closely comparable to those found in substituted benzimidazoles[12]. IR spectral data show that N-benzenesulphonyl L(+) glutamic acid and N-benzenesulphonyl L(−) aspartic acid exhibit bands in the region 1700–1724 cm^{-1} due to COOH. But in these three compounds (R^1H_2, R^2H_2 and R^3H_2) no band in this region is observed. In addition bands in the regions 1570–1613 cm^{-1} and 1360–1369 cm^{-1} due to COO^- antisymmetrical and symmetrical stretches respectively are observed[12-13]. These indicate the zwitterion structures for the compounds which may be represented as in Fig. 2.

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1. Perrin, D. D. and Sharma, V. S., *J. Chem. Soc.*, 1967, A, p. 724.
2. Csovari, S. and Lichner, D., *Med. Radiol*, 1969, 14, 28.
3. Rogozina, E. M., Popov, D. K. and Ponikrova, T. M., *Zh, Obshch. Khim.*, 1968, 38, 1959.
4. Chawla, I. D. and Andrews, A. C., *J. Inorg. Nucl. Chem.*, 1970, 32, 91.
5. Morris, P. J. and Martin, R. B., *J. Am. Chem. Soc.*, 1970, 92, 1543.
6. Michaitiois, M. S. and Martin, R. B., *Ibid.*, 1969, 91, 4683.
7. Ghosh, N. N. and Nandi, M. M., *J. Indian Chem. Soc.*, 1976, 53, 274.
8. Hedin, S. G., *Ber. dt. chem. Ges.*, 1890, 23, 3197.
9. Ghosh, N. N. and Nandi, M. M., *J. Indian Chem. Soc.*, 1976, 53, 331.
10. — and Dasgupta, M., *Z. Anorg. Allg. Chem.*, 1970, 375, 315.
11. — and Nandi, M. M., *J. Indian Chem. Soc.*, 1976, 53, 317.
12. Williams, D. H. L. and Flemming, J., *Spectroscopic Methods in Organic Chemistry* McGraw-Hill, New York, 1966.
13. Bellamy, L. J., *The Infrared Spectra of Complex Molecules,* Methuen, London, 1954.

ISOLATION AND CHARACTERISATION OF ANTHOCYANIN PIGMENT FROM PHOSPHORUS-DEFICIENT MAIZE PLANTS

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ABSTRACT

Phosphorus-deficiency in maize (*Zea mays* var. Ganga–5) resulted in the accumulation of anthocyanin pigment in leaves. The accumulating pigment was extracted in methanol–HCl (99 : 1) and a part of it was hydrolyzed to separate the aglycone (anthocyanidin) and the sugar moieties. The purified anthocyanin pigment and its aglycone were subjected to chromatographic and spectrophotometric analyses and the pigment was identified as cyanidin-3-glycoside, a monoside. Sugar moiety was identified as rhamnose. On the basis of these studies, the accumulating pigment was characterized as cyanidin-3-rhamnoside.

INTRODUCTION

IDENTIFICATION of anthocyanin pigment in maize has so far been accomplished only in the aleurone layer of seeds[1] and the anthers of Pr strains[2]. But no information is available on the identity of the pigment accumulating in the leaves of phosphorus-deficient maize. It was, therefore, decided to undertake the identification of the accumulating pigment.

EXPERIMENTAL

Phosphorus-deficiency was developed in maize (*Zea mays* var. Ganga-5) plants, grown by sand culture technique[3,4], by supplying them 0·3 ppm of phosphorus in the nutrient medium, instead of 62 ppm supplied for normal growth. After 40 days of growth, the pigment was extracted from the leaves in methanol–HCl (99 : 1 v/v) and purified chromatographically[5]. A part of the purified pigment was acid hydrolyzed[6] and the aglycone (anthocyanidin) was collected in 2–3 ml of amyl alcohol and purified[5]. The aqueous layer of the hydrolysate contains large amount of hydrochloric acid besides the sugar fraction. Before analysis, the acid was removed from the sugar sample[7].

The *Rf* values of the anthocyanim pigment and its aglycone were studied by descending chromatography on Whatman No. 1 paper. The solvents

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used for anthocyanin pigment were : BAW (Butanol-acetic acid-water ; 4 : 1 : 5 v/v, upper layer), BuHCl (Butanol-2-N HCl ; 1 : 1 v/v, upper layer) and 1% HCl. The solvents used for the aglycone were : Forestal (Conc. HCl-acetic acid-water ; 3 : 30 : 10 v/v), Formic (Conc. HCl-formic acid-water ; 2 : 5 : 3 v/v) and BAW. The absorbance of the anthocyanin pigment and its aglycone were recorded in u.v. and visible parts of the spectrum. Finally the bathochromic spectral shift of the pigment was studied in the presence of aluminium ions at pH 2–4. All the experiments dealing with characterisation of anthocyanin pigment, were done in the dark. The sugar fraction of the pigment was also identified chromatographically[3] and by studying the spectrum of the aniline-oxalate complex of the sugar[9].

