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Studies in the Sphingolipids Series. XXIII.* Synthesis and Resolution of *erythro* and *threo*-C_{20}-Sphingosines

(Mrs.) B. Majhofer-Oreščanin and M. Proštenik

Department of Biochemistry, Institute »Ruđer Bošković«, Zagreb, Croatia, Yugoslavia

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The synthesis of DL-*trans*-2-amino-1,3-dihydroxy-4-eicosenes (VII) by condensation of 2-octadecyne-1-al (IV) with nitroethanol in the presence of potassium carbonate and subsequent reduction of the two nitrodiols (V) is described. The resolution of the DL-*threo*-base (VIIa) into the optical isomers was effected with D-tartaric acid. The DL-*erythro*-base (VIIb) was resolved with L-glutamic acid. Natural C_{20}-sphingosine is most probably the *trans*-D(+)-*erythro*-base.

The structure of C_{18}-sphingosine (*trans*-D(+)-*erythro*-2-amino-1,3-dihydroxy-4-octadecene), a long-chain base widely distributed in animal tissues, discovered in 1879 by Thudichum, is well established.[1] The four possible stereoisomeric forms of the *trans*-base have been prepared synthetically.[2,3] The recent detection of C_{20}-sphingosine (2-amino-1,3-dihydroxy-4-eicosene) (VII) in horse and bovine brain revealed new views in the sphingolipide chemistry.[4] An extensive investigation in the C_{20}-series furnished the syntheses of the optically active C_{20}-dihydrosphingosines[5] and C_{20}-sphingines.[6]

$$\begin{array}{l} CH_3(CH_2)_{14}—CH=CH—CH—CH_2 \\ \qquad\qquad\qquad\quad | \quad\ | \quad\ | \\ \qquad\qquad\qquad\quad OH\ NH_2\ OH \end{array} \qquad \text{VII}$$

Continuing the studies on the stereochemistry of the new base we have prepared the four optically active forms of VII. The results obtained are presented in this paper.

To our experience, the most suitable general route which has repeatedly been applied to the synthesis of the sphingolipide bases consists in the condensation of nitroethanol with an appropriate long-chain aldehyde under alkaline conditions.[3,5,7,8] For this purpose 2-octadecyne-1-al (IV) was prepared following the method described earlier[8] and characterized as 2,4-dinitrophenylhydrazone. The acetylenic aldehyde was condensed with nitroethanol to yield a mixture of the *erythro* and *threo*-forms of 2-nitro-1,3-dihydroxy-4-eicosyne (V). The less soluble *threo*-nitrodiol crystallized from the ether-pentane solution in a pure condition. The *erythro*-isomer was obtained by working up the mother liquor. The infrared spectra of both isomeric forms differed considerably. The successive reduction of the two nitrodiols with zinc in hydrochloric acid and

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then with lithium aluminium hydride afforded DL-*threo* (VIa) and DL-*erythro*-2-amino-1,3-dihydroxy-4-eicosyne (VIb) and *trans*-DL-*threo* (VIIa) and *trans*-DL-*erythro*-2-amino-1,3-dihydroxy-4-eicosene (VIIb) respectively. Strong absorption bands at 970 cm^{-1} were indicative for the *trans*-ethylenic structure. For further characterization all bases thus obtained were converted into the crystalline oxalates, which showed to be particularly suitable for purification purposes. On the whole, the *threo*-compounds could easily be purified and prepared in a good yield, while the purification of the *erythro*-compounds was less satisfactory and connected with a considerable loss of material. In some cases the *erythro*-forms did not give the correct elemental analyses.

