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Studies in the Sphingolipids Series. IX.* Synthesis of DL-2-Amino-1,3,4-trihydroxyoctadecane and of Its C_4-Methyl Ether**

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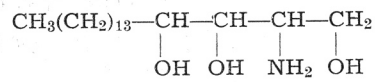
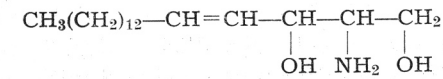
Received June 14, 1957

DL-2-Amino - 1,3,4 - trihydroxyoctadecane (IXa) and its C_4-methyl ether (IXb) were prepared in a series of reactions through the following intermediates: 2-Methoxyhexdecanoic acid (III) → Ethyl 2-methoxyhexadecanoylacetoacetate (IV) → Ethyl (2-p-nitrophenylhydrazono)-2,3-dioxo-4-methoxyoctadecanoate (V) → Ethyl 2-acetamido-3-oxo-4-methoxyoctadecanoate (VI) → Ethyl 2-acetamido-3-hydroxy-4-methoxyoctadecanoate (VII) → 2-Amino-3-hydroxyoctadecanoic acid 1,4-lactone (VIII) → IX. The crude base mixture was precipitated with oxalic acid and the mixture of oxalates crystallized fractionally. Two oxalates of IXa were obtained probably representing two purified racemic forms. IXa was prepared as a free base. The *N*-benzoyl derivative of IXa was also prepared.

In a recent brief communication[1] we have reported the synthesis of DL-2-amino-1,3,4-trihydroxyoctadecane (I). The base can exist in eight stereoisomeric forms (4 racemic pairs) owing to the presence of three asymmetric carbon atoms. One of the optically active isomers should be identical with »phytosphingosine«, a polyfunctional base isolated from inositol lipids of corn by Carter et al.[2] The base is closely related to sphingosine (II) and similar compounds found in the animal kingdom.



I $CH_3(CH_2)_{13}$—CH(OH)—CH(OH)—CH(NH_2)—CH_2(OH)



II $CH_3(CH_2)_{12}$—CH=CH—CH(NH_2)—CH_2(OH)

The investigation of I and similar bases containing three hydroxyl and one amino group isolated from other natural sources[3–9] is being extensively carried out in this laboratory.

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* Paper VIII, M. Proštenik and N. Krvavica, *Croat. Chem. Acta* **29** (1957) 101.

** Abstracted in part from the Doctor's thesis presented by N. Ž. Stanaćev to the Faculty of Technology, University of Zagreb, 1957.

This present publication reports the full experimental details of the preparation of racemic I as well as of its C_4-methyl ether. The synthetic route to be described below is represented by formulas III—IX.

The first stage of the synthesis consisted in the condensation of 2-methoxyhexadecanoic acid (III) via the chloride with ethyl sodioacetoacetate. The introduction of the amino nitrogen into the resulting crude β-keto ester was effected by means of the Japp-Klingemann reaction[10], a procedure which has not been sufficiently explored in preparative organic chemistry. It has, however been repeatedly applied in the synthesis of sphingosine bases[11–13]. For this purpose IV was treated most satisfactorily at *p*H 6.5 and in the presence of small quantities of ammonium chloride with diazotized *p*-nitraniline to yield the *p*-nitrophenylhydrazono ester V. *p*-Nitraniline is employed instead of aniline in order to obtain the better crystalline intermediate suitable for purification.

