Phytochemistry, 1971, Vol. 10, pp. 471 to 472. Pergamon Press. Printed in England.

NYMPHAECEAE

ANTHOCYANINS IN LEAVES OF *NYMPHAEA CANDIDA*

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(*Received* 8 *June* 1970)

Abstract—Delphinidin-3-galactoside, cyanidin-3-galactoside and a pigment tentatively identified as delphinidin-7-galactoside have been isolated from the leaves of *Nymphaea candida*.

IN CONTINUATION of our work on the distribution of anthocyanins in plants, the leaves of *Nymphaea candida* have now been investigated. They have a reddish shade, especially on the underside, and this pigmentation is due to anthocyanins. 3-Mono-galactosides of delphnidin and cyanidin were identified by their hydrolysis products, their spectral properties and their R_f-values. A third pigment (band II) which is rather unstable is also a delphinidin mono-galactoside, since its hydrolysis products were delphinidin and galactose and only delphinidin was obtained after controlled hydrolysis. It was decolourized rapidly by ferric chloride (free 3-hydroxyl),[1] had an E_{440}/E_{max} ratio of 18 per cent (free 5-hydroxyl)[2] and showed a bathochromic shift upon addition of ethanolic aluminium chloride (a free 3′,4′-dihydroxyl grouping).[2] While not precluded by this test, substitution in the 5′-position is unlikely since no anthocyanins containing sugars in this position have been described. Thus, the only possible hydroxyl group remaining is in the 7-position suggesting the pigment to be delphinidin-7-mono-galactoside. This pigment has previously been reported to occur in *Bladhia sieboldii* (Myrsinaceae).[3] Its identification has since been doubted, owing to the known instability of anthocyanidin 7-glycosides;[4] however, recently, other examples of this type of anthocyanin have been reported, namely cyanidin-7-arabinoside from apple skin[5] and delphinidin-7-glucoside from the leaves of *Cotinus coggygria*.[6]

A fourth anthocyanin (band I) was only obtained in trace amount insufficient for identification. It is a cyanidin glucoside which might be acylated, since, when chromatographed in BuHCl, two spots were always obtained.[7]

EXPERIMENTAL

The leaves of *Nymphaea candida* were collected in the north-western part of Dalecarlia. The dried leaves were first pre-extracted with ether and chloroform and then with methanol containing 1% conc. HCl. The pigments were separated by PC on Whatman No. 3 in *n*-BuOH–2 N HCl (1:1, v/v, top layer) giving four bands which were further purified by PC in water–conc. HCl (97:3, v/v) and in 15% HOAc. The anthocyanins

[1] A. LEON, A. ROBERTSON, R. ROBINSON and T. R. SESHADRI, *J. Chem. Soc.* 2672 (1931).
[2] J. B. HARBORNE, *Biochem. J.* **70**, 22 (1958).
[3] P. Y. YEH and P. K. HUANG, *Tetrahedron* **12**, 181 (1961).
[4] J. B. HARBORNE, *Phytochem.* **2**, 85 (1963).
[5] B. H. SUN and F. J. FRANCIS, *J. Food Sci.* **32**, 647 (1968).
[6] S. S. TANCHEV and C. F. TIMBERLAKE, *Phytochem.* **8**, 2367 (1969).
[7] J. B. HARBORNE, *J. Chromatog.* **1**, 473 (1958).

were recrystallized from aqueous ethanol containing 7% conc. HCl. Details of their identification are summarized in Table 1. Hydrolysis, partial hydrolysis of the anthocyanins and identification of their hydrolysis products were performed as described earlier.[8] The pigments II, III and IV were monoglycosides since controlled hydrolysis gave only two spots, (the aglycone and the unchanged pigment). The $FeCl_3$ test was carried out according to Leon, *et al.*[1] Only pigment II was decolourized (less than 30 min).

Acknowledgement—We wish to thank Professor Arne Fredga for his kind interest in this work and for collecting the plant material.

TABLE 1. IDENTIFICATION OF ANTHOCYANINS OF *Nymphaea candida*

	Absorption spectra			*After total acid hydrolysis		R_f values (× 100) in.†			
Band	λ_{max} (nm)		$\frac{E_{440}}{E_{vis.\ max}}$	Aglycone	Sugar	A	B	C	D
I	531,	283	0·22	Cyanidin	Glucose	34	33	03	27
II	543,	281	0·18	Delphinidin	Galactose	35	35	18	39
III	530,	283	0·23	Cyanidin	Galactose	36	24	07	27
IV	541,	278	0·17	Delphinidin	Galactose	24	10	03	17

* In MeOH containing 0·01% conc. HCl.
† Determined by PC on Whatman No. 1.
A = *n*-BuOH–HOAc–H_2O (4:1:5, by vol., top layer);
B = *n*-BuOH–2N HCl (1:1, v/v top layer);
C = HCl–water (3:97, v/v);
D = HOAc–HCl–water (15:3:82, by vol.)

[8] G. BENDZ and Å. HAGLUND, *Acta Chem. Scand.* **21**, 2286 (1967).

Phytochemistry, 1971, Vol. 10, pp. 472 to 475. Pergamon Press. Printed in England.

PRIMULACEAE

HIRSUTIN AND GOSSYPETIN IN *DIONYSIA*

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(*Received* 15 *July* 1970)

Abstract—An earlier flavonoid survey of the Primulaceae has now been extended to a further eight *Dionysia* and seven *Primula* species. As a result, hirsutin has been identified for the first time outside *Primula* as the violet pigment in flowers of *Dionysia archibaldii*, *D. bryoides*, D. *curviflora* and *D. microphylla*. Gossypetin, earlier found as a yellow pigment in flowers of *D. aretioides* is now reported also in *D. bornmuelleri* and *D. paradoxa*. Other characteristic *Primula* markers, namely flavone in the farina and 3′,4′-dihydroxyflavone in the leaf, were present in all species now examined. These results bring the total number of chemical characters of *Primula* present in *Dionysia* to five and correlate with the close morphological relationship known to exist between the two genera. The presence of primetin in the farina of *P. chionantha* has now been confirmed.