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Studies in the Sphingolipids Series. XIII.* On the Ceramides and Ceramide Esters of C_{20}-Phytosphingosine and C_{20}-Phytosphingosine Anhydro Base of Yeast

B. Ries-Lešić and M. Proštenik

Department of Biochemistry, Institute »Ruđer Bošković«, Zagreb, Croatia, Yugoslavia

Received January 23, 1960

The *N* and *O*-acylations of C_{20}-phytosphingosine anhydro base (Ia), C_{20}-anhydro cerebrin (IIa) and C_{20}-cerebrin (IIIa) with long-chain fatty acid chlorides have been examined. The reactions have been carried out in both quinoline and *N,N*-dimethyl formamide. The ceramides and ceramide esters thus obtained are represented by formulas I—III.

The long-chain fatty acid amides of sphingosine (ceramides) such as *N*-palmitoyl, *N*-stearoyl, *N*-lignoceryl and *N*-cerebronylsphingosine have been reported either as constituents of liver and spleen lipids or as hydrolysis products of sphingolipide preparations[1]. The existence in spleen of a sphingomyelin in which the second hydroxy group of the sphingosine is esterified with a long-chain fatty acid has been assumed by Thannhauser and Reichel[2]. On the other hand, *N*-lignoceryl, *N*-cerebronyl, *N*-(2-hydroxyhexacosanoyl) and *N*-(2,3-dihydroxytetracosanoyl) derivatives of homologous C_{18} and C_{20}-4-hydroxydihydrosphingosines, *i.e.* C_{18} and C_{20}-phytosphingosines, have been isolated from yeast, corn, soybean and other plant lipids[3–6].

Although some ceramides of the sphingosine and dihydrosphingosine series have been prepared synthetically by Reichel and Thannhauser[7] no synthetic work has been done yet on ceramides and ceramide esters of phytosphingosines. This problem seems to be of interest since one may suppose *inter alia* the occurrence of the ceramide esters in the native lipids. On the other hand, it was also desirable to work out a satisfactory method of approaching the synthesis of compounds of this type, particularly of cerebrin and anhydro cerebrin.

In our experiments the starting materials were yeast cerebrin (IIIa) — purified through its tetraacetyl derivative —, anhydro cerebrin (IIa) and C_{20}-phytosphingosine anhydro base (Ia). It has been established recently that the yeast bases represent a mixture of C_{18} and C_{20}-homologues respectively[6,8,9]. The isolation procedure originally proposed by Reindel *et al.* yielded the C_{20}-anhydro compound which was evidently not contaminated with a noticeable amount of the C_{18}-compound[8,10]. The C_{20}-compounds only were employed in this investigation.

The synthesis of ceramides and corresponding esters of Ia, IIa and IIIa was effected by two methods. According to Reichel and Thannhauser[7] the *N,O*-acylation can be carried out in ether solution in presence of quinoline.

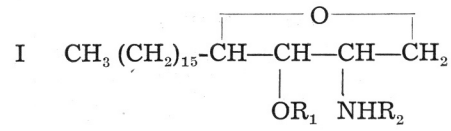
* Paper XII: M. Proštenik, B. Majhofer-Oreščanin, M. Munk-Weinert, and B. Ries-Lešić, *Croat. Chem. Acta* **32** (1960) 11.

The ester group can then be selectively hydrolyzed to yield the *N*-acyl compound. By this way mono-, di- and tetraacyl derivatives of IIa and IIIa have been obtained.

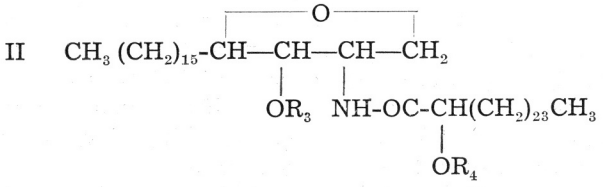
The acylation with oleyl chloride of IIa afforded the monooleyl ester (IIb). Since IIa has two hydroxy groups the position of the acyl rest remains to be elucidated.

