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SHORT COMMUNICATION

ANTHOCYANINS IN *SALIX* SPECIES

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Abstract—The anthocyanin pattern of *Salix* bark shows some differences between species. *S. daphnoides* and *S. alba* contain only cyanidin 3-glucoside; *S. phylicifolia, S. nigricans, S. calodendron* and *S. viminalis* contain both cyanidin and delphinidin 3-glucosides; *S. triandra* and *S. amygdalina* contain delphinidin, cyanidin and petunidin 3-glucosides.

INTRODUCTION

As EMPHASIZED by Binns and Blunden,[1] chemical differences between species of the genus *Salix* are of value, since morphological identification of the plants is often difficult.[2,3] Although differences in the contents of phenolic glycosides and leucoanthocyanidins in leaves have been demonstrated, the findings for many species are not in agreement.[1,4–6] Other components investigated include the piperidine imino-acids in leaves[1,5] and flavonoids in leaves and bark.[7] Some willow species are characterized by various attractive shades of brown, red and violet exhibited by the bark. This prompted a study of the bark anthocyanins as a possible additional aid to identification. Since they did not appear to have been investigated previously, we have made a preliminary survey of their occurrence in some of the most colourful of the 250 varieties of willow present in the Long Ashton collection.

RESULTS AND DISCUSSION

Two species (*Salix daphnoides* and *S. alba* var. *vitellina nova*) contained only cyanidin 3-glucoside, four species (*S. phylicifolia, S. nigricans, S. calodendron* and *S. viminalis*) contained both cyanidin and delphinidin 3-glucosides, while *S. triandra* (six varieties examined) contained small amounts of petunidin 3-glucoside in addition to delphinidin 3-glucoside (the main component) and cyanidin 3-glucoside (Table 1). The Dutch selection, *S. amygdalina* var. Zwarte Driebast, also contained the three latter anthocyanins, thus supporting the view that *S. amygdalina* and *S. triandra* are synonomous.[8] Further, *S. daphnoides* clones (*a*), (*b*),

[1] W. W. BINNS and G. BLUNDEN, *Phytochem.* **8**, 1235 (1969).
[2] A. R. CLAPHAM, T. G. TUTIN and E. F. WARBURG, *Flora of the British Isles*, 2nd edition, p. 582, Cambridge University Press, Cambridge (1962).
[3] A. REHDER, *Manual of Cultivated Trees and Shrubs*, 2nd edition, p. 83, The Macmillan Company, New York (1951).
[4] H. THIEME, *Pharmazie* **20**, 436, 570 (1965).
[5] W. W. BINNS, G. BLUNDEN and D. L. WOODS, *Phytochem.* **7**, 1577 (1968).
[6] J. JAGGI and E. HASLAM, *Phytochem.* **8**, 635 (1969).
[7] J. M. JARRETT and A. H. WILLIAMS, *Phytochem.* **6**, 1585 (1967).
[8] A. REHDER, *Manual of Cultivated Trees and Shrubs*, 2nd edition, p. 93, The Macmillan Company, New York (1951).

TABLE 1. DISTRIBUTION OF ANTHOCYANINS IN THE BARK OF *Salix* SPECIES

Species	Anthocyanin*		
	Cyanidin 3-glucoside	Delphinidin 3-glucoside	Petunidin 3-glucoside
S. daphnoides	1	—	—
S. daphnoides var. *acutifolia*	1	—	—
S. daphnoides clone "a" (*S. triandra*)	1	—	—
S. daphnoides clone "b" (*S. triandra*, var. French Purple)	1	—	—
S. daphnoides clone "c" (*S. caerulea*)	1	—	—
S. daphnoides clone "d" (*S.* × *laurina*)	1	—	—
S. alba var. *vitellina nova*	1	—	—
S. phylicifolia	1	1	—
S. nigricans	1	1	—
S. calodendron	1	2	—
S. viminalis	1	1	—
S. triandra var. Black Maul	2	1	3
S. triandra var. Brown Maul	2	1	3
S. triandra var. Black Top	3	1	3
S. triandra var. Black Spaniard	3	1	3
S. triandra var. Trustworthy	2	1	3
S. triandra var. Newkind	2	1	3
S. amygdalina var. Zwarte Driebast	1	1	3

* 1 = main component(s), 2 = secondary component, 3 = minor component or trace(s).

(*c*) and (*d*), although received as *S. triandra*, *S. triandra* var. French Purple, *S. caerulea* and *S.* × *laurina* respectively, were subsequently assigned morphologically as *S. daphnoides*. The occurrence of only cyanidin 3-glucoside in their bark now supports this designation. The findings are therefore of value as an aid to identification.

Leaf colour is not as prominent as bark colour. Thus the only leaves showing appreciable visible colour when young (i.e. June) were those of *S. viminalis pubescens*, *S. fragilis* × *alba russelliana* and *S. triandra* × *purpurea*. These three willows, which were received from three different sources, are morphologically similar; it is therefore of interest that each contained only cyanidin 3-glucoside. This glucoside has been found previously in leaf galls and young leaves of *S. fragilis*.[9]

EXPERIMENTAL

The outer layer of bark was stripped from shoots and extracted with MeOH–HCl (97:3, v/v) for 18 hr or more in a refrigerator. The extracts were filtered, concentrated, ether-extracted, streaked on sheets of Whatman No. 3 paper and chromatographed successively in three solvents: (1) BAW (*n*-BuOH:acetic acid:water, 4:1:5,v/v); (2) 2% acetic acid or acetic acid:conc. HCl:water (15:3:82,v/v); and (3) BAW, with intermediate and final elution from the paper with MeOH–HOAc–H_2O (94:3:3, v/v). After the first chromatographic separation, bands of identical R_f, which were common to a number of samples, were sometimes combined, purified further and found to contain only one anthocyanin. The purified anthocyanins were identified by *a*. R_f values in comparison with authentic samples, *b*. spectral measurements[10,11] with and without $AlCl_3$, and *c*. controlled and complete acid hydrolysis, with identification of anthocyanidins and sugars by the usual chromatographic methods.[12] Some double-banding was observed during final purification in BAW; this phenomenon, which did not affect the conclusions, is being further investigated.

[9] G. BLUNDEN and S. B. CHALLEN, *Nature* **208**, 388 (1965).
[10] J. B. HARBORNE, *Biochem. J.* **70**, 22 (1958).
[11] J. B. HARBORNE, *Phytochem.* **2**, 85 (1963).
[12] J. B. HARBORNE, *Biochem. J.* **74**, 262 (1960).