FRACTIONATION OF THE PHOSPHOLIPIDS OF *Helianthus annuus*

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UDC 547.953

The phospholipid complex of Helianthus annuus (sunflower), the composition of which has been established by us previously [1] was fractionated in the following way: 1723 mg were dissolved in 5 ml of chloroform, and the phospholipid fraction was precipitated with an excess of ethanol (60 ml). The precipitated solution was kept at –12° C for 3 h, and then the precipitate was filtered off and washed with cold ethanol (3 × 5 ml). The ethanol-soluble fraction was evaporated in a current of nitrogen, and dried in a vacuum drying chest (40-45° C) to constant weight. Yield 1380 mg (80.1%). The alcohol-insoluble fraction was dissolved in chloroform and then the solvent was driven off under the same conditions. Yield 343 mg (19.9%).

TABLE 1. Results of the Column Chromatography of the Phospholipids

Fraction No.	Eluent	Ethanol-soluble fractions*				Ethanol-insoluble fraction†			
		amt. of eluent, ml	yield of frac.		lipids	amt. of eluent, ml	yield of frac.		lipids
			mg	% obtained			mg	% obtained	
I	Chloroform	120	Traces	—	Chloroform-methanol	45	None	—	—
II	Chloroform-methanol (9:1)	250	104	7,5	Cerebrosides, phosphatidic acids	60	66	19,6	Cerebrosides phosphatidic acids
III	Chloroform-methanol (4:1)	250	90	6,6	Glycolipids, phosphatidylinositols	180	168	49,6	Phosphatidylinositols, glycolipids
IV	Chloroform-methanol (1:1)	300	416	30,3	Phosphatidylethanolamines, phosphatidylinositols, glycolipids	100	97	28,7	Phosphatidylinositols phosphatidylethanolamines, glycolipids
V	Chloroform-methanol (1:4)	500	760	55,6	Phosphatidylcholines	70	6	1,8	Phosphatidylcholines
VI	Methanol	250	Traces	—	Chloroform	50	Traces	—	The same
		Total 1370 mg (99.2%)				Total 337 mg (98.2%)			

*Initial amount 1380 mg; column: d = 24 mm, h = 700 mm, 60 g of silica gel

†Initial amount 343 mg; column: d = 16 mm, h = 500 mm, 15 g of silica gel

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 518-519, July-August, 1971. Original article submitted April 24, 1971.

The two fractions were dissolved in suitable amounts of chloroform and chromatographed in two parallel columns filled with a suspension of silica gel (type KSK, 100-150 mesh) in chloroform and were eluted with the same solvent. The rate of flow of the solvent was kept at 2-2.5 ml/min. To elute the substances from the column, solvents were used in the sequence given by Dyatlovitskaya et al. [2]. The fractions collected had a volume of 10 ml each, and their qualitative composition was monitored in a thin layer of silica gel on plates (2.5 × 7.5 cm) in two parallel solvent systems: chloroform–methanol–water (65 : 25 : 4) [3], and chloroform–methanol–25% ammonia (14 : 16 : 1) [4]. The substances were revealed on the chromatograms with 50% H_2SO_4 with subsequent carbonization of the spots. Fractions similar in composition were combined. The results of the chromatographic fractionation of the phospholipids are given in Table 1.

The technique of the fractionation of the phospholipid complex of the sunflower that has been described enabled us to obtain a pure fraction of phosphatidylcholines (see V in Table 1) immediately after column chromatography. The purity of the fraction was evaluated by physicochemical investigations [5]. The other fractions were purified by rechromatography in a thin layer of silica gel in the systems of solvents which we have described previously [5].

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