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Studies in the Sphingolipids Series. XV.* Partial Synthesis of Anhydro Cerebrin of Yeast

M. Proštenik and B. Ries-Lešić

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

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The partial synthesis of anhydro cerebrin (IIa) — a compound resulting by the acid catalyzed release of yeast cerebrin (I) — was effected. The synthetic IIa and its diacetyl derivative (IIc) were identical with the authentic samples.

In recent years tremendous progress has been made towards the elucidation of structural, synthetic and other problems in the sphingolipids field. Five sphingolipid bases such as sphingosine, dihydrosphingosine, C_{18} and C_{20}-phytosphingosine and C_{18}-dehydrophytosphingosine have been found in animal and in plant tissue[1]. They are constituent bases of the complex lipids. Among the least investigated lipids of the plant origin belong those which contain phytosphingosines. The excellent contribution of Carter and his coworkers offers more information on the native form of this class of compounds[2]. The lipid obtained by the alkaline hydrolysis of yeast fat is represented by the formula I which is based on the work of several investigators. Anhydro cerebrin of the structure IIa can readily be obtained by the acid catalyzed release of I. The cleavage of IIa with boiling 10% sulphuric acid and working up the reaction mixture according to Reindel *et al.* yields anhydro C_{20}-phytosphingosine (III)[3-6].

In the present work we have carried out the partial synthesis of anhydro cerebrin (IIa). Our attention was turned to this problem inasmuch as no report could be found in the literature dealing with the synthesis of sphingolipids.

The *N*-acylation of III — obtained from natural yeast cerebrin in a two-stage procedure — was attempted involving different methods. The most suitable appeared to be that of Reichel and Thannhauser[7] who operate in ether solution and in the presence of quinoline. The acylating agent was 2-acetoxyhexacosanoyl chloride which also originated from yeast cerebrin. The monoacetyl derivative (IIb) was obtained in a satisfactory yield by refluxing the ether-quinoline solution of the reactants for 4 hrs. When IIb was treated with acetic anhydride in pyridine the diacetyl compound (IIc) was formed which could readily be purified by crystallization. The alkaline hydrolysis of IIc yielded synthetic anhydro cerebrin (IIa).

For the sake of direct comparison the diacetyl derivative (IIc) was prepared in a good yield by acetylation of IIa obtained from cerebrin according to

* Paper XIV: M. Proštenik and B. Ries-Lešić, *Naturwiss.* **47** (1960) 377.

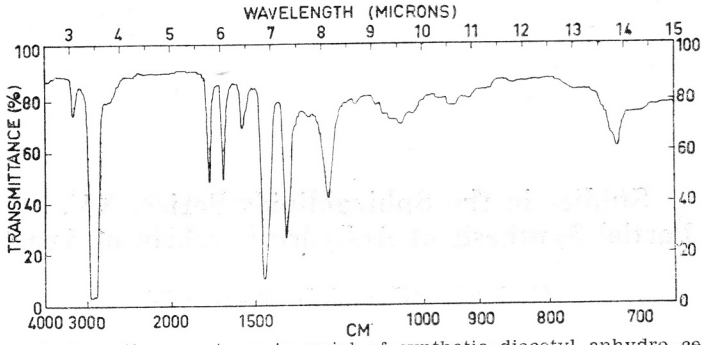


Fig. 1. Infrared absorption spectrum in nujol of synthetic diacetyl anhydro cerebrin (IIc)

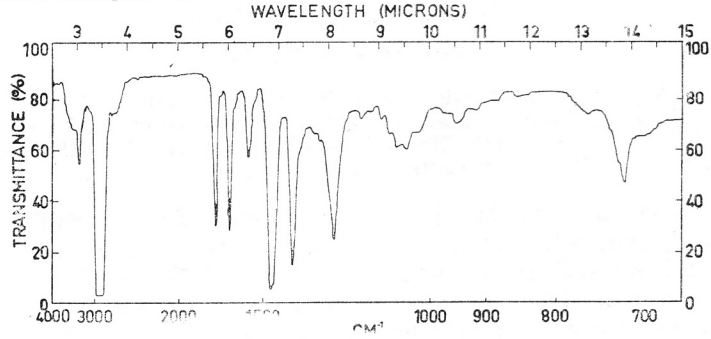


Fig. 2. Infrared absorption spectrum in nujol of analytical diacetyl anhydro cerebrin (IIc)

