STUDIES OF ACETAL PHOSPHOLIPIDES OF BRAIN

III. THE FATTY ALDEHYDES PRESENT IN CRYSTALLINE ACETAL α-PHOSPHOLIPIDE OF BRAIN*

BY S. J. THANNHAUSER, NICHOLAS F. BONCODDO, AND GERHARD SCHMIDT

(From the Research Laboratories of the Boston Dispensary, the Joseph H. Pratt Diagnostic Hospital, and Tufts College Medical School, Boston, Massachusetts)

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Feulgen and Bersin (1) obtained from the hydrolysis products of the acetal phospholipide present in muscle a mixture of thiosemicarbazones. They suggested that these thiosemicarbazones are derivatives of palmitic and stearic aldehydes, although they gave no analytical data supporting this view. Anchel and Waelsch (2) obtained from hydrolysates of lipid mixtures from different tissues, including brain, p-carboxyl phenylhydrazones as well as carboxymethoximes of fatty aldehydes. The analysis of a mixture of these derivatives suggested the presence of C₁₆ and C₁₈ fatty aldehydes. Separation of the two different aldehyde derivatives was not carried out because of the small amounts of material. Klenk (3) reported the hydrolysis of an acetal-containing substance which was apparently a mixture of acetal phosphatidic acid and acetal phospholipide. The hydrolysis yielded an aldehyde which he converted into the corresponding fatty acid. The titration of this fatty acid gave the equivalent weight of stearic acid. Leupold (4) prepared in Klenk’s laboratory a mixture of methyl acetals by boiling the total phospholipide mixture of brain with methanolic hydrochloric acid. After conversion of the methyl acetal mixture to the corresponding fatty acids, palmitic, stearic, and oleic acids were isolated.

Evidence concerning the nature of the aldehyde groups of our crystalline acetal phospholipide from brain (5) will be reported in this paper. Two different procedures were applied for the separation and identification of the aldehydes: (1) separation of the free aldehydes by microdistillation in a high vacuum, and (2) conversion of the aldehyde mixture to the corresponding fatty acids and separation of the methyl esters of the fatty acids by microdistillation in a high vacuum.

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EXPERIMENTAL

Preparation of Aldehyde Mixture—The ether solution of the aldehyde mixture obtained by catalytic splitting of the acetal phospholipide with mercuric chloride in aqueous solution (6) contained mercuric chloride. The removal of the mercuric chloride from the ether solution occasioned difficulties and could only be achieved with considerable losses of the aldehydes. It was found to be much more convenient to prepare the aldehydes by hydrolysis of the crystalline acetal phospholipide with 20 per cent acetic acid.

3 gm. of acetal phospholipide were emulsified with 120 cc. of water. After addition of 30 cc. of glacial acetic acid the mixture was placed in a water bath at 37° for 2 days, and the aldehydes, which formed a floating precipitate, were exhaustively extracted with ether in a separatory funnel. The ether extracts were pooled, washed with 1 N aqueous sodium hydroxide until they gave a neutral reaction to litmus, and dried with sodium sulfate. The extract was concentrated to dryness in vacuo (weight 1.6 gm.). This aldehyde mixture was solid at room temperature.

Identification of Aldehydes—It was found to be impossible to separate mixtures of relatively small amounts of C₁₆ and C₁₈ hydrazones or oximes by fractional crystallization. The ranges of the melting points of the substituted hydrazones and oximes are within 1° to 2° of each other, and their carbon values are likewise too similar to permit a satisfactory identification of the compounds.

It was then attempted to separate the aldehyde mixture by fractional microdistillation under a high vacuum (7). 1.7 gm. of the aldehyde mixture were distilled at 0.0025 mm. pressure and four fractions were obtained. During the distillation extensive polymerization took place in all fractions. Only small quantities of unpolymerized aldehydes of each fraction could be isolated in the form of oximes. The oximes of all fractions gave analytical figures which agreed with those of palmital oxime.

$$C_{16}H_{31}NO. \quad \text{Calculated.} \quad C \ 75.3, \ H \ 11.18, \ N \ 5.49$$
$$\text{Found.} \quad " \ 75.31, " \ 12.6, " \ 5.42$$

The melting point of an authentic sample of $C_{16}H_{31}NO$ was 87°; found, 87°.