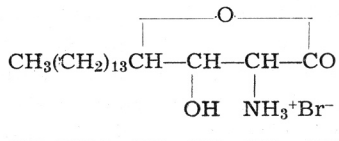
III $CH_3(CH_2)_{13}CH(OCH_3)COOH$

IV $CH_3(CH_2)_{13}CH(OCH_3)CO{-}CH(CO{-}CH_3)COOC_2H_5$

V $CH_3(CH_2)_{13}CH(OCH_3)CO{-}C(=N{-}NH{-}C_6H_4{-}NO_2)COOC_2H_5$

VI $CH_3(CH_2)_{13}CH(OCH_3)CO{-}CH(NH{-}CO{-}CH_3)COOC_2H_5$

VII $CH_3(CH_2)_{13}CH(OCH_3)CH(OH){-}CH(NH{-}CO{-}CH_3)COOC_2H_5$

VIII 

IX $CH_3(CH_2)_{13}CH(R){-}CH(OH){-}CH(NH_2){-}CH_2OH$

a) R = OH, b) R = OCH_3

Reduction of V with zinc powder in a mixture of glacial acetic acid and acetic anhydride gave a well crystallized ethyl 2-acetamido-3-oxo-4-methoxyoctadecanoate (VI). The latter when reduced with sodium borohydride in methanol solution was converted into the crude oily ethyl 2-acetamido-3-hydroxy-4-methoxyoctadecanoate (VII) which was hydrolized without further purification by refluxing it with 48% hydrobromic acid. From the reaction mixture a crystalline substance was obtained which did not contain methoxyl groups and on hydrogenation behaved as a saturated compound. Thus, it is evident that the demethylation proceeded normally without the formation of double bonds. To this substance the structure of 2-amino-3-hydroxyoctadecanoic acid

1,4-lactone hydrobromide (VIII) was attributed as the most probable. The lactone VIII was reduced with lithium aluminum hydride and the resulting crude base mixture precipitated with oxalic acid. The first crop of crystals melted after repeated crystallizations at 200—202° (decomp.). From the mother liquid the more soluble oxalate, m. p. 218—220° was isolated. When treated with aqueous potassium hydroxide the lower melting oxalate decomposed easily into two basic fractions: one was soluble and the other insoluble in chloroform. Both fractions gave after repeated crystallizations two bases (IXa) melting at 112—118° and at 136—138° respectively. The lower melting base gave a crystalline *N*-benzoyl derivative. These results seem to indicate that the series of reactions described above furnished at least two racemic pairs of IXa which, apparently, were not entirely pure.

The C_4-methyl ether IXb was obtained by reducing V with lithium aluminum hydride and subsequent hydrogenation of the oily intermediate in the presence of palladium on barium sulphate as a catalyst. The base was isolated as the oxalate, m. p. 174—176°.

In conclusion, the synthesis and the isolation of that racemate which would contain the natural isomer seem to be connected with considerable difficulties despite of the fact that the noncatalytic reduction VI → VII is assumed to be a stereospecific reaction.

EXPERIMENTAL

The melting points are uncorrected.

2-Methoxyhexadecanoic Acid (III)

Pure palmitic acid (m. p. 63°) was brominated according to Marvel[14] and the 2-bromo acid esterified with methanol in the usual manner.

To a mixture of methyl 2-bromohexadecanoate (231 g., 0.659 mole) and absolute methanol (250 ml.) a solution of sodium methoxide prepared from metallic sodium (18.4 g., 0.8 gatome) and absolute methanol (200 ml.) was gradually added. The reaction mixture was then refluxed for 3 hrs. To the reaction mixture which contained sodium bromide separated during the reaction a solution of sodium hydroxide (40 g.) in water (200 ml.) was added and refluxed for additional 1 hr. After cooling it was poured into 100% sulphuric acid (500 ml.). The colourless precipitate was filtered, dried and recrystallized from petroleum ether to give 141.2 g. (74.8%) of colourless crystals, m. p. 72—73°. A sample for analysis was recrystallized once more from the same solvent; m. p. 73—74°.

Anal. 57.4 mg. subst.: 12.23 ml. 0.1 N $Na_2S_2O_3$
$C_{17}H_{34}O_3$ (286.44) calc'd.: OCH_3 10.84%
found: OCH_3 10.99%

Ethyl 2-methoxyhexadecanoylacetoacetate (IV)

