[CONTRIBUTION FROM THE INSTITUTE OF PHYSIOLOGY AND THE INSTITUTE OF CHEMISTRY, MEDICAL FACULTY, UNIVERSITY OF ZAGREB]

STUDIES IN THE SPHINGOLIPIDS SERIES. II. SYNTHESIS OF ENANTIOMERIC SPHINGINES

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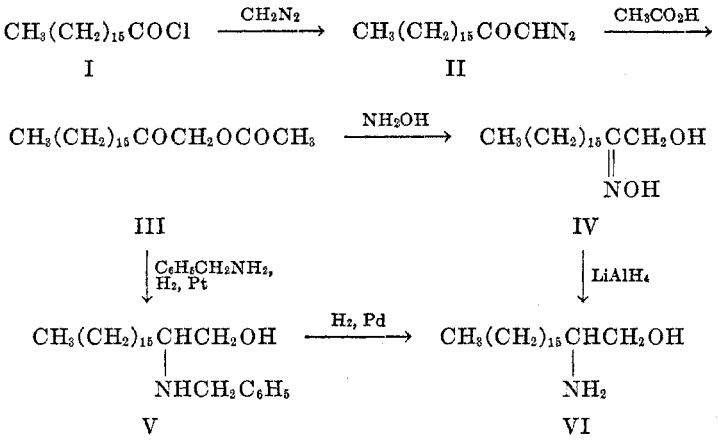
Recieved April 23, 1953

Sphingine was isolated in 1916 as a degradation product of sphingosine by Levene and West (1, 2). In a careful study which appeared recently Carter and Humiston (3) demonstrated that this base could be prepared by hydrogenation of triacetylsphingosine in the presence of a plantinum catalyst. They described the properties of the levorotatory base itself as well as the properties of the diacetyl, N-acetyl, and N-benzoyl derivatives. Further investigations about the configuration of the asymmetric carbon atom lead to the conclusion that the base belonged to the D-series. The formation of sphingine by catalytic reduction of N-acetyl-O-methylsphingosine and of hexaacetylphrenosine was also reported by Carter, *et al.* (4, 5). Racemic sphingine (1-hydroxy-2-aminoöctadecane) (VI) was synthesized by Carter, Norris, and Rockwell (6) by hydrogenation of methyl 2-aminoöctadecanoate in the presence of a large amount of Raney nickel catalyst at 1500 p.s.i. and 110–115°.

In this paper we will describe a new synthesis of racemic sphingine as well as its resolution into both optically active forms.



Heptadecanoic acid was treated with thionyl chloride and the resulting acid chloride (I) was converted into 1-diazo-2-octadecanone (II) by means of diazomethane. On treatment of II with glacial acetic acid 1-acetoxy-2-octadecanone (III) was obtained. The latter was converted to racemic sphingine in two different ways. The reaction of III with hydroxylamine gave a nicely crystalline oxime A (IV) and a partly crystalline, waxy solid B which was probably a mixture of IV and its acetyl derivative. Both A and B were reduced separately with lithium aluminum hydride giving the same base, racemic sphingine (VI). On the other hand the hydrogenation of III in 20 % methanolic benzylamine in the presence

of Adams platinum catalyst with subsequent hydrolysis gave 1-hydroxy-2-benzylaminoöctadecane (V) which was debenzylated catalytically to racemic sphingine using the palladium on barium sulphate catalyst.

The racemic base thus obtained was characterized as its D-tartarate, dibenzoyl-D-tartarate, N-acetyl, and N-benzoyl derivatives. A sample of the free base mixed with DL-sphingine prepared according to Carter, Norris, and Rockwell (6) gave no melting point depression.

The resolution of the racemic base has been effected through its salts with L-(+)-glutamic acid. This acid was used as a resolving agent for amino alcohols by Tishler, *et al.* (7) and was applied successfully for the resolution of racemic dihydrosphingosine by Grob and Jenny (8).

