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Studies in the Sphingolipids Series. XVIII.* Synthesis and Resolution of 1-Hydroxy-2-aminoeicosane (C_{20}-Sphingine)

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The preparation of racemic 1-hydroxy-2-aminoeicosane (C_{20}-sphingine) is described. The racemic base was resolved into the optical antipodes *via* the diastereomeric L-glutamates. *N*-Acetyl- and *O,N*-diacetyl derivatives of the enantiomeric bases are described. D-Configuration is assigned to the (—) base.

D(—)-C_{18}-Sphingine (1-hydroxy-2-aminoöctadecane) is a well known degradation product of C_{18}-sphingosine. The synthesis of both optically active forms has been described in a paper by Sunko and Proštenik where also the relating references can be found[1]. The synthesis of radioactive DL-1-^{14}C-sphingine has also been reported[2].

Most recently, Proštenik and Majhofer-Oreščanin reported the occurrence of C_{20}-sphingosine (1,3-dihydroxy-2-amino-4-eicosene) in both horse and beef brain. It has also been shown that the new base could be reduced into C_{20}-sphingine[3]. Since one could suppose very similar properties — such as melting points and, particularly, specific rotations — of C_{18} and C_{20}-sphingosine, it was of interest to prepare a simpler molecule of C_{20}-sphingine in order to compare its properties with those of C_{18}-sphingine. Undoubtedly, this knowledge would facilitate the improvement of the separation procedures of homologous bases from natural mixtures.

The starting material was very carefully purified stearic acid. This was successively converted, in a series of known reactions[1], into the following compounds: stearoyl chloride, 1-diazo-2-nonadecanone, methyl and ethyl nonadecanoate, nonadecanoic acid, nonadecanoic acid chloride, 1-diazo-2-eicosanone, 1-acetoxy-2-eicosanone, 1-hydroxy-2-eicosanone oxime, and finally 1-hydroxy-2-aminoeicosane (DL-C_{20}-sphingine). Resolution of the racemic base into the optical antipodes was effected highly satisfactorily by means of L-glutamic acid. Both (+) and (—)-base were converted into the nicely crystalline *N*-acetyl and *N,O*-diacetyl derivatives. The melting points and specific rotations of C_{18} and C_{20}-bases and derivatives are listed in Table I.

On the basis of a striking similarity in optical properties of the two bases it is tentatively concluded that the (—)-base has the D-configuration.

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* Paper XVII: B. Majhofer-Oreščanin and M. Proštenik, *Tetrahedron*, 12 (1961) 56.

TABLE I

Comparison of Melting Points and Specific Rotations of C_{18} and C_{20}-Sphingines and Derivatives

Compound	C_{18}-Sphingine[1]		C_{20}-Sphingine	
	M. p., °C	$[\alpha]_D$ in °	M. p., °C	$[\alpha]_D$ in °
DL-Base	79—80		86—87	
N-Acetyl	104—107		112—113	
N,O-Diacetyl			97—98	
N-Benzoyl	92.5—94		102—103	
(—)-Base	84—86	— 4.92	90—90.5	— 5.3
(+)-*N*-Acetyl	97—98.5	+11.74	103—104	+10.4
(+)-*N,O*-Diacetyl	101—103	+22.0	109—110	+18.82
(+)-Base	85—86	+ 4.82	89—91	+ 5.33
(—)-*N*-Acetyl	97—98	12.27	105	—10.1
(—)-*N,O*-Diacetyl	100—103	—23.9	109—110	—18.58

We wish to point out that the experimental data are described in more detail since many essential improvements in relation to the preparation of the C_{18}-base have been achieved.

EXPERIMENTAL

The melting points reported are uncorrected values. Rotations were measured in chloroform.

1-Diazo-2-nonadecanone

The diazoketone was prepared according to Grundmann[4] starting with stearoyl chloride (30,5 g.) and diazomethane (from 36 g. of nitrosomethylurea). The yield was 30.7 g. (98.8%) of a product melting at 69—70°.

