A NEW CLASS OF PHOSPHATIDES ISOLATED FROM SOFT WHEAT FLOUR*

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In the course of an investigation into the composition of the phosphatides of soft wheat flour, two substances, A and B, were obtained which corresponded to no known phosphatides. It has now been established by chemical analyses and synthesis, that A is the monosubstituted fatty acid amide (PEA) of phosphatidylethanolamine, and B, the monosubstituted fatty acid amide of lysophosphatidylethanolamine (LPEA).

The phosphatides were isolated by successive column chromatography over silicic acid, alumina, and silica gel G of a benzene extract of soft wheat flour. Details of the isolation procedure will be published elsewhere. The best yield obtained to date was one gram of A and B from 9 Kg. of flour or about 4% of the tot phosphatides extracted from the flour. Thin layer chromatography on activated silica gel G in various solvent systems established that A and B were not identical with lecithin, phosphatidylethanolamine, cardiolipin, phosphatidylglycerol, sphingomyelin, lysolecithin, lysocephalin, phosphatidylinositol, phosphatidylserine, phosphatidic acid, or cerebrosides.

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As A always had a component associated with it that defied all attempts at removal, efforts to identify the unknowns were concentrated on B. This material migrated as a single substance in four different solvent systems on thin layer chromatography. Tests for a free amino group (Rouser *et al.*, 1961), sugar (Hawk *et al.*, 1947), aldehydogenic group (Norton, 1959; Rapport and Alonzo, 1955; Williams *et al.*, 1962), and an ether linkage (Hanahan *et al.*, 1963) were negative. On analysis by the methods of Snyder and Stephens (1959) for fatty acid ester and Hirata and Appleman (1959) for phosphorous, a fatty acid ester to phosphorous ratio of 1.0:1.0 and a phosphorous content of 4.2% were obtained. The glycerol content by the method of Renkonen (1962) was 11.5%, nitrogen content, 2.0%, by the method of Kirk (1950). The theoretical values of LPEA, assuming the average fatty acid to be oleic are, fatty acid ester to phosphorous ratio, 1.0:1.0, phosphorous, 4.18%, glycerol, 12.3%, nitrogen, 1.88%.

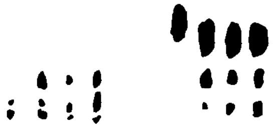
Mild alkaline hydrolysis of B by Dawson's method (1960) gave a phosphorous compound in the polar phase that accounted for 90% of the parent phosphorous. On paper chromatography the polar phase hydrolysis product gave R_f values of .79 and .84 in a phenol solvent (Spencer and Dempster, 1963) and a butanol solvent (Kates and Eberhardt, 1957), respectively, close to the values obtained for glycerylphosphorylcholine. However, choline could not be detected in the hydrolysis product. Moreover, the hydrolysis product was soluble in $CHCl_3$ alone, unlike glycerylphosphorylcholine. Values of 2.8% nitrogen and 6.4% phosphorous were obtained for the hydrolysis product, the corresponding theoretical values for LPEA being 2.92% and 6.46, respectively. From 62 mg. of B on mild alkaline hydrolysis, 40 mg., equivalent to 65% of the parent compound, was obtained in the polar phase, the theoretical value for LPEA being 64.5%.

Infrared spectra of the mild alkaline hydrolysis product and of the parent compound, B, gave peaks at 3.03, 6.04, and 6.4 microns, ascribable to a monosubstituted amide linkage (Fig. 2). Sphingosine was undetectable in the hydrolysis product by either of two methods (Sanstry and Kates, 1964; Sweeley and Moscatelli, 1959). Ethanolamine was the only nitrogen containing compound detected by automated amino acid analysis, thin layer, and paper chromatography.

PEA was synthesized from the reaction of oleoyl chloride with a stoichiometric amount of phosphatidylethanolamine isolated from egg yolk by silicic acid and DEAE column chromatography (Rouser et al., 1961). The reaction was carried out at room temperature in CCl_4 solvent. Isolation of the desired product was achieved by column chromatography on silica gel G. Analysis of the product gave values of 3.04% phosphorous and 1.2% nitrogen. The theoretical values for PEA, assuming the average fatty acid to be oleic, are 3.08% and 1.38, respectively.

