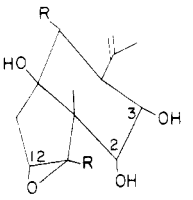
presence of carboxyl or carbomethoxyl as the only carbonyl functions.

The diacid and its diester must be XI and XII. The formation of these substances is regarded merely as the twofold hydrolysis or methanolysis of the dilactone, etc., but the non-conversion of XI to picrotoxic acid, together with the point iv above, requires special comment, once again in terms of a

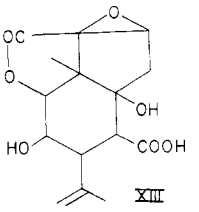


XI, R = COOH
XII, R = COOMe

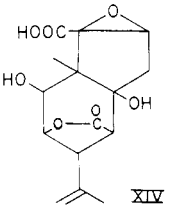
conformational argument. We note that XI and XII are exceptions to the series discussed hitherto, for the cyclohexane ring can be represented in the *chair* form, where, especially in consideration of the conjoined *equatorial* character of the bulky carboxyl and isopropenyl functions, non-bonded interactions should be minimal. But when this *chair* is attained the C-3 hydroxyl is similarly *equatorial* and thus not properly disposed for epoxide opening (*vide supra*); it is necessary to assume that the barrier for reversion to the *boat* (as in X), with an *axial* C-3 hydroxyl, is sufficient to preclude reaction under the conditions. In this view the outcome of competition in formation of picrotoxic acid and of the diacid depends upon whether the C-3 alkoxide ion survives in the *axial* conformation long enough to displace at C-12[18]; we find it satisfying to note then that the methanolysis product ratio, methyl picrotoxate/dimethyl picrotoxinindicarboxylate, increases markedly with increasing methoxide ion concentration.[7] Provided proton exchange with the solvent is rapid compared to other processes, the instantaneous concentration of the intermediate C-3 *axial* alkoxide (of XIII) will be higher when there is more base. Obviously enough, moreover, the hydrolysis of α-picrotoxininic acid (I) to the diacid XI is not accompanied by formation of picrotoxic acid because the C-3 hydroxyl is already *equatorial* in I and need never become *axial* in the course of the change involved.

Acknowledgment.—We wish to thank Miss Beatrice Bonné for technical assistance and S. B. Penick & Co. for generous gifts of picrotoxin.

(18) The reaction course (VII → X *vs.* VII → XI) may well also depend upon the state of the C-14 lactone at the time the C-3 hydroxyl is liberated, for it is attractive to consider that the C-14 lactone can remain intact in the production of picrotoxic acid (*via* the anion of XIII), but the *chair* form of the diacid XI will surely be more easily attained after saponification of this lactone. The acid-catalyzed preparation of picrotoxic acid from VII involves XIII, protonated at the epoxide. We observe further: (i) that picrotoxinindicarboxylic



XIII



XIV

acid (XI) does not relactonize to XIII because in the diacid the C-2 hydroxyl is *axial* upon the *chair*, at some distance from the *quasi-equatorial* C-14 carboxyl, so that relactonization would require reversion to a *boat*, or *half-boat*, with attendant increase in strain; (ii) that spontaneous reconstitution of the lactone (XIV) is not observed for similar reasons; (iii) that no compounds to which the structures XIII and XIV can be attributed are described in the literature.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, NEW YORK STATE PSYCHIATRIC INSTITUTE AND COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY]

Synthesis of Several Phosphorylated Derivatives of Dihydrosphingosine[1]

BY BENJAMIN WEISS

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Dihydrosphingosine-1-phosphate and 3-O-methyldihydrosphingosine II-1-phosphate were prepared from sphingosine and 3-O-methylsphingosine II, respectively, by the following sequence of reactions: (1) hydrogenation to the dihydro form, (2) carbobenzoxylation to block the amino group, (3) phosphorylation with diphenylphosphoryl chloride and (4) removal of the protective groups by catalytic hydrogenolysis. Dihydrosphingosine-1,3-diphosphate was obtained by reduction of N-carbobenzoxy-1,3-bis-(diphenylphosphoryl)-sphingosine which was prepared by successive carbobenzoxylation and diphenylphosphorylation of sphingosine. The dihydrosphingosine-1,3-diphosphate had to be separated from sphingine-1-phosphate which resulted from the hydrogenolysis of the allylic carbon–oxygen bond of N-carbobenzoxy-1,3-bis-(diphenylphosphoryl)-sphingosine.

