# NOTES

## *Selective Reduction of Phosphatides with Lithium Aluminum Hydride. II. Reductive Cleavage of Phosphatidic Acid*

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In a previous paper[1] we had reported that the reductive cleavage of lecithin with lithium aluminum hydride in anhydrous ether gave rise to L, $\alpha$-glycerylphosphorylcholine, a finding which was later confirmed by De Haas and Van Deenen[2] by the resynthesis of L, $\alpha$-lecithins and L, $\alpha$-lysolecithins from L, $\alpha$-glycerylphosphorylcholine prepared by our method. A similar cleavage of the two acyl groups of the phosphatidic acid molecule may be attained by controlling reaction conditions so as to give L, $\alpha$-glycerylphosphoric acid if no further reduction of the phosphate moiety occurs.

The method described by Kates[3] was followed for the preparation of the sodium salt of phosphatidic acid by subjecting purified (by column chromatography) egg yolk lecithin to the action of the lecithinase C of carrot roots and for the liberation of the free acid. The analytical results, the examination by paper chromatography,[4] and analyses for phosphorus and ester bond[5] indicate that the product so obtained consisted of approximately 70% phosphatidic acid and of some such as impurities fatty acids and their esters.

Difficulty was experienced in purifying the sample because phosphatidic acid is known to be highly labile; it is hydrolyzed even on exposure to moist air* or its ethanol solution is allowed to stand at room temperature.[6]

Since phosphatidic acid is a monoester of orthophosphoric acid, an extra mole of the hydride reagent would be required to compensate its acidity in addition to the one mole needed for the cleavage of the two fatty acid residues. Therefore, mole ratios higher than 1:2 (phosphatidic acid: lithium aluminum hydride) were considered in the present study; the reaction time was varied from 10 to 30 min. within a temperature range of 8~15°C. The procedure employed for reduction of the free acid in anhydrous ether with lithium aluminum hydride was essentially the same as that described earlier.[1] The aqueous phase separated from the reaction mixture was passed through a small column of Amberlite IR-112 and

1) C. Urakami, H. Okura and M. Okada, This Bulletin, 33, 144 (1960).
2) G. H. De Haas and L. L. M. Van Deene, *Tetrahedron Letters*, No. 22, 7 (1960).
3) M. Kates, *Can. J. Biochem. Physiol.*, 33, 575 (1955).
4) F. M. Heunnekens et al., *J. Biol. Chem.*, 206, 443 (1954).
5) F. C. Bauer, Jr., and E. F. Hirsch, *Arch. Biochem.*, 20, 242 (1949).
6) J. Olley, *Chem. & Ind.*, 1954, 1069.
* Private commuication from Baer.

the eluate and the ethereal phase initially separated from the reaction mixture were analyzed for phosphorus, α-glycerylphosphoric acid-phosphorus[7,8] and ester bond.[5] The best conditons found from these studies appeared to be a mole ratio of phosphatidic acid to the hydride reagent of 1:2, at 10°C, and for 20 min; a typical example of the results obtained is shown in Table I.

TABLE I. RECOVERY OF PHOSPHORUS IN THE AQUEOUS AND ETHEREAL PHASES

| | | |
|---|---|---|
| Reaction conditions: | | |
| Rhosphatidic acid: $LiAlH_4$=1:2, 10°C, 20 min. | | |
| Sample wt., 18.2 mg. (equivalent to 492 μg. of α-GP-P) | | |
| Reaction products: | | |
| Aqueous phase | $P_i$, μg. | 0.0 |
| | α-GP-P, μg.[a] | 456 ± 6 |
| | % | 92.6 (97.9) |
| | α-GP-P, μg.[b] | 473 ± 2 |
| Ethereal phase | Ester[c] | trace |
| | Total P | — |

$P_i$ stands for inorganic phosphorus, α-GP-P α-glycerylphosphoric acid-phosphorus.

a) Burmaster's method.[7]

b) Voris' method.[8]

c) The quantitative method[5] was employed; a trace amount detected may be due to the presence of fatty acid esters. The figures in parentheses are corrected for a loss of phosphorus during the course of elution through a column of Amberlite IR-112, the average recovery of the lithium salt of glycerylphosphoric acid being 94.5%.

An attempt to isolate the reaction product in the aqueous phase according to the method of Long and Maguire[9] was made with 200 mg. of the phosphatidic acid, but an amount of the barium salt of glycerylphosphoric acid obtained was so small that its rigorous purification could not be attained. Consequently, examination of its optical rotation was not possible (its specific rotation is known to be small, $[\alpha]_D = -1.2$[10]). However, the analytical results revealed that the sample isolated contained 77% α-glycerylphosphoric acid, the chief impurity being barium carbcnate; the amount of periodate consumed was equivalent to the content of α-glycerylphosphoric acid-phosphorus determined by Burmaster's method,[7] and no β-glycerylphosphoric acid-phosphorus was detected.

The fact that L, α-lecithin when treated similarly with the hydride reagent, gives rise to L, α-glyceylphosphoric acid[1] provides the reasonable assumption that the α-glycerylphosphoric acid obtained here retains the L-configuration of the natural acid. Further work is indicated, of course, but this method, involving a single-step reaction appears, to be preferable for the structural determination of naturally-occurring phosphatidic acids to the diazometholysis recommended by Baer,[11] which requires hydrogenation previous to interaction with diazomethane.

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7) C. F. Burmaster., *J. Biol. Chem.*, **164**, 233 (1946).
8) L. Voris et al., ibid., **133**, 491 (1940).
9) C. Long and M. F. Maguire, *Biochem. J.*, **54**, 612 (1953).
10) E. Baer, *J. Biol. Chem.*, **185**, 615 (1950).
11) E. Baer and J. Maurukas, ibid., **212**, 39 (1955).