

Table 1. CITRATE AND SUCCINOXIDASE ACTIVITIES OF MITOCHONDRIAL FRACTIONS FROM LOCAL VARIETIES OF *Mangifera indica* (PRECLIMACTERIC)

Variety	O <sub>2</sub> uptake/ $\mu$ l./h/mg protein	
	Succinate	Citrate
'Kalapahar'	30	9
'Totapuri'	52	19
'Chittor Yamini'	62	11
'Titar Pasand'	85	8
'Lal Pasand'	30	5
'Pairi'	40	9

(The values are averages of four independent observations)

Each Warburg flask contained: phosphate 30  $\mu$ moles; sucrose 800  $\mu$ moles; substrate 20  $\mu$ moles; cytochrome *c* 4 mg; MgSO<sub>4</sub>·7H<sub>2</sub>O, 10  $\mu$ moles; final volume 3.2 ml., pH 7.4, temp. 28° air phase.

Table 2. DEHYDROGENASE ACTIVITIES OF MITOCHONDRIAL FRACTION FROM PULP OF 'PAIRI' VARIETY (PRECLIMACTERIC)

Dehydrogenase	Isocitrate	Oxaloacetate + NADH	Diaphorase	NADH Cyt. C reductase
Units*	0.025	0.676	0.932	1.87

(The values are averages of four independent observations)

\* Optical density change/min/mg protein.

Malate and pyruvate oxidations were also studied for the 'Pairi' variety in the Warburg apparatus and the respiration rates obtained were between 5 and 10  $\mu$ l. O<sub>2</sub>/h/mg protein. The dehydrogenase activities are reported in Table 2. Isocitrate and oxaloacetate + NADH reactions were followed in a Beckman 'DU' spectrophotometer<sup>5</sup>. Diaphorase and NADH-cytochrome *c* reductase were assayed by the method of Mahler *et al.*<sup>6</sup>.

The results presented here have shown the possibility of isolating an actively respiring mitochondrial fraction from the mango fruit. Further, these mitochondria showed respiratory control when tested with the vibrating platinum electrode technique<sup>7</sup> with succinate as substrate in the presence of adenosine diphosphate. Further work on respiratory control and the biochemical properties of mitochondria during various stages of ripening of the fruit is in progress.

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<sup>1</sup> Tager, J. M., *Nature*, **182**, 1521 (1958).

<sup>2</sup> Lieberman, M., *Science*, **127**, 189 (1958).

<sup>3</sup> Weiskich, J. T., and Bonner, W. D., *Plant Physiol.*, **38**, 594 (1963).

<sup>4</sup> Hulme, A. C., Hones, J. D., and Wooltorton, L. S. C., *Phytochemistry*, **3**, 173 (1964).

<sup>5</sup> *Methods in Enzymology*, edit. by Colowick, S. P., and Kaplan, N. O., **1**, 707 (Academic Press, 1955).

<sup>6</sup> Mahler, H. R., Sarkar, N. K., Vernon, L. P., and Alberty, R. A., *J. Biol. Chem.*, **199**, 585 (1958).

<sup>7</sup> Chance, B., *Regulation of Cell Metabolism*; Ciba Foundation Symposium, 91 (Little, Brown and Company, Boston, 1959).

## Anthocyanins in Ferns

THE water-soluble red pigments present in young fern fronds do not seem to have been investigated since Price, Sturgess, Robinson and Robinson<sup>1</sup> in 1938 reported that unusual flavylum salts, resembling 6-hydroxypelargonidin or 6-hydroxycyanidin in their colour properties, occurred in eight ferns. Recent phytochemical interest in the phenolic constituents of lower plants<sup>2</sup> and the discoveries of luteolinidin (I, R = OH) in the moss *Bryum*<sup>3</sup> and of an unidentified anthocyanidin in a fungus<sup>4</sup> suggested that the fern pigments would bear re-investigation.

Two ferns, *Adiantum veitchianum* and *Pteris quadriaurita*, closely allied to those examined by Price *et al.*<sup>1</sup>, were investigated. Extracts of juvenile fronds were hydrolysed with acid and the aglycones produced compared by chromatographic and spectral analysis with authentic anthocyanidins (Table 1). The two aglycones present in *A. veitchianum* were identified as apigeninidin (I, R = H) and luteolinidin (I, R = OH) and that of *Pteris* as luteolinidin. Both these pigments were readily