Since the sphingolipide fraction of monkey brain is labeled after perfusion with either 1-C^{14}-acetate or 1-C^{14}-octanoate,[2] it is of interest to study *in vitro* the synthesis of glyco- and phosphosphingosides by brain tissue so that greater insight may be gained into the intimate mechanisms of sphingolipide metabolism. In order to pursue such studies, it was necessary to prepare derivatives of sphingosine to be used as substrates. Sphingosine-1-phosphate and dihydrosphingosine-1-phosphate were particularly desirable but, after many attempts, the synthesis of sphingosine-1-phosphate was not achieved. It is hoped, however, that in the forthcoming enzymatic investigations the dihydrosphingosine-1-phosphate which was prepared may replace the unsaturated monophosphate ester until such time as it becomes available. Sphingosine phosphate has been identified by means of solvent extraction and paper chromatog-

(1) This investigation was supported in part by research grant No. B-344 (C5 and C6) from the Institute of Neurological Diseases and Blindness of the National Institutes of Health, Public Health Service.

(2) B. Weiss, *J. Biol. Chem.*, **223**, 523 (1956).

raphy in a partial alkaline hydrolysate of sphingomyelin by Rouser, *et al.*[3]

Although methods have been described recently for the synthesis of racemic dihydrosphingosine (1,3-dihydroxy-2-aminoöctadecane),[4-7] naturally occurring sphingosine[8] was employed as the starting material throughout the present investigation since current preparative methods for isolating the base from natural sources are more convenient than existing synthetic procedures. In addition, it was necessary to obtain the natural, optically active form of the derivative. Sphingosine and related bases were isolated from beef spinal cord sphingolipides[9] after hydrolysis with methanolic sulfuric acid according to the procedure of Carter and co-workers.[10] The resulting mixture of bases was fractionated by means of the sulfate salts[11] into sphingosine (and dihydrosphingosine) and the O-methyl ethers of sphingosine which are formed during hydrolysis of the sphingolipides. The O-methyl ethers in ether solution were treated directly with anhydrous hydrogen chloride. The product, after recrystallization from ethanol–ethyl acetate (1:1), melted at 143° in agreement with the melting point of 3-O-methylsphingosine II hydrochloride described by Carter, *et al.*[11]

At the outset of this study, the synthesis of the various dihydrosphingosine derivatives was undertaken first because less difficulty was anticipated in their preparation as a result of the absence of the allylic system which is present in derivatives of the unsaturated base. In the first of the sequence of steps in the synthesis of dihydrosphingosine-1-phosphate, sphingosine was treated with carbobenzoxy chloride in ether solution in the presence of aqueous alkali to yield N-carbobenzoxysphingosine. The product, which crystallized readily from cyclohexane, was phosphorylated in the usual manner in pyridine with diphenylphosphoryl chloride[12] to give N-carbobenzoxy-1,3-bis-(diphenylphosphoryl)-sphingosine. This compound was a sirup and resisted all attempts at crystallization.

Hydrogenation of the sirup in glacial acetic acid in the presence of both platinum[13] and palladium[14] catalysts yielded the corresponding dihydrosphingosine-1,3-diphosphate. In addition, sphingine-1-phosphate was formed in significant amount as a result of the hydrogenolysis of the allylic carbon–oxygen bond, a finding first demonstrated by Carter, *et al.*,[11,15] to occur with 3-O-substituted sphingosines. When the hydrogenation was conducted in absolute ethanol, the reduction proceeded to only 35 to 40% of theory. Separation of the reduction products, dihydrosphingosine-1,3-diphosphate and sphingine-1-phosphate, was accomplished by successive fractionation of the mixture from hot glacial acetic acid and 85% ethanol, solvents in which the monophosphate ester is predominantly soluble.

