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Studies in the Sphingolipids Series. XII.* Structure of the Cerebrin Anhydro Base of Yeast (C₂₀-Phytosphingosine Anhydro Base)

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It was confirmed that the cerebrin anhydro base from yeast (IIa) isolated according to the procedure established by Reindel *et al.*^{2,3} has a C₂₀-structure rather than C₁₈-structure as one might conclude erroneously by simple comparison of physical constants of both bases. The tetrahydrofuran structure was also demonstrated by means of the IR spectroscopy. For this purpose following new compounds derived from C₂₀-anhydro base were prepared: N,O-diacetyl (IIb), N,O-ditosyl (IIc), O-acetyl-N-benzoyl (IIId) and N-phthaloyl derivative (III); in addition, 2-hexadecyl-3,4-dihydroxy-tetrahydrofuran (IV) — a deamination product of the base was described.

Carter, Celmer, Lands, Mueller, and Tomizawa¹ have discovered that phytosphingosine — a base of plant sphingolipides, obtained from corn, soybean and other phosphatides — has a structure of an C₁₈ amino alcohol (Ia).** On the other hand, Reindel *et al.*^{2,3} allotted a C₂₀-structure to the base isolated from yeast (cerebrin base, Ib). The existence and preparation of the corresponding anhydro bases (IIa and C₁₈-base) was also reported by both schools. Recently, the C₂₀-structure of cerebrin base — isolated exactly according to the Reindel's procedure — was confirmed in our laboratories and the correct distribution of four contiguous functional groups established as well (Ib)⁴. It was announced at the same time that the base might be a mixture of the C₁₈ and C₂₀-compounds. This was confirmed experimentally by Oda and Kamiya⁵ by means of the reverse phase column chromatography of fatty acids obtained by the oxidation of the yeast base, and by Sweeley⁶ who analyzed the oxidation products by virtue of gas chromatography. More recently, O'Connell and Tsien⁷ have described in detail the isolation and identification of phytosphingosine anhydro base of corn phosphatides. The base — described for the first time in a free condition — has a C₁₈-chain and the physical constants were in good agreement with those reported for the C₂₀-base (cerebrin anhydro base, IIa).

* Paper XI: D. Keglević, A. Kisić, N. Krvavica-Feretić, and M. Proštenik, *Croat. Chem. Acta* 31 (1959) 41.

** The name *phytosphingosine* was proposed by the same authors preferentially for the C₁₈-base. Since that time the C₂₀-base has been found with certainty in nature and there are indications for the occurrence of other homologues. From now on we shall term all homologous bases with the same distribution of four functional groups as C₁₈-phytosphingosine, C₂₀-phytosphingosine *etc.*

In this paper we wish to contribute additional data in order to support the C_{20} -formulation of the yeast anhydro base as isolated previously^{2,3}. The only product which could be isolated by Reindel's procedure was the lower melting isomer, m. p. 88—89°, of the C_{20} -base. It is interesting to mention on this occasion that the C_{18} and C_{20} -anhydro bases may exist each in two isomeric forms — as a lower and a higher melting one. In paper chromatographic assay on Whatman No. 1 paper impregnated with silicic acid IIa appeared to be homogeneous showing a single spot⁸.

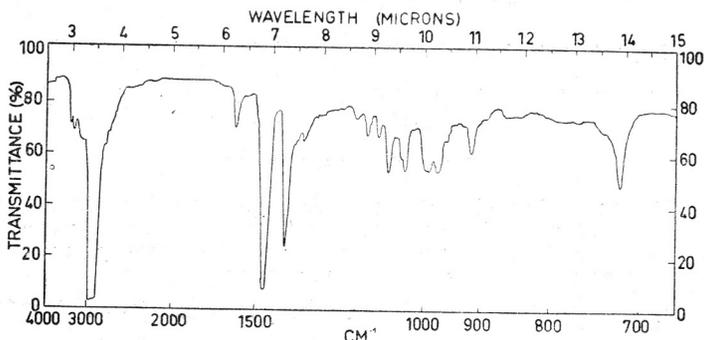


Fig. 1. Infrared absorption spectrum in nujol of C_{20} -phytosphingosine anhydro base (IIa)

The base IIa was converted into several hitherto either unknown or insufficiently characterized derivatives such as *N,O*-diacetyl (IIb), *N,O*-ditosyl (IIc), *O*-acetyl-*N*-benzoyl (II d) and *N*-phthaloyl (III) compound. The *N,O*-ditosyl derivative prepared from the lower melting base (m. p. 88—89°) was reduced with lithium aluminium hydride in order to remove either the C_3 -hydroxy group, or — what was more probable — only the *O*-tosyl group. Reactions like this usually proceed with retention of the *N*-tosyl groups. Unexpectedly, both tosyl groups were split off simultaneously, and the higher melting isomer (m. p. 105°) of IIa was obtained. The mixed melting point with the lower melting base was 97°. Deamination of IIa by means of nitrous acid proceeded smoothly yielding the nitrogen-free 2-hexadecyl-3,4-dihydroxytetrahydrofuran