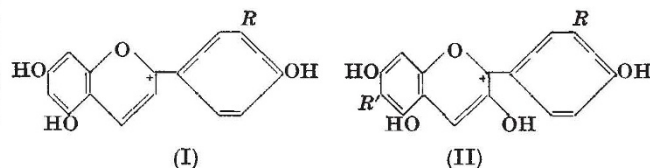
Table 1. CHROMATOGRAPHIC AND SPECTRAL PROPERTIES OF FERN ANTHOCYANIDINS

Pigment	R <sub>F</sub> value in solvent*				MeOH-HCl $\lambda_{max}$ (m $\mu$ )	MeONa $\lambda_{max}$ (m $\mu$ )
	A	B	C	D		
<i>Pteris</i> aglycone	0.61	0.64	0.42	0.43	498†	555
<i>Adiantum</i> aglycones	0.64	0.64	0.42	0.43	498†	555
	0.79	0.75	0.78	0.56	475	532
Apigeninidin	0.78	0.75	0.78	0.56	476	532
Luteolinidin	0.61	0.64	0.42	0.43	497†	555
6-Hydroxypelargonidin	0.57	0.56	0.58	0.24	497	Unstable
6-Hydroxycyanidin	0.30	0.32	0.39	0.21	518	Unstable

\* Solvent A is the Forestal mixture, B is HCO<sub>2</sub>H-HCl-H<sub>2</sub>O (9 : 2 : 3), C is butanol-acetic acid-water (4 : 1 : 5) and D is ethyl acetate-HCO<sub>2</sub>H-2NHCl (85 : 9 : 6). Separations using A, B and C were carried out on Whatman No. 1 paper, those with D on silica gel.

† Also gives an AlCl<sub>3</sub> shift of 52 m $\mu$ .

differentiated by R<sub>F</sub> value from pigments having similar colour properties, that is, 6-hydroxypelargonidin (II, R = H, R' = OH) and 6-hydroxycyanidin (II, R = R' = OH), both of which were available for comparison (Table 1). That these two anthocyanidins occur in some unusual combined form and not as simple sugar derivatives follows from the fact that (a) the natural pigments were more resistant than usual to acid hydrolysis, and (b) the pigments in the frond extracts differed in R<sub>F</sub> value from the known apigeninidin and luteolinidin 5-glucosides<sup>5</sup>. The two pigments in *Pteris*, for example, had R<sub>F</sub> values that were higher in both alcoholic and aqueous solvents (0.71 and 0.66 in butanol-2 N HCl (1 : 1), 0.38 and 0.28 in 1 per cent aq. HCl) than either luteolinidin or its 5-glucoside (0.60 and 0.27 in butanol-HCl, 0.03 and 0.12 in 1 per cent HCl, respectively).



The occurrence in both ferns and mosses of these rare anthocyanidins, which are biogenetically related to the flavonols rather than to the flavonols, is of considerable phytochemical interest. It suggests that these plants lack the ability to add a 3-hydroxyl group to a specific C<sub>15</sub>-intermediate to yield the usual type of anthocyanidin such as cyanidin (II, R = OH, R' = H) found in higher plants. The suggestion that apigeninidin and luteolinidin production is a 'primitive' character is not necessarily contradicted by the fact that these rare pigments are also found in the petals of one of the most 'advanced' families of higher plants, the Gesneriaceae<sup>6</sup>. They are presumably produced in this family in response to selection for orange-red flower colour. It should, however, be pointed out that a few ferns do appear to have normal anthocyanins, since cyanidin and pelargonidin glycosides have been reported in *Davallia divaricata*<sup>1</sup> and in *Dryopteris erythrosora*<sup>8</sup>. Furthermore, many ferns contain the flavonols kaempferol and quercetin<sup>7</sup> as well as leucocyanidin<sup>8</sup>. Investigations of the distribution in the Pteridophyta of anthocyanidins, with and without 3-hydroxyl groups, are therefore in progress.

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<sup>1</sup> Price, J. R., Sturgess, V. C., Robinson, R., and Robinson, G. M., *Nature*, **142**, 356 (1938).

<sup>2</sup> *Chemical Plant Taxonomy*, edit. by Swain, T. (Academic Press, London, 1962).

<sup>3</sup> Benz, G., Martensson, O., and Terenius, L., *Acta Chem. Scand.*, **16**, 1183 (1962).

<sup>4</sup> Avadhani, P. N., and Lim, G., *Abst. Tenth Intern. Bot. Congr.*, 326 (1964).

<sup>5</sup> Harborne, J. B., *Phytochemistry*, **2**, 85 (1962).

<sup>6</sup> Hayashi, K., and Abe, Y., *Bot. Mag., Tokyo*, **68**, 209 (1955).

<sup>7</sup> Hegnauer, R., *Chemotaxonomie der Pflanzen*, **1** (1962).

<sup>8</sup> Bate-Smith, E. C., *Biochem. J.*, **58**, 122 (1954).