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Studies in the Sphingolipids Series. XXI.* C_{20}-Sphingosine, a New Long-Chain Base of Animal Origin**

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A new hitherto undescribed sphingolipide base C_{20}-sphingosine has been discovered in the horse and bovine brain lipides. The base is shown to be D-erythro-2-amino-1,3-dihydroxy-4-eicosene (II). The proposed structure is deduced from the following data: 1. gas chromatographic analysis of the products obtained by periodic and chromic acid oxidations (analyzed as aldehyde dimethyl acetals and fatty acid methyl esters), 2. hydrogenolysis of tribenzoyl-C_{20}-sphingosine to C_{20}-sphingine derivatives, 3. elemental analyses of a series of compounds derived from C_{20}-sphingosine, 4. infra-red and specific rotations measurements.

Sphingosine was first prepared in 1879 by Thudichum from the cerebroside phrenosin — a glycolipide isolated from an alcoholic extract of brain tissue.[1] The base is incorporated mainly in cerebrosides, sphingomyelins and gangliosides which are widely distributed in different animal tissues and liquids. Subsequent investigators established later the chemical structure and the stereochemistry of the base as trans-D-erythro-2-amino-1,3-dihydroxy-4-octadecene (I).[2] The work was continued for more than six decades. In 1941 Lesuk and Anderson[3] and in 1942 Carter and Norris[4] reported the occurrence in nature of I as its dihydro derivative — C_{18}-dihydrosphingosine. Both compounds were at that time the only known sphingolipide bases of animal origin.

The present paper deals with the discovery of C_{20}-sphingosine, a new sphingolipide base which was found in horse and bovine brain. The structure of the base has been established as D-erythro-2-amino-1,3-dihydroxy-4-eicosene (II). Some of the results described here were the subject of a preliminary communication.[5] It is to be noted that this investigation was started in relation to another problem. For this reason the majority of compounds described herein appear somewhat unusual. On the whole, the detection of the C_{20}-base is founded upon the failure to obtain in a pure condition certain derivatives of natural C_{18}-sphingosine isolated from horse brain.

$$\begin{array}{lll} CH_3(CH_2)_n—CH = CH—CH—CH—CH_2 & & \\ \qquad\qquad\qquad\quad | \quad\ | \quad\ | & & \\ \qquad\qquad\qquad\quad OH\ \ NH_2\ OH & \text{II} & n = 14 \end{array} \qquad \text{I} \quad n = 12$$

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* Paper XX: A. Kisić and M. Proštenik, *Croat. Chem. Acta* **32** (1960) 229.

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Crude sphingosine sulphate was isolated from horse brain by adopting the known procedures.[6,7] We wish to point out that the sulphate obtained from horse brain represents colourless crystals, which are less soluble in ethanol than the sulphate from bovine brain and could be obtained in better yields. Even more, the starting sphingolipide mixtures from both sources behaved differently, particularly in respect to their solubilities. Indeed, a number of dissimilarities could be noticed in subsequent preparations.

