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STUDIES IN THE SPHINGOLIPIDS SERIES—XXIV*

SYNTHESIS OF C_{18}-PHYTOSPHINGOSINE

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Abstract—The natural stereoisomer of C_{18}-phytosphingosine—D(+)-ribo-2-amino-1,3,4-trihydroxy-octadecane—has been synthesized. The reaction sequence involved first the synthetic preparation of C_{18}-sphingosine—D(+)-erythro-2-amino-1,3-dihydroxy-4-trans-octadecene (I)—by already reported procedures. The tribenzoyl derivative of the latter (II) was then oxidized with perbenzoic acid and the resulting tribenzoylsphingosine epoxide (III) reduced by both $LiAlH_4$ and molecular hydrogen (Pt catalyst). Hydrogenolysis of the N-benzylphytosphingosine (VI) and methanolysis of N-cyclohexanoylphytosphingosine (V) respectively, yielded natural C_{18}-phytosphingosine (VII).

SPHINGOSINE and dihydrosphingosine are the long-chain bases characteristic of sphingolipids of animal sources.[1] Dihydrosphingosine has also been found recently in some plant organisms[2,3]. Phytosphingosine is a C_{18} and C_{20}-base widely distributed in the plant kingdom as the amide with an α-hydroxy long-chain acid.[4–10] The presence of phytosphingosine in animal tissues has not yet been established. Whereas several syntheses of both racemic and optically active forms of sphingosine and dihydrosphingosine are described, the synthetic preparation of an optically active phytosphingosine is not recorded so far. The only synthetic attempt available comprises a preparation of racemic mixtures of the C_{18}[11] and C_{20}-base.[12]

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[9] M. Prošteník and N. Ž. Stanaćev, *Chem. Ber.* **91**, 961 (1958).

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[12] A. Kisić, Thesis, Zagreb (1961).

This paper deals with the synthetic preparation of natural C_{18}-phytosphingosine. The first stage involves the synthesis of C_{18}-sphingosine which is converted in the second stage to the title compound. Phytosphingosine represents a rather complex molecule in view of the presence of three asymmetric carbon atoms. Therefore, the synthesis might give rise to four racemates composed of eight optically active isomers. Recently, Carter and Hendrickson[13] established the configuration of C_{18}-phytosphingosine as D-ribo-2-amino-1,3,4-trihydroxyoctadecane (VII). Since C_{18}-sphingosine as isolated from animal tissues, is D(+)-erythro-2-amino-1,3-dihydroxy-4-*trans*-octadecene (I), the introduction of a new hydroxy group should result in the formation of two epimeric (ribo and lyxo) 2-aminotrihydroxyoctadecanes. The ribo epimer should represent natural C_{18}-phytosphingosine.

In the present investigation two synthetic procedures developed by Grob and Gadient[14] and Shapiro *et al.*[15] respectively, were applied to the preparation of C_{18}-sphingosine (I). The optically active base was benzoylated and synthetic tribenzoyl-C_{18}-sphingosine (II) thus obtained was then converted to the epoxide (III) by the action of perbenzoic acid. A similar reaction sequence applied to tribenzoylsphingosine of natural provenance—from bovine and horse brain—was reported previously in relation to another problem.[16] The epoxidation—as a stereospecific addition—proceeds in the *cis* manner. Thus taking into account the relative position of the oxirane ring to the hydroxyl group at the carbon 3, two *trans*-tribenzoylsphingosine epoxides might result from this reaction. In fact, the reaction product purified by crystallization was homogeneous and was used as such in the next step. The orientation of the oxirane system to the benzoyloxy group at the carbon 3 seemed to proceed stereo-specifically. The stereochemical analysis of this reaction requires additional experimental data and will be discussed at a later date.

The reduction of III in the presence of Adams platinum catalyst (hydrogen uptake 10 moles) furnished predominantly 2-cyclohexanoylamino-1,3-dicyclohexanoyloxy-4-hydroxyoctadecane (IV) which was hydrolysed without further purification to (−)-2-cyclohexanoylamino-1,3,4-trihydroxyoctadecane (V). The substance was obtained in a high yield (92%) and the presence of the 5-hydroxy isomer was not observed. The relative position of the functional groups was indicated by periodate titration. Nearly 1 mole of the oxidant was consumed. The N-cyclohexanoyl derivative (V) when refluxed with 10% methanolic sulphuric acid afforded a base (VII), m.p. 95–97°, which analysed correctly for $C_{18}H_{39}NO_3$; $[\alpha]_D$ +7·7°, in pyridine.

