rotation of the distillate was then determined and the relative amounts of (R)- and (S)-butan-2-ol calculated on the basis of a value of $[M]_D^{52} = -30°$ for (R)-butan-2-ol[6]. The results obtained from three experiments are summarized in Table I. No 2-butyl acetate was produced when the experiment was conducted in the absence of enzyme and no trans-esterification was detected during distillation of butan-2-ol in the presence of *p*-nitrophenyl acetate. The configurational relationship between (—)-2-butyl acetate and (—)-butan-2-ol was checked by esterification of (—)-butan-2-ol, prepared by the method of INGERSOLL[7], with acetic anhydride. No inversion of the sign of rotation was observed.

TABLE I

PROPERTIES OF ESTER FRACTION

Expt.	*% Ester (W/W)**	*α_D***	*(R)-butan-2-ol %*
1	2.84	—0.38	82
2	7.75	—0.78	75
3	10.85	—1.55	86

* Density taken as 0.80.
** Rotation observed in 1-dcm tube at 25°.

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Formation of the cyclic acetal phospholipid during alkaline and enzymic hydrolysis of choline plasmalogen

FEULGEN AND BERSIN[1] and THANNHAUSER *et al.*[2] isolated what was apparently a cyclic acetal phospholipid from the alkaline-hydrolysis products of muscle and brain phospholipids respectively. It is, however, now generally accepted that the natural

aldehydogenic phospholipids (plasmalogens) are derivatives of glycerylphosphorylcholine, ethanolamine or -serine with two fatty residues, one a fatty acid and the other an acid-labile aldehydogenic residue linked to the glycerol moiety as a $\alpha\beta$-unsaturated ether[3-5]. Lysoplasmalogens possessing this $\alpha\beta$-unsaturated ether structure, determined by the iodine method of SIGGIA AND EDSBERG[6], have been isolated from ethanolamine plasmalogen[7] and choline plasmalogen[8] by removal of the acyl group with alkali. The origin and nature of Feulgen's cyclic acetal phospholipid is not clear, and it has been suggested that the identification was mistaken. Preliminary work in this laboratory showed, however, that on hydrolysis of choline plasmalogen either by snake venom[4] or by alkali there was an apparent loss of total aldehyde, as measured by a standard colorimetric technique of hydrolysis in 90 % acetic acid at 50° and condensation with Feulgen reagent[9], which was in fact due largely to formation of some acid-stable derivative.

The course of decomposition of choline plasmalogen was therefore examined using three methods for estimation of aldehyde: (A) hydrolysis in 90 % acetic acid at 50°, (B) hydrolysis in 90 % acetic acid containing 0.5 *N* HCl and 0.0015 *N* $HgCl_2$ at 50°; this was followed in both cases by condensation with Feulgen reagent for 20 min at 0°. (C) hydrolysis in the acetic acid–HCl–$HgCl_2$ reagent at 0° in presence of the Feulgen reagent for 20 min.

The material used was a "lecithin" fraction of ox-heart muscle containing about equal proportions of choline plasmalogen and phosphatidyl choline; the aldehyde value (taken as 100 %) was the same by methods A and B. On treatment of a portion with *Agkistrodon piscivorus* venom (phospholipase A), the aldehyde value by method A decreased gradually with the decrease in fatty acid ester and reached a constant value of 80 % of the original, but the value by method B remained at 100 %.

On saponification of another portion in 0.1 *N* methanolic NaOH at 38° for 15 min the aldehyde value fell to 70 % by method A and to 90 % by method B (the latter result possibly indicating that about 10 % of the aldehyde was split off and polymerised). The aldehydogenic material (molar ratio P:N, 1:1) was recovered quantitatively by chromatography on silicic acid and cellulose columns; it had a molar ratio of aldehyde:P of 0.75:1 by method A and 0.94:1 by method B, and an $\alpha\beta$-unsaturated ether:P ratio of 0.71:1 by the iodine method. It was concluded that the material was a mixture of the (straight-chain) lysoplasmalogen and a presumably cyclic form. A small amount of the lysoplasmalogen was obtained by fractionation on silicic acid, but the two forms could not be separated quantitatively. The more acid-stable form was therefore isolated by decomposition of the lysoplasmalogen in acetic acid; it was contaminated with some substance, not yet identified, which was detected by chromatography on silicic-impregnated paper. This substance contained phosphorus and choline but no aldehyde and is possibly a glyceryl ether. The pure cyclic form was obtained by further fractionation on alumina.

The rates of liberation of aldehyde from choline plasmalogen, the lysoplasmalogen, the cyclic form and the standard dimethyl acetal of palmitaldehyde by method A (Fig. 1) and the aldehyde values for the different compounds obtained by methods A, B and C (Table I) show that two compounds are formed from choline plasmalogen on saponification with alkali or by treatment with snake venom; a lysoplasmalogen with an $\alpha\beta$-unsaturated ether structure, which is more labile to acid than the original plasmalogen, and a cyclic form which is more stable to acid than either the original

plasmalogen or the standard dimethyl acetal of palmitaldehyde. This explains some recent observations[10,11] on the products of alkaline hydrolysis of plasmalogens.

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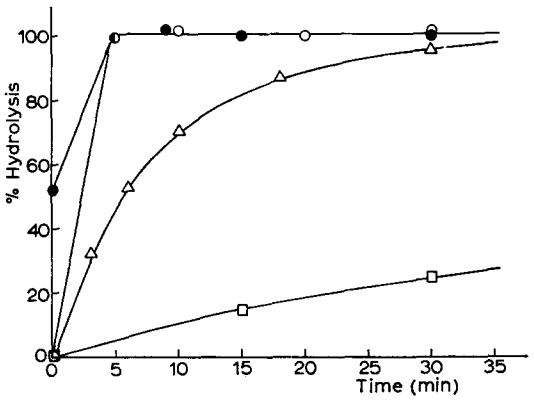

Fig. 1. The course of liberation of aldehyde from intact plasmalogen (△); straight-chain lysoplasmalogen (●); cyclic form (□); dimethyl acetal of palmitaldehyde (○); by method A.

TABLE I

RELATIVE ALDEHYDE VALUES BY DIFFERENT METHODS OF ESTIMATION

Compound	*Aldehyde values by method*		
	A	*B*	*C*
Dimethyl acetal of palmitaldehyde	100	100	68
Choline plasmalogen	100	100	68
Lysoplasmalogen	100	100	100
Cyclic form	33	100	3–5

A. 45 min 90% acetic acid at 50°; 20 min + Feulgen reagent at 0°.
B. 5 min in acetic–HCl–$HgCl_2$ at 50°; 20 min + Feulgen reagent at 0°.
C. 20 min at 0° acetic–HCl–$HgCl_2$ in presence of Feulgen reagent.

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