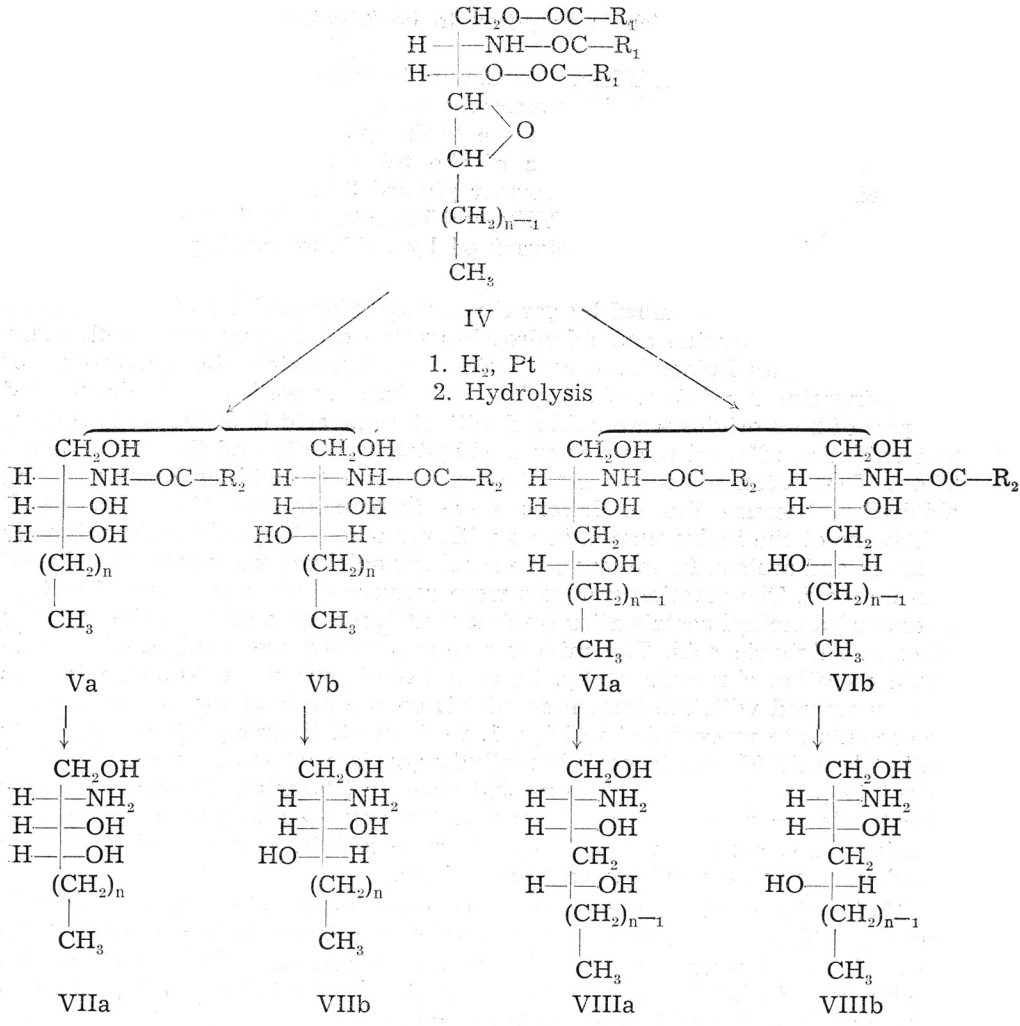
In the first instance, sphingosine thus prepared was converted into the tribenzoyl and triacetyl derivatives. In the course of several runs it was observed that tribenzoyl sphingosine (III) originating from horse brain had the melting point on average 1—2° higher than that obtained from bovine brain. The lower melting point and the increased C,H-values of the triacetyl-sphingosine from horse brain as compared to the normal values shown by the derivative from bovine brain were also indicative of a mixture with a higher base.

Catalytic hydrogenation of tribenzoylsphingosine from the horse brain bases gave the information decisive for the location of the double bond. According to the analytical data, a mixture of *N,O*-dicyclohexanoyl-C_{18}-sphingine and *N,O*-dicyclohexanoyl-C_{20}-sphingine resulted, owing to the hydrogenolysis of the allylic grouping. Partial alkaline hydrolysis furnished a mixture of the *N*-cyclohexanoyl derivatives of both bases. The same result was consistently obtained from different preparations of III from horse bases. A similar procedure has been applied previously to III from bovine brain.[8] In that case the analytical data fit well a C_{18}-sphingine and the corresponding cyclohexanoyl derivatives.

Oxidation of III by means of perbenzoic acid afforded tribenzoylsphingosine epoxide (IV). The infrared spectrum of the epoxide differed from III principally in that it lacked the absorption band at 970 cm^{-1}, which is attributable to the disappearance of the trans-substituted double bond.

Reduction of IV was effected either catalytically by use of Adams platinum catalyst, or by means of lithium aluminium hydride. In the former, exactly 10 moles of hydrogen were taken up to yield a tricyclohexanoyl derivative of an aminotrihydroxy base, which, upon partial alkaline hydrolysis, furnished cyclohexanoylaminotrihydroxyeicosane when started with horse bases, and cyclohexanoylaminotrihydroxyoctadecane, when started with bovine bases, respectively.

