

Isolation and Identification of a Phospholipid

SURAJ PRAKASH BILLA and KRISHNA BAHADUR

Department of Chemistry, University of Allahabad,
Allahabad, India

(Z. Naturforsch. 26 b, 1376 [1971]; received August 2, 1971, revised
October 12, 1971)

A large number of phospholipids have been isolated from plants, e. g. the 12 species of leguminous seeds have been reported by ALFONSO¹ to contain cephalin and lecithins, showing thereby that the seeds are a rich source of phospholipids in plants. According to REWALD² in seeds the amount of cephalin is greater than that of lecithin. The present case of *Lathyrus sativus* is in accordance with the observations of REWALD.

Extraction of Phospholipid: The phospholipids are extracted in a fashion as oils are extracted. The seeds of *Lathyrus sativus* were powdered and extracted using a mixture of chloroform and methanol (2 : 1) in a Soxhlet apparatus. After extraction the mixture of chloroform methanol was distilled off and an oily mass was obtained.

Separation of Phospholipid: The oily mass was mixed with a 0.2% solution of potassium chloride and kept in a separating funnel for two hours. This procedure favoured the separation of the lipid phase from the non-lipid phase. The phospholipid obtained from the lipid layer by treatment with acetone which removed any other lipids which may be present. The phospholipid was washed again thoroughly with a mixture of acetone and water to remove the adhering lipid impurities. The amount of phospholipid was estimated by the method of PAECH and TRACEY³ and found to be 36 per cent.

Purification: The phospholipid was then treated with absolute alcohol to remove other lipids like lecithin if present, filtered off, dissolved in benzene and again filtered. To the filtrate, a 50% aqueous solution of cadmium chloride was added in order to separate it from other organic impurities. The mixture was filtered and the phospholipid was reprecipitated by acetone. It was further purified by means of column chromatography. According to TAUROG et al.⁴ phospholipids can be

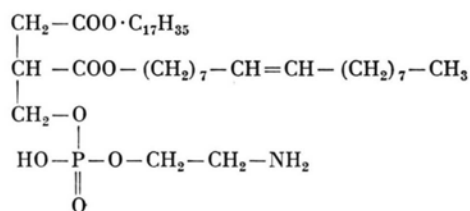
fractionated using magnesia column. In the present case a column of silica gel and magnesium trisilicate (5 : 5) was employed. The amount of cephalin was found to be 65.84 per cent.

Properties: The phospholipid was white when pure and freshly extracted but it became yellowish when in contact with air and finally turned brown on oxidation. It was an aliphatic compound, soluble in benzene, chloroform, methanol but insoluble in acetone and absolute alcohol. It decomposed on heating without well defined melting point. The compound did not react with sodium bicarbonate and Fehling's solution showing the absence of acedic and formyl groups. The compound decolorized solutions of bromine water and potassium permanganate indicating unsaturation. The compound could be titrated against a strong base like potassium hydroxide (RUDY and PAGE⁵).

The compound was saponified and after the saponification fatty acids were obtained which were confirmed by paper chromatography. The fatty acids identified were stearic and oleic.

The infrared spectrum, the mass spectrum and the combustion analysis of the compound were found to be identical with that of an authentic sample of cephalin.

On the basis of above results it may be concluded that the compound consists of stearic and oleic acids which may be present in the form of ester as they were obtained on saponification of the compound. The compound was aliphatic and unsaturated. Thus the compound may be cephalin and the structure of the compound may be given as:



$\text{C}_{41}\text{H}_{80}\text{PO}_8\text{N}$

Calculated	C 67.49	H 10.97	N 1.92	P 4.25	O 15.37,
Found	C 66.39	H 10.82	N 1.98	P 4.38	O 16.43.

Requests for reprints should be sent to Dr. SURAJ PRAKASH BILLA, Department of Chemistry, University of Allahabad, Allahabad, India.

⁵ H. RUDY and I. H. PAGE, Hoppe-Seyler's Z. physiol. Chem. 193, 251 [1930].

¹ A. V. ALFONSO, Rev. Fac. organ [La Plata] 34, 81 [1942]

² B. REWALD, J. Biochem. 36, 822 [1942].

³ K. PAECH and M. V. TRACEY, Modern Methods in Plant Chemistry, Springer-Verlag, Berlin-Göttingen-Heidelberg 1955, Vol. II, p. 367.

⁴ TAUROG et al. J. biol. Chemistry 155, 19 [1944].