2-Methoxyhexadecanoic acid (III) (10 g., 0.035 mole) and freshly distilled thionyl chloride (25 ml.) was left to stand for 24 hrs at room temperature. The excess of thionyl chloride was distilled off in vacuo at 50—60°. The last traces were removed azeotropically with two 10 ml. portions of absolute benzene. The resulting pale yellow oil (10.64 g.) was used in the next stage without further purification. To a stirred suspension of powdered sodium (6.9 g., 0.3 gatome) in absolute ether (150 ml.) a solution of freshly distilled ethyl acetoacetate (45.5 g., 0.35 mole) in absolute ether (100 ml.) was added. Stirring was continued for 1 hr. at reflux temperature and then for 12 hrs. at room temperature. The solution of 2-methoxyhexadecanoyl chloride (73 g., 0.245 mole) in absolute ether (100 ml.) was added in the course of 15 minutes under cooling with ice. The resulting clear, pale yellow solution was stirred for 2 hr.,

then refluxed for additional 1 hr., cooled and poured into 10% hydrochloric acid (50 ml.). The ether layer was separated and the aqueous layer extracted with two 50 ml. portions of ether. The combined ether extracts were washed successively with water, 5% sodium hydrogen carbonate solution, and again with water. After drying with anhydrous sodium sulphate the solvent was distilled off to give 92.5 g. (90.5%) of the crude, yellow ester.

Ethyl (2-p-nitrophenylhydrazono)-2,3-dioxo-4-methoxy-octadecanoate (V)

2-Methoxyhexadecanoylacetoacetate (IV) (10.75 g.) was dissolved in 86% ethanol (250 ml.) and stirred at room temperature with 50% aqueous solution of sodium acetate (30 ml.). After 30 minutes the solution became turbid, 20 g. of solid sodium acetate was added and the solution (I) stirred for additional 15 minutes. *p*-Nitraniline (4.14 g., 0.03 mole) was diazotized with sodium nitrite (2.07 g., 0.03 mole) and conc. hydrochloric acid (9 ml.) in the usual manner. The diazotized solution was adjusted to pH 6.5 with sodium acetate (12 g.) and ammonium chloride (2 g.). The solution thus obtained was poured into solution I while stirring vigorously. The colour of the reaction mixture turned from yellow to red. After stirring for 1 hr. at room temperature the mixture was poured into the same volume of water and the separated oil extracted with three 100 ml. portions of ether. After drying with anhydrous sodium sulphate the solvent was evaporated *in vacuo* at room temperature. The red oil thus obtained was dissolved in 96% ethanol (10 ml.). The yellow crystals (1.3 g., m. p. 123—124°) which separated on standing were discarded. The orange-coloured substance crystallized from the mother liquid at room temperature and was filtered by suction after two days. The yield was 6 g. (34%), m. p. 72—73°. For analysis the product was recrystallized from 96% ethanol; m. p. 73—74°.

Anal. 6.525 mg. subst.: 15.38 mg. CO_2, 4.95 mg. H_2O
4.335 mg. subst.: 0.326 ml. N_2 (21.5°, 743 mm)
$C_{27}H_{43}O_6N_3$ (505.64) calc'd: C 64.13; H 8.57; N 8.31%
found: C 64.32; H 8.49; N 8.52%

Ethyl 2-acetamido-3-oxo-4-methoxyoctadecanoate (VI)

To a vigorously stirred mixture of zinc powder (15 g.), glacial acetic acid (60 ml.) and acetic anhydride (30 ml.) a solution of V (6.5 g., 12.8 mM) in glacial acetic acid (90 ml.) was added dropwise at 15—20°. Stirring was continued for 3 hrs. at room temperature and then for additional 3 hrs. at 40—45°. The reaction mixture was filtered by suction and the yellow-coloured filtrate evaporated to dryness *in vacuo*. The residue was extracted with four 50 ml. portions of hot petroleum ether. The undissolved, colourless crystalline material was identified as diacetyl phenylene diamine, m. p. 275—295° (recorded m. p. 295°). From the cooled filtrate 3.45 g. (64.8%) of a colourless, crystalline substance separated, m. p. 82—84°. For the analysis it was recrystallized twice from petroleum ether; needles, m. p. 83—84°.