Both diastereoisomeric sphingine-L-glutamates which differed considerably in their solubilities were decomposed into the free bases on treatment with

TABLE I

COMPARISON OF MELTING POINTS AND SPECIFIC ROTATIONS OF ENANTIOMERIC SPHINGINES AND DERIVATIVES

COMPOUND	M.P., °C.			$[\alpha]_D$ in °		
	This Paper	Carter, *et al.* (3-5)	Levene, West (1, 2)	This Paper	Carter, *et al.* (3-5)	Levene, West (1, 2)
DL-Sphingine	79–80	81–81.5				
N-Acetyl	104–107					
Diacetyl		98.5–99				
N-Benzoyl	92.5–94					
D(−)-Sphingine	84–86	84–89	83.5–85.5	−4.92	−5.5	−6
(+)-N-Acetyl	97–98.5	101–103		+11.74	+12.0	
(+)-Diacetyl	101–103	108–109	109.5	+22.0	+22.5	+20.44
(+)-N-Benzoyl		112–113			+21.8	
L(+)-Sphingine	85–86			+4.82		
(−)-N-Acetyl	97–98			−12.27		
(−)-N-Diacetyl	100–103			−23.90		

sodium carbonate solution. The more soluble salt gave the levorotatory base which in a mixture with an authentic sample of the natural D-(−)-sphingine, prepared according to Carter and Humiston (3), gave no melting point depression. The free bases and a number of derivatives which were prepared are summarized in Table I and their melting points and specific rotations are compared with those reported by different authors. All these data leave no doubt as to the identity of the synthetic base with the natural one.

EXPERIMENTAL

The melting points are uncorrected.

Heptadecanoic acid chloride (I). Margaric acid (m.p. 59–60°; 54.1 g.) and pure thionyl chloride (50 ml.) were heated on the steam-bath for 2 hours. The excess of thionyl chloride was removed *in vacuo* and the crude, brown-colored acid chloride was distilled under reduced pressure, b.p. $_3$ 164°, yield 53 g. (92%).

1-Diazo-2-octadecanone (II). A solution of 53 g. (0.183 mole) of I in equal volume of dry ether was added slowly to a stirred ether solution of diazomethane (prepared from 100 g. of nitrosomethylurea) at 0–5°. After removal of the solvent *in vacuo* at room temperature there remained 54 g. of a slightly yellow-colored diazoketone, m.p. 57–59°. It was used without further purification for the next step.

1-Acetoxy-2-octadecanone (III). The decomposition of the diazoketone II was carried out by dissolving it in 150 ml. of glacial acetic acid in the presence of 2 g. of dry sodium acetate at 60–70°. For the completion of the reaction the solution was refluxed for 1 hour and left to cool slowly at room temperature. The crystalline, slightly colored product was filtered and recrystallized first from 140 ml. of glacial acetic acid and then from 450 ml. of 95% ethanol with addition of charcoal which removed most of the color. The product separated on cooling and was filtered and dried. Yield 49 g. (82%), m.p. 74–77°. For analysis 0.3 g. was dissolved in 6 ml. of a mixture of benzene-petroleum ether (1:1) and chromatographed through a column of 10 g. aluminum oxide (activity IV). Elution with the same solvent mixture yielded 170 mg. of a fraction with m.p. 76–78°, which after recrystallization from petroleum ether-benzene (10:1) melted at 76.5–78°.

Anal. Calc'd for $C_{20}H_{38}O_3$: C, 73.57; H, 11.73.

Found: C, 73.68; H, 11.62.

1-Hydroxy-2-octadecanone oxime (IV). Dry sodium acetate (16.8 g.) and hydroxylamine hydrochloride (10 g.) were ground finely in a mortar and were suspended in absolute ethanol (150 ml.). This mixture was added to a hot solution of 32.65 g. (0.1 mole) of III in absolute ethanol (200 ml.). The mixture was refluxed for 10 hours. The main part of ethanol was distilled off and the resulting slurry was diluted with 100 ml. of water and extracted with 200 ml. of ether. The ether solution was washed with water, dried over sodium sulphate, and evaporated to dryness. The crude residue was dissolved in 100 ml. of warm petroleum ether (50–70°) and the solution was filtered in order to remove some undissolved inorganic material. The clear, yellow-colored solution deposited on cooling 7.6 g. (26% yield) of colorless crystals which were filtered, washed with a small quantity of high-boiling petroleum ether, and dried; m.p. 87–89°. On recrystallization of a small amount from ether-high-boiling petroleum ether it melted at 91–92°.

