[CONTRIBUTION FROM THE CHEMICAL INSTITUTE, MEDICAL FACULTY, UNIVERSITY OF ZAGREB]

STUDIES IN THE SPHINGOLIPIDS SERIES. I. SYNTHESIS OF RACEMIC 2-AMINO-1,3-DIHYDROXYOCTADECANE

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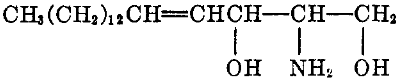
Recent investigation of the structure of sphingosine (I) and dihydrosphingosine show that the amino group is to be found on the second C-atom and the two hydroxy groups on the first and third C-atoms (1). Carter, Norris, and Rockwell (2) have published the results of their experiments indicating a route to the synthesis of dihydrosphingosine and compounds related to it, which consists of the reduction of the methyl esters of α-amino-β-hydroxy fatty acids to the corresponding aminodiols. Following this route Crosby (3) has realized the first synthesis of racemic dihydrosphingosine (2-amino-1,3-dihydroxyoctadecane) having prepared the intermediate aminohydroxy acid by the reduction of ethyl α-phenylhydrazono-β-ketostearate. Three papers treating the synthesis of racemic 2-amino-1,3-dihydroxyoctadecane have recently been published. Gregory and Malkin (4) obtained the racemic base by the reduction of the methyl ester of α-amino-β-hydroxystearic acid by lithium aluminum hydride and Grob, Jenny, and Utzinger (5) obtained it by a route involving catalytic reduction of 2-nitro-1,3-dihydroxyoctadecane, in the final steps. The synthesis by Fisher (6) closely resembles the method of Gregory and Malkin.

We wish to show in this paper that compounds of type VIII can be obtained in another way using the α-bromo fatty acids as a starting material. Their halides can easily be converted, by means of the diazomethane synthesis, into 1,3-dibromo-2-alkanones which on treatment with silver acetate or benzoate gave the 1,3-diacyloxy-2-alkanones. The preparation of several 1,3-diacetoxy-2-alkanones by this route has recently been described (7).

Starting from α-bromomargaric acid [2-bromoheptadecanoic acid (8)], the 1,3-dibromo-2-octadecanone (IVa) was obtained. The reaction of IVa with silver acetate in boiling acetic acid gave the diacetoxyketone (Va) which was difficult to purify. Better results were obtained on heating IVa with silver benzoate and benzoic acid yielding the dibenzoxyketone (VIa). This was hydrogenated at 60° and 1500 p.s.i. in the presence of ammoniacal methyl alcohol and a Raney nickel catalyst. Treatment of the reaction product yielded a brown, tarry mixture of bases which could not be purified by crystallization. If this conformed to expectations it should consist of diastereoisomeric forms of 2-amino-1,3-dihydroxyoctadecane (VIIIa) as well as secondary bases always appearing after reductions (Mignonac). The base, which could not be isolated in a pure state, was characterized by four well-crystallized derivatives. The oxalate, m.p. 196° (decomp.); the triacetyl derivative, m.p. 98–99°; and the tribenzoyl derivative, m.p. 146–147°; were prepared directly from the crude mixture of bases. The N-benzoyl derivative, m.p. 114°, was prepared by partial hydrolysis of the tribenzoyl derivative.[1] VIa treated with hydroxylamine hydrochloride in the presence of sodium acetate gave a resinous, crude oxime (VIIa). This was reduced with lithium aluminum hydride giving a resinous base, which was converted into the oxalate, m.p. 196° (decomp.). There was no melting point depression in the mixture with the oxalate described above. All derivatives have sharp melting points, which leads to the conclusion that there is only one purified racemic form present in each of them. It is probable that this racemate contains the optically active dihydrosphingosine which occurs in nature. At the present time we are unable to give any conclusion about the stereochemistry of the synthetic base.

