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ANTHOCYANINS IN *SALIX* SPECIES: A NEW ANTHOCYANIN IN *SALIX PURPUREA* BARK

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Abstract—Cyanidin 3-glucoside and delphinidin 3-glucoside (minor component) occurred in the bark of *Salix purpurea* (29 cultivars examined), *S. fragilis* (3 cvs), *S. americana* (4 cvs), *S. rubra* (1 cv.), *S. incana* (1 cv.), *S. aegyptica* (1 cv.) and *S. alba* (3 cvs) and several hybrids. A previously unrecorded anthocyanin was also present in appreciable amounts in the bark of eleven *S. purpurea* cultivars, notably those with small leaves. In other large-leaved cultivars of *S. purpurea* (18) it occurred only in traces or was not detected with certainty. A trace was found in one *purpurea* hybrid, but it was not detected in four others. Small amounts of the new anthocyanin were also found in *S. incana* (1 cv.), *S. fragilis* cv. Basfordiana, *S. americana* cv. Cordata and one hybrid, but were absent from other cultivars of *S. fragilis* (2) and *S. americana* (2), and also from *S. alba* (3), *S. aegyptica* (1), *S. daphnoides* (1), *S. rubra* (1), *S. triandra* (1), *S. viminalis* (1) and three hybrids. The new anthocyanin is unique in containing fructose as well as glucose and is based upon a previously undescribed anthocyanidin, possibly dimeric in nature, which is provisionally named purpurinidin.

INTRODUCTION

SOME willow species are characterised by their attractive autumnal and winter bark colourings of brown, red and violet. A preliminary examination of the anthocyanins present in the bark of several coloured species grown at Long Ashton has been reported previously.[1] The observed differences in anthocyanin pattern between some species were of value as an aid to identification, often difficult on morphological criteria alone. During an extension of this work it was noticed that some cultivars of *Salix purpurea* contained an additional anthocyanin which had not been previously described, and which appeared to be a glycoside of a new anthocyanidin, provisionally named purpurinidin. All the available *purpurea* cultivars with coloured bark were therefore examined, as well as some hybrids and other species.

RESULTS

Salix purpurea

All the *S. purpurea* cultivars examined contained cyanidin 3-glucoside, which was the main component except in cultivars 'Angustifolia' and 'Green Dicks' (January sampling) where the new anthocyanin was in excess. Delphinidin 3-glucoside was also uniformly present in small amounts or traces: in only one instance (*S. purpurea* cv. Ferrinea) was it found in an amount similar to that of cyanidin-3-glucoside. The *purpurea* cultivars could be roughly divided into four groups according to their content of purpurindin glycoside compared with that of cyanidin 3-glucoside: (1) Those containing the highest proportion of the unknown anthocyanin (>20% of cyanidin 3-glucoside) included "Leicestershire Dicks', 'Lancashire Dicks,' 'Green Dicks' and 'Angustifolia'. (2) Those containing medium

[1] BRIDLE, P., STOTT, K. G. and TIMBERLAKE, C. F. (1970) *Phytochemistry* **9**, 1097.

proportions (3–15%) included 'Procumbens', 'Richartii', 'Dark Dicks', 'Uralensis', 'Reeks', 'Dicky Meadows', 'Brittany Greens' and also the four cultivars of group 1 whose composition varied according to the date of sampling. (3) Those containing traces (1–3%), such as 'Scharfenbergensis', 'Jagiellonka', 'Welsh', 'Lambertiana' and a red-stemmed unnamed cultivar.* (4) Those where positive detection could not be made (< 1%) included 'Goldstones', 'Pyramidalis', 'Schultz', 'Forbyana', 'Continental Reeks', 'Ferrinea', 'Utilissima', 'Nicholsonii purpurescens', 'French Strain', 'Helix', 'Regalis', 'Kerksii' and a brown-stemmed unnamed cultivar.*

Salix purpurea Hybrids

Cyanidin 3-glucoside was the major component, accompanied by minor amounts of delphinidin 3-glucoside. *S. viminalis* × *purpurea* cv. Abbeys contained a trace (1–2%) of purpurinidin glycoside, but it was not detected in *S. viminalis* × *purpurea* cvs, Mawdesley Seedling A, Harrison's Seedling B, Universal Weide or *S. triandra* × *purpurea*.

Other Species and Hybrids

The new anthocyanin occurred in traces (1–2%) in *S. incana*, *S. fragilis* cv. Basfordiana and *S. americana* cv. Cordata but was not detected in *S. americana*, *S. americana* cvs McKay and American Green U.S.A., *S. aegyptica*, *S. alba* cvs Cardinalis, Vitellina nova and Yelverton, *S. daphnoides*, *S. rubra*, *S. fragilis* cvs, St Reine and Illertissen Clone V, *S. triandra* cv. Black Maul, *S. viminalis* cv. Purbescens, *S. viminalis* × *triandra* cv. Hippophaefolia, *S. Mawdesley* × *americana* and *S. fragilis* × *alba* cv. Russelliana.