The cyclohexanoyl derivatives of both C_{18} and C_{20}-base can appear each in two forms, *i. e.* Va and VIa, and their epimeric structures Vb and VIb. The decision between these structures could partially be reached on the basis of the following data. The oxidation assays with periodic acid of the *N*-cyclohexanoyl derivative of the base from horse brain were negative. In each experiment the unreacted starting compound in almost quantitative yield was recovered. This fact excludes the occurrence of the α-glycol structure, thus suggesting the structures VIa und VIb ($n = 15$) and taking into account the existence of the allylic hydroxy group in II. However, this result is also consistent with formulas possessing the double bond shifted deeper into the hydrocarbon moiety. On the other hand, the *N*-cyclohexanoyl-C_{18}-base (m. p. 83—85°) from bovine brain consumed in average 0.4—0.5 mole of periodic acid. The resulting long-chain aldehyde was



n = 13 or 15
R_1 = phenyl, R_2 = cyclohexyl

identified as *n*-pentadecanal through the thiosemicarbazone[9], thus indicating the presence of Va and Vb. To the unoxidized material, which melted at 98—102°, the most probable structures of VIa and VIb (n = 13) were attributed.

Methanolysis of 2-cyclohexanoylamino-1,3,5-trihydroxyeicosane (VIa and VIb, n = 15) from horse brain afforded 2-amino-1,3,5-trihydroxyeicosane representing probably a mixture of both epimeric forms VIIIa and VIIIb (n = 15). Further evidence supporting the structure of the base mixture was obtained by oxidizing it with periodic acid. Exactly 2 moles of periodic acid were consumed indicating the relative position of the functional groups on the carbon atoms 1, 2, 3 and 5. The compound containing the functional groups on the carbons 1,2,3 and 4 would require 3 moles of periodic acid. The resulting

long-chain aldehyde, which is supposed to be 3-hydroxy-*n*-octadecanal, gave no crystalline derivative.

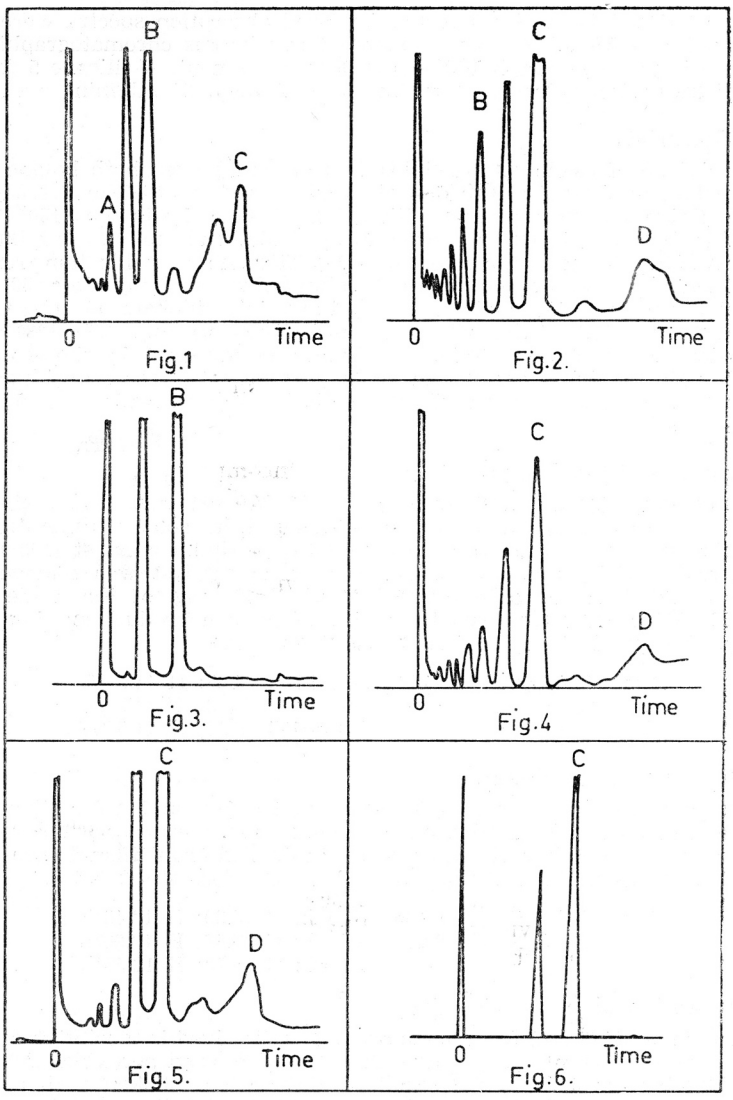
Reduction of IV with lithium aluminium hydride resulted in the formation of 2-benzylamino-1,3,5-trihydroxyeicosane as a main product when started with horse bases. IV from the bovine brain gave 2-benzylamino-1,3,4(or 5)-trihydroxyoctadecane. The location of the third hydroxy group is not determined. Hydrogenolysis of the former yielded 2-amino-1,3,5-trihydroxyeicosane, most probably a mixture of VIIIa and VIIIb (n = 15). The relative position of the functional groups was determined by oxidation with periodic acid as described above.