In the final step of the preparation of dihydrosphingosine-1-phosphate, it was hoped that the monophosphate ester could be prepared from dihydrosphingosine-1,3-diphosphate because secondary phosphate ester linkages are generally more susceptible to hydrolysis than primary phosphate esters. The possibility of racemization at carbon atom 3, resulting in the formation of two diastereoisomers, was considered, but this appeared unlikely, although not conclusively established in this particular case, in view of the general mechanism for the hydrolysis of phosphoric acid esters in which the oxygen–phosphorus bond[16] is cleaved. Thus, when dihydrosphingosine-1,3-diphosphate was treated under the appropriate conditions with a mixture of hydrobromic acid, glacial acetic acid and water (3:1:1), a monophosphate ester was obtained along with the stoichiometric formation of inorganic phosphate. The position of the intact phosphate ester group was ascertained by oxidation of the compound with periodic acid and isolation of the resulting cleavage products. According to theory, one mole of periodate was consumed per mole of compound. This observation conclusively confirmed the absence of an O- to N-migration of the phosphate group. Identification of the oxidation products as hexadecanal and phosphoglycolaldehyde, each characterized as the 2,4-dinitrophenylhydrazone, disclosed unequivocally that the locus of attachment of the phosphate group was at carbon atom 1.

Recently, Carter, *et al.*, have assigned to natural sphingosine and dihydrosphingosine the erythro D-configuration.[15,17-19] Since no racemization is known to result from the synthetic procedures employed, the phosphorylated dihydrosphingosine derivatives must bear to the parent compound the same stereochemical relationship.

Since the separation of dihydrosphingosine-1,3-diphosphate from sphingine-1-phosphate was both tedious and time consuming, the synthetic procedure was modified with the intention of eliminating the formation of sphingine-1-phosphate and, thus, of simplifying the isolation, as well as increasing the yield, of the diphosphate ester. This was accomplished by first destroying the allylic system of sphingosine by reduction to dihydrosphingosine and, then, proceeding through the same synthetic pathway used for the unsaturated base. When N-carbobenzoxydihydrosphingosine was phosphorylated, instead of the expected N-carbobenzoxy-1,3-bis-(diphenylphosphoryl)-dihydrosphingosine, a monophosphorylated product re-

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sulted, which upon reduction over platinum yielded directly a monophosphate ester. By means of periodate oxidation, a comparison of this compound with the one obtained by hydrolysis of the diphosphate ester disclosed that the two substances were identical. This result indicated that the intermediate compound in the former instance, prior to reduction, was N-carbobenzoxy-1-diphenylphosphoryldihydrosphingosine.

Since sphingosine readily forms the N-carbobenzoxy-1,3-bis-(diphenylphosphoryl) derivative, it appears that electronic rather than steric factors exert the major influence in these substitution reactions.

In the preparation of 3-O-methyl dihydrosphingosine II-1-phosphate, the corresponding 3-O-methyl ether of sphingosine II was either directly, or after reduction to the dihydro form, treated to the same sequence of reactions as sphingosine. As a result of hydrogenolysis of the allylic carbon–oxygen bond, a mixture was expected in each case after hydrogenation. In the former instance, in which the allylic system remained throughout the preparation until the final reduction, the compounds O-methyl dihydrosphingosine II-1-phosphate and, presumably, sphingine-1-phosphate, which was not isolated in high enough yield for identification, had to be resolved. In the latter case, where the allylic system was destroyed immediately preceding the substitution reactions, separation involved the mixture O-methyl-N-carbobenzoxydihydrosphingosine II and N-carbobenzoxysphingine, which was formed from the unresolved mixed bases, O-methyl dihydrosphingosine II and sphingine, after reduction of O-methyl sphingosine II. Since it was more difficult to resolve the mixture of monophosphate esters, the latter route was preferred for the preparation of O-methyl dihydrosphingosine II-1-phosphate.

Dihydrosphingosine-1-phosphate exhibited no measurable optical activity (0.0549 g. in 1 ml. of 6 *N* HCl and 4 ml. of methanol, 2 dcm. cell); also inactive were dihydrosphingosine-1,3-diphosphate, sphingine-1-phosphate and 3-O-methyl dihydrosphingosine II-1-phosphate. These compounds gave a negative reaction with ninhydrin.