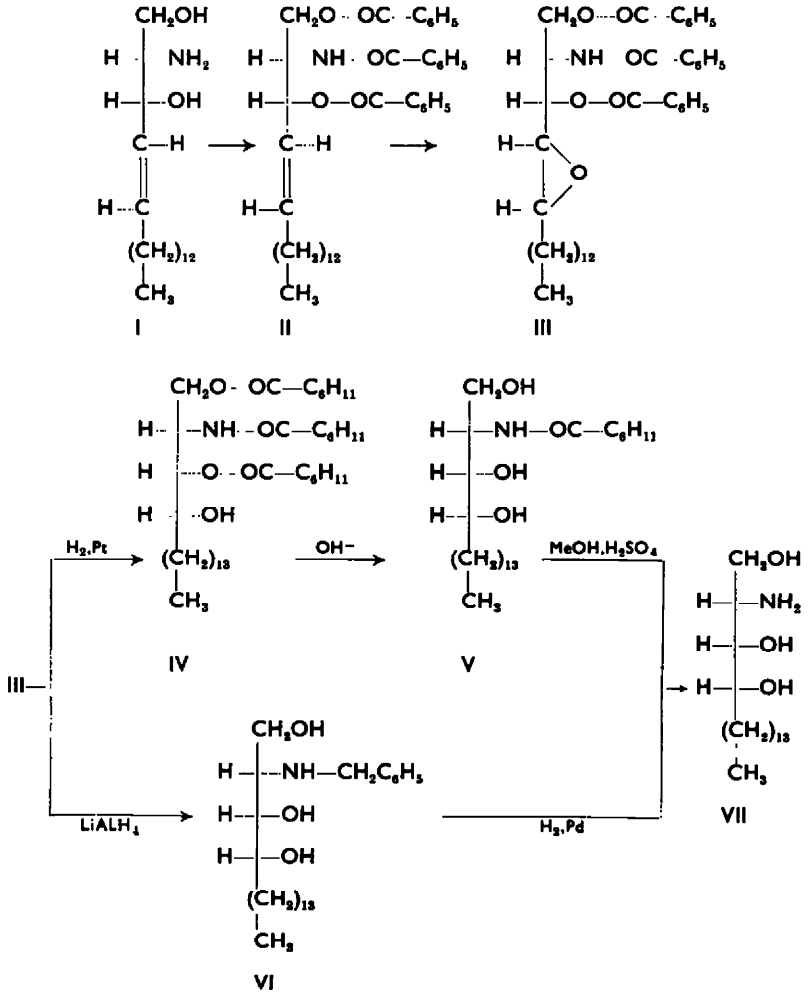
On the other hand, III was reduced with lithium aluminium hydride to give (+)-2-benzylamino-1,3,4-trihydroxyoctadecane (VI). Hydrogenolysis of the latter in the presence of palladium on carbon catalyst yielded the same base (VII), m.p. 96–97° $[\alpha]_D$ +7·9°, in pyridine. By the periodate titration of the base nearly 3 moles of the oxidant were consumed, thus confirming the arrangement of four functional groups on contiguous carbon atoms.

[13] H. E. Carter and H. S. Hendrickson, *Biochemistry* **2**, 389 (1963).
[14] C. A. Grob and F. Gadient, *Helv. Chim. Acta* **40**, 1145 (1957).
[15] D. Shapiro, H. Segal and H. M. Flowers, *J. Amer. Chem. Soc.* **80**, 1194 (1958).
[16] M. Prošteník and B. Majhofer-Oreščanin, *Naturwiss.* **47**, 399 (1960); B. Majhofer-Oreščanin and M. Prošteník, *Croat. Chem. Acta* **33**, 219 (1961).

The presence of the lyxo base, one of the unnatural stereoisomer of C_{18}-phytosphingosine, could not be detected either after catalytic reduction or after reduction by lithium aluminium hydride.

The IR spectra of the bases obtained by both routes were superimposable and identical in all details with the spectrum of natural phytosphingosine prepared from yeast cerebrin.

