DETERMINATION OF THE ESTER GROUPS IN PHOSPHOLIPIDS

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UDC 545.953:665.37

To determine the ester groups in lipids, many workers [1-4] have developed micro methods based on the formation of hydroxamic acids which give a red-violet complex with trivalent iron. The method of Stern and Shapiro [2], designed for the determination of the ester groups in the lipids of human blood, is used most frequently for this purpose. Branän and Ballou [5] have reported the use of this method for investigating bacterial phospholipids, but without details of the analysis.

We have used Stern and Shapiro's method [2] in our own modification to determine the ester groups in the phospholipids that we have isolated from the sunflower [6], in the hydrogenated phosphatidycholines, and on their lyso product obtained on hydrolysis by phospholipase A [7, 8]. Not all classes of phospholipids dissolve in the mixture of ethanol and ether (3 : 1) proposed by Stern and Shapiro [2] and therefore the samples were dissolved first in diethyl ether, and then the mixture mentioned was added. In order to obtain reproducible results the hydroxylaminolysis reaction was performed not at room temperature, but at 40°C. The results of the determination of the ester groups by the hydroxamate method were compared with those of the gravimetric method, for which purpose the fatty acids were isolated after the acid hydrolysis of the phospholipids [6]. Reagents: diethyl ether and ethanol–ether (3 : 1). Aqueous solutions: 4 N HCl; 3.5 N NaOH; 0.37 M $FeCl_3$ in 0.1 N HCl; and 2 M hydroxylamine hydrochloride.

The initial standard solution was prepared by dissolving 295 mg of olive-oil triglycerides in 25 ml of the alcohol–ether mixture (3 : 1). The triglycerides were separated on a column of silica gel (100-150 mesh) by the method of Many and Lakehminarayana [9]. To construct a calibration curve, from the initial standard solution a working solution was prepared which contained 2 μeq of ester in 1 ml of alcohol–ether mixture. For the tests, each of a number of graduated 10-ml test tubes was charged with 0.5-2.5 ml of this solution, 0.5 ml of ether, and the alcohol–ether mixture to a volume of 3.5 ml, and then 0.5 ml of hydroxylamine solution and 0.5 ml of NaOH were added, and the mixture was stirred and was kept at 40°C for 30 min. After the thermostating, 0.5 ml of HCl was added and the tube was shaken, and then 0.5 ml of ferric chloride solution was added, the volume was made up with ether to 6.5 ml, and the mixture was stirred. The optical density was measured after 15-20 min against a blank on an FÉK-M photocolorimeter in a layer 0.1065 cm thick with a green filter.

TABLE 1. Content of Ester Groups in Phospholipids, μeq

Phospholipid	Methods	
	gravimetric	hydroxamate
Phosphatidylcholines	2,12	2,16
Lysophosphatidylcholines	0,98	1,02
Hydrogenated phosphatidylcholines	1,95	2,05
Hydrogenated lysophosphatidylcholines	0,97	1,02
Phosphatidylethanolamines	1,87	1,95
Phosphatidylinositols	1,83	1,87
Bisphosphatidic acids	3,84	4,04

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 655-656, September-October, 1974. Original article submitted April 19, 1974.

A weighed sample containing 3-5 μeq of ester groups was placed in a 10-ml graduated test tube and dissolved in 0.5 ml of diethyl ether with shaking and gentle warming (30-34°C), after which 3 ml of the ethanol–ether mixture was added and then the reagents in the sample sequence and amounts and under the same conditions as for the calibration experiments. Lyso compounds were dissolved directly in the ethanol–ether mixture, and then 0.5 ml of ether was added, followed by the reagents necessary for the hydroxylaminolysis reaction.

The amount of ester groups was determined from the calibration curve (see Table 1).

Consequently, the results of the hydroxamate method are close to those of the gravimetric method. These methods are suitable for determining ester groups in phospholipids and for mutual checking.

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