Additional data obtained by gas chromatographic analysis of the oxidation products of sphingosine and dihydrosphingosine originating from both horse and bovine brain lipides have convincingly demonstrated the occurrence of C_{20}-sphingosine. A mixture of the sphingosine bases as well as the hydrogenated samples of both origins were oxidized with chromic acid in acetic acid solution. The oxidation affected the polar aminodihydroxy moiety and the double bond. The crude reaction mixture consisted of long-chain fatty acids and corresponding aldehydes, because the oxidations were incomplete. On the other hand, oxidations of the hydrogenated bases with periodic acid gave similar mixtures of esters and acetals. In any case, the resulting aldehyde was partially oxidized into the acid. The reaction products were converted into a mixture of methyl esters and dimethyl acetals after treatment with methyl alcohol in the presence of conc. sulphuric acid. The mixtures thus obtained were subjected to gas-liquid partition chromatography. In each experiment the unknown mixtures were compared with the known standards on the basis of the retention data. The results are represented in Figs. 1. to 6. In all figures peak A represents methyl laurate (C_{12}-acid), peak B methyl myristate (C_{14}-acid), peak C methyl palmitate (C_{16}-acid) and peak D methyl stearate (C_{18}-acid). The nearest peaks with the lower retention time belong to the corresponding dimethyl acetals. Stearic acid (peak D) in Fig. 2. (horse brain) and in Figs. 4. and 5. (bovine brain) may originate only from the C_{20}-base.

Under the conditions used in these experiments chromic acid had no apparent affect on the saturated hydrocarbon moiety. This was proved by the oxidation of pure, synthetic C_{18}-dihydrosphingosine.[10] The gas chromatographic record (Fig. 6.) shows only two peaks, which belong to C_{16}-aldehyde acetal and C_{16}-fatty acid ester (peak C), respectively.

By summarizing all these data it becomes evident that both horse and bovine brain bases represent a mixture of at least two compounds — C_{18}-sphingosine (I) and C_{20}-sphingosine (II). The concentration of the C_{20}-base is considerably higher in the horse brain than in the bovine brain. This is probably the reason why — in a series of the reaction stages — the C_{20}-compounds are concentrated more rapidly from the horse brain preparations than from the bovine brain. Therefore, the elemental analyses of the compounds derived from horse brain sphingosine fit better a C_{20}-chain, the others — from bovine brain sphingosine — a C_{18}-chain. In addition, the gas chromatographic data leave no doubt as to the presence of other so far unidentified lower bases.

In spite of the fact that the new base has not been isolated in a pure condition — it contained to a large extent the C_{18}-base — some information about the stereochemistry could be obtained. The erythro structure is assigned



to C_{20}-sphingosine by comparison of the infrared spectra of synthetic D(+)erythro - C_{20}-dihydrosphingosine and natural (+) - dihydrosphingosine (C_{18} + C_{20}) from horse brain. The spectra were identical in all respects and no absorption band characteristic for the threo isomer could be observed.[10] The D-configuration was suggested by the fact that the triacetyl derivatives of both dihydro bases had nearly the same specific rotations.[10]

The stereochemical treatment of the compounds derived from tribenzoyl-sphingosine described here will be the subject of a subsequent communication.