Anal. 6.335 mg. subst.: 15.50 mg. CO_2, 5.90 mg. H_2O
4.600 mg. subst.: 0.145 ml. N_2 (20°, 748 mm)
$C_{23}H_{43}O_5N$ (413.58) calc'd: C 66.79; H 10.48; N 3.39%
found: C 66.77; H 10.42; N 3.61%

2-Amino-3-hydroxyoctadecanoic Acid 1,4-Lactone (VIII)

A solution of VI (4.14 g., 10 mM) in methanol (50 ml.) was added to a solution of sodium borohydride (0.625 g., 15 mM) in methanol (50 ml.) stabilized with 3 drops of 45% aqueous potassium hydroxide. The solution was left for 48 hrs. at room temperature then poured into the same volume of water and extracted four times with ether. The combined ether extracts were washed successively with water, *N* suphuric acid and again with water. After drying the solution the solvent was distilled off and the residual, viscous oil refluxed with 48% hydrobromic acid (50 ml.) for 5 hrs. with occasional stirring. At the beginning of the reaction considerable foaming occured and the semicrystalline substance separated on the surface of the reaction mixture. After cooling somewhat sticky crystals were filtered off and recry-

stallized from ethyl acetate (15 ml.). Thereby 2.48 g. (78.9%) of brown-coloured lactone hydrobromide was obtained, m. p. 120—135°. For analysis the substance was recrystallized once more from the same solvent. Analyzed were samples from two analogous preparations. The substance did not contain methoxyl groups.

Anal. 8.440 mg. subst.: 17.16 mg. CO_2, 7.06 mg. H_2O
8.600 mg. subst.: 17.45 mg. CO_2, 7.18 mg. H_2O
7.695 mg. subst.: 0.234 ml. N_2 (22°, 752 mm)
8.285 mg. subst.: 0.297 ml. N_2 (23.5°, 759 mm)
$C_{18}H_{36}O_3NBr$ (314.39) calc'd: C 54.81; H 9.20; N 3.55%
found: C 55.49; H 9.36; N 3.48%
C 55.37; H 9.34; N 4.00%

While the mother liquid was standing at room temperature some solvent was evaporated spontaneously and a second crop of crystals (25 mg., 4.2%) was obtained. Recrystallization from acetonitrile (3 ml.) gave colourless platelets, m. p. 130—138°.

Anal. 8.730 mg. subst.: 21.87 mg. CO_2, 9.56 mg. H_2O
2.780 mg. subst.: 0.110 ml. N_2 (23.5°, 754 mm)
$C_{18}H_{39}O_3N$ (317.50) calc'd: C 68.03; H 12.37; N 4.41%
found: C 68.36; H 12.26; N 4.52%

Fraction B. — The base insoluble in chloroform was recrystallized from acetonitrile (10 ml.) to give 80 mg. (13.44%) colourless crystals, m. p. 127—135°. Two more crystallizations from acetonitrile (2×20 ml.) gave colourless platelets, m. p. 136—138°. The methoxyl determination was negative.

Anal. 8.540 mg. subst.: 21.46 mg. CO_2, 9.47 mg. H_2O
$C_{18}H_{39}O_3N$ (317.50) calc'd: C 68.03; H 12.37%
found: C 68.58; H 12.41%

DL-*2-Benzamido-1,3,4-trihydroxyoctadecane*

To a suspension of the base IXa, m. p. 112—118° (100 mg.) in ether (50 ml.) *N* natrium hydroxide (10 ml.) and benzoyl chloride (1 ml.) were gradually added with vigorous shaking. After standing for 30 minutes at room temperature the ether layer was separated, washed with water, dried with anhydrous sodium sulphate and the solvent removed in vacuo. The residual semicrystalline oil was dissolved in methanol (20 ml.) 45% aqueous potassium hydroxide (1 ml.) was added and allowed to stand for 18 hrs. at room temperature. After the evaporation of methanol in vacuo, water (10 ml.) was added and extracted with ether. The combined ether extracts were washed with water, dried with sodium sulphate and the solvent removed in vacuo. The solid residue was recrystallized from acetone (3 ml.) to give 55 mg. of a substance melting at 100—105° .One more crystallization yielded colourless needles, m. p. 112—113°.

