identical with an authentic sample. The ester hydrochloride thus obtained was hand stirred with a mixture of ether and an excess of 10% sodium carbonate solution to which a little methanol had been added. After a few minutes, the liberated amino-ester dissolved completely in the ether layer which was washed and concentrated to a low-melting solid. When recrystallized from *n*-hexane, it gave 13.2 g. (41%, based on I) of m.p. 63–65°. A second crystallization raised the m.p. to 64–65°.

Anal. Calcd. for $C_{20}H_{39}O_3N$: C, 70.33; H, 11.51; N, 4.10. Found: C, 70.68; H, 11.65; N, 4.26.

***cis*-2-Phenyl-4-hydroxymethyl-5-(1-pentadecenyl)-2-oxazoline (IV).**—A solution of II (13 g.) and ethyl iminobenzoate hydrochloride (9 g.) in dry chloroform (100 ml.) was boiled under reflux for 3 hours. After a few minutes, ammonium chloride began to precipitate. To the cooled mixture, dry ether (200 ml.) was added, and the precipitate removed by filtration. The filtrate was evaporated and the crude ester III dissolved in dry ether and reduced directly with lithium aluminum hydride, as previously described for the corresponding saturated compound. Crystallization from 5 parts of ethyl acetate gave 8.7 g. (59%) of m.p. 98–99°, which remained constant on further crystallization; infrared spectrum: 3.0, 6.09 and 10.29 μ.

Anal. Calcd. for $C_{25}H_{39}O_2N$: C, 77.87; H, 10.20; N, 3.63. Found: C, 77.90; H, 10.50; N, 3.75.

***cis*-2-Phenyl-4-(β-chloroethylphosphorylmethyl)-5-(1-pentadecenyl)-2-oxazoline (V, Barium Salt).**—A solution

(10) H. E. Baumgarten, THIS JOURNAL, **75**, 1239 (1953).

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

(12) E. Baer, *ibid.*, **75**, 621 (953).

(13) In collaboration with Dr. S. Pinchas of this Institute.

(14) R. N. Jones and C. Sandorff in "Chemical Applications of Spectroscopy," W. West, ed., Interscience Publishers, Inc., New York, N. Y., p. 430.

(15) Analyses were carried out in the Institute's microanalytical laboratory under the direction of Mr. Erich Meier; infrared spectra were measured in chloroform, unless otherwise stated.

of IV (7.8 g.) in dry chloroform (78 ml.) was added during 4–5 minutes at −10 to −15° to a mixture of β-chloroethylphosphoryl dichloride[16] (7.2 ml.) and chloroform (36 ml.) to which a cold solution of pyridine (2.7 ml.) in chloroform (27 ml.) had been added. After stirring for five hours at −5°, the clear pale-yellow solution was transferred by suction, with exclusion of moisture, into a well-stirred 5% barium hydroxide solution (780 ml.) cooled to 10°. Stirring was continued for 1 hour during which time the temperature was allowed to rise slowly to 15–18°. The mixture was then cooled, cold ether (600 ml.) was added at 10° in a thin stream, and the formation of the barium salt was completed by further stirring for one hour at room temperature (20–23°). The presence of an excess of alkali is essential for obtaining a good yield and was controlled by addition of phenolphthalein to the reaction mixture. The organic layer, in which part of the barium was suspended as a white precipitate, was washed with cold water and left overnight at 5°. Filtration gave 6 g. (50%) of a white solid after drying over phosphorus pentoxide. A sample, when dissolved in ethyl acetate with slight warming, crystallized on cooling as a sticky solid; infrared spectrum: 10.35 μ (P → O and *trans*-ethylenic bond)[11] and 6.09 μ (oxazoline).

Anal. Calcd. for $C_{54}H_{86}BaCl_2N_2O_{10}P_2$: C, 54.3; H, 7.3; N, 2.3; P, 5.2; Cl, 5.9; Ba, 11.5. Found: C, 54.1; H, 7.3; N, 2.3; P, 4.7; Cl, 5.7; Ba, 11.4.

Preparation of the Phosphoric Acid V from the Barium Salt.—A solution of the barium salt (2 g.) in chloroform (10 ml.) was added to a stirred ice-cold mixture of ether (50 ml.) and 2 *N* hydrochloric acid (20 ml.). After stirring at 0° for 20 minutes, the precipitate was filtered immediately and washed successively with ether and cold water until the filtrate was free of chloride. The crystalline alkylphosphoric acid thus obtained (1.6–1.7 g.) melted at 103–105°, and showed the band at 6.09 μ due to the oxazoline ring. In hot organic solvents the acid suffers rupture of the ring with formation of VI, and could not, therefore, be recrystallized from ethyl acetate or dioxane in which it dissolved readily on warming.