Many efforts were made, all unsuccessful, to prepare sphingosine-1-phosphate. One of these involved the attempted hydrolysis to remove the protective groupings of N-carbobenzoxy-1,3-bis-(diphenylphosphoryl)-sphingosine under a variety of conditions. Another method concerned the preparation of dibromosphingosine with the expectation of carrying it through the same reaction sequence as dihydrosphingosine. After brominating sphingosine, followed by carbobenzoxylation, the resulting N-carbobenzoxydibromosphingosine spontaneously decomposed upon drying and formed a reddish brown resin. The same result was obtained with N-trifluoroacetyldibromosphingosine. Finally, consideration was given to the synthesis of N-acetylsphingosine-1,3-diphosphate from which it was hoped the monophosphate ester might be obtained by the selective removal of the acetyl and allylic phosphoric acid groupings. When N-acetylsphingosine[10] was treated with phosphorus oxychloride in dimethylaniline, the use of diphenylphosphoryl chloride being precluded as it involved hydrogenation, the uptake of two moles of phosphorus occurred but with the concomitant loss of nitrogen. Since similar results were obtained with N-trifluoroacetylsphingosine and N-trifluoroacetyldihydrosphingosine, it appeared that the loss of nitrogen was due to an attack on the amide bond by the phosphorus oxychloride. This nitrogen-free diphosphate ester released little or no inorganic phosphate upon refluxing with either 6 *N* HCl or with the hydrobromic acid:glacial acetic acid:water mixture (3:1:1) previously used. This result suggested that the allylic and primary phosphate ester groupings are of comparable stability.

Experimental

N-Carbobenzoxysphingosine (I).—To 6.99 g. of sphingosine sulfate, dissolved by gentle warming in 200 ml. of ether and 75 ml. of 0.5 *N* NaOH, was added 5.1 g. of carbobenzoxy chloride in 25 ml. of ether with vigorous stirring during 5 minutes. After stirring the reaction mixture for an additional 15 minutes, the ether layer was removed and the aqueous phase was re-extracted with two 150-ml. portions of ether. The combined ether extracts were washed with 100 ml. of water, and the solvent was removed under reduced pressure at a bath temperature below 50°. The residue was dried *in vacuo* over phosphorus pentoxide and crystallized from cyclohexane (75 ml. per g.); yield 6.27 g. (73%); m.p. 87°. The product gave a negative reaction with ninhydrin and consumed no periodate.

Anal. Calcd. for $C_{26}H_{43}O_4N$ (433.6): C, 71.95; H, 10.00; N, 3.23. Found: C, 72.22; H, 10.19; N, 3.17.

N-Carbobenzoxydihydrosphingosine (II).—6.99 g. of sphingosine-sulfate was dissolved by gentle warming in 200 ml. of ether and 75 ml. of 0.5 *N* NaOH. The ether layer was withdrawn and the aqueous phase was reëxtracted with 150 ml. of ether. The combined ether extracts, after washing with 100 ml. of water, were concentrated to a sirup under diminished pressure at a bath temperature below 50°, and the resulting free base was hydrogenated over 150 mg. of platinum in 20 ml. of ethanol. At the end of the hydrogenation, the reaction mixture was heated, filtered to remove the catalyst and concentrated to near dryness under reduced pressure. The reduced free base, taken up in 200 ml. of ether and 75 ml. of 0.5 *N* NaOH, was treated with 5.1 g. of carbobenzoxy chloride in 25 ml. of ether and processed in the same manner as in preparing I. The crude product, after removal of the ether was dried *in vacuo* over phosphorus pentoxide and crystallized from 70% ethanol (35 ml. per g.); yield 6.24 g. (72%); m.p. 106–107°.

Anal. Calcd. for $C_{26}H_{45}O_4N$ (435.6): C, 71.62; H, 10.41; N, 3.21. Found: C, 71.64; H, 10.64; N, 3.19.