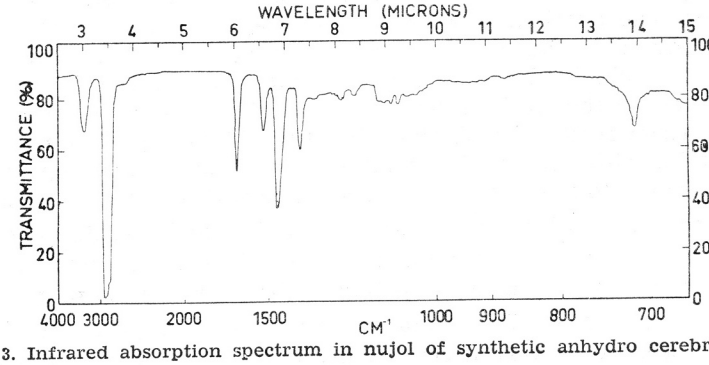


Fig. 3. Infrared absorption spectrum in nujol of synthetic anhydro cerebrin (IIa)

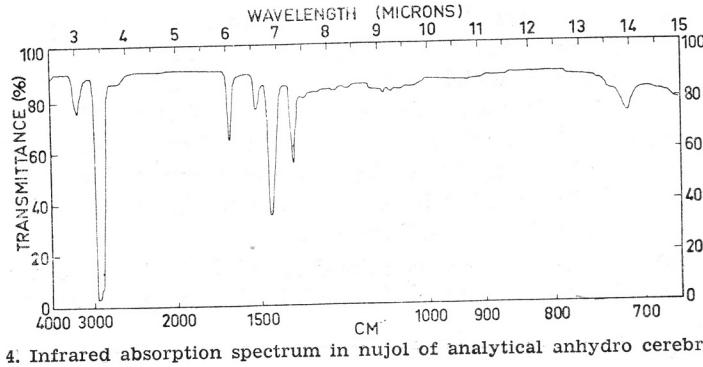


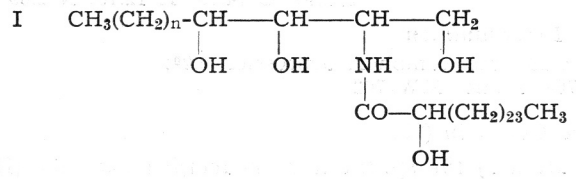
Fig. 4. Infrared absorption spectrum in nujol of analytical anhydro cerebrin (IIa)

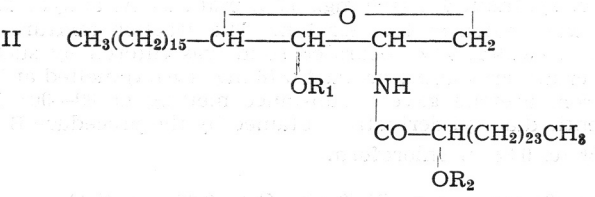
Reindel *et al.* The subsequent hydrolytic cleavage under the identical conditions as used in the hydrolysis of the synthetic product gave *analytical* anhydro cerebrin (IIa). We wish to point out that slight differences in physical properties might be expected since the starting materials were somewhat inhomogeneous. It is namely well known that the hydroxy acid component as isolated from yeast is a mixture of 2-hydroxyhexacosanoic acid and 2-hydroxytetracosanoic acid[8,9]. Therefore it is possible that during the purification of the natural mixture an enrichment of either C_{24} or C_{26} acid may occur. Nevertheless, the properties (melting points, specific rotations, infrared spectra) of synthetic IIa and of its diacetyl derivative (IIc) were identical in all respects with the analytical samples (Table I and Fig. 1.).

