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Synthesis of DL-Erythro and Threo-sphingosine-4,5-^{3}H

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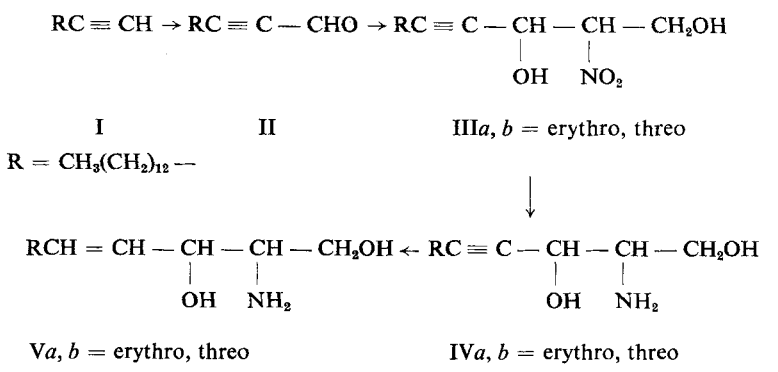
SUMMARY

DL-erythro-*sphingosine and DL*-threo-*sphingosine specifically labelled with ^3H in positions 4 and 5 were prepared. The synthesis of these compounds was based on a modification of a procedure published by Grob and Gadient. Quantitative separation of the sphingosines from acetylenic impurities was accomplished, and the physical constants of pure DL-*erythro *and* threo *sphingosines were determined. D-*erythro *sphingosine was prepared by resolving the DL racemate and it was found identical to the naturally occurring product.*

INTRODUCTION

Studies in this laboratory on the biochemistry of sphingolipids and on the relation of these lipids to lipidoses required the preparation of *erythro* and *threo* sphingosines labelled with tritium [1, 2]. Grob and Gadient [3,4,5,6] have published the synthesis of these non-radioactive materials. In course of the present work, their general procedures were employed but several modifications were necessary in order to prepare the tritiated compounds. The general scheme is as follows : 1-pentadecyne (I) was converted through a Grignard reaction with triethylorthoformate and hydrolysis to 2-hexadecynal (II). This material was condensed with 2-nitroethanol to give the *erythro* and *threo* isomers of 2-nitro-4-octadecyne-1,2-diol (III*a* and *b*). These compounds were reduced to the amino compounds (IV*a* and *b*) using zinc and ethanol. The reduction of IV*a* and *b* with lithium aluminium hydride gave DL-*erythro* and *threo* sphingosines.



Elsner and Paul [7] prepared 1-pentadecyne (I) from 1-bromotridecane using sodium acetylide. In the present work commercially available lithium acetylide-ethylenediamine complex * was used for the preparation of the chemically pure compound. In another approach, the inexpensive 1-pentadecene was brominated and dehydrohalogenated with sodium amide in liquid ammonia. The product contained a small amount of 1-pentadecene which, however, did not interfere in the subsequent reactions. Following Grob and Gadient's [3,4,5,6] procedure this compound was convertedin to 2-hexadecynal (II). No further modification of the original procedure was necessary to obtain the isomeric 2-nitro-4-octadecyne-1,2-diols (III*a* and *b*) but difficulties were encountered when these compounds were reduced [3,4,5,6,8]. However, satisfactory results were obtained when the product was reacted in a hydrochloric acid-ethanol solution with granular zinc for three days.

The reduction of *erythro*-2-amino-4-octadecyne-1,3-diol (IV*a*) with lithium aluminium hydride by the method of Grob and Gadient [3] yields a product softening at 66° C and melting at 72° C. Thin-layer chromatography showed that this compound contains about 30 percent unreacted acetylenic material which is difficult to eliminate by crystallization. By extending the reaction time to one day, a mixture could be obtained with less acetylenic impurities. Since column chromatographic purification was unsatisfactory, preparative layer chromatography was used for further purification. The product obtained in this manner, yielded after crystallization, chromatographically pure DL-*erythro*-sphingosine which softens at 70° C and melts at 83° C.

Utilizing these procedures but substituting lithium aluminium hydride-^{3}H for the non radioactive reducing agent, the pure tritiated material was synthesized.

The preparation of the DL-*threo*-sphingosine is simpler because of less acetylenic impurities in the final product. This compound melts sharply and

* Foote Mineral Co., Wickliffe, Ohio.

could be readily purified by crystallizing it several times. The tritiated isomer was prepared in a manner analogous to that for the *erythro* compound.