Anal. Calc'd for $C_{18}H_{37}NO_2$: C, 72.19; H, 12.45; N, 4.68.

Found: C, 72.33; H, 12.50; N, 4.46.

The petroleum ether mother liquor after the removal of the product with m.p. 87–89° and evaporation to dryness gave 19.6 g. (65.5% yield) of a yellow, waxy product which could not be crystallized successfully and which probably consisted of a mixture of 1-acetoxy and 1-hydroxy-2-octadecanone oxime. It was designated as oxime B and was reduced separately with lithium aluminum hydride as described below.

1-Hydroxy-2-benzylaminoöctadecane (V). To a clear solution of III (1.69 g., 6 mM) in absolute methanol (90 ml.), pure benzylamine (20 ml.) was added. Adams platinum oxide (100 mg.) (9) in absolute methanol (20 ml.) was reduced separately. The solution of III was then added to the catalyst and the mixture was reduced under atmospheric pressure and room temperature. One mole (about 150 ml.) of hydrogen was absorbed in 2 hours. The reaction proceeded slowly and an additional 50 ml. of hydrogen were take up during the next 5 hours. After removal of the catalyst the solution was made strongly alkaline by the addition of 3 g. of solid potassium hydroxide and was steam-distilled until the distillate practically ceased to be alkaline (phenolphthaline). The emulsion which remained in the distilling flask on cooling deposited a solid precipitate. This precipitate was extracted with ether (300 ml.), and the extracts were washed with water and dried over sodium sulphate. The solution was concentrated to about 10 ml., cooled, and the resulting crystals were filtered and dried. The crude product (1.34 g., 60% yield, m.p. 65–70°) was recrystallized three times from 95% ethanol and melted then at 72–74° with a transition in crystalline form at 57–58°.

Anal. Calc'd for $C_{25}H_{45}NO$: C, 79.94; H, 12.08; N, 3.67.

Found: C, 79.68; H, 11.84; N, 3.73.

1-Hydroxy-2-aminoöctadecane (DL-*sphingine*) (VI). *A. By lithium aluminum hydride reduction of the oxime* IV. A solution of 2.19 g. (7.3 mM) of crystalline oxime A in 100 ml. of absolute ether was added dropwise to a solution of 1.12 g. (30 mM) of lithium aluminum hydride in 100 ml. of absolute ether. The mixture was then refluxed for 5 hours. The excess of hydride was hydrolyzed by the cautious addition of water (10 ml.), and the ether solution was filtered and the white precipitate washed with ether. The combined extracts were dried over sodium sulphate. After removal of the solvent there remained 1.78 g. (85% yield) of a crude, yellowish-colored product melting at 75–76°. Several recrystallizations from high-boiling petroleum ether gave 1.48 g. of DL-sphingine (m.p. 77–78°). For analysis 500 mg. were dissolved in 50 ml. of boiling anhydrous ether, filtered, and the solution brought to about 10 ml. by evaporation. The substance crystallized in glistening plates, m.p. 79–80°.

Anal. Calc'd for $C_{18}H_{39}NO$: C, 75.72; H, 13.77; N, 4.91.

Found: C, 75.78; H, 13.73; N, 4.86.

A solution of 5.14 g. of oxime B in 100 ml. of absolute ether reduced in the same manner with 2.7 g. of lithium aluminum hydride in 300 ml. of ether gave 3.03 g. (62% yield) of crude DL-sphingine which was recrystallized from 100 ml. of high-boiling petroleum ether and melted at 76–77°.