Ethyl Nonadecanoate

The diazoketone (30.5 g., 0.1 mole, m. p. 69—70°) was suspended in 200 ml. of absolute ethanol and placed in a 1000-ml. three-necked round-bottomed flask fitted with a mechanical stirrer, a droping funnel and a condenser. A solution of silver benzoate (2 g.) in triethylamine (18 g.) was then added dropwise over a period od 70 min. The reaction mixture turned black and the evolution of nitrogen started immediately. The reaction mixture was then refluxed for 15 min. and filtered by aid of charcoal. The solvent was removed by distillation *in vacuo*, the residue dissolved in ether (200 ml.) and washed successively with *N* hydrochloric acid, sodium hydrogen carbonate and water. After drying over sodium sulphate and removal of the solvent the crude ester was purified by distillation. The yield was 25.3 g. (78.4%), b. p. 210—225° at 12 mm. Hg.

Methyl Nonadecanoate

The Wolff rearrangement of the diazoketone was carried out in methanol exactly as described in the foregoing preparation. Thereby, the ester, b. p. 215—225° at 12 mm. Hg., was obtained in a 81.6% yield.

Nonadecanoic Acid

Alkaline hydrolysis of the methyl or ethyl ester in the usual manner furnished pure nonadecanoic acid, m. p. 67—68°, yield 95—98%.

1-Diazo-2-eicosanone

Nonadecanoic acid chloride was prepared by heating the acid (10 g., m. p. 67—68°) with thionyl chloride (15 ml.) at 70° for 1 hr. After removal of the excess of

thionyl chloride *in vacuo* the procedure was repeated with a new quantity of the reagent. The yield of the acid chloride, b. p. 223° at 14 mm. Hg., was 8.2 g. (77.2%). It was dissolved in absolute ether and treated in the conventional manner with the ethereal solution of diazomethane (prepared from 13.4 g. of nitrosomethylurea). Evaporation of the solvent to dryness *in vacuo* yielded 8.1 g. (97%) of the diazoketone, yellow leaflets, m. p. 69—71°. It was used without further purification for the next reaction step.

1-Acetoxy-2-eicosanone

A sample of 1-diazo-2-eicosanone (8 g., 24.8 m*M*) was added in small portions to glacial acetic acid (30 ml.) containing potassium acetate (1 g.) at 70°. For the completion of the reaction the solution was refluxed for 1 hr. After cooling to room temperature the reaction mixture was poured into water (150 ml.), the separated solid extracted with ether, the solution washed thoroughly with water and dried over sodium sulphate. The solvent was then distilled off to yield 8 g. (91%) of the crystalline solid, m. p. 79—81°. One crystallization from 95% ethanol (150 ml.) with addition of charcoal yielded 6.7 of a product melting at 81—82.5°. For analysis the substance was dissolved in benzene and chromatographed through activated aluminium oxide (Fluka). Colourless crystals, m. p. 82—83°.

Anal. 7.520 mg. subst.: 20.55 mg. CO_2, 7.97 mg. H_2O
$C_{22}H_{42}O_3$ (354.56) calc'd: C 74.52; H 11.94%
found: C 74.57; H 11.86%

1-Hydroxy-2-eicosanone oxime

A solution of the acetoxy ketone (4 g. 11.3 m*M*, m. p. 81—82°) and hydroxylamine acetate (prepared from 10 g. of hydroxylamine hydrochloride and 15 g. of crystalline sodium acetate) in absolute ethanol (150 ml.) was refluxed for 15 hrs. The excess of ethanol was distilled off, water (100 ml.) was added and the mixture extracted with ether. The solution was then washed with water, dried over sodium sulphate and evaporated to dryness. The crude oxime (3.7 g., 100%, m. p. 87—89°) was recrystallized from 1.3 l. of petroleum ether (b. p. 50—70°) (refluxing for at least 1 hr. is necessary). The analytical sample melted at 91—93°.

