Selective Reduction of Phospholipids with Lithium Aluminum Hydride I. Ovolecithin

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Recently, Long and Maguire[1] have reported a method of determination of the structure of the phosphatidylcholines, involving a long series of reactions which leads to the formation of L, α-glycerophosphoric acid; Baer and Maurukas[2] have shown another method involving the diazomethanolysis which is simpler but applicable only to the phosphatidylserines and cephalins. Therefore, it seems desirable to find a new method which is simple in itself to be applicable even when small amounts of phosphatides are available from natural sources.

Since the mechanism of reduction by lithium aluminum hydride is generally considered to be analogous to that of Grignard reaction, the reductive cleavage of the two fatty acid ester linkages in the phosphatidylcholine molecule would take place at an equal rate and with the retention of the configuration. On the other hand, the cleavage of the linkage between the glyceryl or choline and the phosphoryl would not be so easy as the previous case since approach of the nucleophilic reagent to the P^+ to attain a coordination at the transition state may be interfered by the presence of the oxygen atoms with a high electron density around the P^+. The cleavage of the linkage between the phosphoryl and choline may, however, be effected if the mechanism proposed by Gaylord[3] is assumed to be involved. When a group such as —N—C—O is present, a quasi ring is formed by coordination of the nitrogen atom with AlH_3[4] and of the hydrogen atom of the AlH_3 with the electron pair on the oxygen atom. The adjacent carbon atom being positive in character owing to the electrostatic effect of the cationic nitrogen would then react with the hydride ion, H^-, present in the solution[4], resulting in cleavage of the C—O bond. Since a six-membered ring would be equally stable as the five-membered, as illustrated by Gaylord, this mechanism may be applicable to the case of the phosphatidylcholine molecule. In this case however, the reaction would not take place so readily as the cleavage of the fatty acid ester for the following two reasons; firstly, the weakly acidic AlH_3 must overcome the zwitterionic effect in its approach to the nitrogen atom; and secondly, the electrostatic effect would be much weaker on the β-carbon atom. Therefore, under carefully controlled reaction conditions, it may be possible to carry out the cleavages in a stepwise manner to give L, α-glycerophosphorylcholine in the first step and L, α-glycerophosphoric acid in the following. Complicated reactions may, however, occur in the last step, as demonstrated by Karrer and Jucker[5].

The sample of lecithin employed was purified according to the method described by Rhodes and Lea[6] and Hanahan, et. al.[7]

TABLE I

HYDROGENOLYSIS OE OVOLECITHIN WITH LITHIUM ALUMINUM HYDRIDE

Expt. No.	I	II	III
Reaction time (min.)	10	30	60
Temperature (°C)	14	16	16
Mole ratio of L.: $LiAlH_4$	1:2	1:2	1:2
Total choline, calcd.	19.1	17.8	17.8
Total choline[8] in aq. phase	18.0	19.1	19.6
Free choline[9] in aq. phase	0.8	4.1	4.1

L. stands for lecithin.

The reaction was carried out in anhydrous ether. No significant amount of organic phosphorus was found in the ether phase nor inorganic phosphorus in the aqueous phase.

1) C. Long and M. F. Maguire, *Biochem. J.*, **57**, 223 (1954).
2) E. Baer and J. Maurukas, *J. Biol. Chem.*, **212**, 39 (1955).
3) N. G. Gaylord, *Experientia*, **X**, 356 (1954).
4) N. L. Paddock, *Nature*, **167**, 1070 (1951).
5) P. Karrer and E. Jucker, *Helv. Chim. Acta*, **35**, 1586 (1952).
6) D. N. Rhodes and C. H. Lea, *Biochem. J.*, **65**, 526 (1957).
7) D. J. Hanahan, M. S. Turner and M. E. Jayko, ibid., **192**, 623 (1951).
8) D. Glick, *J. Biol. Chem.*, **156**, 643 (1944).
9) G. Schmidt, L. Hecht, P. Fallot, L. Greenbaum and S. J. Thannhauser, ibid., **197**, 601 (1952).

As shown in Table I, glycerophosphorylcholine appears to be formed in 95% yield, accompanied by an insignificant amount of free choline under the conditions of Expt. I. The "free choline" designated in the table, however, should be questioned according to the mechanism discussed above which should give rise to trimethylethylamine. Thus, its structure remains to be investigated.

The product was isolated according to the procedure described by Baer and Kates[10], after passing the aqueous solution through a column of a mixture of IRC—50 and Dowex-3. Since only a limited amount of the product was available, a concentrated solution of the aqueous phase was used for the following determinations: P: choline, 1.0:1.02; absorption of a periodate solution, 97% of theory; and $(\alpha)_D^{24}$ -2.96 ± 0.2(in water), $(\alpha)_D^{23}$ -2.85 ± 0.1[10]. On drying the crystalline cadmium complex of the product, two moles of water was found to be lost.

Anal. Found(amorphous): P, 5.24. Calcd. for $(C_8H_{22}O_7NP)_2(CdCl_2)_3$: P, 5.62.
Found (crystalline): C, 19.66; H, 5.32. Calcd. for $(C_8H_{22}O_7NP) \cdot CdCl_2 \cdot 2H_2O$: C, 19.46; H, 5.30.
Found (dehydrated crystals): C, 21.56; H, 4.80. Calcd. for $C_8H_{22}O_7NP \cdot CdCl_2$: C, 20.97; H, 4.84.

These results show that the glycerophosphorylcholine isolated has the L,α-configuration. It also suggests that the method can be utilized in obtaining L,α-glycerophosphoric acid from phosphatidic acids, and this is now under investigation.

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10) E. Baer and M. Kates, *J. Am. Chem. Soc.*, **70**, 1394 (1948).