TABLE I

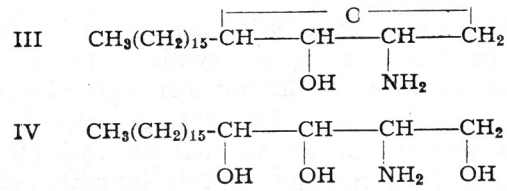
Compound	M. P., °C	$[\alpha]_D$
Anhydro Cerebrin Synth.	115 — 116	+ 14.2
Diacetyl	89 — 90	+ 17.3
Anhydro Cerebrin Anal.	115.5 — 116	+ 16.6
Diacetyl	90.5 — 91.5	+ 15.7

In conclusion it is to be mentioned that the described synthesis was accomplished with considerable difficulties. So far all attempts to synthesize cerebrin (I) itself from 2-acetoxyhexacosanoyl chloride and C_{20}-phytosphingosine (IV) in an analogous manner were unsuccessful.





a) $R_1 = R_2 = H$
b) $R_1 = H$, $R_2 = CH_3CO$
c) $R_1 = R_2 = CH_3CO$



EXPERIMENTAL

The melting points are uncorrected. The infrared absorption spectra were measured on Perkin-Elmer Model 134 spectrophotometer.

Starting Materials

2-Hydroxyhexacosanoic acid was obtained by hydrolysis of yeast cerebrin previously purified through its tetraacetyl derivative according to the procedure of Reindel *et al.*[3] The hydroxy acid thus prepared was supposed to be of at least 90% purity[8]. The acetylation and subsequent treatment with thionyl chloride[4] yielded crude, oily 2-acetoxyhexacosanoic acid chloride which was used in the following preparations without further purification.

N-(2-Acetoxyhexacosanoyl) C_{20}-Phytosphingosine Anhydro Base, Monoacetyl Anhydro Cerebrin (IIb)

A sample of C_{20}-phytosphingosine anhydro base (III) (236 mg., 0.72 m*M*, m. p. 88—89°) was dissolved in warm freshly distilled quinoline (10 ml.). To this solution 2-acetoxyhexacosanoic acid chloride (1.0 g., 2.16 m*M*) dissolved in dry ether (40 ml.) was added dropwise in the course of 45 min. The reaction mixture was then stirred at reflux for additional 4 hrs. To the cooled solution ether (100 ml.) was added and quinoline was removed by shaking with 2*N* hydrochloric acid. The solution was then washed with water to neutral reaction, the organic layer dried over sodium sulphate and concentrated *in vacuo*. The yellow, sticky residue was dissolved in warm methanol (200 ml.) and treated with charcoal. After cooling the clear solution in the refrigerator colourless crystals (524 mg., 60.7%, m. p. 58—60°) separated. Three more crystallizations from methanol gave a substance melting at 70—71.5°.

Anal. 6.516 mg. subst.: 17.85 mg. CO_2, 7,08 mg. H_2O
9.145 mg. subst.: 0.167 ml. N_2 (24°, 755 mm.)
$C_{48}H_{93}NO_5$ (764.23) calc'd.: C 75.43; H 12.26; N 1.83%
found: C 74.75; H 12.16; N 2.09%

Molecular Weight Determination

1.2 mg. subst.; 10.4 mg. camphor; depression 6.2°;
calc'd.: MW 764; found: MW 702.

Diacetyl Anhydro Cerebrin (IIc)

A. *By acetylation of the synthetic monoacetyl derivative* (IIb)

A mixture of synthetic IIb (400 mg., 0.523 m*M*), acetic anhydride (2 ml.) and dry pyridine (4 ml.) was heated at 100° for 1 hr. The reaction mixture was then cooled and poured into ice-water. The separated solid was filtered by suction and washed with water. The crude, crystalline product (376 mg., 89.5%) melted at 74—76°. Repeated crystallization from acetone gave a substance melting at 89—90°. Mixture melting point with authentic diacetyl derivative obtained by the procedure B (m. p. 90.5—91.5°) 89°. $[\alpha]_D^{26}$ + 17.3° (c, 0.38 in chloroform).