3-O-Methyl-N-carbobenzoxysphingosine II (III).—7.00 g. of 3-O-methylsphingosine II hydrochloride, dissolved in 200 ml. of ether and 75 ml. of 0.5 *N* NaOH, was treated with 5.1 g. of carbobenzoxy chloride, and the reaction mixture was processed in the same manner as in the preparation of I. The crude product, after drying *in vacuo* over phosphorus pentoxide, was crystallized from cyclohexane (35 ml. per g.) and gave a negative reaction with ninhydrin; yield 7.44 g. (83%); m.p. 67°.

Anal. Calcd. for $C_{27}H_{45}O_4N$ (447.6): C, 72.38; H, 10.13; N, 3.13. Found: C, 72.72; H, 10.11; N, 3.14.

3-O-Methyl-N-carbobenzoxydihydrosphingosine II (IV).—7.00 g. of 3-O-methylsphingosine II hydrochloride was dissolved in 60 ml. of 90% ethanol and hydrogenated over 150 mg. of platinum oxide. Following the reduction, the reaction mixture was treated according to the procedure employed in the preparation of II except that the final dried crude product was crystallized three times from cyclohexane (35 ml. per g.); yield 6.02 g. (67%); m.p. 63–64°.

Anal. Calcd. for $C_{27}H_{47}O_4N$ (449.6): C, 72.06; H, 10.54; N, 3.11. Found: C, 72.76; H, 10.55; N, 3.10.

N-Carbobenzoxy-1,3-bis-(diphenylphosphoryl)-sphingosine (V).—To 6.0 g. of I in 40 ml. of anhydrous pyridine, surrounded by an ice-bath, was added 10.80 g. of diphenyl-

phosphoryl chloride during 5 minutes with constant agitation of the reactants. The reaction mixture was stored overnight at 5°, allowed to attain room temperature and slowly poured into a liter of crushed ice-water. After standing 1 hr., the aqueous fluid was decanted from the sirup and replaced by 500 ml. of crushed ice–water. The water was removed after 30 minutes and the product, which was taken up in 450 ml. of petroleum ether (60–70°), was washed successively with 200 ml. of dilute hydrochloric acid and three 150-ml. portions of water. The petroleum ether was removed under diminished pressure, and the resulting colorless sirup was dried *in vacuo* over paraffin and phosphorus pentoxide; yield 10.38 g. (83%).

Anal. Calcd. for $C_{50}H_{61}O_{10}NP_2$ (898.0): N, 1.56; P, 6.90. Found: N, 1.51; P, 6.55.

N-Carbobenzoxy-1-diphenylphosphoryldihydrosphingosine (VI).—6.0 g. of II was treated in the same manner as was V except that 7.38 g. of diphenylphosphoryl chloride was used. The precipitate, which formed upon pouring the reaction mixture into a liter of crushed ice–water, was removed by filtration and washed on the filter with 500 ml. of ice–water. The product was dried *in vacuo* over phosphorus pentoxide and crystallized from 75% ethanol (30 ml. per g.); yield 7.56 g. (82%); m.p. 55°.

Anal. Calcd. for $C_{38}H_{54}O_7NP$ (667.9): N, 2.10; P, 4.64. Found: N, 2.12; P, 4.62.

3-O-Methyl-N-carbobenzoxy-1-diphenylphosphorylsphingosine II (VII).—7.00 g. of III was treated with 6.0 g. of diphenylphosphoryl chloride under the conditions employed in preparing V. The product, which separated as a solid upon addition of the reaction mixture to crushed ice–water, was removed by filtration, dissolved in petroleum ether and successively washed and concentrated to a sirup as in preparing V. The sirup was taken up in 100 ml. of 33% aqueous acetone and chilled in an acetone–Dry Ice bath; the precipitate was removed by filtration and dried *in vacuo* over phosphorus pentoxide; yield 9.4 g. (85%); m.p. 47°.

Anal. Calcd. for $C_{39}H_{54}O_7NP$(679.9): N, 2.06; P, 4.56. Found: N, 2.05; P, 4.44.

3-O-Methyl-N-carbobenzoxy-1-diphenylphosphoryldihydrosphingosine II (VIII).—6.0 g. of IV was treated with 5.2 g. of diphenylphosphoryl chloride under the same conditions employed in preparing V. The final product, which had been washed and dried as in preparing V, was a colorless sirup, yield 7.3 g. (80%).

