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STUDIES IN THE SPHINGOLIPIDS SERIES—XVII*

SYNTHESIS AND RESOLUTION OF ERYTHRO AND THREO-C_{20}-DIHYDROSPHINGOSINES†

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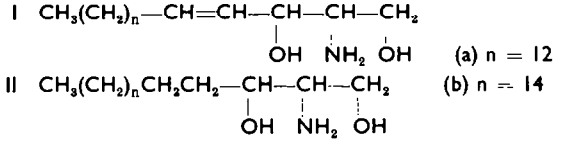
Abstract—Erythro and threo-1,3-dihydroxy-2-amino-eicosane have been obtained by addition of nitroethanol to octadecanal and subsequent reduction of both erythro and threo-nitrodiols. Resolution with L-glutamic acid gave four optically active forms of the base. The D-configuration has been assigned to the +-erythro-isomer.

SPHINGOSINE (Ia) is one of the eight stereoisomeric 1,3-dihydroxy-2-amino-4-octadecenes. Its saturated derivative, dihydrosphingosine (IIa), is one of the four possible isomers and has a structure of D+-erythro-1,3-dihydroxy-2-amino-octadecane. Both bases have been found in animal tissue and all stereoisomeric forms have been prepared synthetically.[1] Recently, we have discovered the third base of animal origin, C_{20}-sphingosine, which was isolated from horse and beef brain.[2] The new base has been characterized as 1,3-dihydroxy-2-amino-4-eicosene (Ib). So far there is no experimental evidence as to the stereochemistry of the C_{20}-base.



In continuation of our work on the new base we wish to describe first a synthesis of the four optically active forms of its dihydro derivative, C_{20}-dihydrosphingosine (IIb). This problem is of interest for several reasons. First, it is reasonable to suppose the occurrence of C_{20}-dihydrosphingosine in nature through analogy with C_{18}-dihydrosphingosine. On the other hand, the knowledge of the optical isomers of the dihydro base may offer an additional approach to the stereochemistry of the C_{20}-bases.

Of the many syntheses of C_{18}-sphingosine and C_{18}-dihydrosphingosine reported within the past few years the most practical appear to be those of Grob *et al.*[3,4] They consist in the first stage in the reaction of hexadecen-2-al-1 and hexadecanal-1 respectively with nitroethanol. Similar reaction scheme for the synthesis of C_{18}-dihydrosphingosine was proposed some twenty years ago by Seydel[5].

* Part XVI, see reference 2.

† Presented at the I. Congress of Pure and Applied Chemistry of Yugoslavia. Abstracts of papers, p. 131. Zagreb-Rijeka-Beograd, June (1960).

‡ Taken from a thesis by Mrs. B. Majhofer-Oreščanin in partial fulfillment for the Ph.D. University of Zagreb (1960).

[1] For recent reviews covering this field see H. E. Carter *et al.*, *Canad. J. Biochem. Physiol.* **34**, 320 (1956); *Fed. Proc.* **16**, 817 (1957).

[2] M. Prošteník and B. Majhofer-Oreščanin, *Naturwiss.* **47**, In press (1960).

[3] C. A. Grob, E. F. Jenny and H. Utzinger, *Helv. Chim. Acta* **34**, 2249 (1951).

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

[5] P. V. Seydel, *Zur Kenntnis des Sphingosins* Ph.D. Thesis, Zürich (1941).

In the present work the synthesis of the four 1,3-dihydroxy-2-aminoeicosanes (IIb) was carried out by the route indicated in Chart 1. The starting material was octadecanal-1 which was prepared in 66 per cent yield by the Rosenmund reduction of stearoyl chloride. Addition of nitroethanol to the aldehyde thus obtained in the presence of potassium carbonate furnished a mixture of DL-erythro- and DL-threo-1,3-dihydroxy-2-nitroeicosanes. Both nitrodiols A and B could easily be separated due to