Finally it was decided to convert the aldehyde mixture into the corresponding fatty acids and to fractionate the methyl esters of these acids by high vacuum distillation. The equivalent weight of the free fatty acids could be expected to show definitely whether or not C₁₆ and C₁₈ aldehydes are present in the α-acetal phospholipide.

The conversion of the aldehyde mixture to the fatty acids was carried out over the oximes and nitriles (3).

$$R-CH=NOH \rightarrow R-C≡N \rightarrow R-COOH$$
1.7 gm. of the aldehyde mixture were dissolved in 10 cc. of 95 per cent alcohol, and 4 gm. of hydroxylamine hydrochloride, 24 cc. of water, and 16 cc. of 10 per cent sodium hydroxide were added to the solution. After further addition of 100 cc. of 95 per cent alcohol the solution was heated on the steam bath for 15 minutes. The oximes crystallized overnight in the refrigerator and were recrystallized from 10 cc. of hot 95 per cent alcohol (weight 0.88 gm.; m.p. 78–93°).

The total amount of the recrystallized oximes was refluxed with 20 cc. of acetic anhydride on a steam bath for 2½ hours. A calcium chloride tube was attached to the condenser. The solution was concentrated under diminished pressure to dryness and dried in the desiccator.

The dried nitriles were boiled with 10 cc. of 33 per cent aqueous sodium hydroxide for 2 hours. After diluting the mixture with 200 cc. of water the soaps were converted to the free fatty acids with 1 N hydrochloric acid. The fatty acids were extracted with ether, dried over sodium sulfate, filtered, and the ether was removed by distillation (weight 0.8 gm.). The fatty acids were transformed into the methyl esters by heating with 25 cc. of 7.5 per cent methanolic sulfuric acid (by volume) for 4 hours on a steam bath. After cooling, the methyl esters were extracted with petroleum ether. The petroleum ether was washed twice with water to remove any traces of sulfuric acid. The solution of the esters was dried with sodium sulfate and concentrated to dryness in vacuo (weight 0.75 gm.). The methyl esters were fractionated by distillation at 0.002 mm. pressure (Fraction I, 0.2 gm.; Fraction II, 0.15 gm.; Fraction III, 0.15 gm.).

The saponification of the methyl esters was carried out by dissolving each fraction in 15 cc. of methanol, adding 1 cc. of 33 per cent aqueous sodium hydroxide, and heating on the steam bath for ½ hour. The free fatty acid of Fraction I was repeatedly recrystallized from a small volume of petroleum ether at 0°; m.p. 60°; molecular weight by titration with 0.1 N sodium ethylate, 250. (Palmitic acid, m.p. 61°; molecular weight, 256.)

The free fatty acid of Fraction III was repeatedly recrystallized from a small volume of petroleum ether at 0°; m.p. 67°; molecular weight by titration with 0.1 N sodium ethylate, 279. (Stearic acid, m.p. 69°; molecular weight 284.) The fatty acid of Fraction II proved to be mainly palmitic acid contaminated by traces of stearic acid; m.p. 63°. The iodine number of the pooled fatty acids was 1.01.

**SUMMARY**

The fatty aldehydes present in crystalline acetal α-phospholipide of brain were converted into the corresponding fatty acids.

Palmitic and stearic acids were identified by high vacuum distillation of their methyl esters.
The isolated crystalline acetal α-phospholipide of brain does not contain unsaturated fatty aldehydes.
The amount of palmitic aldehyde in the acetal α-phospholipide is larger than that of stearic aldehyde.

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