[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MEDICAL FACULTY, UNIVERSITY OF ZAGREB, AND THE DEPARTMENT OF BIOCHEMISTRY, INSTITUTE "RUGJER BOŠKOVIČ"]

Studies in the Sphingolipids Series. IV.[1] Determination of the Configuration of the Amino Carbon Atom in Sphingosine[2]

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Received December 8, 1955

The configuration of the amino carbon atom in sphingosine (I) has been determined by a direct chemical method. For this purpose sphingosine was converted *via* the hitherto undescribed (+)-1-chloro-2-benzoylaminoöctadecane (III) into (+)-2-aminoöctadecane (V). The hexahydrophthaloyl derivative of V, m.p. 44–45°, $[\alpha]_D$ +6.31°, proved to be identical with (+)-2-hexahydrophthaloylaminoöctadecane (VII), m.p. 43–44°, $[\alpha]_D$ +5.59°, prepared synthetically starting with natural L-alanine. On the basis of these results the D-configuration is assigned to the carbon atom 2 in sphinogsine, which is in full agreement with that obtained by other authors.[3,4] In connection with recent statements concerning the spatial structure of the ethylenic double bond[5,6] and the *erythro* relation of the C_2—NH_2 to C_3—OH,[4,7,8] this investigation represents the final proof that natural sphingosine has the structure of *trans*, *erythro*-D-1,3-dihydroxy-2-aminoöctadecene-4.

The problem of the configuration of the asymmetric centers in natural sphingosine (I) could be solved by different routes. 1. Sphingosine could be converted by suitable reactions into derivatives, which would allow a comparison of physical properties with those of similar compounds of already known configuration. 2. The molecule of I could be degraded to smaller units without affecting the centers of asymmetry, and the optically active fragment could be compared with the identical compound accessible from some other source of known configuration. 3. The molecule of I or of some of its derivatives could be prepared synthetically starting with asymmetric compounds of known configuration—by methods which exclude the possibility of inversion—and the synthetic compound compared with the natural one.

The first route which gives the indirect proof of the configuration was successfully applied by Carter and Humiston.[3] They converted I into (−)-2-benzoylaminoöctadecanoic acid. On the basis of characteristic shifts in optical rotation of the acyl derivatives of D- and L-amino acids on dilution of their dioxane or acetic acid solutions with water, the D-configuration has been assigned to the (−)-2-benzoylaminoöctadecanoic acid thus obtained. Consequently, sphingosine would belong to the D-series in respect to the carbon atom 2.

The second approach was recently realized by Kiss, Fodor, and Banfi.[4] They correlated the configuration of 3-amino-2,4-dihydroxybutyrolactone obtained by the ozonolysis of O,N-diacetylsphingosine, with that of D-erythro-2-amino-3,4-dihydroxybutyric acid.[9]

The third possibility, which would furnish a direct chemical proof for the configuration of I, was so far as we are aware not investigated. In this paper we describe a route, which made possible the determination of the configuration of the amino-carbon atom in I. For this purpose we carried out two series of reactions: 1. The conversion of I into (+)-2-aminoöctadecane, and 2. the synthesis of (+)-2-aminoöctadecane starting with L-alanine.

The first step in the conversion of I into V consisted in the preparation of sphingine.[10] Its benzoyl derivative (II) gave, on treatment with thionyl chloride (+)-1-chloro-2-benzoylaminoöctadecane (III), which was reduced with lithium aluminum hydride into (+)-2-benzylaminoöctadecane (IV). Catalytic hydrogenolysis of IV yielded (+)-2-aminoöctadecane (V), which was converted into the (−)-N-acetyl, (+)-N-phthaloyl (VI), and (+)-N-hexahydrophthaloyl derivative (VII).

Compounds IV, V, and VI were synthesized previously from 2-octadecanone by Munk-Weinert and Proštenik.[11] Thus, a direct comparison of (+)-2-aminoöctadecane obtained from I with the synthetic one was possible.