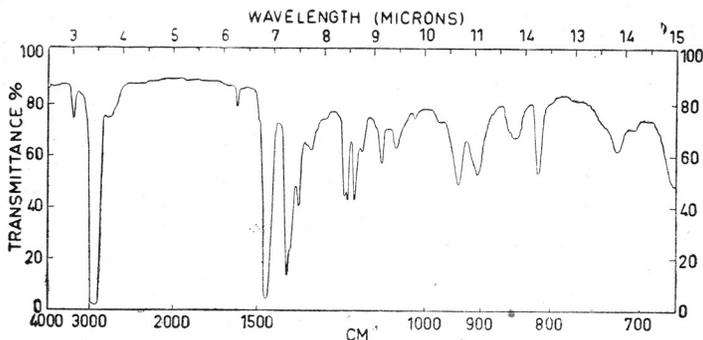
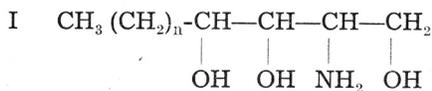


Fig. 2. Infrared absorption spectrum in nujol of *N,O* ditosyl C_{20} -phytosphingosine anhydro base (IIc)

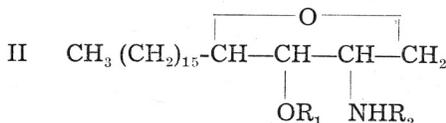
(IV). All these compounds gave good elementary analyses for the C_{20} -formulation. The analytical data exclude the admixture of noticeable quantities of the C_{18} -base.

The presence of the tetrahydrofuran ring in IIa was detected by means of the infrared spectroscopy. The absorption characteristic for the tetrahydrofuran structure occurs at 912 and 1075 cm^{-1} .

In conclusion, the bases isolated from corn and soybean phosphatides and — on the other hand — from yeast using Reindel's procedure are not identical. The former has a C_{18} the latter a C_{20} -chain, although the physical properties such as melting points and specific rotations are nearly identical. Consequently, the comparison of properties of both bases cannot be a reliable means of identification.



a) $n = 13$, b) $n = 15$

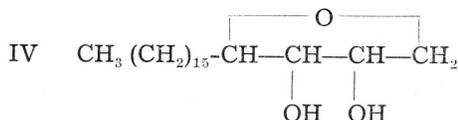
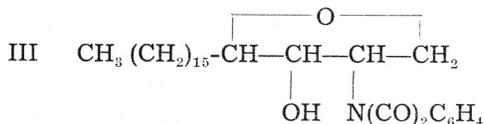


a) $R_1 = R_2 = \text{H}$

b) $R_1 = R_2 = \text{CH}_3\text{CO}$

c) $R_1 = R_2 = p\text{-CH}_3\text{C}_6\text{H}_4\text{SO}_2$

d) $R_1 = \text{CH}_3\text{CO}$, $R_2 = \text{C}_6\text{H}_5\text{CO}$



EXPERIMENTAL

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

Starting Materials

The preparation of C_{20} -phytosphingosine anhydro base (IIa) employed in this investigation has been achieved starting with crude yeast cerebrin following exactly the two-stage procedure given by Reindel *et al.*^{3,3}

N,O-Ditosyl C_{20} -Phytosphingosine Anhydro Base (IIc)

C_{20} -Phytosphingosine anhydro base (IIa, 200 mg., m. p. 88–89⁰), toluene sulphochloride (512 mg.) and pyridine (1.5 ml.) were mixed and left to stand at room temperature for 24 hrs. The mixture was then poured onto 10 g. of crushed ice and

cooled in the refrigerator for 2–3 hrs. During this time the oily phase crystallized completely. The substance was collected and washed with cold water. One crystallization from methanol of the crude product gave 350 mg. of colourless powder, m. p. 64–65°. One more crystallization from the same solvent raised the m. p. to 75–76°. The analytical sample melted — after four additional crystallizations — at 79–80°. $[\alpha]_D^{18}$ — 18.8° (c, 0.8 in methanol).

Anal. 7.935 mg. subst.: 18.74 mg. CO₂, 5.92 mg. H₂O
 7.870 mg. subst.: 0.147 ml. N₂ (20.5°, 763 mm)
 C₂₄H₅₃NO₆S₂ (635.93) calc'd.: C 64.22; H 8.40; N 2.20%
 found: C 64.45; H 8.35; N 2.18%

C₂₀-Phytosphingosine Anhydro Base, m. p. 105°

A 400 mg. sample of the ditosyl derivative IIc (m. p. 79–80°) was suspended in 40 ml. of dry ether and added to a solution of 400 mg. of lithium aluminium hydride in 40 ml. of ether. After refluxing for 5 hrs. the reaction mixture was treated carefully with water, the ether phase separated by decantation and the solid residue washed with ether. The combined ether extracts were evaporated to dryness yielding 200 mg. of a waxy solid, m. p. 82–84°. The crude substance was recrystallized four times from methanol. The crystals melted at 105° indicating the identity with the higher melting form. The mixed melting point with the lower melting isomer (m. p. 88–89°) was 97°.