3-O-Benzoxy-1-β-chloroethylphosphorylsphingosine (VI).—Opening of the oxazoline ring of the above phosphate by heating with organic solvents was accompanied by losses due to partial decomposition of the molecule; the following method of hydrolysis gave more satisfactory results.

The product of m.p. 103–105° (2.4 g.) was dissolved with slight warming in dioxane (36 ml.) and 2 *N* hydrochloric acid (4 ml.), and the solution left overnight at 20°. The addition of ice and water precipitated a white solid which was extracted with ether. The thoroughly washed ether solution was evaporated under reduced pressure to a sticky viscous mass (2.2 g.), which was dried by azeotropic distillation with chloroform (*in vacuo*) at a temperature not exceeding 40–45°; infrared spectrum: main band at 5.80 μ, due to the ester carbonyl group; no absorption at 6.1 μ (oxazoline).

Acylation of VI. a. N-Palmitoyl-3-O-benzoxy-1-β-chloroethylphosphorylsphingosine (VIIa).—To a mixture of the preceding ester (2.0 g.), chloroform (20 ml.) and pyridine (5 ml.), prepared at 5°, was added dropwise, with stirring, a solution of palmitoyl chloride (2.1 ml.) in chloroform (15 ml.). The clear solution was allowed to stand overnight at 5° and poured into a mixture of concentrated hydrochloric acid (5 ml.) and ice-water (50 ml.). The chloroform was washed twice with cold *N* hydrochloric acid, then with water until neutral, filtered and evaporated under reduced pressure to dryness. When the waxy solid thus obtained was warmed with methanol (40 ml.), a small quantity (100–200 mg.) of an insoluble product remained behind whose infrared spectrum was indicative of a fatty acid anhydride. Alternatively, the same by-product crystallized rapidly in fine needles when the methanol solution was allowed to stand at room temperature for 10–20 minutes. The filtrate, or the supernatant, was cooled to 5°, the precipitate filtered and washed with a little cold methanol. The crude product was purified through the barium salt as follows. It was dissolved in dioxane (30 ml.) and ether (70 ml.), and the solution stirred for 30 minutes with a slight excess of 5% barium hydroxide solution in the presence of phenolphthalein. Water was then added, the upper layer washed, filtered from a slight precipitate and evaporated *in vacuo*. The residue crystallized from methanol at 5°, giving 1.8 g., m.p. 89–93°. This was dissolved in ether and a little chloroform, and the solution shaken several times with *N* hydrochloric acid to remove completely the barium ions. The thoroughly washed solvent was evaporated *in vacuo*, and the residue was recrystallized from methanol. The free acid melted at 84–87°; infrared spectrum: main bands at 5.82, 6.08 and 10.4 μ.

Anal. Calcd. for $C_{43}H_{75}ClNO_7P$: C, 65.88; H, 9.65; Cl, 4.5; N, 1.78; P, 3.95. Found: C, 65.54; H, 9.54; Cl, 4.69; N, 1.91; P, 3.75.

b. N-Stearoyl-3-O-benzoxy-1-β-chloroethylphosphorylsphingosine (VIIb) was prepared similarly and isolated as barium salt, which was recrystallized from methanol and a little ethyl acetate; m.p. 86–89°, yield: 55%; infrared spectrum: main bands at 5.85, 6.08 and 10.4 μ.

Anal. Calcd. for $C_{90}H_{158}BaCl_2N_2O_{14}P_2$: C, 61.77; H, 8.98; Ba, 7.78; Cl, 4.02; N, 1.6; P, 3.52. Found: C, 61.44; H, 8.80; Ba, 7.43; Cl, 4.08; N, 1.65; P, 3.44.

c. N-Lignoceroyl-3-O-benzoxy-1-β-chloroethylphosphorylsphingosine (VIIc) was prepared from 1.65 g. of ester VI and 1.95 g. of lignoceroyl chloride. After treatment of the slurry with hydrochloric acid, the chloroform was evaporated to about 15–20 ml. and cooled at 5° for 1–2 hours. The crystalline product of m.p. 78–80° (lignoceric anhydride, 0.8 g.) was washed with a little cold chloroform, and the filtrate evaporated under reduced pressure to dryness. The remaining semi-solid (2.5 g.) was dissolved in warm methanol (100 ml.) and the solution decanted from an insoluble oil (0.1 g.). After standing at 24°, a small portion of crystals separated which were filtered off by gravity. The filtrate was allowed to stand overnight at 15–20°, the crude product (1.65 g.) of m.p. 70° was dissolved in warm ether (50 ml.) and a few ml. chloroform, and treated with 5% barium hydroxide solution. The barium salt (1.55 g.), obtained after crystallization from a mixture of ethyl acetate (12 ml.) and methanol (8 ml.), was treated as above with hydrochloric acid, and the free acid crystallized at room temperature from methanol; m.p. 70–73°; infrared spectrum: 5.80, 6.08 and 10.4 μ.