Anal. 8.750 mg. subst.: 22.61 mg. CO_2, 8.06 mg. H_2O
6.225 mg. subst.: 0.177 ml. N_2 (22°, 753 mm)
$C_{25}H_{43}O_4N$ (421.60) calc'd: C 71.22; H 10.28; N 3.32%
found: C 70.60; H 10.38; N 3.26%

DL-*2-Amino-1,3-dihydroxy-4-methoxyoctadecane* (IXb)

A solution of V (2 g., 3.7 mM) in absolute ether (20 ml.) was added dropwise to a solution of lithium aluminum hydride (1.05 g.) in absolute ether (50 ml.). After the vigorous reaction has subsided the mixture was refluxed for 2 hr. and allowed to stand overnight at room temperature. The excess of lithium aluminum hydride was decomposed by addition of water, the ether layer separated and the solvent distilled off *in vacuo*. The red-coloured oil thus obtained (1.5 g.) was dissolved in a mixture of absolute ethanol (50 ml.) and absolute ether (50 ml.). After the addition of conc. hydrochloric acid (3.43 ml.) the solution was hydrogenated in the presence of 5% palladium on a carbon catalyst (preparated from 1. g. of palladium chloride). After 90 minutes 94 ml. of hydrogen was absorbed (21°, 749 mm), calc'd: 91.5 ml. The catalyst was filtered off and the pale yellow filtrate evaporated in vacuo at 40°. The residual oil (1.3 g.) was treated with ethyl acetate (25 ml.) and the separated yellow-coloured crystals of *p*-phenylene diamine dihydrochloride (110 mg., m. p. 265°) were removed by filtration. The mother liquid was brought to dryness *in vacuo*, the residue shaken with 2*N* natrium carbonate and with three 50 ml. portions of ether. The combined ether extracts were washed with water, the solvent distilled off *in vacuo*, the residual oil (880 mg.) dissolved in absolute ethanol and precipitated with conc. solution of oxalic acid in absolute ethanol. The brown-colored sticky crystals (395 mg.) were recrystallized from absolute ethanol (25 ml.); m. p. 171—173°. One more crystallization from the same solvent — the solution being previously

filtered through cellite — yielded colourless platelets, m. p. 174—176° (decomp.). Analyzed were samples from two analogous preparations.

Anal. 8.470 mg. subst.: 19.81 mg. CO_2, 8.48 mg. H_2O
4.450 mg. subst.: 10.46 mg. CO_2, 4.45 mg. H_2O
6.775 mg. subst.: 0.250 ml. N_2 (27.3°, 745 mm)
4.990 mg. subst.: 0.178 ml. N_2 (25°, 749 mm)
13.62 mg. subst.: 2.26 ml. 0.1 N $Na_2S_2O_3$
$C_{20}H_{42}O_5N$ (376.54) calc'd: C 63.94; H 11.24; N 3.72; OCH_3 8.24%
found: C 63.83; H 11.20; N 4.11; OCH_3 8.57%
C 64.14; H 11.19; N 4.03%

Acknowledgment. We are indebted to Mrs. M. Munk-Weinert from our microanalytical laboratory for carrying out the microanalyses.

IZVOD

Studije u redu sfingolipoida IX.
Sinteza DL-2-amino-1,3,4-trihidroksi-oktadekana i njegova C_4-metil-etera

N. Ž. Stanaćev i M. Proštenik

DL-2-Amino-1,3,4-trihidroksi-oktadekan (IXa) i njegov C_4-metil-eter (IXb) pripravljeni su preko ovih intermedijara: 2-Metoksi-heksadekan kiselina (III) → Etil 2-metoksi-heksadekanoil-acetoacetat (IV) → Etil (2-p-nitrofenilhidrazono)-2,3-diokso-4-metoksi-oktadekanoat (V) → Etil 2-acetamido-3-okso-4-metoksi-oktadekanoat (VI) → Etil 2-acetamido-3-hidroksi-4-metoksi-oktadekanoat (VII) → 2-Amino-3-hidroksi-oktadekan kis. 1,4-lakton (VIII) → IX. Sirova smjesa baza taložena je oksalnom kiselinom, a smjesa oksalata frakcionirano kristalizirana. Dobivena su dva oksalata baze IXa, vjerojatno pročišćeni racemički oblici. IXa je pripravljen i kao slobodna baza, koja je karakterizirana i u obliku *N*-benzoil-derivata.

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Primljeno 14. lipnja 1957.

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