Pure DL-*erythro*-sphingosine was resolved using a method published by Shapiro and co-workers [9] who synthesized by a different procedure triacetyl-D-*erythro*-sphingosine. Surprisingly, we found no optical rotation for the D-compound. After acetylation, the resulting triacetyl-D-*erythro*-sphingosine showed the same rotation as the acetylated natural product. It has been concluded that the slight leavorotation published by Seydel [10] for this sphingosine was attributable to by-products.

Experimental *

1-Pentadecyne (I).

A. *From 1-bromotridecane.*

To lithium acetylide-ethylenediamine 38.7 g (0.42 mole) was added under an argon atmosphere, 220 ml of dimethylsulfoxide. The mixture was stirred and 100 g (0.38 mole) of 1-bromotridecane was added over a period of 15 min. The temperature was maintained at 25° C by cooling the reaction with ice water. After 6 hours the mixture was decomposed with 300 ml of ice water and extracted with ether. The etheral solution was washed with 2 N hydrochloric acid, dried over sodium sulfate and evaporated. The resulting oil 65 g (85% yield) was distilled through a 35 cm *Vigreux* column at 90-92° (3 mm); mp 15° C; n^{25}D 1.4409; $\nu_{max}^{0.1\ mm}$ 3340 (s) (RCH=CH—str.), 2900 (s), 2130 (m) (RC=CH—) 1460, 1380, 1230 and 720 cm^{-1}.

B. *From 1-pentadecene.*

One mole (160 g) of bromine was added with stirring at —5° C in 1.5 hr to a solution of 200 g (0.95 mole) of 1-pentadecene in 400 ml of ether. The mixture was kept at 0° C for 16 hr in the dark and was then added in 2 hr to a solution of sodium amide in liquid ammonia prepared from 50.5 g (3.5 moles) of sodium [11]. The mixture was stirred for 3 hr. More ether (1.5 l) was added and the mixture was allowed to attain room temperature. Over a 24 hr period after adding 1 kg of ice, the solution was neutralized with cold 6 N hydrochloric acid. The ether layer was separated, dried, and evaporated. The residual oil was distilled twice and 160 g (80%) of an oil was obtained. Infrared showed very weak bands at 910, 990 and 1650 cm^{-1} indicating the presence of a small amount of pentadecene.

* Melting points were corrected. They were obtained on a Thomas-Hoover melting point apparatus.

DL-*erythro* and *threo*-2-amino-4-octadecyne-1,3-diol, (IV*a* and *b*).

General procedure. Ten g (0.03 mole) of DL-*erythro* or *threo*-2-nitro-4-octadecyne-1,3-diol (III*a* or *b*) in 50 ml of ethanol and 30 g granular (20-30 mesh) zinc was added to a mixture of 80 ml of concentrated hydrochloric acid and 200 ml of ethanol kept at 5° C. In 30 min the temperature rose to 25° C which was maintained for three days. The mixture was filtered to remove traces of unreacted zinc and the ethanol was distilled at room temperature *in vacuo*. To the remaining acid solution was added 100 ml of ice and 200 ml of concentrated ammonium hydroxide solution and the mixture was extracted with chloroform. The extract was washed with water, dried and evaporated. The residue when recrystallized from 100 ml of chloroform-methanol (3 : 10) yielded 75-80% IV*a* or *b*. These products were recrystallized from isopropyl ether. Mp erythro 77-78° C [3]. Threo 83-84° C, (83-84° C [3]). Erythro ν_{max}^{KBr} 3 400, 3 300, 2 950, 2 880 (all *s*), 1 600, 1 460, 1 380, 1 320, 1 270, 1 150, 1 085, 1 070 (all *m*), 10 30 (*st*), 978, 875, 800 and 720 (all *m*) cm^{-1}. Threo ν_{max}^{KBr} 3 400 (*m*), 3 100 (*m*), 2 900 (*s*), 2 850 (*s*), 1 600, 1 460 and seven other *m* bands, 1 060 (*s*), 982, 875, 800 and 720 cm^{-1} all *m* bands.

DL-*erythro* and *threo*-trans-2-amino-4-octadecene-1,3-diol, (V*a* and *b*).