B. By hydrogenolysis of the benzylamino compound V. The catalyst was prepared on shaking 500 mg. of palladium hydroxide on barium sulphate (10) in 10 ml. of 95% ethanol in a hydrogen atmosphere. A solution of 470 mg. (1.25 mM) of V in 20 ml. of 95% ethanol was added and was reduced at atmospheric pressure and room temperature until the reaction was practically complete. The hydrogen uptake was 44 ml. or 138% of the amount required for one mole of hydrogen. After removal of the catalyst the solution was evaporated to dryness and 330 mg. (92.5% yield) of crude product was obtained (m.p. 72–74°). One recrystallization from 10 ml. of high-boiling petroleum ether gave 280 mg. (78.5% yield) of VI melting at 78–80° which gave no melting point depression in a mixture with DL-sphingine prepared by the reduction of the oxime.

*N-Benzoyl-*DL-*sphingine* (VIa). A sample of VI (1 g.) was benzoylated according to Carter and Humiston (3). After two recrystallizations from 95% ethanol and one crystallization from acetonitrile the product melted at 92.5–94°.

Anal. Calc'd for $C_{25}H_{43}NO_2$: N, 3.60; Found: N, 3.81.

*N-Acetyl-*DL-*sphingine* (VIb). A sample of VI (300 mg.) was acetylated with an acetic anhydride–pyridine mixture and the crude diacetyl derivative was hydrolyzed with methanolic potassium hydroxide according to Carter and Humiston (3). There was obtained 180 mg. of a crude N-acetyl derivative, m.p. 98–101°. Two recrystallizations from absolute methanol raised the m.p. to 104–107°.

Anal. Calc'd for $C_{22}H_{41}NO_2$: C, 73.34; H, 12.62; N, 4.28.

Found: C, 73.28; H, 12.30; N, 4.33.

DL-*Sphingine-*D-*tartarate* was prepared from VI (60 mg.) and D-tartaric acid (40 mg.) in absolute methanol. The salt which precipitated immediately was recrystallized from methanol; m.p. 150–155° (decomp.).

Anal. Calc'd for $C_{40}H_{84}N_2O_8$: N, 3.89. Found: N, 3.96.

DL-*Sphingine-dibenzoyl-*D-*tartarate*. This salt was prepared in an analogous manner and was recrystallized twice from acetone, m.p. 143–144°.

Anal. Calc'd for $C_{54}H_{92}N_2O_{10}$: N, 3.02. Found: N, 3.02.

Resolution of the racemic 1-hydroxy-2-aminoöctadecane. In a preliminary experiment 104.6 mg. of L-glutamic acid was dissolved in 12 ml. of hot 50% ethanol and to this solution 201.8 mg. of VI in 6 ml. of hot 95% ethanol was added. Upon cooling the turbid solution with ice, a crystalline precipitate was formed which was filtered off, washed with ethanol, and dried. Thus 150 mg. (47.5% yield) of the L-glutamic acid salt was obtained (m.p. 143–146°). Recrystallization from aqueous ethanol (35 ml. of ethanol and 2 ml. of water) gave 104 mg. of the L-glutamic acid salt melting at 149–151°.

Anal. Calc'd for $C_{23}H_{48}N_2O_5$: N, 6.48. Found: N, 6.40.

The mother liquor from the above experiment, when evaporated *in vacuo* to a small volume, upon standing deposited 45 mg. of a salt, m.p. 137–139°. Recrystallization from methanol gave the diastereoisomeric salt, m.p. 135–137°.

Anal. Calc'd for $C_{23}H_{48}N_2O_5$: N, 6.48. Found: N, 6.37.

In a second run, from 1.4 g. of VI in 40 ml. of 95% ethanol and 726 mg. of L-glutamic acid in 30 ml. of water and 50 ml. of 95% ethanol, 1.235 g. of crude salt (m.p. 146–148°) was obtained. Recrystallization from 240 ml. of 90% ethanol gave 880 mg. (83% yield) of pure L-(+)-sphingine-L-glutamate, m.p. 150–152°. The mother liquors deposited on standing at −5 to −10° for a few hours 280 mg. of D-(−)-sphingine-L-glutamate, m.p. 135–137°. The filtrate was evaporated *in vacuo* at 40–45° to dryness and the residue was recrystallized from 100 ml. of 95% methanol. After standing for a few days a second crop (292 mg.) of the salt, m.p. 135–137° was obtained. Total yield 572 mg. (54% calculated for one isomer).