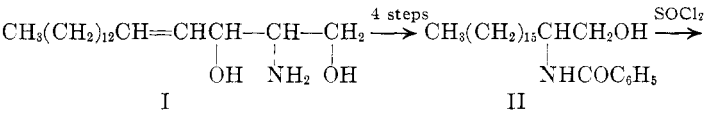
Sunko and Proštenik[12] have found recently, that the Bowman ketone synthesis[13,14] could be extended to the preparation of α-amino ketones and related compounds, when α-phthaloylamino acid chlorides were used as starting materials. The reaction now was applied for the synthesis of V. The condensation of N-phthaloyl-L-alanyl chloride with ditetrahydropyranyl sodiotetradecylmalonate

(1) Paper III, Munk-Weinert, Sunko, and Proštenik, *J. Org. Chem.*, **19**, 378 (1954).
(2) Presented at the XIVth International Congress of Pure and Applied Chemistry, Zürich, July 21–27, 1955.
(3) Carter and Humiston, *J. Biol. Chem.*, **191**, 727 (1951).
(4) Kiss, Fodor, and Banfi, *Chemistry & Industry*, 517 (1954); *Helv. Chim. Acta*, **37**, 1471 (1954).
(5) Mislow, *J. Am. Chem. Soc.*, **74**, 5155 (1952).
(6) Fodor and Kiss, *Nature*, **171**, 651 (1953).
(7) Carter, Shapiro, and Harrison, *J. Am. Chem. Soc.*, **75**, 1007, 4705 (1953).
(8) Jenny and Grob, *Helv. Chim. Acta*, **36**, 1454 (1953).
(9) Hamel and Painter, *J. Am. Chem. Soc.*, **75**, 1362 (1953).
(10) Munk-Weinert, Sunko, and Proštenik, *J. Org. Chem.*, **19**, 378 (1954).
(11) Munk-Weinert and Proštenik, *Arhiv kem.*, **26**, 89 (1954).
(12) Sunko and Proštenik, *Arhiv kem.*, **26**, 7 (1954).
(13) Bowman, *J. Chem. Soc.*, 325 (1950).
(14) Bowman and Fordham, *J. Chem. Soc.*, 3945 (1952).

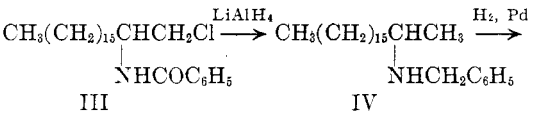
TABLE I

COMPARISON OF MELTING POINTS AND SPECIFIC ROTATIONS OF (+)-2-AMINOÖCTADECANE AND DERIVATIVES

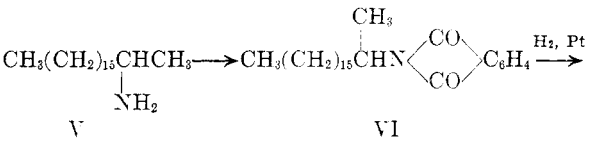
Compound	M.p., °C. This paper From sphingosine	M.p., °C. This paper From L-alanine	M.p., °C. Munk-Weinert, Prošteník[7]	$[\alpha]_D$ in ° This paper From sphingosine	$[\alpha]_D$ in ° This paper From L-alanine	$[\alpha]_D$ in ° Munk-Weinert, Prošteník[7]
(+)-2-Aminoöctadecane	75–78		76–80	+ 2.78		+ 2.80
(−)-N-Acetyl	90–91		90–91	− 4.60		− 4.75
(+)-N-Phthaloyl	60–61		61–62	+10.93		+10.67
(+)-N-Hexahydrophthaloyl	44–45	43–44	42–43	+ 6.31	+5.59	+ 6.54