The specific rotation of the synthetic base corresponds to that of natural phytosphingosine ($[\alpha]_D$ +8·05°, in pyridine). Surprisingly, the rotation of phytosphingosine has not been reported by earlier investigators. For this reason we prepared it from yeast cerebrin by a modified procedure which enables easy separation of phytosphingosine from other compounds, particularly from phytosphingosine anhydro base. The procedure is based on a relatively great difference in solubilities of both principal bases in light petroleum. The product thus obtained was a mixture of C_{18} and C_{20}-phytosphingosine, the latter being the major component.[17] It was compared as



[17] A. Kisić and M. Proštenik, *Croat Chem. Acta* **32**, 229 (1960).

such with the synthetic C_{18}-base, taking into account the well known similarities in physical properties of C_{18} and C_{20}-sphingolipide bases.

All these data strongly suggest a structure of a D-ribo base (C_{18}-phytosphingosine). However, the synthetic product may contain a small quantity of the epimeric lyxo base. This presumption is hard to verify, particularly if the physical properties of the latter—for the time being unknown—do not differ markedly from those of the ribo isomer. We are now attempting to synthesize the other stereoisomers of C_{18}-phytosphingosine.

EXPERIMENTAL

All m.ps are uncorrected. Microanalyses were carried out by Mrs. M. Munk-Weinert, Mrs. N. Zambeli and Mrs. J. Zake. IR spectra were recorded in nujol on a Perkin-Elmer Model 137 spectrophotometer by Mr. T. Magjer. Optical rotations were measured on a Zeiss-Winkel Kreispolarimeter 0·01 instrument and are average values of a large number of single measurements.

Tribenzoyl-C_{18}-sphingosine (II)

Racemic C_{18}-sphingosine (D-erythro-2-amino-1,3-dihydroxy-4-*trans*-octadecene (I) was prepared by the method of Grob and Gadient.[14] By applying the conversion of the intermediary formed threo-nitrodiol to the erythro-nitrodiol via the benzal derivative a satisfactory over-all yield based on 2-hexadecyne-1-al was obtained. Minor quantities of racemic C_{18}-sphingosine were prepared starting with *trans*-2-hexadecenoic acid according to Shapiro *et al.*[15] The base obtained was resolved by means of L-glutamic acid.[16] Optically active C_{18}-sphingosine ($[\alpha]_D^{25}$ +3·3° ± 1°, in pyridine) was benzoylated as reported by Carter *et al.*[18] Tribenzoyl derivative: m.p. 120–122°, $[\alpha]_D^{25}$ −10·7° ± 1°, in $CHCl_3$.

trans-*Tribenzoyl-C_{18}-sphingosine epoxide* (III)

The epoxide (m.p. 134–135°) was prepared from synthetic tribenzoyl-C_{18}-sphingosine (yield 64%) as reported in a previous paper.[16]

(+)-2-*Cyclohexanoylamino*-1,3,4-*trihydroxyoctadecane* (V)

A sample of III (2·25 g) was suspended in 95% ethanol (100 ml) and hydrogenated 3 hr at room temp in the presence of Adams Pt catalyst (600 mg) in a Parr autoclave. The catalyst was removed and the filtrate evaporated *in vacuo* to dryness. The crude, syrupy product was dissolved in 1N methanolic KOH and heated to 40° for 1 hr. The solvent was partly evaporated *in vacuo*, the residue diluted with water and extracted exhaustively with ether. The combined ether extracts yielded a crude product which after one crystallization from acetonitrile melted at 94–97° (1·45 g, 92%). Further crystallizations from the same solvent did not raise the m.p. $[\alpha]_D^{25}$ +4·2° ± 1° (*c* 2·3, in pyridine). (Found: C, 70·23; H, 11·75. $C_{25}H_{49}NO_4$ requires: C, 70·21; H, 11·55%). IR bands at: 850(w), 883(w), 900(m), 950(m), 1078(s), 1118(w), 1160(w), 1220(m), 1265(w), 1340(w), 1565(s), 1625(s), 1650(s), 3350(s), and 3470(s) cm^{-1}.

Oxidation of the N-*cyclohexanoyl derivative* (V) *with periodic acid*

To a solution of V (100 mg) in methanol (10 ml), 0·2M methanolic solution of periodic acid (10 ml) was added. The reaction mixture was left at room temp for 3 hr. The estimated consumption of periodic acid was 93% of the quantity calculated for 1 mole of the compound.