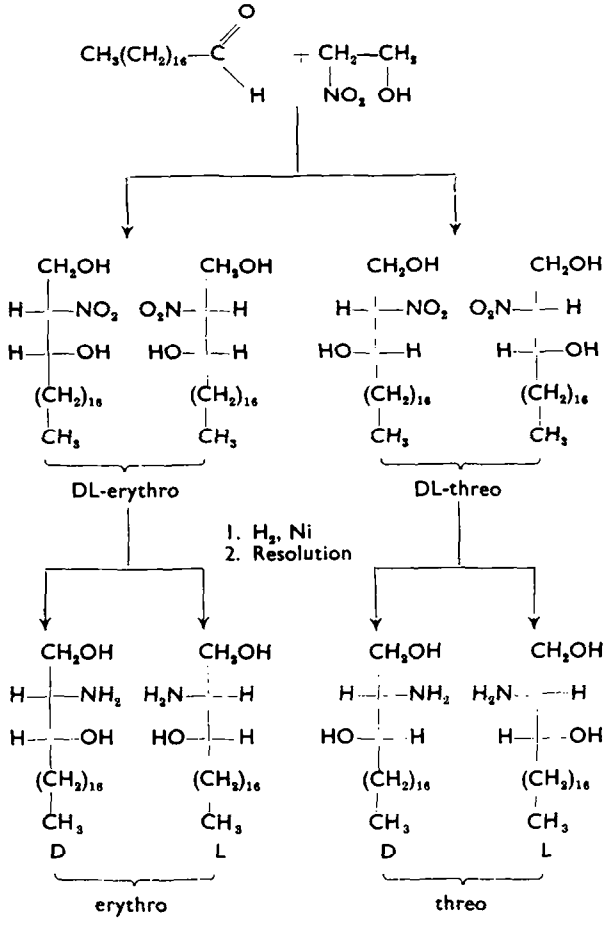


Chart 1.

the different solubility in a solvent mixture ether–pentane. The less soluble threo-isomer was obtained in an analytically pure condition. The yield of separated and purified nitrodiols was 56 per cent. The nitrodiols A (DL-threo-isomer) and B (DL-erythro-isomer) were hydrogenated separately in the presence of Raney nickel catalyst. Very good yields of pure threo-1,3-dihydroxy-2-aminoeicosane were obtained, while the erythro-aminodiol was purified less satisfactorily. However, the racemic aminodiols were best purified by converting them into the sparingly soluble oxalates. Treatment of the latter with potassium hydroxide followed by extraction with chloroform furnished the analytically pure bases.

The resolution into the optically active compounds of both racemic aminodiols thus prepared was effected by means of L-glutamic acid. This acid has repeatedly been applied as a very efficient resolving agent in the field of the sphingolipid bases.[6–9] One crystallization from 80 per cent ethanol of the less soluble glutamic acid salt was usually sufficient to attain the optically pure compound. The filtrates contained the more soluble glutamates. Their optical purity was satisfactory even when the filtrates were evaporated to dryness without further crystallizations.

TABLE 1. COMPARISON OF MELTING POINTS AND SPECIFIC ROTATIONS OF OPTICALLY ACTIVE AND RACEMIC 1,3-DIHYDROXY-2-AMINOEICOSANES AND THEIR DERIVATIVES

Compound	M.p.	$[\alpha]_D$ in °
DL-Threo-base	99·5–100·5	
Oxalate	189–191	
DL-Erythro-base	81–83	
Oxalate	201–205	
+-Threo-base	108·5–109·5	+17·6
Glutamate I	153–156	
—-Threo-base	108–109	−17·7
Glutamate II	169–171	
+-Erythro-base	89–91	+9·7
Glutamate III	151–156	
—-Erythro-base	85–90	−9·9
Glutamate IV	155–167	

Furthermore, it was of interest to provide evidence as to the stereochemistry of synthetic C_{20}-dihydrosphingosines. In assigning threo and erythro structures to the synthetic C_{20}-bases following types of evidence were considered: chemical reactions, physical properties and infra-red spectra. The assignment of structure was based on the agreement in the relative behaviour of the C_{20}-isomers as compared to the C_{18}-isomers. Thus, from a consideration of melting points, infra-red spectra and analogy of chemical preparations, we conclude that the enantiomers melting at 108·5–109·5° and 108–109° showing $[\alpha]_D = +17{\cdot}6°$ and $-17{\cdot}7°$ respectively are the threo-forms, while the others melting at 89–91° and 85–90° showing $[\alpha]_D = +9{\cdot}7°$ and $-9{\cdot}9°$ are the erythro-forms. The melting points and specific rotations of the described compounds are summarized in Table 1.