EXPERIMENTAL

All melting points are uncorrected. Infrared absorption spectra were measured on a Perkin-Elmer Model 137 spectrophotometer. The gas chromatographic analyses were done using an Aerograph Wilkens A-90 C instrument. A silicone 5 foot column was run at 230° with hydrogen at a flow rate of about 30 ml./min.

Starting Materials

The mixture of crude sphingolipids was prepared from both bovine and horse brain according to the procedure described by Carter *et al.*[6] The average yield of the sphingolipide mixture starting with 5 kg. of horse brain was 150—175 g. The methanolysis according to Carter's procedure[7] yielded the crude base mixture which was converted into the sulphate, m.p. 140—190° after one crystallization from absolute ethanol. Yield: 7—8 g. of the purified sphingosine sulphate from 50 g. of the sphingolipide mixture. Benzoylation of sphingosine sulphate (5 g.)[7] from horse brain gave 3.7 g. (45%) of the tribenzoyl derivative (III), m.p. 121—123° after three crystallizations from ethanol. Triacetylsphingosine prepared in the usual manner melted after six crystallizations from acetone at 91—93°. Triacetylsphingosine from bovine brain bases melted after six crystallizations from acetone at 99°, reported m.p. 101—103°.[7]

Dicyclohexanoyl-C_{20}-sphingine

Tribenzoylsphingosine (III) from horse brain (450 mg., m.p. 121—123°) was suspended in ethanol (20 ml.) and hydrogenated at room temperature and at atmospheric pressure in the presence of Adams platinum catalyst (100 mg.). After 2 hrs. 11 moles of hydrogen were taken up. The catalyst was removed and the filtrate evaporated in vacuo to dryness to yield 400 mg. of the crude substance, m.p. 86—89°. Four crystallizations from ethanol gave a product melting at 89—90°. Pure dicyclohexanoyl C_{18}-sphingine melts at 90—91°.[8]

Anal. $C_{32}H_{59}NO_3$ (505.75) calc'd.: C 75.62; H 11.57%
$C_{34}H_{63}NO_3$ (533.80) calc'd.: C 76.48; H 11.89%
found : C 76.34; H 11.82%

N-Cyclohexanoyl C_{20}-sphingine

Dicyclohexanoyl derivative (150 mg., m.p. 89—90°) and 1*N* methanolic NaOH (7 ml.) were heated at 40° for 1 hr. After cooling, the separated crystals were filtered off and washed thoroughly with water. Recrystallization from ethanol gave a colourless product (85 mg.), m.p. 111—112°. Lit. m.p. of the C_{18} derivative: 115—116°.[8]

Anal. $C_{25}H_{49}NO_2$ (395.65) calc'd.: C 75.89; H 12.48%
$C_{27}H_{53}NO_2$ (423.70) calc'd.: C 76.46; H 12.60%
found : C 76.20; H 12.57%

Tribenzoylsphingosine Epoxide (IV)

A sample of III (3.7 g.) from horse brain was dissolved in a solution of perbenzoic acid in chloroform (30 ml., prepared from 6 g. of dibenzoyl peroxide). After standing in a refrigerator for 5 days, the reaction mixture was shaken with a solution of ferrous sulphate and washed with water. During removing the solvent, a red coloured substance crystallized out. It was recrystallized several times from ethanol with addition of charcoal. Colourless needles, m.p. 133—134.5°; yield 2.35 g. (62%).

Anal. $C_{39}H_{49}NO_6$ (627.79) calc'd.: C 74.61; H 7.87%
$C_{41}H_{53}NO_6$ (655.84) calc'd.: C 75.08; H 8.15%
found : C 75.47; H 8.23%

The epoxide prepared in the same manner from III (6.5 g.) of bovine brain melted at 132—132.5°; yield 4 g. (60%).***

*** Tribenzoylsphingosine epoxide from bovine brain was first prepared by Dr. N. Ž. Stanaćev in the Department of Chemistry, Medical Faculty, University of Zagreb.