The racemic *erythro*-aminodiol (VIIb) was resolved without difficulties into the optically active antipodes by means of L-glutamic acid. On the contrary, all attempts to resolve the *threo*-isomer (VIIa) with the same agent failed. In this case the complete resolution was achieved with D-tartaric acid. The four possible active isomers of the *trans*-series having a satisfactory optical purity were thus obtained. (Table I). The structure of *trans*-(+)-*erythro*-2-amino-1,3-dihydroxy-4-eicosene is tentatively assigned to C_{20}-sphingosine of horse and bovine brain. Configurational assignments in the series of C_{20}-sphingosines are based on the mode of chemical reactions, physical properties and infrared spectra as discussed previously in case of the synthetic C_{20}-dihydrosphingosines.[5]

TABLE I

Melting Points and Specific Rotations of Optically Active trans-*2-Amino-1,3-dihydroxy-4-eicosenes and Their Derivatives*

Compound	M. p. in °C	$[\alpha]_D$ in °
(+)-*erythro*-Base	69—76	+3.8
Glutamate I	165—179 (decomp.)	
Triacetyl	98—99.5	—11.4
(—)-*erythro*-Base	66—72	—3.5
Glutamate II	145—157 (decomp.)	
(+)-*threo*-Base	97—100	+2.6
Tartarate I	176—181 (decomp.)	
(—)-*threo*-Base	97—99	—2.3
Tartarate II	135—145 (decomp.)	
(—)-*erythro*-Dihydrobase	81—84	—11.4
(+)-*threo*-Dihydrobase	105—107	+18.3

EXPERIMENTAL

All melting points are uncorrected. Infrared absorption spectra were measured on a Perkin-Elmer Model 134 spectrophotometer.

1-Bromopentadecane (I)

The substance was prepared from silver palmitate following the method of Hunsdiecker.[9]

1-Heptadecyne (II)

The alkyne was obtained in 65% yield according to the general procedure described by Jenny and Meier[10] starting with I and sodium acetylenide; b. p. 120° at 0.5 mm. Hg, n_D^{22} 1.4430.

2-Octadecyne-1-al Diethyl Acetal (III)

To a solution of the Grignard reagent prepared from magnesium turnings (7 g.) and ethyl bromide (46 g.) in tetrahydrofurane (65 ml.) placed in a 500 ml. round bottomed flask equipped with a mechanical stirrer a solution of II (49 g.) in tetrahydrofurane (45 ml.) was dropwise added. The flask content was refluxed for 6 hrs. Upon addition of a solution of ethyl orthoformate (43 g.) in tetrahydrofurane (45 ml.) it was refluxed for additional 30 hrs. The reaction mixture was dissolved in ether (500 ml.) and treated with 2 *N* ammonium chloride (10 ml.). Distillation of the ether layer furnished the acetal (48.5 g., 70%), b. p. 155—165° at 0.3 mm. Hg; n_D^{22} 1.4496.

Anal. $C_{22}H_{42}O_2$ (338.56) calc'd.: C 78.04; H 12.50%
found: C 77.64; H 12.19%

2-Octadecyne-1-al (IV)

A mixture of III (47 g.), water (37 ml.), concentrated hydrochloric acid (4 ml.) and dioxane (110 ml.) was placed in a 500 ml. three-necked round bottomed flask fitted with a mechanical stirrer a dropping funnel and a condenser, and heated to 110° in a nitrogen atmosphere for 7 hrs. Upon cooling the mixture was diluted with ether, the organic layer separated and washed successively with 2 *N* sodium carbonate and then with water to the neutral. The solvent was removed and the residue distilled *in vacuo*. Colourless liquid (33.2 g., 90%), b. p. 134—138° at 0.2 mm. Hg, u_D^{24} 1.4607, which crystallized upon standing.

2,4-Dinitrophenylhydrazone. The substance was prepared in the usual manner in 91% yield. Orange-coloured crystals from ethanol, m.p. 51—53.5°.

Anal. $C_{24}H_{36}N_4O_4$ (444.56) calc'd.: C 64.84; H 8.16; N 12.60%
found: C 64.77; H 8.20; N 12.39%

DL-threo *and* DL-erythro-*2-Nitro-1,3-dihydroxy-4-eicosyne* (V)

