The Synthesis of Neutral Plasmalogens

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The *cis*- and *trans*-isomers of the di-*O*-stearoyl and di-*O*-palmitoyl esters of 1-*O*-hexadec-1′-enyl-L-glycerol and 1-*O*-tetradec-1′-enyl-L-glycerol and the *cis*- and *trans*-isomers of 1-*O*-octadec-1′-enyl-2,3-di-*O*-stearoyl-L-glycerol were obtained by separation of the *cis–trans* mixtures by t.l.c. on silica gel–silver nitrate. The *cis*-isomers of the di-*O*-palmitoyl derivatives were also obtained free from the *trans*-isomers by fractional crystallisation. 1-*O*-Heptadec-2′-enyl-2,3-*O*-isopropylideneglycerol is rapidly degraded by potassium t-butoxide in dimethyl sulphoxide at 50° but 1-*O*-hexadec-1′-enyl-2,3-di-*O*-methylglycerol is comparatively stable under these conditions.

NEUTRAL plasmalogens occur in small quantities in many mammalian tissues [1] and in higher concentrations in the tissues of some fish. A neutral plasmalogen fraction has been isolated from the lipids of the shark, *Hydrolagus colliei*, and characterised [2] as a 2,3-di-*O*-acyl-1-*O*-alk-1′-enyl-L-glycerol. Helmy and Hack [3] have shown that neutral plasmalogens are present in high concentration in the lipids of the preputial gland of the mouse, but concluded that the main component was a monoacyl di-*O*-alk-1′-enyl-glycerol and that a tri-*O*-alk-1′-enyl-glycerol was also present.

In the present paper we describe the preparation of the *cis*- and *trans*-isomers of the di-*O*-palmitoyl and di-*O*-stearoyl derivatives of 1-*O*-hexadec-1′-enyl-L-glycerol and 1-*O*-tetradec-1′-enyl-L-glycerol and of 2,3-di-*O*-stearoyl-1-*O*-octadec-1′-enyl-L-glycerol by the general procedure described previously.[4] In all cases the pure *cis*- and *trans*-isomers were obtained by t.l.c. on silica gel–silver nitrate, and the pure *cis*-isomer of each of the di-*O*-palmitoyl derivatives could be separated by fractional crystallisation.

Basic hydrolysis of the pure *cis*-isomers of the 1-*O*-alk-1′-enyl-2,3-di-*O*-palmitoyl-L-glycerols will give [4] the pure 1-*O*-alk-*cis*-1′-enyl-L-glycerols, and reacylation of these compounds with any fatty acid (saturated or unsaturated) followed by hydrolysis with lipase [5] will give 2-*O*-acyl 1-*O*-alk-*cis*-1′-enyl-L-glycerols. Slotboom, de Haas, and van Deenen [5b] have shown that a synthetic racemic 2-*O*-acyl 1-*O*-alk-*trans*-1′-enyl-glycerol and a 2-*O*-acyl-1-*O*-alk-*cis*-1′-enyl-L-glycerol, obtained by enzymic hydrolysis of a natural plasmalogen, can be converted into plasmalogens by the standard techniques of phospholipid chemistry, and therefore the neutral plasmalogens described in this Paper should make available fully synthetic plasmalogens with the required stereochemistry.

We have previously [4] reviewed other published methods for the synthesis of 1-*O*-alk-1′-enyl-glycerols and neutral plasmalogens; subsequently Preobrazhenskii and his co-workers [6] have found that their route to the racemic *cis–trans* neutral plasmalogens by pyrolysis of the di-(1,2-di-*O*-acylglycerol) acetals of long-chain aldehydes gives a mixture of 2,3-di-*O*-acyl 1-*O*-alk-1′-enyl-glycerols and 1,3-di-*O*-acyl-2-*O*-alk-1′-enyl-glycerols. Perkins and his co-workers [7] have also shown that the method [8] for the preparation of 1-*O*-alk-1′-ynyl-2,3-*O*-isopropylideneglycerol gives instead the 1-*O*-alka-1′,2′-dienyl-2,3-*O*-isopropylideneglycerol. Preobrazhenskii and his co-workers [9] have described a route for the preparation of derivatives of racemic 1-*O*-alk-1′-enyl-glycerols by the elimination of toluene-*p*-sulphonic acid from derivatives of 1-*O*-(2-*p*-tolylsulphonyloxyalkyl)glycerols [or of iodine from 1-*O*-(2-iodoalkyl)-glycerols] by the action of potassium t-butoxide in t-butyl alcohol. The authors have not established that the isomeric 1-*O*-alk-2′-enyl-glycerols are absent from their products.