In an analogous manner 2-amino-1,3-dihydroxypentadecane (VIIIb) was synthesized starting from 1,3-diacetoxy-2-pentadecanone (Vb) described recently (7). The crude base was characterized as the oxalate, m.p. 198°; the triacetyl derivative, m.p. 92–93°; and the N-acetyl derivative, m.p. 125–126°.



$$CH_3(CH_2)_{12}CH{=}CH\underset{OH}{CH}{-}\underset{NH_2}{CH}{-}\underset{OH}{CH_2}$$

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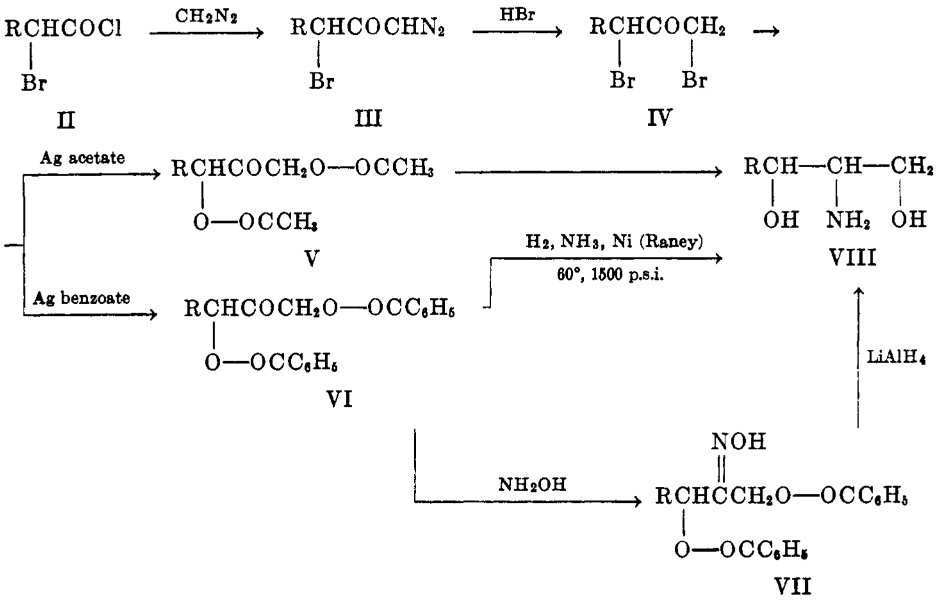

a. R = $(CH_2)_{14}CH_3$
b. R = $(CH_2)_{11}CH_3$

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[1] Melting points of derivatives of the naturally occurring dihydrosphingosine: triacetyl 102–103°, N-acetyl 125–126°, tribenzoyl 144–145°, and N-benzoyl 116–119° (9).

Melting points of the synthetic derivatives reported by different authors: triacetyl 98–100° (G., M.) (4), 67–68° (G., J., U.) (5); N-acetyl 123–125° (G., M.); tribenzoyl 138–140° (G., M.), 145–148° (G., J., U.), 146–148° (F.) (6); oxalate 195.5–196.5° and 232° (G., J., U.).

EXPERIMENTAL

The melting points are uncorrected.

2-Bromoheptadecanoic acid. Starting from heptadecanoic acid [margaric acid, m.p. 60–61°, 115 g. (8)], red phosphorus (5.24 g.), and bromine (176 g.) using the procedure of Hell-Volhard-Zelinsky, 131 g. of the 2-bromo acid, m.p. 52.5°, recrystallized from high-boiling petroleum ether, was obtained.

1,3-Dibromo-2-octadecanone (IVa). The acid chloride (IIa) from 2-bromoheptadecanoic acid (22 g.) was prepared with thionyl chloride (11 ml.). It was dissolved in dry ether (40 ml.) and the resulting solution was added dropwise at 0° with constant stirring to the ether solution of diazomethane (prepared from 19.1 g. of nitrosomethylurea). After standing at room temperature for 2 hours the ether was evaporated under reduced pressure at 25° to dryness and the crude, yellow, crystalline diazoketone (IIIa) was converted without purification into the dibromoketone. For this purpose the diazoketone was dissolved in 30 ml. of glacial acetic acid and to the solution 10 ml. of 66% hydrobromic acid was gradually added under cooling. When the evolution of nitrogen ceased 100 ml. of water was added. The crystalline substance was filtered, and washed with a saturated aqueous solution of sodium bicarbonate and finally with water. On recrystallization from ethanol (3 times) 20.3 g. (75.6% yield) of colorless prisms, m.p. 52–52.5°, were obtained.