Anal. Calcd. for $C_{51}H_{91}ClNO_7P$: C, 68.25; H, 10.15; Cl, 3.93; N, 1.6; P, 3.45. Found: C, 68.57; H, 10.12; Cl, 3.86; N, 1.61; P, 3.10.

N-Palmitoyl-sphingomyelin (IXa).—The pure barium salt of VIIa (1.6 g.) dissolved in dry benzene was treated with an excess of trimethylamine as described earlier for the saturated derivative. The solvent was evaporated *in vacuo*, the residue dissolved in warm methanol (40 ml.) and the solution allowed to stand at room temperature for two hours. A small precipitate was removed, and the filtrate treated for four hours with 2 *N* sodium hydroxide solution (2 ml.). To the gelatinous mass was added *N* methanolic hydrochloric acid (5 ml.), followed by 2 *N* aqueous hydrochloric acid (5 ml.) and acetone (100 ml.). After cooling for 30 minutes, the precipitate was filtered and washed with a mixture of equal volumes of 70% methanol and acetone. To remove completely the barium ions, it was redissolved in methanol (30 ml.) and treated again with hydrochloric acid and acetone. The product thus obtained weighed 0.9 g. after drying over phosphorus pentoxide, and showed no band at 5.8 μ (ester carbonyl). It was dissolved in methanol (100 ml.), the solution filtered and, after addition of distilled water (5 ml.), passed over a column of Amberlite-IRA-45. After evaporation of the solvent, the dry residue (0.7–0.8 g.) was recrystallized first from methanol and acetone and then from butyl acetate giving a slightly hygroscopic powder of m.p. 209–211° (with previous sintering); infrared spectrum: 3.03, 3.42, 3.50, 6.08, 6.43, 6.82, 8.11, 9.18, 9.46, 10.36, 10.88, 12.01 μ.

Anal. Calcd. for $C_{39}H_{81}N_2O_7P$: C, 64.97; H, 11.3; N, 3.9; P, 4.3. Found: C, 65.21; H, 11.53; N, 3.94; P, 4.13.

By the same procedure and in similar yields were prepared IXb and IXc. The crude products were first recrystallized from methanol and acetone (2.5:7.5).

N-Stearoyl-sphingomyelin (IXb) was recrystallized from butyl acetate; m.p. 209–210°.

Anal. Calcd. for $C_{41}H_{85}N_2O_7P$: C, 65.69; H, 11.44; N, 3.74; P, 4.13. Found: C, 65.75; H, 11.47; N, 3.66; P, 3.94.

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

N-Lignoceroyl-sphingomyelin (IXc) crystallized from butyl acetate; m.p. 213–216° (with strong sintering at 180–190°); infrared spectrum: 3.01, 3.42, 3.50, 6.10, 6.42, 6.78, 8.12, 9.41, 10.33, 10.81, 12.01 μ.

Anal. Calcd. for $C_{59}H_{91}N_2O_7P$: C, 67.75; H, 11.7; N, 3.36; P, 3.7. Found: C, 67.73; H, 11.6; N, 3.49; P, 3.7.

REHOVOTH, ISRAEL

[CONTRIBUTION FROM PARKE, DAVIS AND COMPANY'S MULTIPLE FELLOWSHIP IN MEDICINAL CHEMISTRY, MELLON INSTITUTE]

Diazoacetic Esters of Hydroxyamino Acids[1]