To a cold solution of freshly distilled IV (37 g.) in methanol (50 ml.), nitroethanol (10 g.) and potassium carbonate (2.5 g.) were added. The mixture was shaken for 15 min. and allowed to stand in the refrigerator for 15 min. The mixture was then treated with water, acidified with concentrated hydrochloric acid and extracted with ether. Evaporation of the solvent yielded 45.5 g. of a viscous residue which was dissolved in ether (30 ml.). After addition of pentane (500 ml.) and standing at room temperature for 24 hrs. the resulting precipitate of the *threo*-isomer (nitrodiol *A*, 16 g., m. p. 76—78°) was collected. The filtrate was concentrated to a small volume and cooled in a refrigerator for 48 hrs. Thus, the crystalline *erythro*-isomer (nitrodiol *B*, 12 g., m. p. 40—49°) was obtained. The yield on both isomers was 38.5 g. (57%). For analysis the *threo*-isomer was recrystallized from pentane; m. p. 78—82°.

Anal. $C_{20}H_{37}NO_4$ (355.5) calc'd.: C 67.57; H 10.49; N 3.94%
found: C 67.42; H 10.31; N 3.95%

The *erythro*-racemate was obtained in the analytically pure condition by recrystallization from a mixture chloroform and pentane (1:5); m. p. 48—50°.

Anal. $C_{20}H_{37}NO_4$ (355.5) calc'd.: C 67.57; H 10.49; N 3.94%
found: C 67.66; H 10.57; N 4.04%

DL-threo-*2-Amino-1,3-dihydroxy-4-eicosyne* (VIa)

A solution of the *threo*-nitrodiol *A* (V, 11 g) in ethanol (70 ml.) was added with stirring to the cold mixture of *conc.* hydrochloric acid (25 ml.) and ethanol (25 ml.). The mixture was then treated with zinc dust (20 g.) in small portions. Three 3 ml. portions of concentrated hydrochloric acid were added at this reaction stage. Stirring was continued for 1 hr. at room temperature, the inorganic precipitate filtered, washed with water and ethanol, the filtrate made alkaline by addition of 10 *N* sodium hydroxide and extracted with ether. The combined extracts were washed with water to the neutral. After drying with sodium sulfate the solvent was evaporated to dryness to give 6.5 g. (64%) of the crude base. Several crystallizations from acetonitrile gave a product melting at 88—90°.

Anal. $C_{20}H_{39}NO_2$ (325.5) calc'd.: C 73.79; H 12.08; N 4.30%
found: C 73.92; H 12.08; N 4.35%

Oxalate. The substance, recrystallized from glacial acetic melted at 198—199° (decomp.).

Anal. $C_{21}H_{40}NO_4$ (370.53) calc'd.: C 68.07; H 10.88; N 3.78%
found: C 68.22; H 11.09; N 4.00%

DL-erythro-*2-Amino-1,3-dihydroxy-4-eicosyne* (VIb)

The *erythro*-base was prepared analogously starting with the *erythro*-nitrodiol *B* (V, 12 g.). The yield was 10.8 g. (98%), m. p. 54—60°. Recrystallization from acetonitrile gave a product melting at 72—75°.

Anal. $C_{20}H_{39}NO_2$ (325.5) calc'd.: C 73.79; H 12.08; N 4.30%
found: C 74.21; H 12.41; N 4.26%

Oxalate. The substance was recrystallized from ethanol; m. p. 179—182°.

Anal. $C_{21}H_{40}N_4$ (370.53) calc'd.: C 68.07; H 10.88; N 3.78%
found: C 68.34; H 11.12; N 3.85%

DL-trans-threo-*2-Amino-1,3-dihydroxy-4-eicosene* (VIIa)

A sample of VIa (5.75 g.), lithium aluminium hydride (2.5 g) and tetrahydrofurane (120 ml.) were refluxed for 4 hrs. The reaction mixture was cooled in an ice-bath and treated successively with water (2.5 ml.), 15% sodium hydroxide (2.5 ml.) and again with water (7.5 ml.). The solid material was removed and the filtrate evaporated *in vacuo* to dryness. The crude base (4.7 g., 82%, m. p. 72—85°) was recrystallized four times from acetonitrile; m. p. 92—95°.