In respect to the infra-red spectra there exist remarkable differences between the threo and erythro-isomers, which were observed consistently in both the C_{18} and C_{20}-series (Fig. 1). This observation offers a reliable means of assignment of erythro or threo structure.

Moreover, it is possible to assign the D-configuration to the +-erythro-C_{20}-dihydrosphingosine and, consequently, the L-configuration to the —-enantiomer. Namely, the stereochemistry of the C_{18}-bases has been completely elucidated by earlier workers. It has been well known that natural C_{18}-sphingosine and C_{18}-dihydrosphingosine possess the D+-erythro-configuration. The close agreement in physical properties of the +-erythro-C_{20}-base and natural dihydrosphingosine from horse brain—which represents a mixture of C_{18} and C_{20}-bases—is seen in Table 2 and Fig. 1.

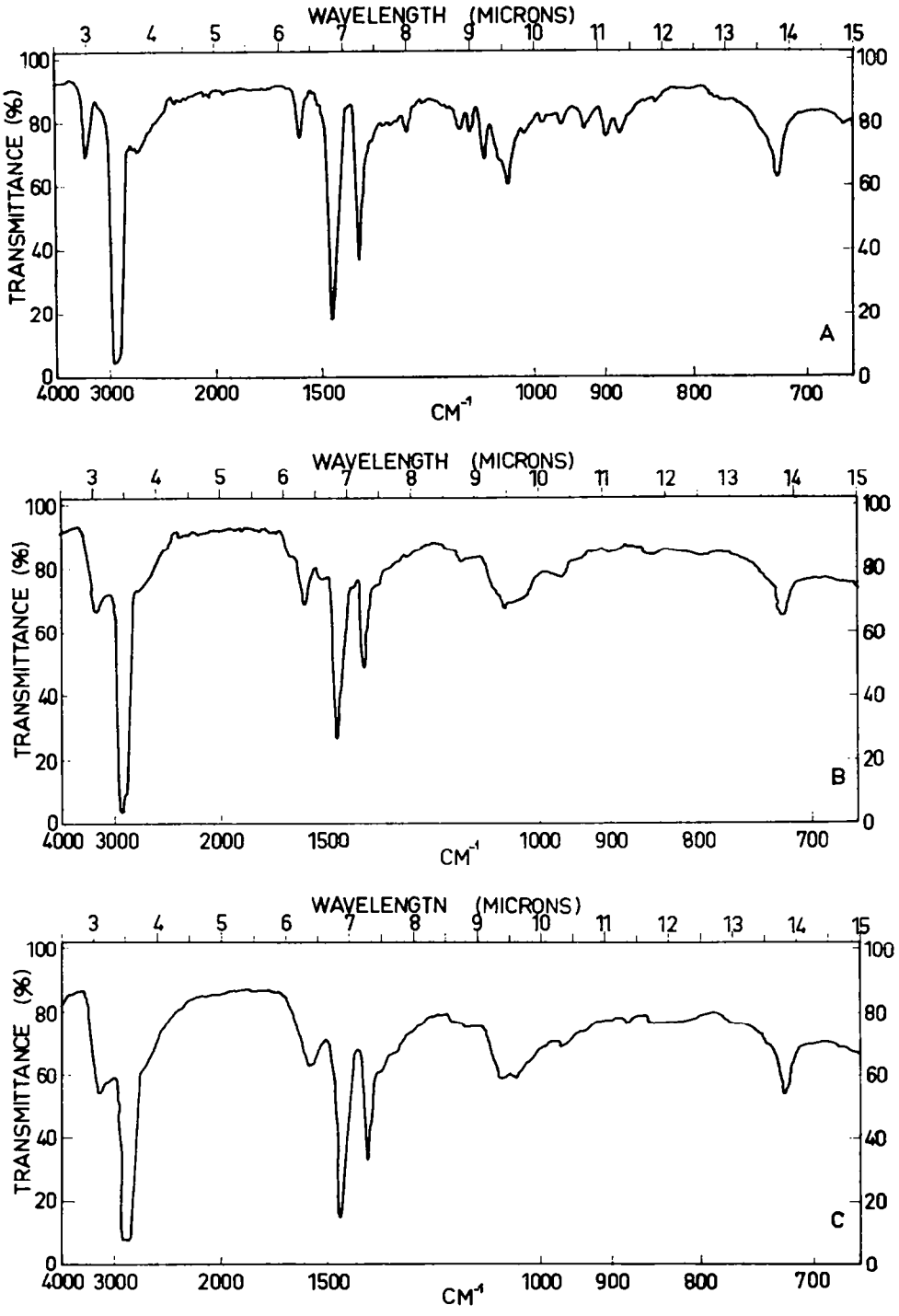


FIG. 1. Infra-red spectra in Nujol of: A—synthetic +-threo-C_{20}-dihydrosphingosine, B—synthetic +-erythro-C_{20}-dihydrosphingosine, C—natural +-dihydrosphingosine (from horse brain).

EXPERIMENTAL

All melting points are uncorrected. Rotations were measured in chloroform. Microanalyses were carried out by Mrs. M. Munk-Weinert (Department of Chemistry, Medical Faculty, University of Zagreb). Infra-red absorption spectra were measured on a Perkin-Elmer Model 134 spectrophotometer by Mr. T. Magjer.

Octadecanal-1. The substance was obtained by the Rosenmund reduction of stearoyl chloride. The acid chloride was prepared in the usual manner by heating stearic acid and thionyl chloride at 70° for 1 hr and distillation of the reaction product; b.p. 195–202°/15 mm. Stearoyl chloride (18 g) was dissolved in dry xylene (80 ml), 5% palladium on barium sulphate catalyst (8 g) was added and reduced

TABLE 2. COMPARISON OF MELTING POINTS AND SPECIFIC ROTATIONS OF SYNTHETIC +-ERYTHRO-C_{20}-DIHYDROSPHINGOSINE AND DIHYDROSPHINGOSINE FROM HORSE BRAIN

Compound	M.p.	$[\alpha]_D$ in °
+-Erythro-C_{20}-Base (synth.)	89–91	+9.7