Results and Discussion

The results of the present work are summarized in Table I. The *Rf* values of the extracted pigment in different solvents and the λ_{max} values in u.v. and visible range of the spectrum tallied, very closely with those of 3-monosidic glycosides of cyanidin. The pigment also underwent a bathochromic spectral shift, the value being very close to that obtained with standard cyanidin glycosides. This indicated that the pigment had free *o*-dihydroxylic groupings, which further strengthened its identity as cyanidin based pigment. Unlike 5-glycoside and 3, 5-diglycoside, this pigment does not fluoresce in u.v. light and gives dull colour. It was thus taken to be an additional proof for the pigment being a 3-glycoside, a monoside.

The *Rf* values of the aglycone fraction in different solvents and the λ_{max} values in u.v. and visible range of the spectrum also tallied exactly with those of authentic cyanidin. Finally, the *Rf* value of the sugar fraction, the colour of its aniline oxalate complex and the spectral absorbance of the complex were exactly similar to those observed with standard rhamnose.

From the results of the present work, it can be seen that the pigment extracted from the leaves of phosphorus-deficient maize plants has the following structural features (Fig. 1) : (*i*) The aglycone part of the pigment is cyanidin. (*ii*) The pigment is a 3-glycoside, a monoside. (*iii*) The sugar present in the pigment is rhamnose. It is, therefore, evident that the extracted pigment

Table I

Properties of the anthocyanin pigment extracted from maize leaves

Anthocyanin pigment	*Rf* (× 100) in			λ_{max} in		$AlCl_3$ shift (nm)
	BAW	BuHCl	1%HCl	U.V.	Visible	
Anthocyanin from *Zea mays*	31	42	10	284	533	46
Cyanidin based pigment	31	44	9	284	533	45

Anthocyanidin pigment	*Rf* (× 100) in			λ_{max} in	
	Forestal	Formic	BAW	U.V.	Visible
Anthocyanidin from *Zea mays*	49	21	64	277	535
Authentic anthocyanidin	49	21	68	277	535

Sugar	*Rf* (× 100) in Benzene-Butanol-Pyridine-Water (3 : 10 : 5 : 4 v/v)	λ_{max} in the range 330-550 nm
Sugar from the extracted pigment	83	370
Standard rhamnose	83	370

is cyanidin-3-rhamnoside. Thus a novel pigment is being reported from the maize plants.

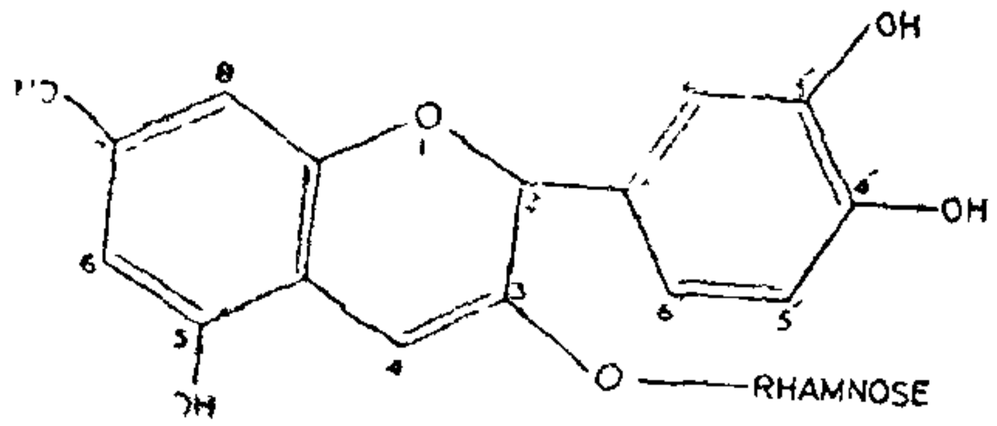

FIG. 1. Cyanidin-3-rhamnoside.

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1. Harborne, J. B. and Gavazzi, G., *Phytochem.*, 1969, 8, 999.
2. Styles, E. D. and Ceska, O., *Ibid.*, 1972, 11, 3019.
3. Hewitt, E. J., *Sand and Water Culture Methods used in Plant Propagation*, Commonwealth Agricultural Bureax, Farnham Royal. Buck. England, 1966, p. 56.
4. Arnon, D. I. and Hoagland, D. R., *Soil Sci.*, 1940, 50, 463.
5. Harborne, J. B., *Biochem. J.*, 1957, 70, 22.
6. —, *Comparative Biochemistry of Flavonoids*, Academic Press, London, 1967, p. 1.
7. Saini, A. S., *J. Chromat.*, 1966, 24, 484.
8. Hais, I. M. and Macek, K., *Paper Chromatography*, Publishing House of Czechoslovak Academy of Sciences, Prague, 1963, p. 289.
9. Horborne, J. B., *Phytochemical Methods—A Guide to Modern Techniques of Plant Analysis*. Chapman and Hall, Ltd., London, 1973, p. 59.