The Preferential Cleavage of the β-Ester Linkage of Egg Yolk Lecithin by Treatment with Alkoxides and Laurylamine

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Marinetti¹⁾ reported that the treatment of lecithin with sodium methoxide at 0°C gave rise to a mixture of lysolecithins consisting of a somewhat larger proportion of the β-isomer. This implies that the α-ester linkage is preferentially cleaved by methoxide ions. Since no further, more detailed study has yet been reported, it is now of interest to ascertain the relative extent of the cleavage of the two fatty acid ester bonds by alkoxides with different steric requirements.

Highly-purified egg yolk lecithin, 3.23 μmol, was treated with an equivalent amount of an alkoxide in the corresponding alcohol at 0°C, except in the case of *t*-butoxide, where a temperature of 40°C was used, and in the case of laurylamine, where the reaction was carried out in methanol at 37°C. Two hundred and fifty μ*l* aliquots containing 25 μg of phosphorus were removed at intervals, and the lysolecithin formed was separated from the unreacted lecithin on a preparative silica-gel thin-layer chromatoplate, using chloroform-methanol-water (65 : 25 : 4, by vol) as the developing solvent. The fatty acids of the lysolecithin were transmethylated according to the procedure of Bowyer *et al.*,²⁾ and the esters were analyzed by gas-liquid chromatography using a hydrogen-flame ionization detector. The positional distribution of fatty acids in the lecithin was determined by employing snake venom, which is known to release those fatty acids only in the β-position.³⁾ The analytical results showed that the fatty acid composition of the lecithin was 85.1% saturated acids (a large amount of palmitic acid and a small amount of stearic acid) and 14.9% unsaturated acids in the α-position, and 3.7% saturated and 96.3% unsaturated fatty acids (a large amount of oleic acid and a small amount of linoleic acid) in the β-position. The total amount of the saturated acids of the lecithin was 58.0%, while that of the unsaturated acids was 42.0%. Each experiment was run more than three times.

Since the separation of α-lysolecithin from its β-isomer can not be readily accomplished at present, the fatty acid composition of the lysolecithin isolated from a reaction mixture is used in estimating the relative extent of cleavage of the α- and β-ester linkages. The calculation is based on the above analytical results; *i. e.*, the presence of 85.1% and 3.7% saturated acids in the isolated lysolecithin indicates a 100% and a 0% cleavage, respectively, of the β-ester linkage, and similarly, the presence of 96.3% and 14.9% unsaturated acids indicates a 100% and 0% cleavage of the α-ester respectively. Accordingly, the 77.2% portion of saturated acids and the 22.8% portion of unsaturated acids in the lysolecithin isolated from a reaction mixture of the lecithin and *t*-butoxide, for example, would indicate a 90.4% cleavage of the β-ester and a 9.6% cleavage of the α-ester respectively. The results, shown in Fig. 1, show that the *n*-alkoxides, propoxide and butoxide, cleave the β-ester bond preferentially in early stages of alcoholysis but both ester linkages in a random manner in later stages, whereas *i*-propoxide and *t*-butoxide give a highly preferential cleavage of the ester (90% throughout the reaction time studied (5 hr)) and laurylamine (79% even after 20 hr of reaction).

The present results are not in accord with the latest proposal made by Hancock⁴⁾ concerning the alkaline hydrolysis of the ester, according to which a preferential cleavage of the α-ester bond should result, provided that an ionic effect of the phosphorylchloline residue remains the same on both ester bonds. A solvent effect, *i. e.*, the unsaturated acids esterified chiefly on the β-carbon atom are more readily released because of their high solubilities in organic solvents than are the saturated acids present in the α-position, may not be a major controlling factor in the reaction, since a random cleavage occurred in the case of *n*-alkoxides. Furthermore, no such effect has been made apparent in the case of the hydrolysis of monoglycerides in an organic solvent.⁵⁾ Therefore, it appears that certain conformations of the lecithin molecule are involved in directing the course of the reaction.

The present observations suggest, therefore, that the lecithin molecule in the alcohols employed exists chiefly in the trans-trans and trans-gauche

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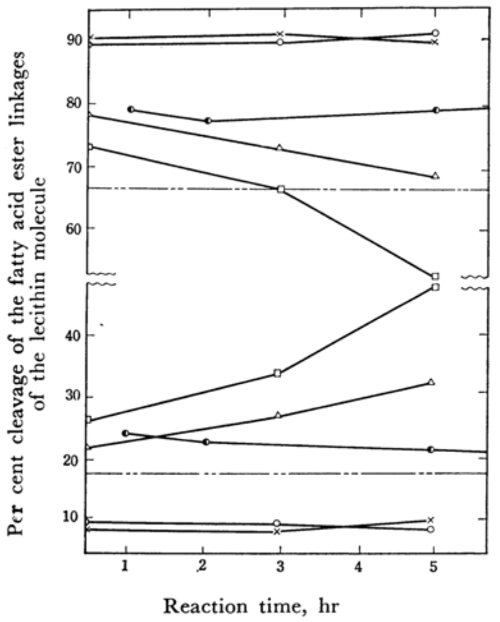
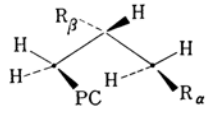


Fig. 1. The relative extent of the cleavage of the α- or β-ester bond of egg yolk lecithin by treatment with alkoxides and laurylamine. The curves in the upper half indicate the percentage of cleavage of the β-ester linkage, as calculated from the amount of the saturated fatty acid remaining in the isolated lysolecithin, while the curves in the lower half show the percentage of cleavage of the α-ester linkage, as calculated from the amount of the unsaturated fatty acid in the isolated lysolecithin. The dotted lines denote a 1:1 cleavage of the α- and β-ester bonds. □—□ *n*-propoxide, △—△ *n*-butoxide, ○—○ *i*-propoxide, ×—× *t*-butoxide, ◐—◐ laurylamine.

conformations, as represented by I and II respectively, although the gauche-gauche conformation has been proposed for lecithin in a crystalline form.[6] The trans-trans conformation, I, is, however, considered to be more important than the trans-gauche conformation, II, in leading to the highly preferential cleavage of the β-ester bond, particularly by isopropoxide and *t*-butoxide, since the group on the α'-carbon atom in the former exerts the greatest and the least interference on the group attached to the α- and β-carbon atoms respectively. In this connection, it may be pointed out that a ring structure, rather than an extended form, of the phosphorylcholine group at the α'-position[7] may also exert additional interference with the reaction center of the group in the α-position.



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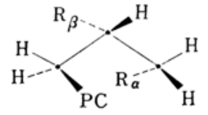

II

R_α and R_β represent the fatty acid residues at the α- and β-positions respectively while PC indicates the phosphorylcholine residue.

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