(+)-2-*Benzylamino*-1,3,4-*trihydroxyoctadecane* (VI)

A sample of III (2 g), $LiAlH_4$ (2·5 g) and absolute ether (150 ml) were refluxed for 5 hr. The excess of the hydride was then hydrolysed by cautious addition of water, the ether phase separated by decantation and the inorganic residue extracted repeatedly with ether. The combined extracts furnished 1·1 g (85%) of the crude base (m.p. 50–56°) which was recrystallized twice from acetonitrile; m.p. 55–58°, $[\alpha]_D^{20}$ +8·7° ± 1° (*c* 1·0, in pyridine). (Found: C, 73·85; H, 11·10. $C_{25}H_{45}NO_3$

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

requires: C, 73·66; H, 11·13%). IR bands at: 698(s), 748(s), 835(m), 880(w), 905(w), 970(m), 1050(s), 1110(w), 1205(w), 1250(w), 1348(m), 1500(w) and 3430(s) cm^{-1}.

D(+)-*Ribo*-2-*amino*-1,3,4-*trihydroxyoctadecane* (C_{18}-*phytosphingosine*; VII)

A. *By methanolysis of* V. A solution of the N-cyclohexanoyl derivative (V, 560 mg) in 10% methanolic H_2SO_4 (20 ml) was refluxed for 24 hr and then made strongly alkaline by addition of 45% KOH. Extraction with ether gave 345 mg (83%) of the crude base, m.p. 85–90°. After two crystallizations from acetonitrile it melted at 95–97°; $[\alpha]_D^{20}$ +7·7° ± 1° (*c* 1·0, in pyridine). IR bands at: 920(w), 950(w), 1065(s), 1240(w), 1553(m), 1630(w) and 3450(s) cm^{-1}.

B. *By hydrogenolysis of* VI. A solution of VI (850 mg) in ethanol (50 ml) was hydrogenated in the presence of 5% Pd-C catalyst (2·5 g) at room temp and atm. press. After 5 hr, 40 ml (calc'd: 47 ml) of H_2 was taken up. The reaction mixture yielded 590 mg (87%) of the base which was recrystallized twice from acetonitrile; m.p. 96–97°, $[\alpha]_D^{20}$ +7·9° ± 1° (*c* 1·2, in pyridine). (Found: C, 68·24; H, 12·56; N, 4·14. $C_{18}H_{39}NO_3$ requires: C, 68·03; H, 12·37; N, 4·41%). The IR spectrum was superimposable with that of the base obtained by the procedure A.

The *oxalate* of the base was prepared in the usual manner and recrystallized from glacial acetic acid; m.p. 195–197°.

Oxidation of VII *with periodic acid*

The base (50 mg) dissolved in methanol (5 ml) was oxidized with 0·2M methanolic periodic acid (5 ml). The uptake of the oxidant was estimated against a blank at various intervals and found to be 1·7, 2·8 and 2·8 moles (95%) at the end of 0·5 hr, 1 hr and 3 hr, respectively.

Preparation of phytosphingosine from yeast cerebrin

The methanolysis of 20 g yeast cerebrin (N. V. Philips-Roxane, Pharmazeutisch-chemische Industrie DUPHAR, Amsterdam) and isolation of a mixture of crude bases was carried out by adopting the known procedures.[7] The somewhat sticky base mixture (2·25 g) was suspended in boiling light petroleum (50–70°, 200 ml) and the mixture allowed to stand for a few min until impure, almost insoluble phytosphingosine had settled. The supernatant solution contained the anhydro base. The undissolved substance was filtered off while hot (1·32 g) and recrystallized from acetonitrile. Four crystallizations gave a product (0·65 g) m.p. 106° with sintering from 96°. The base thus obtained was practically insoluble in boiling light petroleum. $[\alpha]_D^{20}$ +8·05° ± 1° (*c* 1·0, in pyridine); $[\alpha]_D^{20}$ +7·3° ± 1° (*c* 1·1, in $CHCl_3$). (Found: C, 69·31; H, 12·35. $C_{30}H_{43}NO_3$ calc.: C, 69·51; H, 12·51%). Thin-layer chromatography on Silica Gel G (Merck) according to Sambasivarao and McCluer[19] showed a single spot. IR bands at: 920(w), 950(w), 1065(s), 1240(w), 1555(m), 1630(w) and 3450(s) cm^{-1}.

Oxalate M.p. 197–199°, from glacial acetic acid. (Found: C, 64·45; H, 10·97; N, 3·52. $C_{21}H_{44}NO_6$ requires: C, 64·58; H, 11·36; N, 3·59%).

[19] K. Sambasivarao and R. H. McCluer, *J. Lipid Res.* **4**, 106 (1963).