Anal. 8.055 mg. subst.: 21.59 mg. CO₂, 9.00 mg. H₂O
 6.490 mg. subst.: 0.253 ml. N₂ (25°, 736 mm)
 C₂₀H₄₁NO₂ (327.54) calc'd.: C 73.33; H 12.62; N 4.28%
 found: C 73.15; H 12.50; N 4.33%

N,O-Diacetyl C₂₀-Phytosphingosine Anhydro Base (IIb)

A mixture of IIa (200 mg., m. p. 88–89°), acetic anhydride (1 ml.) and pyridine (2 ml.) was heated at 100° for 2 hrs. The reaction mixture was then poured into cold water (15 ml.), the separated colourless, crystalline solid filtered by suction and washed thoroughly with water. The crude product (190 mg., 75.7%, m. p. 73–75°) was recrystallized from petroleum ether and melted at 76–77°.

Anal. 6.071 mg. subst.: 15.62 mg. CO₂, 6.02 mg. H₂O
 C₂₄H₄₅NO₄ (411.61) calc'd.: C 70.03; H 11.20%
 found: C 70.21; H 11.10%

O-Acetyl-N-benzoyl C₂₀-Phytosphingosine Anhydro Base (IID)

The acetylation of the N-benzoyl derivative (100 mg., m. p. 105°)^{2,3} with acetic anhydride (0.5 ml.) and pyridine (1 ml.) was performed in a known way. The crude product was crystallized three times from ethanol and melted at 89–90°.

Anal. 5.365 mg. subst.: 14.51 mg. CO₂, 4.97 mg. H₂O
 C₂₉H₄₇NO₄ (473.67) calc'd.: C 73.53; H 10.00%
 found: C 73.81; H 10.37%

N-Phthaloyl C₂₀-Phytosphingosine Anhydro Base (III)

The mixture of IIa (1.31 g., 4 mM, m. p. 88–89°), phthalic anhydride (592 mg.) and toluene (150 ml.) was refluxed under continuous separation of water formed during the reaction. The reaction was completed in 2 hrs. Evaporation of the solvent at 50–60° *in vacuo* to dryness gave 1.9 g. of the crude product melting at 102–107°. Three crystallizations from ethanol yielded the analytically pure compound melting at 102–103.5°.

Anal. 8.060 mg. subst.: 21.73 mg. CO₂, 6.80 mg. H₂O
 8.010 mg. subst.: 0.216 ml. N₂ (24°, 765 mm)
 C₂₈H₄₃NO₄ (457.63) calc'd.: C 73.48; H 9.47; N 3.06%
 found: C 73.57; H 9.44; N 3.12%

2-Hexadecyl-3,4-dihydroxytetrahydrofuran (IV)

To a solution of IIa (600 mg., m. p. 88—89°) in glacial acetic acid (15 ml.) solid sodium nitrite (210 mg.) was added in small portions. A strong evolution of nitrogen took place. After standing at room temperature overnight the solution was diluted with water (50 ml.), the resulted precipitate extracted with ether and the extracts dried with sodium sulphate. Evaporation of the solvent gave a somewhat sticky residue which was recrystallized from methanol. The yield of the colourless substance — m. p. 51—52° — amounted to 400 mg. (67%). It was crystallized again from methanol to give a product melting at 57—59°.

Anal. 8.275 mg. subst.: 22.83 mg. CO₂, 8.96 mg. H₂O
 C₂₀H₄₀O₃ (328.54) calc'd.: C 73.12; H 12.27%
 found: C 73.58; H 12.12%

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IZVOD

Studije u redu sfingolipoida. XII.
 Struktura anhidro-cerebrinske baze iz kvasca
 (C₂₀-fitosfingočin anhidro baze)

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Na temelju novih analitičkih podataka potvrđeno je, da anhidro-cerebrinska baza (IIa) pripremljena iz kvasčeva cerebrina točno prema podacima Reindel-a i sur.^{2,3} ima lanac C₂₀, a ne C₁₈. Uspoređivanjem fizikalnih svojstava (tališta i specifičnih skretanja), što su objavili neki autori^{4,7}, moglo bi se pogrešno zaključiti da su identične. Tališta i specifična skretanja ne mogu biti kriterij za identifikaciju bliskih homolognih baza cerebrinskoga reda. Dokazana je također tetrahidrofuranska struktura anhidrocerebrinske baze s pomoću IR spektroskopije.

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