Anal. $C_{20}H_{41}NO_2$ (327.5) calc'd.: C 73.33; H 12.62; N 4.28%
found: C 73.64; H 12.76; N 4.02%

Oxalate. Crystallization from a mixture ethanol-glacial acetic acid (10 : 1) gave a product melting at 195—197° (decomp.).

Anal. $C_{21}H_{42}NO_4$ (372.5) calc'd.: C 67.70; H 11.36; N 3.76%
found: C 67.93; H 11.35; N 3.87%

DL-trans-erythro-*2-Amino-1,3-dihydroxy-4-eicosene* (VIIb)

The *erythro*-base was prepared analogously from the *erythro*-racemate VIb (2.7 g.). The crude base (2 g., 75%, m. p. 51—53°) was recrystallized four times from acetonitrile; m. p. 72—77°.

Anal. $C_{20}H_{41}NO_2$ (327.5) calc'd.: C 73.33; H 12.62; N 4.28%
found: C 73.23; H 12.42; N 4.12%

Oxalate. The substance was recrystallized twice from ethanol and four times from glacial acetic acid; m. p. 196—198° (decomp.).

Anal. $C_{21}H_{42}NO_4$ (372.5) calc'd.: C 67.70; H 11.36; N 3.76%
found: C 67,42; H 11.45; N 3.77%

Resolution of DL-trans-threo-*2-Amino-1,3-dihydroxy-4-eicosene*

D-Tartaric acid (0.92 g.) was dissolved in hot 95% ethanol (30 ml.) and to this solution the DL-*trans-threo*-base VIIa (2 g.) in hot 95% ethanol (50 ml.) was added. Upon cooling the crystalline precipitate was filtered off (1.2 g., m. p. 154—178° decomp., tartarate I). The mother liquor was concentrated *in vacuo* to a half of its initial volume and left to stand in the refrigerator for 24 hrs. The precipitate was filtered by suction (1.1 g., m. p. 130—150°, tartarate II). The yield on both diastereoisomeric salts was 2.3 g. (93%). For analysis the tartarates were recrystallized three times from ethanol.

Tartarate I, m. p. 176—181° (decomp.).
Anal. $C_{22}H_{44}NO_5$ (402.6) calc'd.: N 3.48%
found: N 3.56%

Tartarate II, m. p. 135—145° (decomp.).
Anal. $C_{22}H_{44}NO_5$ (402.6) calc'd.: N 3.48%
found: N 3.21%

trans-(+)-threo-2-*Amino-1,3-dihydroxy-4-eicosene*

The tartarate I (1.2 g.) was decomposed with 2 *N* sodium carbonate (50 ml.) and the base was extracted with chloroform (500 ml.). The extracts were evaporated *in vacuo* to dryness to yield 0.5 g. (52%) of the crude base melting at 90—92°. Three crystallizations from acetonitrile gave a product melting at 97—100°, $[\alpha]_D^{22}$ + 2.6° ± 1° (c, 1.7 in pyridine).

Anal. $C_{20}H_{41}NO_2$ (327.5) calc'd.: C 73.33; H 12.62; N 4.28%
found: C 73.61; H 12.56; N 4.30%

trans-(—)-threo-2-*Amino-1,3-dihidroxy-4-eicosene*

The tartarate II (0.97 g.) was decomposed in the same manner as described above yielding 0.34 g. (43%) of the crude base, m. p. 94—97°, which was recrystallized from acetonitrile; m.p. 97—99°, $[\alpha]_D^{25}$ — 2.3° ± 1° (c, 2.5 in pyridine).

Equal amounts of both enantiomeric *threo*-bases were mixed and the mixture was crystallized from acetonitrile. The product melted at 92—94° and showed no depression with the racemic base VIIa (92—95°).

(+)-threo-2-*Amino-1,3-dihydroxyeicosane*

A solution of the *trans*-(+)-*threo*-base (100 mg.) in ethanol was hydrogenated in the presence of Adams platinum catalyst. The hydrogen uptake was 7 ml. at room temperature and at atmospheric pressure. The base was recrystallized twice from acetonitrile; m.p. 105—107°, $[\alpha]_D^{25}$ + 18.3° ± 2° (c, 0.38 in chloroform) (lit. m.p. 108.5—109.5, $[\alpha]_D^{26}$ + 17.6° ± 1°)[5].