Anal. Calcd. for $C_{39}H_{56}O_7NP$ (681.9): N, 2.05; P, 4.54. Found: N, 2.00; P, 4.28.

Separation of Dihydrosphingosine-1,3-diphosphate and Sphingine-1-phosphate (IX).—8.0 g. of V was dissolved in 25 ml. of glacial acetic acid containing 2.2 g. of platinum oxide and 500 mg. of palladium oxide and hydrogenated at slightly greater than atmospheric pressure and at room temperature. The consumption of hydrogen ceased, when 10 to 20% in excess of the theoretical 18 moles had been taken up. The reaction mixture was heated in a boiling water-bath and filtered with suction. The catalyst was washed repeatedly with five 15-ml. portions of hot glacial acetic acid. To the combined filtrate and washings, after cooling to room temperature, were added successively two volumes of water, 5 *N* NaOH to *p*H 5.0 to 6.0 and 75 ml. of 1.0 *M* barium acetate. The copious precipitate was removed by centrifugation and washed successively on the centrifuge with 200 ml. of ethanol, ethanol:ether (1:1) and ether; yield 4.30 g.

1.50 g. of the mixed bases was suspended in 30 ml. of glacial acetic acid and heated in a boiling water-bath for 2 minutes with occasional stirring. The hot solution was centrifuged, and the procedure repeated by resuspending the precipitate in the same volume of glacial acetic acid.

The final precipitate was washed successively with 20 ml. of water, ethanol and ether and air dried. The glacial acetic acid supernatant liquids from the previous centrifugations were combined and, after cooling to room temperature, diluted with an equal volume of water. The solution was further cooled in an ice-bath, and the resulting precipitate was washed successively by centrifugation with 30 ml. of water, ethanol and ether.

The precipitate, insoluble in 50% acetic acid, was heated with 100 ml. of 85% ethanol at the boiling point in a hot water-bath for 5 minutes with occasional agitation. The hot solution was centrifuged, and the supernatant liquid, after attaining room temperature, was cooled in an ice-bath. The flocculent precipitate was removed by centrifugation, and the supernatant liquid discarded. The precipitate, insoluble in hot 85% ethanol, was refractionated four additional times with 100-ml. portions of 85% ethanol in the same manner. The combined precipitates, soluble in hot 85% ethanol, were washed with two 30-ml. portions of ethanol and redissolved in 10 ml. of hot glacial acetic acid. The hot solution was centrifuged, any undissolved material being discarded, and an equal volume of water was added to the supernatant liquid after cooling to room temperature. The solution was further cooled in an ice-bath, and the precipitate, collected by centrifugation, was washed successively with 25-ml. portions of water, ethanol and ether; yield of sphingine-1-phosphate, 310 mg. The compound consumed no iodine or periodate.

Anal. Calcd. for $C_{18}H_{40}O_4NP$ (365.0): C, 59.17; H, 11.05; N, 3.84; P, 8.49. Found: C, 59.07; H, 11.03; N, 3.85; P, 8.38.

The insoluble precipitate remaining from the fractionation with hot 85% ethanol was combined with the glacial acetic acid insoluble precipitate, derived in the first stage of the separation of dihydrosphingosine-1,3-diphosphate from sphingine-1-phosphate and washed with 30 ml. of ethanol. The precipitate was suspended in 10 ml. of glacial acetic acid and heated for 2 minutes with occasional stirring in a boiling water-bath. The hot solution was centrifuged and the precipitate washed successively with 25-ml. portions of water, ethanol and ether; yield of monobarium salt of dihydrosphingosine-1,3-diphosphate, 540 mg. The compound consumed no iodine or periodate.

Anal. Calcd. for $C_{18}H_{39}O_8NP_2Ba$ (596.33): C, 36.22; H, 6.59; N, 2.35; P, 10.39; Ba, 23.03. Found: C, 37.12; H, 6.89; N, 2.31; P, 10.35; Ba, 22.79.