General procedure. To a stirred mixture of 455 mg (12 mmoles) of powdered lithium aluminium hydride in 70 ml dry tetrahydrofuran was added under an argon atmosphere 594 mg (2 mmoles) of either DL-erythro or threo-2-amino-4-octadecyne-1,3-diol (IV*a* or *b*). The mixture was then heated in an oil bath slowly to 80° C (external) and kept at this temperature with moderate stirring for one day. The flask was then cooled with ice and the reaction mixture was decomposed with 6 ml of a tetrahydrofuran-water (1 : 1) mixture in 1 hr and filtered. The inorganic material was extracted with 150 ml of hot chloroform. The pooled solutions were filtered, evaporated and the residue was crystallized from isopropyl ether. Yields : 460 mg (77%) for DL-*erythro*-sphingosine mp 66° C (softening)-77° C. For the *threo* isomer 560 mg (93%); mp 88-93° C. Recrystallized twice from isopropyl ether mp 97-98.5° C. (Lit. [5] 95-97° C) (small needles). It is not very soluble in cold chloroform.

Preparative layer chromatography of DL-*erythro*-sphingosine, (V*a*).

Thin-layer chromatography was performed on Silica Gel G plates according to Sambasivaro and McCluer [12]. (Alternatively Silica Gel H was used which gave similar results.) R_f values (average) in this system :

DL-*erythro*-sphingosine	0.52
DL-*threo*-sphingosine	0.42
DL-*erythro*-dihydroxy-amino-octadecyne	0.67
DL-*threo*-dihydroxy-amino-octadecyne	0.62

Using methanol-water (95 : 5) as the developer a more satisfactory separation could be achieved. R_f values :

DL-*erythro*-sphingosine	0.31-0.38 (some trailing)
DL-*erythro*-dihydroxy-amino-octadecyne	0.64

By spotting plates with mixtures having up to 50% content of dihydroxy-amino-octadecyne as little as 1% of this acetylenic impurity could be detected in the sphingosines and the composition of a mixture could be determined semiquantitatively.

For quantitative separations, 60 mg of a mixture containing 5-10% of the acetylenic impurity were applied on a 600 mμ Silica Gel H plate. Both of the above described solvent systems were used alternatively. Small strips in the middle and on both edges of the plate were sprayed with ninhydrine in butanol [12] *. The areas corresponding to DL-*erythro*-sphingosine were scraped out and the gel was placed in a glass column and was eluted with 500 ml of chloroform-methanol (1 : 1). After evaporation of the solvents the residue was redissolved in a small amount of chloroform and filtered. Crystallization from isopropyl ether or benzene gave after removal of chloroform about 70% product mp 70° C (softening)-83° C. Anal. Calcd. for $C_{18}H_{37}NO_2$: C, 72.18; H, 12.45. Found : C. 72.41; H, 12.13.**

The solubilities of DL-*erythro*-sphingosine in different solvents are the following. Diethylether 4° C, 0.8%, at 25° C, above 2% : Isopropyl ether 4° C, 0.5%; Benzene 80° C, 0.5%, at 6° C, 0.2% : Methanol 4° C, above 10% : Acetone 4° C, above 10%.

The infrared spectra for the *erythro* (Fig. 1) and *threo* (Fig. 2) isomers are different. They both show the out of plane bending vibrations around 972 cm^{-1} indicating trans configurations. The acetylenic starting materials (IV*a erythro* and *b*, *threo*) both contain bands at 978 and 983 cm^{-1} which could be mistaken for the trans bands.

DL-*erythro* and *threo*-sphingosine-4,5-^{3}H.

The general procedure was followed but lithium aluminium hydride-^{3}H, 100 mC was used. The final specific activity obtained for the *erythro*-sphingosine was 1.1 mC/mmole and for the *threo* isomers 3.6 mC/mmole. The melting points, mixed melting points and the infrared spectra were identical for the radioactive and for the unlabelled sphingosines. Thin-layer chromatograms showed no impurities. Also scannings of the plates in a Vanguard plate scanner showed no radioimpurities.

* Silica Gel HF-254 + 366 plates showed at 366 mμ light blue spots. This allowed detection and quantitative separation of the sphingosines. Separation of traces of the fluorescent dye from the purified product complicates however this approach.