Anal. 6.815 mg. subst.: 18.20 mg. CO_2, 7.44 mg. H_2O
2.245 mg. subst.: 0.087 ml. N_2 (21°, 750 mm)
$C_{20}H_{41}NO_2$ (327.54) calc'd: C 73.33; H 12.66; N 4.28%
found: C 72.88; H 12.22; N 4.44%

1-Hydroxy-2-aminoeicosane (DL-C_{20}-Sphingine)

A sample of the oxime (1.6 g., 4.88 m*M*, m. p. 90—92°) was suspended in absolute ether (50 ml.) and added dropwise to a solution of lithium aluminium hydride (750 mg.) in absolute ether (50 ml.). After the vigorous reaction has subsided the mixture was refluxed for 7 hrs. The excess of hydride was hydrolized by cautious addition of water. The flask content was then evaporated to dryness and the resulting solid extracted with three 50 ml. portions of boiling chloroform. Removal of the solvent *in vacuo* gave crude amino alcohol, m.p. 80—93°. Recrystallization from petroleum ether (b. p. 60—80°) yielded 750 mg. (49%) of glistening leaflets, m. p. 86—87°.

Anal. 8.765 mg. subst.: 24.52 mg. CO_2, 10.48 mg. H_2O
5.680 mg. subst.: 0.226 ml. N_2 (21°, 750 mm)
$C_{20}H_{43}NO$ (313.55) calc'd: C 76.60, H 13.82, N 4.47%
found: C 76.34, H 13.38, N 4.54%

*Diacetyl-*DL-C_{20}*-sphingine*

A sample of the base (400 mg., 1.28 m*M*) was acetylated with an acetic anhydride — pyridine mixture. The crude diacetyl derivative (450 mg., 88.8%, m.p. 97—99°) was recrystallized from acetonitrile and melted at 97—98°.

Anal. 8.280 mg. subst.: 21.88 mg. CO_2, 8.76 mg. H_2O
7.355 mg. subst.: 0.219 ml. N_2 (21.5°, 750 mm)
$C_{24}H_{47}NO_3$ (397.62) calc'd: C 72.49; H 11.91; N 3.52%
found: C 72.11; H 11.84; N 3.41%

N-Acetyl-DL-C_{20}-sphingine

The diacetyl derivative (300 mg.) was hydrolized with methanolic potassium hydroxide to give 250 mg. (91.1%) of a product melting at 111—112°. After one crystallization from methanol it melted at 112—113°.

Anal. 9.090 mg. subst.: 24.68 mg. CO_2, 10.34 mg. H_2O
7.090 mg. subst.: 0.254 ml. N_2 (21°, 750 mm)
$C_{22}H_{45}NO_2$ (355.59) calc'd: C 74.31; H 12.75; N 3.94%
found: C 74.09; H 12.72; N 4.10%

N-Benzoyl-DL-C_{20}-sphingine

A sample of the base was benzoylated in the usual manner. The substance was recrystallized from 95% ethanol, m.p. 102—103°.

Anal. 9.180 mg. subst.: 26.14 mg. CO_2, 9.34 mg. H_2O
7.930 mg. subst.: 0.253 ml. N_2 (24°, 753 mm)
$C_{27}H_{47}NO_2$ (417.65) calc'd: C 77.64; H 11.34; N 3.35%
found: C 77.71; H 11.39; N 3.63%

N-Phthaloyl-DL-C_{20}-sphingine

A sample of the base (500 mg., 1.63 m*M*, m. p. 86—87°), phthalic anhydride (236 mg.) and toluene (50 ml.) were refluxed for 2 hrs. The water formed during the reaction was removed azeotropically. After the completion of the reaction the mixture was evaporated *in vacuo* to dryness and the crystalline residue (700 mg., 99%, m.p. 67—70°) recrystallized from acetonitrile. The analytical sample melted at 72—73°.

