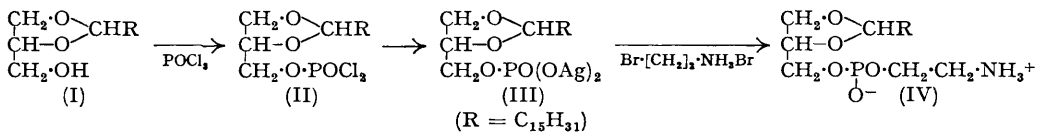
562. *Plasmalogens. Part I. A Synthesis of 2-Aminoethyl 2 : 3-O-Hexadecylideneglycerophosphate.*

By M. J. Egerton and T. Malkin.

An unambiguous method for the synthesis of plasmalogens is described. Long and short *X*-ray spacings of " palmital-plasmalogen " have been determined.

Plasmalogens were discovered in brain and skeletal muscles by Stepp, Feulgen, and Voit, (*Biochem. Z.*, 1927, **181**, 284), who found that on treatment with acids or mercuric chloride they yielded a steam-volatile substance; this gave an intense Schiff's reaction, and Feulgen, Imhauser, and Behrens (*Z. physiol. Chem.*, 1929, **180**, 161) found it to be a mixture of long-chain fatty aldehydes, mainly hexadecanal (palmitaldehyde) and octadecanal (stearaldehyde). Feulgen and Bersin later (*ibid.*, 1939, **260**, 217) showed that plasmalogens were acetal phosphatides, closely related to phosphatidylethanolamine, namely, 2-aminoethyl 2 : 3-*O*-hexa- or -octa-decylidene-1-glycerophosphate. Although Feulgen and Bersin obtained a mixture of 2-aminoethyl esters of 1- and 2-glycerophosphoric acid by the action of warm mercuric chloride solution on the plasmalogen, the question of the existence of the 2-glycerophosphoric isomer is an open one, owing to the well-known 1 ⟶ 2 migration of glycerophosphates: analogy with lecithin and kephalin, and the optical activity of plasmalogens, reported by Thannhauser, Boncoddo, and Schmidt (*J. Biol. Chem.*, 1951, **188**, 417), indicate the 2 : 3-acetal structure.

In 1941, Bersin, Moltmann, Nafziger, Marchand, and Leopold (*Z. physiol. Chem.*, **269**, 241) published a synthesis of the 2-aminoethyl ester of 2 : 3-*O*-hexadecylidene-1-glycerophosphoric acid, according to the scheme:



but doubts may be expressed regarding the nature of their final product. Thus, the acetal (I) was made by the direct condensation of glycerol and hexadecanal which could give rise to *cis*- and *trans*-forms of 1 : 2- and 1 : 3-acetals (cf., *e.g.*, Hibbert and Carter, *J. Amer. Chem. Soc.*, 1928, **50**, 3376; Verkade and van Roon, *Rec. Trav. chim.*, 1942, **61**, 831). The melting point was not recorded for (I), which was stated to be crystalline, and the evidence that it yielded 1-*O*-methylglycerol on methylation and hydrolysis does not appear to us to be satisfactory proof of homogeneity. Moreover, in the analogous synthesis of the 2-aminoethyl ester of phosphatidic acid (kephalin), Bevan and Malkin (*J.*, 1951, 2667) found that the action of 2-bromoethylammonium bromide on disilver phosphatidate gave only a trace of the desired product. Bersin *et al.* claim a 60% yield, but state that their product readily breaks down into plasmalogenic acid (III; H in place of Ag) and 2-hydroxyethylamine on crystallisation. This, however, is not the normal behaviour of an aminoethyl ester, and it seems probable that their product was rather a hydroxyethylamine salt, or at least, highly contaminated with this. No melting point or description was given for the synthetic product, and only nitrogen was determined.

In view of these uncertainties, we have synthesised (I) by condensing hexadecanal with α-monolaurin and removing the lauroyl group with ethanolic sodium hydroxide, to which the acetal is stable. (Protection of the 1-hydroxyl group by acetyl or benzoyl was not so satisfactory.) The acetal was then converted by phosphorus oxychloride into the plasmalogenoyl dichloride (II) which was condensed with 2'-hydroxyethylphthalimide. Removal of the phthaloyl group with aqueous hydrazine yielded a crystalline product which gave correct analyses for the plasmalogen, a positive Feulgen–Schiff's aldehyde test, and a positive ninhydrin test.

During this acetal condensation we also obtained what appears to be the geometric isomer, but as the melting points of the two pairs of isomers are very similar we prefer to reserve judgment pending *X*-ray examination.

Work on the synthesis of plasmalogens from this isomer, and from the 1 : 3-acetal is in progress.