L-(+)-*Sphingine* (VII). L-Glutamate with m.p. 150–152° (2.8 g.) was decomposed with 2 *N* sodium carbonate solution and the base was extracted exhaustively with much ether. The combined extracts were washed with water, dried over potassium carbonate, and evaporated to a volume of about 50 ml. The amino alcohol separated on standing and cooling with ice in colorless, glistening plates. It was filtered by suction and dried *in vacuo*. Yield, 1.45 g. or 78.4%, m.p. 84–85°. For analysis it was recrystallized from high-boiling petroleum ether, m.p. 85–86°; $[\alpha]_D^{20}$ +4.82° (*c*, 3.76 in chloroform).

Anal. Calc'd for $C_{18}H_{39}NO$: C, 75.72; H, 13.77; N, 4.91.
Found: C, 75.53; H, 13.62; N, 4.85.

(−)-*Diacetyl*-L-*sphingine* (VIIa). L-Sphingine (135 mg.) was acetylated with acetic anhydride (1 ml.) and pyridine (1 ml.) according to Carter and Humiston (3). For analysis it was recrystallized twice from 95% ethanol and once from absolute methanol; yield 90 mg., m.p. 100–103°; $[\alpha]_D^{22}$ −23.9° (*c*, 2.046 in chloroform).

Anal. Calc'd for $C_{22}H_{43}NO_3$: N, 3.79. Found: N, 3.87.

(−)-*N-Acetyl*-L-*sphingine* (VIIb). The diacetyl derivative (80 mg.) was hydrolyzed as described by Carter and Humiston (3). After recrystallization from methanol 50 mg. of pure VIIb was obtained, m.p. 97–98°; $[\alpha]_D^{21}$ −12.27° (*c*, 2.737 in chloroform).

Anal. Calc'd for $C_{24}H_{41}NO_2$: N, 4.28. Found: N, 4.45.

D-(−)-*Sphingine* (VIII). The L-glutamate with m.p. 135–137° (570 mg.) was decomposed in the same manner as described above for the other salt yielding 374 mg. of sphingine, m.p. 82–84°. Recrystallization from high-boiling petroleum ether gave colorless plates melting at 83–85°; $[\alpha]_D^{23}$ −4.92° (*c*, 3.64 in chloroform). Mixed with a sample of natural (−)-sphingine, m.p. 84–89°, it gave no melting point depression.

Anal. Calc'd for $C_{18}H_{39}NO$: C, 75.72; H, 13.77; N, 4.91.
Found: C, 76.21; H, 13.67; N, 4.79.

Equal amounts of both enantiomeric bases were mixed and the mixture was crystallized from ether. The product melted at 78–80.5° and showed no depression with racemic sphingine.

(+)-*Diacetyl*-D-*sphingine* (VIIIa). The preparation was the same as described for the L-compound. M.p. 101–103°; $[\alpha]_D^{21}$ +22.0° (*c*, 2.154 in chloroform).

Anal. Calc'd for $C_{22}H_{43}NO_3$: N, 3.79. Found: N, 3.81.

(+)-*N-Acetyl*-D-*sphingine* (VIIIb) was prepared as was VIIb; m.p. 97–98.5°; $[\alpha]_D^{21}$ +11.74° (*c*, 2.49 in chloroform).

Anal. Calc'd for $C_{20}H_{41}NO_2$: N, 4.28. Found: N, 4.40.

Equal amounts of VIIb and VIIIb were mixed and were recrystallized from absolute methanol. The product melted at 104–108° and gave no depression with N-acetyl-DL-sphingine (VIb).

Acknowledgment. We are indebted to Mrs. M. Munk-Weinert from our microchemical laboratory and to Mrs. I. Guštak-Mašek from the Institute of Organic Chemistry, Technical Faculty, University of Zagreb, for the microanalyses.

SUMMARY

1. A new synthesis of racemic sphingine (1-hydroxy-2-aminoöctadecane, VI) was described.

2. The resolution of the racemic base into both enantiomeric forms was effected.

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