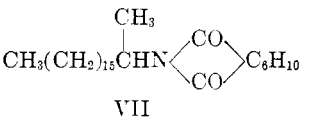
I II



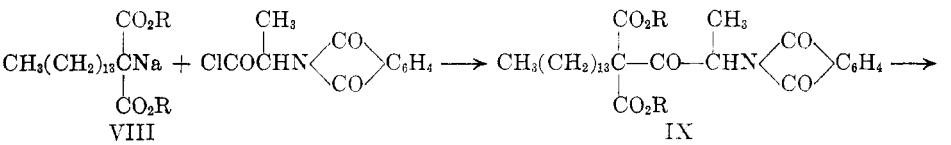
III IV



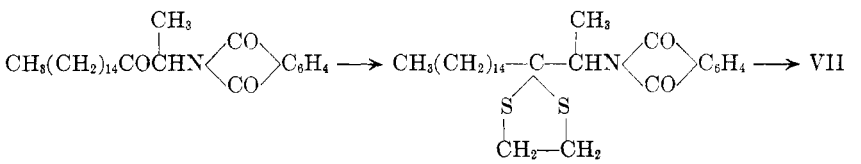
V VI



VII



VIII IX



X R = 2-Tetrahydropyranyl XI

(VIII) led to (−)-2-phthaloylamino-3-octadecanone (X). The latter substance was converted into the ethylene thioketal derivative (XI), which by boiling the ethanol solution with freshly prepared Raney nickel W4 catalyst[15] gave (+)-hexahydrophthaloylaminoöctadecane (VII), identical with the compound prepared from I.

Thus, in both cases, *i.e.* by the degradation of I, and by the synthesis with L-alanine, we obtained the same dextrorotatory hexahydrophthaloyl derivative of V. The comparison of the physical constants of V and of its derivatives obtained by different routes is given in Table I. In view of the identity in the magnitude of the rotations of V, obtained from I as well as by the resolution of the inactive base,[11] it would appear, that the conversion of I *via* the (+)-1-chloro-2-benzoylaminoöctadecane (III) is not attended with remarkable racemization. When prepared from L-alanine VII had a rotatory power $[\alpha]_D$ +5.59°, *i.e.* somewhat lower than that of the active compound, $[\alpha]_D$ +6.54°, prepared by the resolution of the inactive base.

According to the proposal of Barrow and Ferguson,[16] Reihlen, Knöpfle, and Sapper,[17] and also of Karrer and Dinkel,[18] the D-configuration is assigned to V. In our case, if in the L-alanine the carboxyl

(15) Pavlic and Adkins, *J. Am. Chem. Soc.*, **68**, 1471 (1946); Adkins and Pavlic, *J. Am. Chem. Soc.*, **69**, 3039 (1947).

(16) Barrow and Ferguson, *J. Chem. Soc.*, 410 (1935).

(17) Reihlen, Knöpfle, and Sapper, *Ann.*, **534**, 247 (1938).

(18) Karrer and Dinkel, *Helv. Chim. Acta*, **36**, 122 (1953).

group is transformed into the hexadecyl residue, the opposite *i.e.* D-configuration should be assigned to the (+)-2-aminoöctadecane (V) produced. This configuration is in full agreement with that deduced by other methods.[3,4]

In this way sphingosine is brought for the first time in direct correlation with natural amino acids. This fact represents at the same time a strong support to the opinion expressed by different authors,[3,19] that L-serine may serve as the precursor in the biosynthesis of the sphingosine bases.

EXPERIMENTAL

The melting points are uncorrected.

I. PREPARATION OF (+)-2-AMINOÖCTADECANE FROM SPHINGOSINE

(−)-*Sphigine* was prepared from tribenzoylsphingosine according to Munk-Weinert, Sunko; and Prošstenik.[10] The benzoylation of the base following the procedure of Carter and Humiston[3] gave the pure (+)-N-benzoylsphingine (II), m.p. 112–114°, $[\alpha]_D^{20}$ +21.9° (*c*, 2 in chloroform).

(+)-*1-Chloro-2-benzoylaminoöctadecane* (III). A solution of 1 g. of II in 75 ml. of absolute ether and 10 ml. of thionyl chloride was refluxed for 3 hours. The solution then was evaporated *in vacuo* to dryness leaving a colorless, crystalline residue, which was purified by crystallization from absolute ethanol. Thereby, 0.6 g. of colorless needles was obtained, m.p. 101–102°, $[\alpha]_D^{20}$ +33.57° (*c*, 1.83 in chloroform).

Anal. Calc'd for $C_{25}H_{42}ClNO$: C, 73.57; H, 10.38; N, 3.44. Found: C, 73.97; H, 10.48; N, 3.64.

