REDUCTIVE DEACYLATION OF CEPHALINS WITH LITHIUM BOROHYDRIDE*

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Recent work in this laboratory indicates the presence of phosphatidylpeptides in the lipids of unbleached wheat flour (Anker et al. 1961). The conversion of these compounds to water soluble derivatives would greatly facilitate characterization of the peptide moieties of these molecules. Phospholipids can, of course, be readily deacylated by the hydrolytic method of Dawson (1954), but it is doubtful that a peptide chain would be unaffected by even these mild alkaline conditions. Although $LiAlH_4$ has been used for the reductive deacylation of lecithin (Urakami and Okura 1960), $LiAlH_4$ will also reduce peptide bonds (Biemann and Vetler, 1960). This reagent would not therefore, be applicable to the selective deacylation of phosphatidylpeptides. Although $LiBH_4$ is generally used for the reduction of aldehydes and ketones, the reduction of a simple methyl ester bond has been noted. Thus Crawhall and Elliot (1955) reported the reduction of the methyl ester of benzoylglycylalanine; under selected conditions employed by these authors, the amide linkages were not affected by $LiBH_4$. These data suggested that $LiBH_4$ might be used for the reductive

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deacylation of a lipopeptide, i.e., a compound containing fatty acid ester and phosphodiester linkages, as well as peptide bonds. Since, at this writing, lipopeptides of known composition were not available for investigation, we chose to study the $LiBH_4$ reductive deacylation of cephalins.

In a typical experiment, dipalmitoyl-D,L,-α-glycerylphosphorylethanolamine[1] (215 mg) was added to 100 ml of anhydrous tetrahydrofuran which contained 10 mg $LiBH_4$. The reaction mixture was stirred for 3 hours at 24° at which time methanolic HCl was added to destroy the excess $LiBH_4$ and, concomitantly, neutralize the reaction mixture. Ether (100 ml) and 50 ml H_2O were added, the phases separated and taken to dryness in vacuo.

Cetyl alcohol (m.p. 48-48.5°, uncorr., reported 49-50°) was isolated from the ether soluble residue in ca. 87% yield. The p-nitrobenzoate derivative was prepared and recrystallized from aqueous ethanol, (m.p. 58°, uncorr., reported 58.5°).

When subjected to analysis by the automatic amino-acid analysis technique of Spackman, Moore and Stein (1958), the water soluble residue was found to contain glycerylphosphorylethanolamine and ethanolamine in a molar ratio of 5:1. These compounds exhibited elution patterns identical with those obtained with authentic ethanolamine and glycerylphosphorylethanolamine prepared by the procedure of Dawson (1954). Evidence that the major compound was glycerylphosphorylethanolamine was further substantiated by comparative paper chromatographic studies as described by Baer (1959).

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[1]Obtained from Mann Research Laboratories, Inc.

Finally, to test the applicability of this method to the reductive deacylation of a mixture of cephalins, a soybean cephalin preparation (Scholfield and Dutton, 1955) was subjected to $LiBH_4$ reduction at 0° for 18 hours. Again glyceryl-phosphorylethanolamine appeared as the major reaction product.

These studies, then, show that lipid ester bonds will undergo reductive cleavage by $LiBH_4$ under conditions which, reportedly, do not affect peptide linkages. $LiBH_4$, therefore, might well become an important tool in the characterization of lipopeptides, proteolipids and/or lipoproteins whose characterization, heretofore, has suffered from a lack of a specific water-solubilizing degradation technique.

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