Anal. Calc'd for $C_{18}H_{34}Br_2O$: C, 50.71; H, 8.04.
Found: C, 50.82; H, 7.95.

1,3-Diacetoxy-2-octadecanone (Va). A mixture of IVa (3.34 g., 0.01 mole), silver acetate (5.01 g., 0.03 mole) and glacial acetic acid (20 ml.) was refluxed for 3 hours. Silver bromide and the excess of silver acetate were filtered off. To the filtrate 50 ml. of water was added, and the solution was neutralized with sodium bicarbonate and extracted with ether. After drying the solution over sodium sulphate and removing the solvent, 3.32 g. of a viscous, brown oil was obtained. It was dissolved in 20 ml. of low-boiling petroleum ether and chromatographed over 80 g. of aluminum oxide, activity III, standardized according to Brockmann. From the benzene eluate 1.4 g. of crystals, m.p. 50–53°, were obtained. Three recrystallizations from high-boiling petroleum ether gave colorless needles, m.p. 56.5°.

Anal. Calc'd for $C_{22}H_{40}O_5$: C, 68.71; H, 10.49.
Found: C, 68.86; H, 10.46.

1,3-Dibenzoxy-2-octadecanone (VIa). A mixture of IVa (18 g.), silver benzoate (29.7 g.), and benzoic acid (30 g.) was heated at 120° for 3 hours with stirring. The brown reaction mixture was dissolved in 150 ml. of chloroform. Silver bromide and the excess of silver benzoate were filtered off, benzoic acid was extracted with a saturated aqueous solution of sodium bicarbonate, and the chloroform solution thus purified was dried over sodium sulphate. After removing the solvent the residue was crystallized once from 96% ethanol giving 8.34 g. (38.8% yield) of colorless prisms, m.p. 70–71°. For the analysis the substance was recrystallized twice more, m.p. 72–73°.

Anal. Calc'd for $C_{32}H_{44}O_6$: C, 75.55; H, 8.72.
Found: C, 75.67; H, 8.91.

2-Amino-1,3-dihydroxyoctadecane (VIIIa). A solution of VIa (11 g.) in methanol saturated with ammonia (400 ml.) was hydrogenated for 12 hours at 60° and 1500 p.s.i. with shaking in the presence of a Raney nickel catalyst (prepared from 25 g. of alloy). The catalyst was then filtered off and after evaporation of the methanol the residue was refluxed with aqueous 1 *N* sodium hydroxide (200 ml.) for 3 hours The extraction with ether gave 5.4 g. of a yellow, tarry product (83% yield).

Oxalate. The crude base mixture (370 mg.) was dissolved in absolute ethanol (2 ml.) to which a solution of oxalic acid (46 mg.) in ethanol was added. The precipitate was suspended in ethanol (250 ml.) and boiled under reflux. Water was added drop by drop to the boiling suspension until solution of the precipitate had been effected. On cooling 65 mg. of colorless crystals, m.p. 196° (decomp.) were obtained.

Anal. Calc'd for $C_{38}H_{80}N_2O_8$: C, 65.85; H, 11.63; N, 4.04.
Found: C, 65.74; H, 11.95; N, 4.16.

Triacetyl derivative. The crude base mixture (300 mg.), dry pyridine (1 ml.), and acetic anhydride (1 ml.) were heated on a steam-cone for an hour and then evaporated to dryness *in vacuo.* To the residue 0.1 *N* sulphuric acid was added, the mixture was extracted with ether, and the ether solution washed with water and dried. On evaporation of the solvent there remained 315 mg. of a brown, crystalline mass which, after six recrystallizations from high-boiling petroleum ether, gave 46 mg. of colorless needles, m.p. 98–99°.

Anal. Calc'd for $C_{24}H_{45}NO_5$: C, 67.42; H, 10.61; N, 3.28.

Found: C, 67.63; H, 10.56; N, 3.56.

Tribenzoyl derivative. The crude base mixture (5.1 g.), dry pyridine (30 ml.), and benzoyl chloride (15 ml.) were allowed to stand at room temperature for 24 hours. The mixture was then poured into ice-cold water, the separated oil was extracted with ether, and the ether solution washed successively with ice-cold 1 *N* sulphuric acid, water, ice-cold 0.1 *N* sodium hydroxide, and finally with water. Evaporation of the ether gave a brown, viscous oil to which high-boiling petroleum ether (50 ml.) was added. The separated crystals (1.07 g.) melted at 110–139°. Six recrystallizations from acetone gave 540 mg. colorless needles, m.p. 146–147°.