Anal. $C_{39}H_{49}NO_6$ (627.79) calc'd.: C 74.61; H 7.87%
found : C 74.89; H 7.83%

2-Cyclohexanoylamino-1,3,5-trihydroxyeicosane (VIa and VIb, n = 15)

A sample of IV (500 mg.) from horse brain was suspended in 95% ethanol (30 ml.) and hydrogenated in the presence of Adams platinum catalyst (100 mg.). After 3 hrs. a theoretical amount (10 moles) of hydrogen was taken up at atmospheric pressure and at room temperature. The catalyst was removed and the filtrate evaporated *in vacuo* to dryness. The crude, syrupy product was, dissolved without further purification, in 1*N* methanolic KOH (20 ml.) and heated to 40° for 1 hr. The solvent was partly evaporated *in vacuo*, the residue poured into cold water and the separated solid extracted with ether. The ether solution was washed with water to neutral and the solvent distilled off. The residue was recrystallized four times from acetonitrile to give a colourless substance, m.p. 91—93°.

Anal. $C_{25}H_{49}NO_4$ (427.65) calc'd.: C 70.21; H 11.55%
$C_{27}H_{53}NO_4$ (455.70) calc'd.: C 71.16; H 11.72%
found : C 71.32; H 11.42%

Different preparations gave products which melted over a range of about 30° (78—108°) and all analyses fit well the C_{20}-chain.

2-Cyclohexanoylamino-1,3,5-trihydroxyoctadecane (VIa and VIb, n = 13)

The compound prepared similarly from IV (1.3 g.) of bovine brain melted at 83—85°; yield 828 mg. (93%).

Anal. $C_{25}H_{49}NO_4$ (427.65) calc'd.: C 70.21; H 11.55; N 3.28%
found : C 70,23; H 11.75; N 3.01%

Oxidation of the N-*cyclohexanoyl Derivatives with Periodic Acid*

A. To a solution of the *N*-cyclohexanoyl compound (100 mg., m.p. 103—108°) from horse brain in methanol (10 ml.) a 5% methanolic solution of periodic acid (10 ml.) was added. No consumption of periodic acid was observed after 3, 5, and 24 hrs., even after heating to 50° (titration with *N*/10 $Na_2S_2O_3$). The solution was diluted with an equal volume of water and shaken with hexane. The substance suspended in the hexane phase was filtered off and crystallized from acetonitrile; m.p. 102—107°. On the basis of the melting point and analytical data (found: C 71.59; H 11.39%) it was identified as the starting material. The oxidation assays were repeated with the *N*-cyclohexanoyl derivatives of various preparations with the same result.

B. Analogously, a sample of the *N*-cyclohexanoyl derivative (400 mg.) from bovine brain was oxidized with periodic acid. 44% of the quantity of oxidant calculated for 1 mole of the compound was consumed. The fraction insoluble in hexane (216 mg.) melted at 98—102° and represented a part of the unreacted starting material (2-cyclohexanoylamino-1,3,5-trihydroxyoctadecane).

Anal. $C_{25}H_{49}NO_4$ (427.65) calc'd.: C 70.21; H 11.55%
found : C 70.12; H 10.90%

Taking into account the unreacted, recovered starting material, the consumption of periodic acid was 92% of the quantity calculated for 1 mole. Evaporation of the hexane solution furnished a crystalline residue (65 mg.), which was treated with a solution of thiosemicarbazide hydrochloride (50 mg.) in acetic acid (3 ml.) in the usual manner.[9] The resulting thiosemicarbazone was recrystallized three times from methanol; m.p. 96—99°. The mixed melting point with authentic *n*-pentadecanal thiosemicarbazone (m.p. 97—99°) was unchanged. The mixed melting point with *n*-tetradecanal thiosemicarbazone and *n*-hexadecanal thiosemicarbazone, respectively, showed a depression of about 10°.

2-Benzylamino-1,3,5-trihydroxyeicosane

A suspension of IV (3.7 g.) from horse brain was added dropwise to a solution of lithium aluminium hydride (3.7 g.) in ether (250 ml.). The mixture was refluxed for 5 hrs. and the excess of the hydride hydrolized by cautious addition of water.

The ether phase was separated and the residual solid material extracted with three 75 ml. portions of ether. Evaporation of the solvent gave the crude base (2.37 g., m.p. 70—72°) which after several crystallizations from acetonitrile melted at 83—87°. The base was converted into the oxalate, m.p. 145—150° from ethanol. Neither the base nor its oxalate gave correct elemental analyses.