** Anal. Service Unit, National Institute of Arthritis and Metabolic Diseases, NIH.

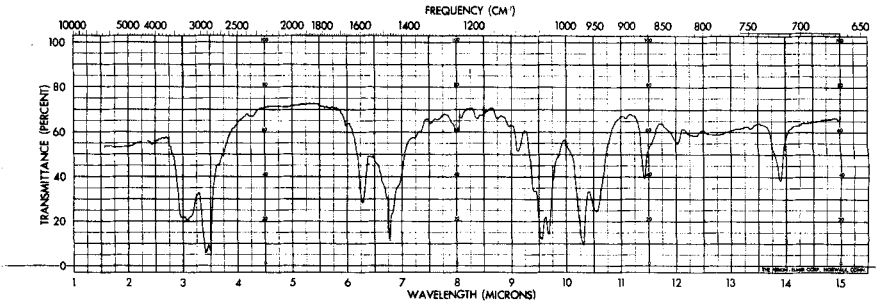


FIG. 1. DL-erythro-sphingosine.

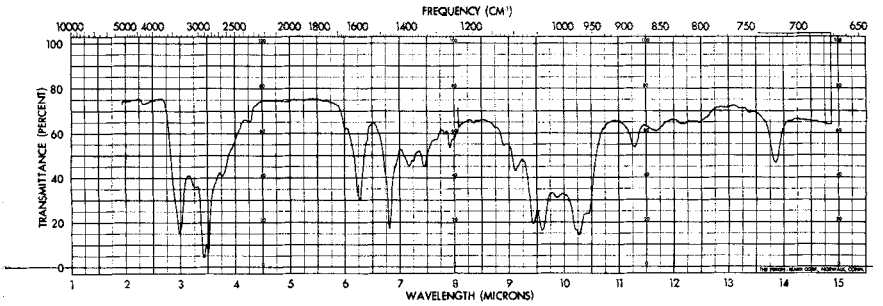


FIG. 2. DL-threo-sphingosine.

Radioactive measurements, were carried out in a *Packard Tricarb* model 3003 liquid scintillation spectrometer. The scintillation solvent contained 4g PPO and 100 mg POPOP in 1 liter of toluene [13]. External standard was used for quenching correction. Samples were also counted on planchets in a model D 47 (windowless) Nuclear Chicago gas flow counter.

D-*erythro*-sphingosine (V*a*).

D-glutamic acid, D-*erythro*-sphingosine salt was prepared from DL-*erythro*-sphingosine [9]. This diastereoisomer was recrystallized from 90% ethanol. It becomes waxy at 139° C and decomposes at 178° C.

A solution of 78 mg of this compound in 0.5 ml of ethanol was decomposed with 10 ml of 0.05 N sodium hydroxide solution and extracted with chloroform. The solution was dried, the solvent evaporated and the residue was crystallized from isopropyl ether. D-*erythro*-sphingosine 44 mg (84%); mp 70° C (softening)-83° C was obtained. $[\alpha]^{25}D$ 0° $\pm$ 0.5° (*c* 2.0, $CHCl_3$).

The triacetyl-D-*erythro*-sphingosine which was prepared from this product had the same rotation, melting point and infrared spectra as the one obtained from the naturally occurring sphingosine. This compound was

deacetylated in dioxane with 0.5 N barium hydroxide solution according to Tripton [14]. The isolated D-*erythro*-sphingosine showed no observable rotation.* Seydel [10] found $[\alpha]^{22}D$—3.4° (*c* 2.0, $CHCl_3$).

The D-*erythro*-sphingosine used for comparisons was prepared from naturally occurring glycolipids according to Carter and co-workers [15, 16]. This product was used for the preparation of the triacetylated sphingosine according to the procedure of Grob and Gadient [3]. D-triacetyl-*erythro*-sphingosine was recrystallized until the mp. 102-103.5° C was reached. $[\alpha]^{25}D$ —14° (*c* 1.0, $CHCl_3$). Hydrolysis of this product [13] yielded D-*erythro*-sphingosine, 86% yield, showing some impurities on thin-layer chromatography.

Preparative layer chromatography gave a chromatographically pure compound, mp 70° C (softening)-83° C. Sublimes at 120-130° C (0.05 mm). The sublimed D-*erythro*-sphingosine was identical to the above described, products.

ACKNOWLEDGEMENTS

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* The optical rotation of this product was also determined by Dr. N. Mitchel from Cary Scientific Institute. He found no activity from 6 025 Å to 2 500 Å. Under the experimental conditions the rotation was less than 2 millidegrees in the sodium D line region.

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