An alternative method described recently by Weiss and Raizman[11] using *N,N*-dimethylformamide as a solvent in presence of traces of pyridine appears to be more suitable for the preparation of *N*-monoacyl derivatives.

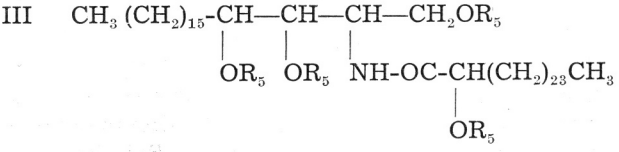
The amides and esters of long-chain fatty acids thus prepared are represented by formulas I — III.



a) $R_1 = R_2 = H$
b) $R_1 = H$, $R_2 = CH_3(CH_2)_{16}CO$
c) $R_1 = CH_3CO$, $R_2 = CH_3(CH_2)_{16}CO$
d) $R_1 = H$, $R_2 = CH_3(CH_2)_{14}CO$
e) $R_1 = H$, $R_2 = CH_3(CH_2)_{15}CH(OH)CO$



a) $R_3 = R_4 = H$
b) $R_3 = H$, $R_4 = CH_3(CH_2)_7CH=CH(CH_2)_7CO$ or
$R_3 = CH_3(CH_2)_7CH=CH(CH_2)_7CO$, $R_4 = H$
c) $R_3 = H$, $R_4 = CH_3(CH_2)_{16}CO$ or
$R_3 = CH_3(CH_2)_{16}CO$, $R_4 = H$
d) $R_3 = R_4 = CH_3(CH_2)_{16}CO$



a) $R_5 = H$
b) $R_5 = CH_3(CH_2)_{16}CO$

EXPERIMENTAL

The melting points are uncorrected.

N-Stearoyl C_{20}-Phytosphingosine Anhydro Base (Ib)

A 400 mg. (1.22 m*M*) sample of C_{20}-phytosphingosine anhydro base (Ia, m. p. 88—89°) prepared according to Reindel *et al.*[3,4] was dissolved in warm dimethyl

formamide (4 ml.). To this solution dry pyridine (0.1 ml.) and stearoyl chloride (333 mg., 1.09 m*M*) in dimethyl formamide (1 ml.) were added. The reaction mixture was allowed to stand at room temperature for 1 hr. and then at 5° overnight. The separated crude crystalline product was filtered by suction (300 mg., m. p. 94—96°) and recrystallized four times from 95% ethanol. Colourless crystals, m. p. 109—110.5°; $[\alpha]_D^{25}$ + 7.4° (c, 0.35 in chloroform).

Anal. 6.272 mg. subst.: 17.59 mg. CO_2, 7.116 mg. H_2O
7.173 mg. subst.: 0.166 ml. N_2 (21°, 760 mm)
$C_{38}H_{75}NO_3$ (593.98) calc'd.: C 76.83; H 12.72; N 2.35%
found: C 76.79; H 12.69; N 2.45%

Molecular Weight Determination
0.65 mg. subst.; 9.35 mg. camphor; depression 4.2°
calc'd.: MW 594; found: MW 662

O-*Acetyl* N-*Stearoyl* C_{20}-*Phytosphingosine Anhydro Base* (Ic)

N-Stearoyl anhydro base (200 mg.), dry pyridine (1 ml.) and acetic anhydride (0.5 ml.) were heated at 100° for 1 hr. After cooling the reaction mixture was poured into cold water (15 ml.). The separated colourless solid was filtered, washed with water and dried. The melting point was 88.5—89.5° and remained unchanged after crystallization from acetonitrile. $[\alpha]_D^{26}$ — 18.04° (c, 0.205 in chloroform).