2-Benzylamino-1,3,4 (or 5)*-trihydroxyoctadecane*

A sample of IV (500 mg.) from bovine brain when reduced with lithium aluminium hydride as described above furnished the neutral oxalate which after two crystallizations from ethanol melted at 141—145°; yield 336 mg. (91%).

Anal. $C_{26}H_{46}NO_5$ (452.64) calc'd.: C 68.98; H 10.27; N 3.09%
found : C 69.20; H 10.21; N 3.27%

The hydrogen oxalate was prepared by treating the base with a large excess of oxalic acid. After four crystallizations from ethanol it melted at 137—138°.

Anal. $C_{27}H_{47}NO_7$ (497.65) calc'd.: C 65.18; H 9.52%
found : C 65.72; H 9.58%

The free base was obtained from the neutral oxalate (2.4 g.) in the usual manner; yield 1.6 g. after four crystalizations from acetonitrile, m.p. 78-83°.

Anal. $C_{25}H_{45}NO_3$ (407.62) calc'd.: C 73.66; H 11.13%
found : C 73.95; H 11.16%

2-Amino-1,3,5-trihydroxyeicosane (VIIIa and VIIIb, n = 15)

A. A solution of the *N*-cyclohexanoyl derivative (300 mg., m.p. 103—108°) from horse brain in 1*N* methanolic H_2SO_4 (15 ml.) was refluxed for 24 hrs. The reaction mixture was made alkaline with 45% KOH and the resulting precipitate extracted with ether. Removal of the solvent and crystallization from acetonitrile gave a colourless substance, m.p. 67—74° (118 mg., 55%).

Anal. $C_{18}H_{39}NO_3$ (317.50) calc'd.: C 68.03; H 12.37%
$C_{20}H_{43}NO_3$ (345.55) calc'd.: C 69.51; H 12.54%
found : C 69.30; H 11.84%

B. A solution of 2-benzylamino-1,3,5-trihydroxyeicosane (480 mg.) from horse brain in ethanol (30 ml.) was hydrogenated in the presence of 10% palladium on barium sulphate catalyst (300 mg.) at atmospheric pressure and at room temperature. After 4 hrs. the hydrogenolysis was completed, the catalyst filtered off and the solvent evaporated to dryness. The residual crude base (380 mg.) was converted into the neutral oxalate by addition of the ethanolic solution of oxalic acid. The precipitate (285 mg., 65%, m.p. 187—197°) was recrystallized twice from abs. ethanol; m.p. 197—199.5°.

Anal. $C_{19}H_{40}NO_5$ (362.50) calc'd.: C 62.94; H 11.12%
$C_{21}H_{44}NO_5$ (390.55) calc'd.: C 64.58; H 11.36%
found : C 64.56; H 11.39%

The free base was obtained by shaking the oxalate (250 mg.) with 1*N* KOH (20 ml.) and chloroform (500 ml.). Evaporation of the solvent gave the crude base (127 mg.) which was recrystallized for times from acetonitrile; m.p. 81—90°.

Anal. $C_{18}H_{39}NO_3$ (317.50) calc'd.: C 68.03; H 12.37; N 4.41%
$C_{20}H_{43}NO_3$ (345.55) calc'd.: C 69.51; H 12.54; N 4.05%
found : C 69.44; H 12.49; N 4.20%

Oxidation of the Trihydroxy Base with Periodic Acid

The foregoing trihydroxy base (100 mg.) from horse brain consumed 2 moles (110 mg.) HIO_4. Three moles require 167 mg. HIO_4. The hexane extracts of the reaction mixture (48 mg.) were treated with both thiosemicarbazide and 2,4-dinitrophenylhydrazine. In no case were crystalline derivatives obtained.

2-Amino-1,3-4 (or 5)*-trihydroxyoctadecane*

The catalytic hydrogenolysis of the *N*-benzylamino base (1.61 g.) from bovine brain was carried out as described above. The base was converted into the oxalate; m.p. 196—198°.