Experimental

1 : 2-O-*Hexadecylidene*-3-*monolaurin*.—Hexadecanal (5 g., 0·02 mole) and α-monolaurin (7 g., 0·026 mole) were heated with a little toluene-*p*-sulphonic acid at 80—90° for 4 hr. From time to time the pressure was reduced (water-pump) in order to remove the water produced. The cold product was extracted with benzene, and the extract washed with sodium hydrogen carbonate solution and water and dried (Na_2SO_4). After removal of the solvent, several crystallisations from benzene–ethanol yielded the *acetal* as colourless flat prisms (2 g.) which exhibited spherulite formation when viewed through a polarising microscope and had m. p. 51·5—52·5° (Found: C, 75·2; H, 12·1. $C_{31}H_{60}O_4$ requires C, 75·0; H, 12·1%). Evaporation of the mother-liquor left a residue which when recrystallised from methanol gave a crystalline *isomer* (3·5 g.) which did not exhibit spherulite behaviour and melted at 49—50° (Found: C, 75·3; H, 12·0%). The total yield of condensation product was 5·5 g. (51·8%).

1 : 2-O-*Hexadecylideneglycerol*.—The above lauroyl derivative (0·85 g.; m. p. 51·5—52·5°) was refluxed for 6 hr. with ethanol (70 ml.) containing 0·07 g. of sodium hydroxide. After removal of ethanol under reduced pressure, water was added to the residue, and the mixture was extracted with ether. The ethereal solution was well washed with water and dried (Na_2SO_4). After removal of the ether there remained 1 : 2-O-*hexadecylideneglycerol* as an oil which solidified at room temperature, and recrystallised from light petroleum (b. p. 40—60°) 0° as prisms (yield, *ca.* 100%), m. p. 45·5—46·5° (Found: C, 72·4; H, 12·0. $C_{19}H_{38}O_3$ requires C, 72·6; H, 12·1%).

The *isomer* (Found: C, 72·6; H, 12·0%), obtained in a similar manner from the lauroyl derivative of m. p. 49—50°, melted at 45—46°, and at 43—46° on admixture with the previous product.

2-*Aminoethyl* 2 : 3-O-*Hexadecylidene*-1-*glycerophosphate* (*Plasmalogen*).—The method of synthesis is the same as that used by Bevan and Malkin (*loc. cit.*) for the synthesis of kephalins. The apparatus should be oven-dried, and the solvents and reagents treated as follows: Chloroform to be freed from alcohol with concentrated sulphuric acid, dried ($CaCl_2$; P_2O_5), and distilled shortly before use. Pyridine to be dried (KOH) and fractionated. Phosphorus oxychloride to be fractionated.

To phosphorus oxychloride (342 mg., 0·0022 mole) in chloroform (2·5 ml.) in an ice-cooled 50-ml. three-necked flask fitted with mechanical stirrer, dropping funnel, and calcium chloride tube, pyridine (1 ml.) was added slowly with vigorous stirring. The flask was then placed in a bath at 10—15° and 1 : 2-*O*-hexadecylideneglycerol, m. p. 45·5—46·5° (700 mg., 0·0022 mole) in chloroform (11 ml.) and pyridine (0·11 ml.) was added dropwise with continued stirring during 1 hr. Stirring was then continued for 30 min. with the bath at 25° and for a further 30 min. at 45°. The bath-temperature was then reduced to 10—15° and 2′-hydroxyethylphthalimide (425 mg., 0·0022 mole) in chloroform (16 ml.) was added to the vigorously stirred mixture during 1 hr. Stirring was then continued at 30° and at 40° for 30 min. each. The bath-temperature was then reduced to 10—15°, and a few drops of water were added to the reaction mixture, which was then vigorously stirred for 30 min. The solvent was next removed completely (water-pump and finally mechanical pump at <40°). The residue was triturated with N-hydrochloric acid at 0—10° and filtered, the residue being washed with cold water until free from mineral acid. The precipitate (dried on a filter) was dissolved in neutral 2-methoxyethanol (25 ml.) (mechanical stirrer; <40°), and then 0·42N-sodium hydroxide (4·8 ml.) was added dropwise with vigorous stirring during 1 hr. (sufficient to form a monosodium salt on the assumption of a 90% yield of phthalimido-derivative). Hydrazine hydrate (223 mg., 0·0022 mole; 50% w/w) was added to the mechanically stirred solution, the temperature of which was slowly raised. Frothing which began to occur during the addition of sodium hydroxide ceased at this stage, and the mixture was gently refluxed for 1 hr. and left overnight. The precipitate which separated was filtered off and washed with a little neutral 2-methoxyethanol, after which it was extracted with boiling ether. The residual *plasmalogen*, recrystallised from methanol–ethanol, gave a colourless microcrystalline powder (200 mg., 21%), which softened at about 206° and formed a meniscus at 223° with some decomposition. It gave a positive blue colour

with 2% ethanolic ninhydrin and a positive Feulgen–Schiff's test (Found: C, 57·4; H, 9·8; N, 3·4. $C_{21}H_{44}O_6NP$ requires C, 57·7; H, 10·1; N, 3·2%). *X*-ray spacings: long, 38·9 Å; short, 5·64 (mod. strong, 4·54 (strong), and 3·83 Å (mod. strong).

One of us (M. J. E.) thanks the City of Gloucester Education Authority for a maintenance grant.

THE UNIVERSITY, BRISTOL. [*Received, May 14th,* 1953.]