Anal. 9.065 mg. subst.: 25.29 mg. CO_2, 8.53 mg. H_2O
8.370 mg. subst.: 0.241 ml. N_2 (28°, 747 mm)
$C_{28}H_{45}NO_3$ (443.65) calc'd: C 75.79; H 10.23; N 3.16%
found: C 76.13; H 10.52; N 3.21%

N-(o-Carboxybenzoyl)-DL-C_{20}-sphingine

A solution of the *N*-phthaloyl derivative (150 mg., 0.34 m*M*, m.p. 72—73°) in 95% ethanol (5 ml.) was refluxed with 17% ethanolic potassium hydroxide (2.5 ml.) for 10 min. The solution was poured into aqueous hydrochloric acid (1:1, 20 ml.), the crystalline precipitate filtered by suction and washed thoroughly with water. Yield: 150 mg. (96.1%) of colourless crystals, m.p. 105—107°. Further crystallization from methanol gave the analytically pure sample melting at 107—108°.

Anal. 9.050 mg. subst.: 24.28 mg. CO_2, 8.40 mg. H_2O
6.985 mg. subst.: 0.194 ml. N_2 (26.5°, 746 mm)
$C_{28}H_{47}NO_4$ (461.66) calc'd: C 72.84; H 10.26; N 3.03%
found: C 73.21; H 10.39; N 3.11%

Resolution of Racemic C_{20}-Sphingine

D-Glutamic acid (740 mg., 5.03 m*M*) was dissolved in boiling 50% ethanol (85 ml.) and to this solution the racemic base (1.57 g., 5 m*M*, m.p. 86—87°) in boiling 95% ethanol (45 ml.) was added. The solution which became turbid rapidly, was allowed to stand at room temperature for 14 hrs. The crystalline precipitate was collected; yield 1.55 g., m. p. 147—153°. Recrystallization from 90% ethanol gave glistening leaflets, 1.15 g., (50%), m. p. 153—155°: glutamate *A*.

Anal. 7.935 mg. subst.: 0.408 ml. N_2 (22.5°, 751 mm)
$C_{25}H_{52}N_2O_5$ (460.68) calc'd: N 6.06%
found: N 5.85%

Both mother liquors from the removal and recrystallization of the less soluble glutamate were evaporated to dryness. The residue (1.1 g.) was crystallized from methanol (170 ml.) to yield 660 mg. of a substance melting at 140—155°.

It was recrystallized from methanol, the insoluble free glutamic acid being removed by filtration; m. p. 139—140°: glutamate *B*.

Anal. 7.210 mg. subst.: 0.394 ml. N_2 (23°, 750 mm)
$C_{25}H_{52}N_2O_5$ (460.68) calc'd: N 6.06%
found: N 6.22%

(+)-C_{20}-*Sphingine*

The glutamate *A* (1 g., m.p. 149—153°) was treated with 2 *N* sodium carbonate solution and the base extracted with chloroform. The extracts were washed with water, dried over sodium sulphate and the solvent evaporated to dryness. The crude base (650 mg., 95.5%) melted at 85—89°. Two crystallizations from petroleum ether gave glistening platelets (403 mg.), m.p. 89—91°, $[\alpha]_D^{24}$ + 5.33° (c 1.50).

Anal. 8.900 mg subst.: 25.25 mg. CO_2, 10.98 mg. H_2O
4.985 mg. subst.: 0.206 ml. N_2 (23°, 750 mm)
$C_{20}H_{43}NO$ (313.55) calc'd: C 76.60; H 13.82; N 4.47%
found: C 76.96; H 13.81; N 4.70%

(—)-*Diacetyl*-C_{20}-*sphingine*

(+)-C_{20}-Sphingine (300 mg., 0.96 m*M*, m.p. 89—91°) was acetylated with acetic anhydride (1.5 ml.) and pyridine (1.5 ml.). Yield: 358 mg. (94.1%), m. p. 108.5—109.5°. A sample for analysis was recrystallized from 90% ethanol; m.p. 109—110°, $[\alpha]_D^{27}$ — 18.58° (c 2.18).