(+)-2-*Benzylaminoöctadecane* (IV). To a solution of 0.5 g. of lithium aluminum hydride in 50 ml. of absolute ether 550 mg. of III in 50 ml. of ether was gradually added. After the addition was complete, the reaction mixture was refluxed for 4 hours. Water was carefully added with shaking and the clear ether solution was decanted from the solid material. The ether was removed and the partially crystalline residue was distilled *in vacuo* giving a colorless oil (410 mg.), b.p. 130–140° (heating block temperature).

Benzoyl-D-alanine salt. A solution of 180 mg. of IV and 96 mg. of benzoyl-D-alanine in 1 ml. of acetone was allowed to stand at 0° overnight. The crystals were collected (84 mg.) and recrystallized twice from acetone. Needles, m.p. 80°, $[\alpha]_D^{23}$ −21.9° (*c*, 2.50 in 96% ethanol).

Anal. Calc'd for $C_{35}H_{56}NO_3$: N, 5.07. Found: N, 5.09.

When benzoyl-L-alanine was used instead of the D-isomer, no crystalline product could be obtained.

(+)-*2-Aminoöctadecane* (V). A solution of IV (500 mg.) in 15 ml. of 96% ethanol was reduced catalytically in the presence of a 10% palladium on barium sulphate catalyst (200 mg.) at atmospheric pressure and at room temperature. After 0.5 hour the calculated amount of hydrogen was absorbed. The catalyst was filtered off and the filtrate was evaporated *in vacuo* to dryness. Distillation of the residue in a glass tube gave 235 mg. of a crystalline solid, m.p. 75–78°; b.p. 90–100° at 0.01 mm. (heating block temperature); $[\alpha]_D^{20}$ +2.78° (*c*, 4.68 in chloroform).

(−)-*2-Acetylaminoöctadecane.* A mixture of 100 mg. of IV, 1 ml. of pyridine, and 0.5 ml. of acetic anhydride was heated at 100° for 1 hour. After cooling the crystals were collected and recrystallized from 96% ethanol to give colorless leaflets (80 mg.), m.p. 90–91°; $[\alpha]_D^{20}$ −4.60 (*c*, 2.61 in chloroform).

Anal. Calc'd for $C_{20}H_{41}NO$: C, 77.10; H, 13.26; N, 4.50. Found: C, 76.96; H, 13.16; N, 4.57.

(+)-*2-Phthaloylaminoöctadecane* (VI). A mixture of 237 mg. of V and 130 mg. of phthalic anhydride was heated at 140° for 1 hour. After cooling the solid was recrystallized three times from 96% ethanol to give colorless plates (186 mg.), m.p. 60–61°; $[\alpha]_D^{24}$ +10.93° (*c*, 3.02 in chloroform).

Anal. Calc'd for $C_{26}H_{41}NO_2$: C, 78.14; H, 10.34. Found: C, 78.20; H, 10.78.

(+)-*2-Hexahydrophthaloylaminoöctadecane* (VII). A solution of 160 mg. of VI in 20 ml. of 96% ethanol was hydrogenated in the presence of 100 mg. of Adams' platinum oxide catalyst at 21° and at 747 mm. After 3 hours 31 ml. of hydrogen was taken up, the catalyst was filtered off, and the solvent was removed *in vacuo*. The solid residue (160 mg., m.p. 40–42°) was recrystallized twice from 96% ethanol to give colorless needles (70 mg.), m.p. 44–45°; $[\alpha]_D^{23}$ +6.31° (*c*, 3.1 in chloroform).

Anal. Calc'd for $C_{26}H_{47}NO_2$: C, 76.98; H, 11.68; N, 3.45. Found: C, 77.00; H, 11.78; N, 3.62.