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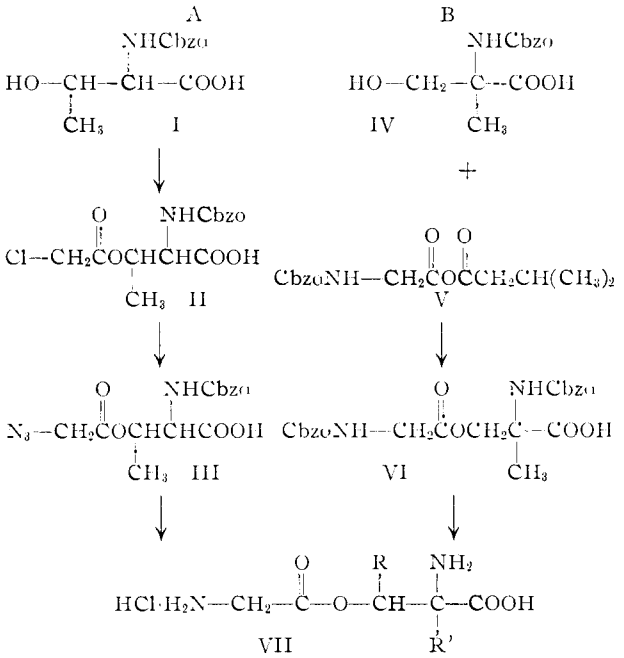
The preparation of the diazoacetic esters of DL-threonine, 2-methyl-DL-serine, 6-hydroxy-DL-norleucine and 4-hydroxy-L-proline are described.

As part of a program on the preparation of compounds for antitumor test, we have studied the effect of replacing the serine part of the azaserine molecule with various hydroxyamino acids. The present paper discusses the diazoacetic esters of DL-threonine, of 2-methyl-DL-serine, of 6-hydroxy-DL-norleucine and of 4-hydroxy-L-proline. The hydroxyamino acids were selected primarily because of their structural relationship to serine. In addition, dextrorotatory 2-methylserine has been reported to be a component of the antibiotic Amicetin,[3] and 6-hydroxy-norleucine has been shown to be similar to an anemia-producing factor of deaminized casein.[4]



The diazoacetic esters were obtained *via* the corresponding glycyl esters VII. The glycyl esters were prepared by *O*-acylation of the appropriate *N*-protected hydroxyamino acid with a reagent having a potential amino group, by procedures similar to those described previously for the synthesis of azaserine,[5] and outlined below for the syntheses of the glycyl esters of threonine (route A) and 2-methylserine (route B).

The *N*-*p*-nitrocarbobenzoxy derivative[6] of 4-hydroxy-L-proline was used for the preparation of the corresponding glycyl ester VII *via* route A in order to obtain crystalline intermediates. The glycyl ester of 6-hydroxynorleucine was prepared according to scheme B.

The glycyl esters of the hydroxyamino acids, as their hydrochloride salts, were diazotized in water at 5°, in the presence of excess nitrite ion at a *p*H of 4.5 to 5.5. The crude reaction mixtures were purified by passage through carbon columns.[7] The yields of the diazoacetic esters were quite low with the exception of the diazoacetic ester of 2-methylserine, which was obtained in 42–65% yields from the pure glycyl ester.

The ultraviolet absorption spectra of the four new diazoacetic esters show a single sharp maximum at 250 mμ and are comparable, on a molar basis, with the ultraviolet absorption spectrum of azaserine. The infrared absorption spectrum of each new diazoacetic ester is characterized, like the infrared spectrum of azaserine, by a strong band at 4.8 μ.

The new diazoacetic esters, and many of the intermediates, were submitted to Sloan-Kettering Institute for test against the Crocker sarcoma-180 tumor in mice, and to the Research Laboratories of Parke, Davis and Co. for various other biological tests. Preliminary reports[8] have indicated that these compounds have no appreciable activity in retarding the growth of tumors in mice at doses which were large in comparison with the minimum effective dose of azaserine.

Acknowledgments.—The authors wish to thank Dr. E. D. Nicolaides for his technical advise and for his interest in this work. They are indebted to Dr. Foil A. Miller and associates for infrared and ultraviolet analyses.

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(3) E. H. Flynn, J. W. Hinman, E. L. Caron and D. O. Woolf, Jr., THIS JOURNAL, **75**, 5867 (1953).

(4) E. Page, R. Gingras and R. Gaudry, *J. Biol. Chem.*, **171**, 831 (1947).

(5) E. D. Nicolaides, R. D. Westland and E. L. Wittle, THIS JOURNAL, **76**, 2887 (1954).

(6) F. H. Carpenter and D. T. Gish, *ibid.*, **74**, 3818 (1952).

(7) S. A. Fusari, R. P. Frohardt, A. Ryder, T. Haskell, D. W. Johannessen, C. C. Elder and Q. R. Bartz, *ibid.*, **76**, 2881 (1954).

(8) C. C. Stock, "Cancer Chemotherapy Screening Data I," *Cancer Research*, **18**, No. 8, Part 2 (1958).