Dihydrosphingosine-1-phosphate (X).—6.75 g. of VI was hydrogenated over 2.0 g. of platinum oxide in 30 ml. of glacial acetic acid under the conditions used in preparing IX. At the end of the reduction, the catalyst was separated from the reaction mixture and washed as in preparing IX. The combined filtrate and washings, after attaining room temperature, were diluted with an equal volume of water, and the resulting precipitate was washed successively on the centrifuge with 100 ml. of water, acetone and ether. When the precipitate had air-dried, the procedure was repeated by dissolving it in 125 ml. of hot glacial acetic acid. After centrifuging the hot solution and diluting the cool supernatant liquid with an equal volume of water, the resulting precipitate was washed successively with 100-ml. portions of water, acetone and ether; yield 3.00 g. (78%).

Anal. Calcd. for $C_{18}H_{40}O_5NP$ (381.0): C, 56.69; H, 10.58; N, 3.67; P, 8.13. Found: C, 57.05; H, 10.50; N, 3.56; P, 7.75.

3-O-Methyl Dihydrosphingosine II-1-Phosphate (XIA). 9.0 g. of VII was reduced under the same conditions, including solvent and catalyst, employed in preparing IX. The catalyst was separated from the reaction mixture and washed as in preparing IX after the hydrogenation. To the combined filtrate and washings, after attaining room temperature, were added successively five volumes of water, 5 *N* NaOH to *p*H 5.0 to 6.0, and 80 ml. of 1 *M* barium acetate. The precipitate was collected by centrifugation and washed successively with 100-ml. portions of water, acetone and ether, yield 4.30 g.

1.0 g. was suspended in 20 ml. of glacial acetic acid and warmed with constant agitation for 5 minutes in a 50° water-bath. The warm solution was centrifuged and the supernatant liquid, after diluting with one and a half volumes of water, was chilled in an ice-bath. The precipitate was removed by centrifugation and washed successively with 20-ml. portions of water, acetone (twice), acetone: ether (1:1) and ether. After air drying, the precipitate was resuspended in 20 ml. of glacial acetic acid and the procedure repeated two additional times. Each residue obtained after decanting the glacial acetic acid supernatant liquid was discarded. The final air-dried precipitate obtained after the third glacial acetic acid fractionation was crystallized from 100 ml. of hot 85% ethanol and washed once by centrifugation with 50 ml. of acetone; yield 0.55 g. The product consumed no iodine or periodate.

Anal. Calcd. for $C_{19}H_{42}O_5NP$ (395.0): C, 57.72; H, 10.72; N, 3.54; P, 7.84; CH_3O, 7.85. Found: C, 57.98; H, 10.72; N, 3.49; P, 7.79; CH_3O, 7.61.

3-*O*-Methyl Dihydrosphingosine-II-phosphate (XIB).—6.0 g. of VIII was hydrogenated over 1.5 g. of platinum oxide in 25 ml. of glacial acetic acid under the same conditions as were used in preparing IX. Following reduction, the reaction mixture was treated exactly as in preparing XIA with the exception that the entire precipitate obtained after reduction was taken through the purification step only once with 40 ml. instead of 20 ml. of glacial acetic acid in a 50° water-bath. The product was crystallized from hot 85% ethanol (200 ml. per g.); yield 2.55 g. (73%).

Anal. Calcd. for $C_{19}H_{42}O_5NP$ (395.0): C, 57.72; H, 10.72; N, 3.54; P, 7.84; CH_3O, 7.85. Found: C, 57.63; H, 10.81; N, 3.46; P, 7.83; CH_3O, 7.65.

Hydrolysis of Dihydrosphingosine-1,3-diphosphate.—One hundred mg. of the monobarium salt of dihydrosphingosine-1,3-diphosphate was refluxed for 24 hr. in 25 ml. of a hydrobromic acid solution, prepared by adding to the compound in the following sequence, in order to effect complete solution, 5 ml. of glacial acetic acid, 15 ml. of concentrated hydrobromic acid (34%) and 5 ml. of water. Upon completion of hydrolysis, the hot reaction mixture was centrifuged and the small insoluble residue discarded. The supernatant liquid, after attaining room temperature, was diluted with an equal volume of water and chilled in an ice-bath. The precipitate was removed by centrifugation and washed on the centrifuge with 30 ml. of water. The supernatant liquid and washing were combined and diluted to the mark in a 100-ml. volumetric flask for inorganic phosphate determinations. The washed precipitate was treated with 20 ml. of hot 85% ethanol and the small undissolved residue discarded after centrifugation of the hot solution. To the cooled supernatant liquid, diluted with an equal volume of water, was added 1 ml. of 1 *M* barium acetate. The resulting precipitate, which consisted of the barium salt of dihydrosphingosine-1-phosphate, was removed by centrifugation of the chilled solution and washed successively with 15-ml. portions of water, ethanol and ether; yield 61.0 mg. (70%).