Anal. 9.335 mg. subst.: 25.62 mg. CO_2, 9.62 mg. H_2O
7.400 mg. subst.: 0.147 ml. N_2 (26°, 751 mm.)
$C_{50}H_{95}NO_6$ (806.26) calc'd.: C 74.47; H 11.87; N 1.73%
found: C 74.90; H 11.54; N 2.24%

B. *By acetylation of natural anhydro cerebrin*

A mixture of anhydro cerebrin (I) (1 g., m. p. 88—89°), acetic anhydride (5. ml.) and pyridine (10 ml.) was heated at 100° for 1 hr. and worked up as described above. The crude diacetyl derivative thus obtained (1.18 g., m. p. 90—92°) was recrystallized twice from 95% ethanol to give a product melting at 90.5—91.5°. $[\alpha]_D^{22}$ + 15.7° (c, 0,35 in chloroform).

Anal. 7.360 mg. subst.: 20.06 mg. CO_2, 7.86 mg. H_2O
$C_{50}H_{95}NO_6$ (806.26) calc'd.: calc'd.: C 74.47; H 11.87%
found: C 74.39; H 11.94%

Anhydro Cerebrin (I)

A. *By hydrolysis of synthetic diacetyl derivative*

A solution of synthetic diacetyl anhydro cerebrin (IIc) (115 mg., 0.142 m*M*) in *N* methanolic potassium hydroxide (15 ml.) was heated on the water-bath at 50° for 1 hr. After cooling the reaction mixture was poured into ice-water, the separated solid was collected, washed thoroughly with water and dried. The crude substance melted at 113.5—114.5°. Three more crystallizations from 95% ethanol raised the m. p. to 115—116°. Mixture melting point with natural anhydro cerebrin (m. p. 115°) 115°. $[\alpha]_D^{27}$ + 14.2° (c, 0.158 in pyridine).

Anal. 7.690 mg. subst.: 21.60 mg. CO_2, 8.85 mg. H_2O
7.820 mg. subst.: 0.157 ml. N_2 (22.5°, 743 mm.)
$C_{46}H_{91}NO_4$ (722.20) calc'd.: C 76.50; H 12.70; N 1.90%
found: C 76.65; H 12.88; N 2.27%

B. *By hydrolysis of natural diacetyl derivative*

The substance was obtained from the diacetyl derivative IIc (200 mg., 0.248 m*M*) and *N* methanolic potassium hydroxide as described above. The crude product (170 mg., 95.5%, m. p. 112—115°) was recrystallized from 95% ethanol. The substance melted then at 115.5—116°. $[\alpha]_D^{26}$ + 16.6° (c, 0.141 in pyridine).

Anal. 8.450 mg. subst.: 23.60 mg. CO_2, 9.69 mg. H_2O
$C_{46}H_{91}NO_4$ (722.20) calc'd.: C 76.50; H 12.70%
found: C 76.22; H 12.83%

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IZVOD

Studije u redu sfingolipoida. XV. Parcijalna sinteza anhidro cerebrina iz kvasca

M. Proštenik i B. Ries-Lešić

Opisana je parcijalna sinteza anhidro cerebrina (IIa) — spoja, koji nastaje iz kvaščeva cerebrina dehidratacijom u metanolu uz sudjelovanje katalitičkih količina sumporne kiseline. Sintetski produkt (IIa) kao i njegov diacetilderivat (IIc) identificirani su po talištima, specifičnim skretanjima i infracrvenim spektrima s autentičnim uzorcima dobivenim iz prirodnog materijala.

BIOKEMIJSKI ODJEL
INSTITUT »RUĐER BOŠKOVIĆ«
ZAGREB

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