Triacetyl	94–96	+17·3*
Dihydrosphingosine (natural)	90–92	+8·8
Triacetyl	91–94	+19·8*

* Carter and Shapiro[8] reported the following data: Natural triacetyl-C_{18}-dihydrosphingosine, m.p. 100–102°, $[\alpha]_D = +18°$ (in chloroform); synthetic triacetyl-C_{18}-dihydrosphingosine, m.p. 98–100°, $[\alpha]_D = +19·2°$ (in chloroform).

The mixed melting points of synthetic and natural base was 89–90°, and of triacetyl derivatives 93–95°.

in the hydrogen atm. After 1 hr 83% of the calculated amount of hydrogen chloride was evolved. Three such runs gave 31 g (66·5%) of the distilled aldehyde, b.p. 200–215°/20 mm. It solidified on standing at room temp.

DL-*Threo and* DL-*erythro*-1,3-*dihydroxy*-2-*nitroeicosane*. To a cooled solution of octadecanal-1 (16 g, 0·059 mole) in methanol (30 ml) nitroethanol (6 g, 0·066 mole) and potassium carbonate (1·4 g) were added. The mixture was shaken for 8 hr and then left to stand at room temp for 24 hr. The reaction mixture was then treated with water and acidified cautiously with methanolic hydrogen chloride. It was extracted with a large volume of ether, the combined ether extracts washed repeatedly with water and dried over sodium sulphate . Evaporation of the solvent yielded 18·7 g of a sticky residue which was dissolved in ether (30 ml). After addition of 300 ml of pentane and standing at room temp overnight, the resulting precipitate of the threo-isomer (nitrodiol A; 4·95 g, m.p. 54–68°; recrystallized from ether–pentane: m.p. 71–85°) was collected. The filtrate was concentrated to a smaller volume and cooled in the refrigerator. A second crop of crystals representing the erythro-isomer (nitrodiol B; 6·9 g, m.p. 46–50°; recrystallized from pentane: m.p. 53–57°) was obtained. The yield on both isomers was 11·85 g (56%). For analysis the threo-racemate was recrystallized three times from ether–pentane; colourless crystals, m.p. 87–88°. (Found: C, 67·15; H, 11·13; N, 3·97. $C_{20}H_{41}NO_4$ requires: C, 66·81; H, 11·49; N, 3·90%).