Anal. Calcd. for $C_{18}H_{38}O_5NPBa$ (516.3): N, 2.71; P, 5.99; inorganic phosphate, 5.19. Found: N, 2.76; P, 5.99; inorganic phosphate, 5.55.

Conversion of Barium Dihydrosphingosine-1-phosphate to Free Acid.—One hundred mg. of barium dihydrosphingosine-1-phosphate was dissolved in 5 ml. of warm glacial acetic acid and, after cooling to room temperature, diluted with an equal volume of water. The resulting precipitate, removed by centrifugation of the chilled solution, was washed twice with 10-ml. portions of water and dried *in vacuo* over phosphorus pentoxide; yield 66.0 mg. (90%).

Anal. Calcd. for $C_{18}H_{40}O_5NP$ (381.0): C, 56.69; H, 10.58; N, 3.67; P, 8.13. Found: C, 56.67; H, 10.62; N, 3.55; P, 7.84.

Periodate Oxidation of Dihydrosphingosine-1-phosphate.—Two hundred mg. of dihydrosphingosine-1-phosphate (0.525 mmole) in a 100-ml. volumetric flask was dissolved in 50 ml. of methanol and 2 ml. of 6 *N* HCl with gentle warming. Following the addition of 3 ml. of 0.4 *M* periodic acid and 10 ml. of water, the solution was diluted to volume with methanol. A blank determination was run simultaneously with the sample. After standing 24 hr. at room temperature, 10-ml. aliquots were withdrawn for titration in the usual manner with standard thiosulfate solution. Prior to titration, 10 ml. of water was added to the sample followed in succession by an excess of solid sodium bicarbonate and 1 ml. of 20% potassium iodide solution. The 0.483 mmole of periodate consumed represented 92% completion of the reaction. The remaining solution was extracted with four 40-ml. portions of *n*-heptane, and the combined extracts were washed once with 25 ml. of water. The concentrate, obtained after removal of the solvent under diminished pressure, was derivatized with 2,4-dinitrophenylhydrazine in the conventional manner, and the isolated 2,4-dinitrophenylhydrazone was recrystallized three times from 95% ethanol; yield 67.0 mg. The product melted at 108° in agreement with the melting point reported for the 2,4-dinitrophenylhydrazone of palmitaldehyde.[20]

Anal. Calcd. for $C_{22}H_{36}O_4N_4$ (420.5): C, 62.78; H, 8.63; N, 13.32. Found: C, 63.14; H, 8.68; N, 13.17.

The solution remaining from the *n*-heptane extraction was treated with an excess of a saturated sodium sulfite solution to remove iodate and periodate. The 2,4-dinitrophenylhydrazine reagent was then added and, after standing 30 minutes at room temperature, the solution was concentrated to approximately 25 ml. under diminished pressure at a bath temperature below 40°. The precipitate, collected by centrifugation of the chilled solution, was washed with three 10-ml. portions of warm water and three 10-ml. portions of warm 95% ethanol and dried over phosphorus pentoxide *in vacuo;* yield of 2,4-dinitrophenylhydrazone of phosphoglycolaldehyde, 15.0 mg.

Anal. Calcd. for $C_5H_9O_3N_4P$ (320.1): P, 9.68. Found: P, 9.72.

This value for phosphorus is in disagreement with the finding of Fleury, *et al.*,[21] who concluded from their analytical data that two moles of phosphoglycolaldehyde react with three moles of 2,4-dinitrophenylhydrazine, the final product being hydrated with eight moles of water. The discrepancy may be accounted for if it is assumed that the third mole of reagent is bound in ionic form, producing a salt with the phosphate group, and is removed during the washing process.

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