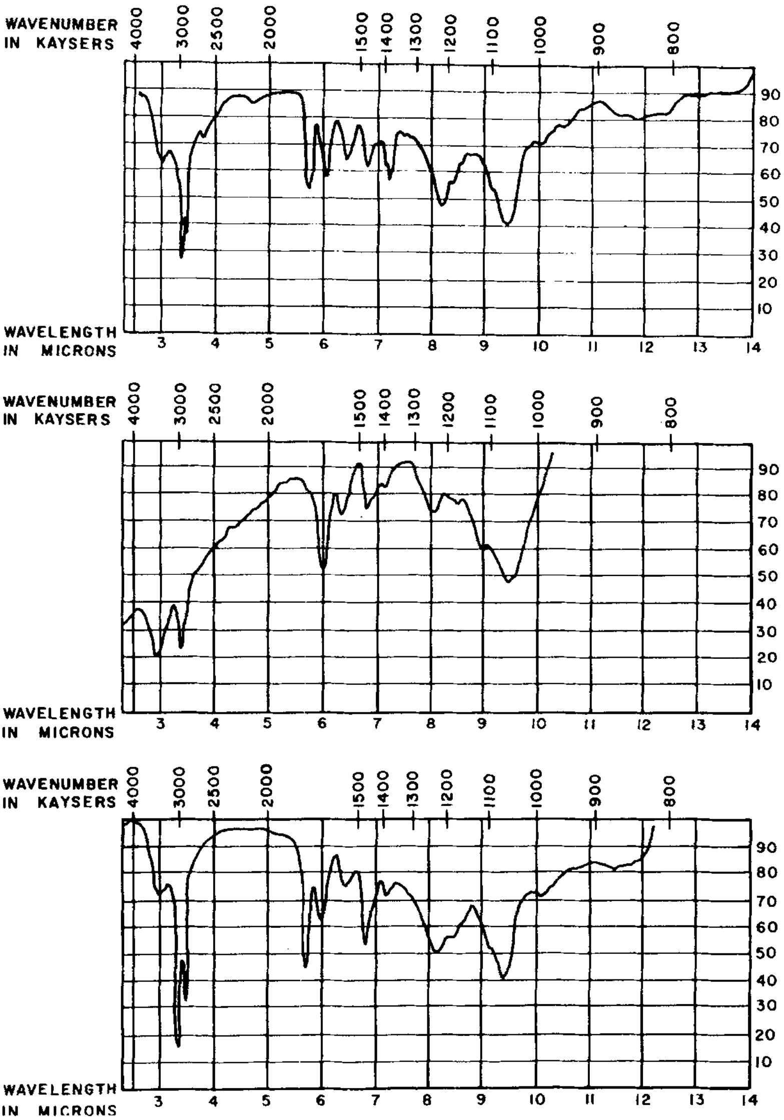
The synthetic compound (SPEA) subjected to thin layer chromatography migrated in three different solvent systems the same distances as substance A. Attempts to obtain LPEA from SPEA by removal of one fatty acid ester with phospholipase A proved futile. However, allowing SPEA to remain adsorbed on a column of neutral alumina at room temperature for 24 hours resulted in conversion of about 50% of SPEA to a substance that migrated the same distance as substance B in three different solvent systems on silica gel G. Conversely, the reaction of oleoyl chloride with substance B gave a compound that behaved identically with SPEA on thin layer chromatography.

The polar phase mild alkaline hydrolysis product from SPEA behaved identically with the product from substance A and from substance B in various solvent systems on paper and silica gel G. Paper chromatography of the polar phase acid hydrolysis products from all three compounds gave the essentially identical patterns as shown in Fig. 1. Substance B gave a higher percentage of beta glycerophosphate than either SPEA or substance A. Presumably, migration of the phosphoryl group would occur more readily on alumina with a lyso structure (substance B) than with the diester structure (substance A) in the isolation procedure from flour. The main components from all three substances were glycerophosphate, ethanolamine, and fatty acids. The lesser hydrolysis products have yet to be identified, but do not include O-phosphory ethanolamine.



Legend for Fig. 1

Tracing of water soluble acid hydrolysis (3N aqueous HCl, 90 minutes reflux) products chromatographed for 15 hours on Whatman no. 1 paper in n-butanol: acetic acid: H_2O, 5:3:1 columns 1-4 are spots positive with the Hanes-Isherwood phosphate detection spray. The first column has the products from α, β glycerophosphate, the second, third, and fourth columns from SPEA, LPEA, and PEA, respectively. The fifth, sixth, seventh, and eighth columns are the ninhydrin positive spots from ethanolamine, SPEA, LPEA, and PEA, respectively.



Legend for Fig. 2

Infrared spectra of top, LPEA in CCl_4; middle, mild alkaline hydrolysis product of LPEA in KBr; bottom, SPEA in CCl_4.

The infrared spectrum of SPEA given in Fig. 2 shows peaks ascribable to a monosubstituted amide link at 3.03, 6.0 and 6.42 microns as does that of substance B. The mild alkaline hydrolysis product had peaks at 2.98, 6.02, and 6.38 microns, the sample having been run in a KBr pellet, while the first two spectra are of samples in CCl_4 solution. The carbonyl ester band at 5.75 microns is more intense relative to the amide link in the spectrum of SPEA than in that of substance B and is missing for the hydrolysis product.

Haining et al., (1960) and Fukui and Axelrod (1961) have reported that rat liver microsomes catalyze the formation of N-acyl amino acids from fatty acids and amino acids. It is tempting to speculate that the microsome system may be capable of synthesis of the PEA type compound as it is (Tzur and Shapiro, 1964) of other phosphatides.

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