Anal. $C_{19}H_{40}NO_5$ (362.50) calc'd.: C 62.94; H 11.12%
found : C 62.59; H 10.82%

Oxidation of Sphingosine and Dihydrosphingosine with Chromic Acid

A. From horse brain. A sample of sphingosine sulphate (1 g.) was dissolved in glacial acetic acid (60 ml.) and oxidized with a 10% solution of chromium trioxide in glacial acetic acid at 60° for 1 hr. The cooled reaction mixture was diluted with water (400 ml.) and extracted with ether. The ether solution was washed with water, the solvent distilled off and the crude residue, consisting of a mixture of the fatty acids and the corresponding aldehydes, refluxed with 3% methanolic H_2SO_4 (20 ml.) for 5 hrs. The solution was poured into an equal volume of water and extracted with ether. Removal of the solvent gave a mixture of methyl esters and dimethyl acetals, which were first distilled in a glass tube at 0.02 mm. Hg. heating block temperature 110—135°, and then subjected to vapor-phase chromatography. In Fig. 1. peak *A* represents C_{12}-acid, peak *B:* C_{14}-acid and peak *C:* C_{16}-acid. The retention data of the corresponding aldehyde dimethyl acetals are somewhat lower.

A sample of dihydrosphingosine (770 mg.) obtained by the catalytic hydrogenation of sphingosine sulphate from horse brain in the usual way, was oxidized with chromic acid, treated with methanol and the resulting mixture of esters and acetals chromatographed as described above. Fig. 2.: peak *B* is a record of C_{14}-acid, peak *C:* C_{16}-acid and peak *D:* C_{18}-acid.

B. From bovine brain. Sphingosine sulphate was oxidized as described above. The chromatographic record is represented in Fig. 3. The major peak *B* corresponds to C_{14}-acid.

Oxidation products of dihydrosphingosine from bovine brain are recorded in Fig. 4. Peak *C:* C_{16}-acid, peak *D:* C_{18}-acid.

Oxidation of Dihydrosphingosine from Bovine Brain with Periodic Acid

Sphingosine was recovered from the oxalate (1.38 g.) and hydrogenated in the usual manner to give dihydrosphingosine base (1.05 g.). A solution of the latter (820 mg.) in methanol was oxidized with 5% methanolic HIO_4 (50 ml.). After 5 hrs. the solution was diluted with water (50 ml.) and extracted with hexane. The combined extracts afforded the crude aldehyde mixture (390 mg.) which was dissolved in glacial acetic acid (8 ml.) and oxidized with bromine (0.2 ml.) at room temperature overnight. The excess of bromine was then removed with a few drops of aqueous $NaHSO_3$ and the mixture evaporated *in vacuo* to dryness. The residue was treated several times with 1 ml. portions of water followed by evaporation to dryness each time. Treatment with ethyl acetate (25 ml.) furnished some insoluble material which was discarded. From the filtrate 380 mg. of the waxy substance was obtained. It was esterified with methanol and sulphuric acid as described earlier and the resulting mixture of esters and acetals submitted to vapor-phase chromatography. Fig. 5. shows the presence of C_{16}-acid (peak *C*) and C_{18}-acid (peak *D*) in admixture with corresponding aldehydes.

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IZVOD

Studije u redu sfingolipoida. XXI. C_{20}-Sfingozin, nova dugolančana baza animalnoga porijekla

B. Majhofer-Oreščanin i M. Prošteník

Nova, do sada neopisana sfingolipoidna baza C_{20}-sfingozin otkrivena je u lipoidnoj frakciji konjskoga i goveđega mozga. Bazi je pripisana struktura D-eritro-2-amino-1,3-dihidroksi-4-eikosena (II). Predložena struktura izvedena je na osnovi ovih podataka: 1. plinsko-kromatografske analize produkata dobivenih oksidacijom s perjodnom i kromnom kiselinom (analizirani su kao dimetilni acetali aldehida i metilni esteri masnih kiselina), 2. hidrogenolize tribenzoil-C_{20}-sfingozina u derivate C_{20}-sfingina, 3. elementarnih analiza većega broja spojeva izvedenih iz C_{20}-sfingozina, 4. infracrvenih spektara i specifičnih skretanja.

BIOKEMIJSKI ODJEL
INSTITUT »RUĐER BOŠKOVIĆ«
ZAGREB

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