Anal. 9.050 mg. subst.: 24.14 mg. CO_2, 9.44 mg. H_2O
7.770 mg. subst.: 0.248 ml. N_2 (23°, 750 mm)
$C_{24}H_{47}NO_3$ (397.62) calc'd: C 72.49; H 11.91; N 3.52%
found: C 72.79; H 11.67; N 3.63%

(—)-N-*Acetyl*-C_{20}-*sphingine*

The foregoing diacetyl derivative (200 mg., 0.5 m*M*, m.p. 109—110°) was hydrolized with *N* methanolic potassium hydroxide to yield 140 mg (78.3%), of a colourless substance, m. p. 102—104°. For analysis it was recrystallized from methanol; m. p. 105°, $[\alpha]_D^{24}$ — 10.1° (c 1.03).

Anal. 8.225 mg. subst.: 22.50 mg. CO_2, 9.29 mg. H_2O
8.030 mg. subst.: 0.290 ml. N_2 (23°, 749 mm)
$C_{22}H_{45}NO_2$ (355.59) calc'd: C 74.31; H 12.75; N 3.94%
found: C 74.65; H 12.64; N 4.10%

(—)-C_{20}-*Sphingine*

The glutamate *B* (300 mg., 0.65 m*M*, m.p. 146—155°) was decomposed in the same manner as described for the glutamate *A* yielding 200 mg. (98%), m.p. 81—88°, of the (—)-base. After recrystallization from petroleum ether colourless platelets, m.p. 90—90.5°, $[\alpha]_D^{24}$ — 5.30° (c 1.51), were obtained.

Anal. 7.725 mg. subst.: 21.77 mg. CO_2, 9.40 mg. H_2O
6.155 mg. subst.: 0.265 ml. N_2 (27°, 739 mm)
$C_{20}H_{43}NO$ (313.55) calc'd: C 76.60; H 13.82; N 4.47%
found: C 76.90; H 13.62; N 4.76%

(+)-*Diacetyl*-C_{20}-*sphingine*

The substance was prepared from the (—)-base in the same manner as described for the enantiomorph. Yield: 78.9%, m.p. 109—110°, $[\alpha]_D^{28}$ + 18.82° (c 2.20).

Anal. 7.790 mg. subst.: 20.78 mg. CO_2, 8.19 mg. H_2O
7.170 mg. subst.: 0.233 ml. N_2 (27°, 748 mm)
$C_{24}H_{47}NO_3$ (397.62) calc'd: C 72.49; H 11.91; N 3.52%
found: C 72.79; H 11.77; N 3.62%

(+)-N-Acetyl-C_{20}-sphingine

The substance was prepared from the (—)-base as was the (—)-*N*-acetyl derivative. Yield: 98.4%, m.p. 103—104°, $[\alpha]_D^{28}$ + 10.4° (c 0.5).

Anal. 7.780 mg. subst.: 21.24 mg. CO_2, 8.96 mg. H_2O
7.880 mg. subst.: 0.280 ml. N_2 (28°, 747 mm)
$C_{22}H_{45}NO_2$ (355.59) calc'd: C 74.31; H 12.75; N 3.94%
found: C 74.50; H 12.89; N 3.96%

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IZVOD

Studije u redu sfingolipoida. XVIII. Sinteza i cijepanje u optičke antipode 1-hidroksi-2-amino-eikozana (C_{20}-sfingina)

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Opisano je pripremanje racemičnoga 1-hidroksi-2-amino-eikozana (C_{20}-sfingina). Racemična baza je cijepana u optičke antipode preko diastereoizomernih soli s L-glutaminskom kiselinom. Opisani su *N*-acetil i *N,O*-diacetil-derivati enantiomernih baza. (—)-Bazi se pripisuje D-konfiguracija.

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