The erythro-racemate could not be purified sufficiently enough to give the correct elementary analysis. It melted at 63–67° and contained a small quantity of octadecanal.

DL-*Threo*-1,3-*dihydroxy*-2-*aminoeicosane*. The threo-nitrodiol A (2 g, m.p. 77–80°) was dissolved in 95% ethanol (30 ml) and hydrogenated in the presence of Raney nickel catalyst prepared from ca. 1 g of alloy. After 1 hr a theoretical amount (3 moles) of hydrogen was taken up at atmospheric press and room temp. The catalyst was removed by filtration and the filtrate evaporated to dryness to yield 1·8 g (97%) of the crude base, m.p. 67–73°. Recrystallization from 95% ethanol gave colourless crystals, m.p. 87–94°. Further purification could be effected by converting the base into the oxalate. The solution of the base (1·59 g) and oxalic acid (580 mg) in absolute ethanol (each in 5 ml) were mixed and cooled in the refrigerator for 5 hr. The precipitate was filtered off and washed twice

[6] C. A. Grob and E. F. Jenny, *Helv. Chim. Acta* **35**, 2106 (1952).
[7] D. E. Sunko and M. Prošťenik, *J. Org. Chem.* **18**, 1523 (1953).
[8] H. E. Carter and D. Shapiro, *J. Amer. Chem. Soc.* **75**, 5131 (1953).
[9] D. Shapiro, H. Segal and H. M. Flowers, *J. Amer. Chem. Soc.* **80**, 1194 (1958).

with boiling ethanol; crystalline solid (750 mg, 50%), m.p. 189–191°. After two crystallizations from glacial acetic acid the m.p. of the oxalate remained unchanged. (Found: C, 67·29; H, 11·87;. $C_{21}H_{44}NO_4$ requires: C, 67·33; H, 11·87%).

The pure threo-base was prepared by shaking the oxalate (1·3 g) with N KOH (25 ml) and chloroform (500 ml). After removal of the solvent 566 mg (49%), m.p. 97–98°, of the colourless solid was obtained. For analysis it was recrystallized three times from methanol; glistening meedles, m.p. 99·5–100·5°. (Found: C, 72·80; H, 12·89; $C_{20}H_{43}NO_2$ requires: C, 72·89; H, 13·15%).

DL-*Erythro*-1,3-*dihydroxy*-2-*aminoeicosane*. The erythro-nitrodiol B (1·98 g, m.p. 59–64°) was reduced in the same manner as described for the threo-isomer. The crude base (1·8 g) was converted into the oxalate which was purified only by washing with hot ethanol due to the insolubility in organic solvents. The yield of the colourless oxalate, m.p. 201–205°, amounted to 1·75 g (65%). (Found: C, 67·68; H, 11·49. $C_{21}H_{44}NO_4$ requires: C, 67·33; H, 11·84%).

The pure erythro-base was obtained from the oxalate (1·7 g) in the conventional way. Thereby, after three crystallizations from acetonitrile followed by two crystallizations from methanol, 840 mg of the analytically pure meedles, m.p. 81–83°, were obtained. (Found: C, 72·68; H, 12·89. $C_{20}H_{43}NO_2$ requires: C, 72·89; H, 13·15%).

Resolution of DL-*threo*-1,3-*dihydroxy*-2-*aminoeicosane*. L-Glutamic acid (950 mg) was dissolved in hot 50% ethanol (100 ml) and to this solution the racemic threo-base (2·1 g, m.p. 98–99°) in hot 95% ethanol (50 ml) was added. The turbid solution was left to stand at room temp for 3 hr. The crystalline precipitate was filtered off; yield 1·3 g, m.p. 146–149°. After concentration of the mother liquor a second crop of crystals (480 mg, m.p. 158–169°) was obtained. Total yield 1·78 g (59%). Recrystallization from 80% ethanol (400 ml) gave glistening leaflets, m.p. 153–156°: glutamate I. (Found: N, 5·92. $C_{25}H_{52}NO_6$ requires: N, 5·88%).