Anal. Calc'd for $C_{39}H_{51}NO_5$: C, 76.31; H, 8.38; N, 2.28.

Found: C, 76.22; H, 8.49; N, 2.29.

N-Benzoyl derivative. A solution of the tribenzoyl derivative (350 mg., m.p. 146–147°) and 1 *N* potassium hydroxide in methanol (20 ml.) was heated on the water-bath at 40° for 45 minutes. The solution was poured in water, and the flocculant precipitate filtered and washed with water. A colorless substance (220 mg.), m.p. 102–112°, was obtained, which after 4 recrystallizations from acetone melted at 114°.

Anal. Calc'd for $C_{25}H_{43}NO_3$: C, 74.03; H, 10.69; N, 3.45.

Found: C, 73.76; H, 10.62; N, 3.42.

Reduction of VIIa with lithium aluminum hydride. Compound VIa (200 mg.), hydroxylamine hydrochloride (50 mg.), sodium acetate (100 mg.), and methanol (20 ml.) were refluxed for 12 hours. The solvent was removed *in vacuo.* To the residue 30 ml. of water was added and the resulting emulsion was extracted with ether. The ether solution gave the crude, oily oxime which, without purification, was dissolved in absolute ether (20 ml.) and reduced with a solution of lithium aluminum hydride (200 mg.) in absolute ether (40 ml.). The mixture was refluxed for 2 hours, and the excess of lithium aluminum hydride decomposed upon the addition of water. The ether layer was dried over sodium sulphate and the solvent distilled off; 90 mg. of yellow resin was obtained. This was dissolved in ethanol and precipitated with an ethanolic solution of oxalic acid. The precipitate (35 mg.) was recrystallized from a boiling mixture of ethanol and water. Colorless crystals resulted, m.p. 196° (decomp.), which were identical with the oxalate of the base VIIIa, prepared by the reduction by Mignonac, giving no melting-point depression in the mixture with the latter.

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Anal. Calc'd for $C_{38}H_{80}N_2O_8$: N, 4.04. Found: N, 4.21.

2-Amino-1,3-dihydroxypentadecane (VIIIb). A solution of Vb (34.2 g., 0.1 mole) in methanol saturated with ammonia (600 ml.) was hydrogenated for 6 hours at 60° and 1500 p.s.i. in the presence of a Raney nickel catalyst (from 50 g. of alloy). There was obtained 21.5 g. of a yellow, tarry product.

Oxalate. The oxalate, recrystallized from boiling ethanol-water, melted at 198° (decomp.).

Anal. Calc'd for $C_{32}H_{68}N_2O_8$: C, 63.12; H, 11.26; N, 4.60.
Found: C, 63.61; H, 11.15; N, 4.70.

Triacetyl derivative. The reaction mixture obtained from the crude base (2 g.) with dry pyridine (4 ml.) and acetic anhydride (4 ml.) was worked up as before. Recrystallization of 670 mg. of crystals from the ether eluate gave colorless prisms, m.p. 92–93°.

Anal. Calc'd for $C_{21}H_{39}NO_5$: C, 65.42; H, 10.20; N, 3.63.
Found: C, 65.66; H, 10.00; N, 3.80.

N-Acetyl derivative. The triacetyl derivative (100 mg.) was hydrolyzed with 1 *N* potassium hydroxide (5 ml.) at 40° for 45 minutes. Recrystallization from methanol gave 55 mg. of colorless needles, m.p. 125–126°.

Anal. Calc'd for $C_{17}H_{35}NO_3$: N, 4.65. Found: N, 4.88.

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SUMMARY

1. The synthesis of racemic 2-amino-1,3-dihydroxyoctadecane (racemic dihydrosphingosine, VIIIa), starting from 2-bromoheptadecanoic acid was described.

2. In an analogous manner the synthesis of racemic 2-amino-1,3-dihydroxypentadecane (VIIIb) was effected.

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