Resolution of DL-trans-erythro-2-*Amino-1,3-dihydroxy-4-eicosene*

L-Glutamic acid (0.61 g.) was dissolved in hot 50% ethanol (60 ml.) and to this solution the DL-*trans-erythro*-base VIIb (1.36 g.) in hot 95% ethanol (50 ml.) was added. The turbid solution was allowed to stand at room temperature for 5 hrs. The precipitate was filtered by suction (1.08 g., m. p. 150—170° decomp., glutamate I). The mother liquor was evaporated *in vacuo* to a small volume. A second crop of crystals was obtained (0.92 g., m. p. 135—155° decomp., glutamate II). Total yield on both diastereoisomeric salts was 2 g. (100%). For analysis the glutamates were recrystallized three times from 80% ethanol.

Glutamate I, m. p. 165—179° (decomp.).
Anal. $C_{25}H_{50}N_2O_6$ (474.67) calc'd.: N 5.90%
found: N 5.99%

Glutamate II, m. p. 145—157° (decomp.).
Anal. $C_{25}H_{50}N_2O_6$ (474.67) calc'd.: N 5.90%
found: N 6.11%

trans-(+)-erythro-2-*Amino-1,3-dihydroxy-4-eicosene*

The glutamate I (1 g.) gave in the usual manner 0.53 g. of the crude base, which was recrystallized three times from acetonitrile; m.p. 70—76°, $[\alpha]_D^{27}$ + 3.8° ± 1° (c, 1.32 in pyridine).

Triacetyl-derivative. The *trans*-(+)-*erythro*-base was acetylated in the conventional way; m.p. 98—99.5° from acetone, $[\alpha]^{24}$ — 11.4° ± 2° (c, 0.42 in

chloroform). Melting points and specific rotations reported for *a)* synthetic triacetyl--C_{18}-sphingosine: 103—104°, $[\alpha]_D^{24}$ — 12.7°,[2] *b)* natural triacetyl-C_{18}-sphingosine: 101—102°, $[\alpha]_D^{25}$ — 11.7°.[11]

trans-(—)-erythro-*2-Amino-1,3-dihydroxy-4-eicosene*

The decomposition of the glutamate II (0.8 g.) yielded 0.43 g. (79%) of the crude base which after crystalization from acetonitrile melted at 66—72°; $[\alpha]_D^{25}$ — 3.5° ± 1° (c, 1.77 in pyridine).

A mixture of equal amounts of both enantiomeric *erythro*-bases was optically inactive.

(—)-erythro-*2-Amino-1,3-dihydroxyeicosane*

Catalytic hydrogenation of the *trans*-(—)-*erythro*-base gave the dihydro-base, m.p. 84°, $[\alpha]_D^{27}$ — 11.4° ± 2° (c, 0.52 in chloroform) (lit. m.p. 85—90°, $[\alpha]_D^{23}$ 9.9° ± 2°)[5].

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IZVOD

Studije u redu sfingolipida. XXIII. Sinteza i cijepanje u optičke antipode *eritro* i *treo*-C_{20}-sfingozina

B. Majhofer-Oreščanin i M. Prošteník

Izvedena je sinteza DL-*trans*-2-amino-1,3-dihidroksi-4-eikosena (VII) kondenzacijom 2-oktadecin-1-ala (IV) s nitroetanolom uz sudjelovanje kalijeva karbonata i daljnjom redukcijom dobivenih racemičkih nitrodiola (V). Cijepanje u optičke antipode DL-*treo*-baze uspjelo je s pomoću D-vinske kiseline, dok je DL-*eritro*-baza rastavljena u antipode s pomoću L-glutaminske kiseline. Tališta i specifična skretanja prikazana su tabelarno. Prirodnom C_{20}-sfingozinu pripada vjerojatno struktura *trans*-D(+)-*eritro*-baze.

BIOKEMIJSKI ODJEL
INSTITUT »RUĐER BOŠKOVIĆ«
ZAGREB

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