The mother liquor after the removal of the less soluble glutamate was concentrated, cooled in the refrigerator over-night and the crystalline precipitate separated by filtration. The diastereoisomeric glutamate was recrystallized three times from 80% ethanol (75 ml each); colourless needles, m.p. 168–171°: glutamate II. (Found: N, 6·14; $C_{25}H_{52}NO_6$ requires: N, 5·88%).

+-*Threo*-1,3-*dihydroxy*-2-*aminoeicosane*. The glutamate I with m.p. 153–156° (1·1 g) was decomposed with 2 N Na_2CO_3 (30 ml) and the base was extracted with much chloroform. The combined extracts were washed with water, and evaporated to dryness to yield 529 mg (69%) of the crude base melting at 107–108°. Three crystallizations from chloroform gave glistening needles, m.p. 108·5–109·5°, $[\alpha]_D^{26} = +17·6° \pm 1°$ (*c*, 0·5). (Found: C, 73·01; H, 13·11. $C_{20}H_{43}NO_2$ requires: C, 72·89; H, 13·15%).

−-*Threo*-1,3-*dihydroxy*-2-*aminoeicosane*. The glutamate II with m.p. 169–171° (450 mg) was decomposed in the same manner as described above yielding 303 mg of the crude base. Crystallization from chloroform gave glistening needles, m.p. 108–109°, $[\alpha]_D^{27} = -17·7° \pm 1°$ (*c*, 0·372). (Found; C, 72·79; H, 13·00. $C_{20}H_{43}NO_2$ requires: C, 72·89; H, 13·15%).

Equal amounts (3 mg) of both enantiomeric threo-bases were mixed and the mixture was crystallized from methanol. The product melted at 99–100° and showed no depression with the starting racemic base.

Resolution of DL-*erythro*-1,3-*dihydroxy*-2-*aminoeicosane*. The DL-erythro-base (840 mg) was resolved with L-glutamic acid (425 mg) in the same manner as described for the threo-racemate. The first crop of crystals (442 mg) melted at 89–110° and was a mixture of the unreacted base and glutamic acid. The second crop of crystals (562 mg) melted at 150–163°. After three crystallizations from 80% ethanol the m.p. was 151–156°: glutamate III. (Found: N, 5·20. $C_{25}H_{52}NO_6$ requires: N, 5·88%).

The mother liquor after the separation of the less soluble glutamate III was evaporated to dryness and the residue (340 mg) recrystallized three times from 80% ethanol; colourless powder, m.p. 155–167°: glutamate IV. (Found: N, 6·03. $C_{25}H_{52}NO_6$ requires: N, 5·88%).

+-*Erythro*-1,3-*dihydroxy*-2-*aminoeicosane*. The base recovered from the glutamate III (m.p. 151–156°) was crystallized from methanol and then from acetonitrile; m.p. 89–91°, $[\alpha]_D^{27} = +9·7° \pm 2°$ (*c*, 0·46).

Triacetyl-derivative. The +-erythro-base (97 mg), acetic anhydride (1 ml) and pyridine (1 ml) were mixed and allowed to stand at room temp for 24 hr. The reaction mixture was evaporated to dryness, dissolved in ether, washed successively with N HCl and water and the solvent removed by distillation. The crude product (38 mg, m.p. 71–74°) was recrystallized three times from acetone; m.p. 94–96°, $[\alpha]_D^{22} = +17·3° \pm 1°$ (*c*, 0·29).

—*-Erythro-1,3-dihydroxy-2-aminoeicosane.* The base obtained from the glutamate IV (m.p. 155–167°) was recrystallized from acetonitrile; colourless powder, m.p. 85–90°, $[\alpha]_D^{23} = -9{\cdot}9° \pm 2°$ (*c*, 0·534).

Equal amounts of both enantiomeric erythro-bases were mixed and the mixture was crystallized from methanol. The product melted at 70–73° and showed no depression with the starting racemic base.

Dihydrosphingosine. Crude sphingosine sulphate isolated from horse brain was treated with N NaOH and the free base extracted with ether. After crystallization from acetonitrile it melted at 90–92°; $[\alpha]_D^{23} = +8{\cdot}8° \pm 2°$ (*c*, 0·57).

Triacetyl-derivative. Prepared as described above and recrystallized from acetone it melted at 91–94°; $[\alpha]_D^{23} = +19{\cdot}8° \pm 1°$ (*c*, 0·83).