Anal. 6.260 mg. subst.: 17.28 mg. CO_2, 6.74 mg. H_2O
5.145 mg. subst.: 0.110 ml. N_2 (25°, 739 mm)
$C_{40}H_{77}NO_4$ (636.02) calc'd.: C 75.53; H 12.20; N 2.20%
found: C 75.33; H 12.05; N 2.38%

Molecular Weight Determination
0.90 mg. subst.; 11.2 mg. camphor; depression 5.1°
calc'd.: MW 636; found: MW 630

N-*Palmitoyl* C_{20}-*Phytosphingosine Anhydro Base* (Id)

The palmitoyl derivative was prepared from the anhydro base (400 mg., 1.22 m*M*, m.p. 88—89°), palmitoyl chloride (300 mg., 1.09 m*M*), pyridine (0.1 ml.) and dimethyl formamide in the same way as described for the stearoyl compound. The crude product (400 mg., 58%) melted at 105—106.5°. One crystallization from ethanol gave colourless crystals, m. p. 110—110.5°; $[\alpha]_D^{21}$ + 10.2° (c, 0.127 in chloroform).

Anal. 5.551 mg. subst.: 15.52 mg. CO_2, 6.34 mg. H_2O
7.431 mg. subst.: 0.177 ml. N_2 (21°, 750 mm)
$C_{36}H_{71}NO_3$ (565.92) calc'd.: C 76.40; H 12.67; N 2.47%
found: C 76.29; H 12.78; N 2.73%

N-*(2-Hydroxystearoyl)* C_{20}-*Phytosphingosine Anhydro Base* (Ie)

To a solution of anhydro base (0.98 g., 3 m*M*, m. p. 88—89°) in warm dimethyl formamide 2-acetoxystearoyl chloride (1 g., 2.7 m*M*) and pyridine (0.39 ml.) were added. The reaction mixture was kept at 5° in the refrigerator overnight and then the crystalline precipitate filtered by suction. The crude yellowish product (0.74 g., m. p. 90—99° was dissolved in methanolic *N* KOH and allowed to stand at room temperature overnight. The solution was then poured into cold water, extracted with ether, the ether layer washed with water, dried over sodium sulphate and evaporated *in vacuo* to dryness. After crystallization of the colourless crystalline residue (m. p. 96—97°) from ethanol the melting point raised to 106—107°; $[\alpha]_D^{22}$ + 6.06° (c, 0.325 in chloroform).

Anal. 5.637 mg. subst.: 15.50 mg. CO_2, 6.18 mg. H_2O
$C_{38}H_{75}NO_4$ (609.98) calc'd.: C 74.82; H 12.39%
found: C 75.01; H 12.27%

Distearoyl Anhydro Cerebrin (IId)

A sample of anhydro cerebrin (IIa, 4 g., 5.53 m*M*, m. p. 115.5—116°)[3,4] was dissolved in warm freshly distilled quinoline (35 ml.) dried previously with solid potassium hydroxide. A solution of stearoyl chloride (12 ml.) in dry ether (40 ml.) was then added dropwise in the course of 45 minutes with stirring. The reaction mixture was refluxed for 4 hrs. with continuous agitation. After that time the cooled solution was extracted with ether (400 ml.), the ether layer washed successively with 2 *N* hydrochloric acid and water to the neutral reaction. The ether solution was left to stand at room temperature overnight and the separated substance filtered by suction. The crude product (4.99 g., 72%, m. p. 65—66°) was recrystallized from a mixture of ether and methanol (2:1) and melted finally at 68—68.5°. To the original ether mother liquid ten volumes of methanol were added causing the separation of an additional crop of crystals melting at the same temperature; $[\alpha]_D^{22} + 3.36°$ (c, 0.624 in chloroform).