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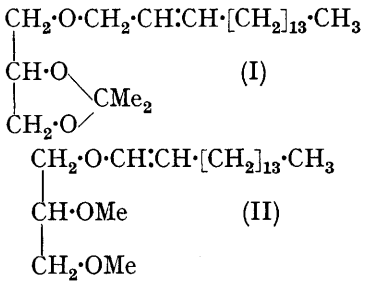
[7] G. K. Chacko, K. Schilling, and E. G. Perkins, *J. Org. Chem.*, 1967, **32**, 3698.

[8] M. V. Berezovskaya, I. K. Sarycheva, and N. A. Preobrazhenskii, *Zhur. obshchei Khim.*, 1964, **34**, 543.

[9] See ref. 9 of previous paper; [4] E. A. Parfenov, G. A. Serebrennikova, and N. A. Preobrazhenskii, *Zhur. org. Khim.*, 1967, **3**, 1566, 1766, 1955; G. A. Serebrennikova, T. I. Ryaplova, E. A. Parfenov, and N. A. Preobrazhenskii, *ibid.*, 1967, **3**, 1958.

We have previously[10] shown that the action of potassium t-butoxide in dimethyl sulphoxide at 100° on the γ-substituted allyl ether (I) caused elimination of heptadecadiene. Other workers[11] have subsequently observed eliminations of γ-substituted allyl ethers under similar conditions. We have investigated this reaction at 50° and have found that compound (I) is completely degraded in 30 min. Under the same conditions the enol ether (II) was unaffected during 2 hr., but at 100° compound (II) was degraded in about 3 hr. The ease



(I)

(II)

of degradation of the alk-2-enyl ether (I) and the comparative stability of the alk-1-enyl ether (II) at 50° suggest that, if a mixture of the two isomers is produced in the elimination reaction described by Preobrazhenskii and his co-workers,[9] then the alk-2-enyl ether could be removed from the product by the above treatment.

EXPERIMENTAL

T.l.c. was carried out as described previously.[10] Specific rotations were measured at 22—24° with a Bendix Automatic Polarimeter.

Tetradecanal Dimethyl Acetal.[12]—This compound was prepared from tetradecanol (Koch-Light Ltd., 'not less than 95% by g.l.c.') by the method described[4] for octadecanal dimethyl acetal.

Di(Glycerol 1,2-Carbonate) Acetals of Aldehydes.—The following acetals were prepared from the corresponding dimethyl acetals by the method described previously:[4] racemic tetradecanal di(glycerol 1,2-carbonate) acetal, m.p. 61—64° (Found: C, 61·2; H, 8·9. Calc. for $C_{22}H_{38}O_8$: C, 61·4; H, 8·9%); *tetradecanal di-*D-(*glycerol 1,2-carbonate*) *acetal*, m.p. 69—71°, $[\alpha]_D$ +17·1° (*c* 1 in $CHCl_3$) (Found: C, 61·4; H, 8·9%), and *hexadecanal di-*D-(*glycerol 1,2-carbonate*) *acetal*, m.p. 79—80°, $[\alpha]_D$ +16·3° (*c* 0·8 in $CHCl_3$) (Found: C, 63·0; H, 9·4. $C_{24}H_{42}O_8$ requires C, 62·85; H, 9·2%).

1-O-*Alk*-1′-*enyl Glycerols.*—The following compounds were prepared from the di(glycerol 1,2-carbonate) acetals as described previously:[4] *cis–trans*-1-*O*-tetradec-1′-enyl-L-glycerol, m.p. 33—36° (Found: C, 71·5; H, 11·7. Calc. for $C_{17}H_{34}O_3$: C, 71·3; H, 12·0%) and *cis–trans*-1-*O* hexadec-1′-enyl-L-glycerol, m.p. 45—47° (Found: C, 72·7; H, 11·9. Calc. for $C_{19}H_{38}O_3$: C, 72·6; H, 12·2%).

2,3-*Di*-O-*acyl* 1-O-*Alk*-1′-*enyl* L-*Glycerols.*—The following esters were prepared (*ca.* 80%) by acylation of the mixture of *cis*- and *trans*-isomers of the 1-*O*-alk-1′-enyl L-glycerols with hexadecanoyl or octadecanoyl chlorides [prepared from the corresponding acids (B.D.H. Ltd., 'not less than 98% by g.l.c.') by the action of thionyl chloride] as described previously.[4] The *cis*- and *trans*-isomers were separated by preparative t.l.c. on silica gel–silver nitrate (4 : 1) plates by applying the mixed isomers (200 mg.) as a streak on a layer (20 × 20 × 0·2 cm.) and developing with ether–light petroleum 1 : 9. The separated isomers were visible on the plates in daylight; in each case the *trans*-isomer had the greater mobility.