II. SYNTHESIS OF (+)-2-AMINOÖCTADECANE FROM L-ALANINE

(−)-2-Phthaloylamino-3-octadecanone (X). A solution of ditetrahydropyranyl tetradecylmalonate prepared from tetradecylmalonic acid (45 g., 0.15 mole) in dry benzene (150 ml.) was added in the course of a few minutes to a vigorously stirred suspension of sodium powder (3.2 g.) in benzene (100 ml.). Some cooling was necessary in order to prevent the temperature from rising above 35°. The stirring was continued for two hours; in due course practically all sodium had reacted. To the stirred sodium malonate solution phthaloyl-L-alanyl chloride[20] (32 g., 0.136 mole) in benzene (150 ml.) was added dropwise. During the addition the temperature was held below 35°. Stirring was continued for 7 hours. The reaction mixture then was heated on a steam-bath, and after refluxing for 10 minutes, glacial acetic acid (10 ml.) was added. A vigorous evolution of carbon dioxide occurred, and the mixture was refluxed for an additional 2 hours. After cooling, water (200 ml.) was added, the benzene layer was separated, washed with water, and the solvent was evaporated. The resulting oil was dissolved in hot petroleum ether (500 ml., b.p. 70–80°); phthaloyl-L-alanine (8.3 g.) separated upon cooling. The filtrate was evaporated and the residue was crystallized from 96% ethanol (120 ml.). After a second crystallization from the same solvent 14 g. of the crude phthaloylamino ketone, m.p. 68–71°, was obtained. For analysis the product was repeatedly crystallized from ethanol, and then from petroleum ether to give colorless needles, m.p. 78–79°; $[\alpha]_D^{18}$ −3.2° (*c*, 5 in chloroform); $[\alpha]_D^{18}$ −3.1° (*c*, 10 in chloroform).

Anal. Calc'd for $C_{26}H_{39}NO_3$: C, 75.50; H, 9.50; N, 3.39. Found: C, 75.96; H, 9.93; N, 3.52.

Oxime. Prepared in the usual manner with methanolic hydroxylamine acetate, and crystallized from methanol and then from petroleum ether (b.p. 70–80°), this derivative had m.p. 93–93.5°.

Anal. Calc'd for $C_{26}H_{40}N_2O_3$: C, 72.85; H, 9.41; N, 6.54. Found: C, 72.68; H, 9.65; N, 6.51.

(+)-*2-Hexahydrophthaloylaminoöctadecane* (VII). A suspension of X (845 mg.) in chloroform (2.5 ml.) and ethanedithiol (2.5 ml.) was saturated with dry hydrogen chloride at 0–5°. After standing for 15 hours in the refrigerator, the mixture cleared up. Some ether was added, and the solution was washed successively with sodium hydrogen carbonate solution, water, 2 *N* hydrochloric acid, and water, dried over sodium sulphate, and evaporated *in vacuo*. The residual oil (750 mg.) could not be induced to crystallize, and was submitted without further purification to the Raney nickel desulfuration. The crude thioketal (735 mg.) was dissolved in warm ethanol (50 ml.), a suspension of freshly prepared W4 Raney nickel catalyst[15] (10 ml. in ethanol) was added, and the mixture was refluxed for 7 hours. After adding some

(19) Sprinson and Coulon, *J. Biol. Chem.*, **207**, 585 (1954).

(20) Balenović, Cerar, and Fuks, *J. Chem. Soc.* 3316 (1952).

charcoal the catalyst was removed by filtration, and the solution was evaporated to dryness. A nearly colorless oil was obtained which crystallized on standing. For analysis it was recrystallized from 96% ethanol to give colorless needles, m.p. 43–44°; $[\alpha]_D^{21}$ +5.59° (*c*, 3.04 in chloroform).

Anal. Calc'd for $C_{26}H_{47}NO_2$: C, 76.99; H, 11.68; N, 3.45. Found: C, 77.06; H, 11.37; N, 3.45.

2-o-(Carboxyhexahydrobenzoylamino)octadecane. A solution of VII in ethanol was heated with 17% potassium hydroxide solution on the steam-bath for 10 minutes. After cooling and acidification with diluted (1:1) hydrochloric acid, the separated crude product was recrystallized from 70% ethanol. It had m.p. 77–80° (with softening from 60°).

Anal. Calc'd for $C_{26}H_{49}NO_3$: C, 73.70; H, 11.66. Found: C, 74.01; H, 11.75.

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