Anal. 6.787 mg. subst.: 19.56 mg. CO_2, 7.72 mg. H_2O
$C_{82}H_{159}NO_6$ (1255.1) calc'd.: C 78.47; H 12.77%
found: C 78.62; H 12.72%

Monooleyl Anhydro Cerebrin (IIb)

To a solution of IIa (2.75 g., 3.8 m*M*) in quinoline (20 ml.) oleyl chloride (2.66 g., 8 m*M*) dissolved in absolute ether (40 ml.) was added dropwise. The mixture was refluxed for 4 hrs. and then worked up as described above. From the ether-methanol solution 1.22 g. colourless substance, m. p. 46—48° separated. The melting point was unchanged after recrystallization from acetone.

Anal. 7.370 mg. subst.: 21.01 mg. CO_2, 8.26 mg. H_2O
8.410 mg. subst.: 0.118 ml. N_2 (24°, 746 mm)
$C_{64}H_{123}NO_5$ (986.62) calc'd.: C 77.90; H 12.56; N 1.41%
found: C 77.80; H 12.55; N 1.58%

Molecular Weight Determination
1.45 mg. subst.; 12.45 mg. camphor; depression 4.5°
calc'd.: MW 987; found: MW 1035

Monostearoyl Anhydro Cerebrin (IIc)

The monooleyl compound (190 mg.) was dissolved in 96% ethanol (20 ml.) and hydrogenated in presence of Adams' platinum catalyst (50 mg.) at room temperature and at atmospheric pressure. After the theoretical amount of hydrogen was taken up, the catalyst was removed by filtration and the solvent evaporated to yield 155 mg. colourless substance, m. p. 59—61°. After recrystallization from acetone it melted at 64—65°.

Anal. 9.215 mg. subst.: 26.26 mg. CO_2, 10.41 mg. H_2O
8.885 mg. subst.: 0.122 ml. N_2 (25°, 739 mm)
$C_{64}H_{125}NO_5$ (988.64) calc'd.: C 77.74; H 12.74; N 1.41%
found: C 77.77; H 12.64; N 1.53%

Molecular Weight Determination
1.05 mg. subst.; 13.05 mg. camphor; depression 3.6°
calc'd.: MW 989; found: MW 897

Tetrastearoyl Cerebrin (IIIb)

A sample of cerebrin (IIIa, 0.276 m*M*, m. p. 140°)[3,4] purified through its tetraacetyl derivative was dissolved in quinoline (15 ml.). Five milliliters of stearoyl chloride dissolved in ether (20 ml.) were added dropwise to the agitated solution of cerebrin. The reaction mixture was worked up as described above. The ether solution furnished on standing 0.6 g. of a crude substance which was recrystallized first from acetone and then from ether-methanol (2:1). The analytical sample melted at 78—79°.

Anal. 5.120 mg. subst.: 14.75 mg. CO_2, 5.75 mg. H_2O
$C_{118}H_{229}NO_9$ (1806.01) calc'd.: C 78.47; H 12.78%
found: C 78.62; H 12.57%

Acknowledgment. We are indebted to Mrs. M. Munk-Weinert (Department of Chemistry, Medical Faculty, Univ. of Zagreb) for the microanalyses.

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IZVOD

Studije u redu sfingolipoida. XIII. O ceramidima i ceramidskim esterima C_{20}-fitosfingozina i C_{20}-fitosfingozin anhidro baze iz kvasca

B. Ries-Lešić i M. Prošťenik

Ispitane su mogućnosti *N* i *O*-acilacija C_{20}-fitosfingozin anhidro baze (Ia), C_{20}-anhidro cerebrina (IIa) i cerebrina (IIIa) kloridima viših masnih kiselina i 2-hidroksi-kiselina. Acilacije su izvedene ili u kinolinu ili u *N,N*-dimetil-formamidu. Dobiveni amidi i esteri prikazani su formulama I — III.

BIOKEMIJSKI ODJEL
INSTITUT »RUĐER BOŠKOVIĆ«
ZAGREB

Primljeno 23. siječnja 1960.