(a) 2,3-*Di*-O-*palmitoyl*-1-O-*tetradec*-1′-*enyl*-L-*glycerol.* The cis-*isomer* had m.p. 45—46°, $[\alpha]_D$ −3·3° (*c* 1 in $CHCl_3$) (Found: C, 77·1; H, 12·1. $C_{49}H_{94}O_5$ requires C, 77·1; H, 12·4%). The trans-*isomer* had m.p. 47·5—49° (Found: C, 77·2; H, 12·4%). The *cis*-isomer (720 mg.) was obtained free from the *trans*-isomer after two recrystallisations of the mixed isomers (1 g.) from acteone (10 parts v/w) at 20°.

(b) 2,3-*Di*-O-*stearoyl*-1-O-*tetradec*-1′-*enyl*-L-*glycerol.* The cis-*isomer* had m.p. 51—52°, $[\alpha]_D$ −4·2° (*c* 1·1 in $CHCl_3$) (Found: C, 77·75; H, 12·5. $C_{53}H_{102}O_5$ requires C, 77·7; H, 12·5%). The trans-*isomer* had m.p. 54—55° (Found: C, 77·3; H, 12·6%). The *cis*-isomer could not be obtained free from the *trans*-isomer by fractional crystallisation from light petroleum or acetone.

(c) 1-O-*Hexadec*-1′-*enyl*-2,3-*di*-O-*palmitoyl*-L-*glycerol.* The cis-*isomer* had m.p. 54·5—55·5°, $[\alpha]_D$ −4·3° (*c* 1·3 in $CHCl_3$) (Found: C, 77·2; H, 12·1. $C_{51}H_{98}O_5$ requires C, 77·4; H, 12·5%). The trans-*isomer* had m.p. 52—54° (Found: C, 77·3; H, 12·2%). The *cis*-isomer (620 mg.) was obtained free from the *trans*-isomer after two recrystallisations of the mixed isomers (1 g.) from light petroleum (100 parts v/w) at 0°.

(d) 1-O-*Hexadec*-1′-*enyl*-2,3-*di*-O-*stearoyl*-L-*glycerol.* The cis-*isomer* had m.p. 54—55°, $[\alpha]_D$ −3·6° (*c* 1·1 in $CHCl_3$) (Found: C, 77·9; H, 12·3. $C_{55}H_{106}O_5$ requires C, 77·9; H, 12·6%). The trans-*isomer* had m.p. 55·5—57° (Found: C, 78·1; H, 12·4%). The pure *cis*-isomer could not be obtained from the mixed isomers by fractional crystallisation from light petroleum or acetone.

(e) 1-O-*Octadec*-1′-*enyl*-2,3-*di*-O-*stearoyl*-L-*glycerol.* The cis-*isomer* had m.p. 58—60°, $[\alpha]_D$ −4·1° (*c* 0·75 in $CHCl_3$) (Found: C, 78·1; H, 12·4. $C_{57}H_{110}O_5$ requires C, 78·2; H, 12·7%). The trans-*isomer* had m.p. 54—56° (Found: C, 78·0; H, 12·4%). The *cis*-isomer (620 mg.) was obtained almost free from the *trans*-isomer after two recrystallisations of the mixed isomers (1 g.) from light petroleum (100 parts v/w) at 0°.

Racemic cis–trans-1-O-*Hexadec*-1′-*enyl*-2,3-*di*-O-*methylglycerol* (II).—Racemic *cis–trans*-1-*O*-hexadec-1′-enylglycerol (1 g.) was methylated with sodium hydride (500 mg.) and methyl iodide (3 ml.) in dry tetrahydrofuran (50 ml.) at room temperature. When the initial reaction had moderated the solution was heated under reflux for 1 hr. Methanol was added to the cooled solution to decompose the excess of sodium hydride, and the solvent was evaporated off under reduced pressure. The residue was extracted with ether and the extract was washed with water, dried (K_2CO_3), and evaporated to give the dimethyl ether (II) as an oil (1 g.), n_D^{22} 1·4508 (Found: C, 73·7; H, 12·1. Calc. for $C_{21}H_{42}O_3$: C, 73·6; H, 12·4%).

Action of Potassium t-Butoxide in Dimethyl Sulphoxide on

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Compounds (I) *and* (II).—The dimethyl ether (II) (100 mg.) was added to a solution of potassium t-butoxide (100 mg.) in dry dimethyl sulphoxide (2 ml.) at 50°. T.l.c. (ether–light petroleum, 1 : 9) showed that the starting material (R_F 0·35) was not degraded during 2 hr. Under the same conditions racemic *cis–trans*-1-*O*-heptadec-2′-enyl-2,3-*O*-isopropylideneglycerol [10] (I) (R_F 0·4) was completely degraded to heptadecadiene [10] (R_F 1) in 30 min. At 100° in the same reaction medium, compound (II) was almost completely degraded in 2 hr. to an unsaturated product (R_F 1) which was not further investigated.

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