With the exception of *S. daphnoides*, *S. triandra* and its hybrid with *S. viminalis*, all the above cultivars contained cyanidin 3-glucoside accompanied by small amounts or traces of delphinidin 3-glucoside. *S. viminalis* × *triandra* cv. Hippophaefolia, exhibited the characteristic pattern of *S. triandra*, viz. delphinidin 3-glucoside with a smaller amount of cyanidin 3-glucoside and a trace of the component (of intermediate R_f in BAW) previously identified as petunidin 3-glucoside.[1] In contrast to previous findings,[1] *S. alba* cv. Vitellina nova contained a small amount (10%) of delphinidin 3-glucoside in addition to cyanidin 3-glucoside; a similar pattern occurred with *S. alba* cvs. Cardinalis and Yelverton. Some seasonal variation in anthocyanin composition was therefore indicated, but it did not affect *S. triandra* cv. Black Maul, *S. viminalis* or *S. daphnoides*, whose patterns agreed with those previously found.[1] The latter thus remains the only species of those so far examined which contains solely cyanidin 3-glucoside.

Variation in Content of Purpurinidin Glycoside

The best source of the new anthocyanin was *S. purpurea* cv. Leicestershire Dicks in which the content (expressed as a percentage of cyanidin 3-glucoside) was 14% (September), 56% (October–December) and 32% (February). The amount in Lancashire Dicks varied from 13 to 33%. Green Dicks showed the greatest variation in content from 8% (October) to 180% (January—similar to cv. Angustifolia) but since only the shoot tips were coloured it was not a good source.

Nature of Purpurinidin Glycoside

While a complete characterization has not yet been made, this anthocyanin possesses a number of unique features. The orange glycoside contains fructose and glucose, is readily

* Received from Institüt für Obstbau, Stuttgart Hohenheim.

hydrolysed by cold N HCl to a red intermediate anthocyanin containing glucose only and is based upon a violet aglycone. Fructose has not been found previously in anthocyanins,* possibly because it is unstable under the usual hydrolysis conditions (hot acid). Both purpurinidin glycoside and the intermediate glucoside are highly resistant to decolorisation by sulphur dioxide, and with increasing pH change to their corresponding anhydrobases without intermediate carbinol base formation. These features, which are characteristic of 4-substituted flavylium salts,[2] indicate that the anthocyanins may be dimers linked through their 4-positions.[3]

TABLE 1. PROPORTION OF PURPURINIDIN GLYCOSIDE AND MEAN LEAF LENGTH*

Group	No. of cultivars examined	Main cultivars	% of purpurinidin glycoside relative to cyanidin 3-glucoside	Mean leaf length (mm)
1	4	{Leicestershire Dicks, Lancashire Dicks}	>20	69
2	7	{Dark Dicks, Brittany Greens}	3–15	73
3	5	{Lambertiana, Welsh}	1–3	103
4	13	{Goldstones, French Strain}	<1	123

* Measurements made on a representative sample of 15 leaves taken in September from the mid-point of a total of ten 1-yr-old coppice shoots.

DISCUSSION

The taxonomy of the genus *Salix* is complex, for whilst morphological criteria can be sufficient to differentiate species, the differentiation of hybrids or cultivars within a species is often extremely difficult, demanding the examination of material at different seasons and from different habitats.[4,5] For these reasons differences in chemical constituents are proving of increasing value in assigning the parentage of chance hybrids and of characterizing cultivars.[5] The results presented here on the detection of distinct proportions of purpurinidin glycoside in a range of cultivars of *S. purpurea* is therefore a useful addition to identification within the species. These cultivars of *S. purpurea* exhibit a wide range in leaf size (mean length: 65–130 mm) and though leaf size is not a precise diagnostic feature (varying with site, nutrition, position on the shoot and age of shoot), cultivars of *S. purpurea* are often designated as small or large leafed types. It is therefore of some interest that, when leaf measurements were made of the cultivars comprising each Group, the small leafed cultivars (mean leaf length 69 mm) showed a high proportion of purpurinidin glycoside, whereas large leafed cultivars (mean leaf length 123 mm) contained a low proportion (Table 1). This observation helps to substantiate the view that broad differences

* Since writing, fructose has been reported in *Mentha piperita*. (SHAKHOVA, M. F. (1971) *Rast. Resur.* **7**, 407).

[2] TIMBERLAKE, C. F. and BRIDLE, P. (1968) *Chem. Ind. (London)* 1489.

[3] BRIDLE, P. and TIMBERLAKE, C. F. *Rep. Long Ashton Res. Stn. for* 1971, 171.

[4] CLAPHAM, A. R., TUTIN, T. G. and WARBURG, E. F. (1962) *Flora of the British Isles*, 2nd Edn, p. 582, Cambridge University Press, Cambridge.

[5] BINNS, W. W. and BLUNDEN, G. (1969) *Phytochemistry* **8**, 1235.

exist within the species. Also, of a taxonomic interest is the considerable difference in proportion of purpurinidin glycoside detected in December between Leicestershire Dicks (56%) and Lancashire Dicks (33%). Hitherto these two small leaved, red stemmed bushy cultivars had appeared so morphologically similar that their existence as two separate cultivars had seemed questionable.

Petunidin 3-glucoside was again found only in the species *S. triandra* cv. Black Maul and a trace in its hybrid with *S. viminalis* (cv. Hippophaefolia) but not in its hybrid with *S. purpurea*. The *triandra* × *purpurea* hybrid examined possessed cyanidin 3-glucoside, delphinidin 3-glucoside and no detectable purpurinidin glycoside. A similar pattern of constituents was detected in *S. purpurea* cv. Kerksii, *S. americana*, *S. americana* cvs MacKay and American Green U.S.A., *S. viminalis* cv. purbescens, *S. Mawdesley* × *americana* and *S. fragilis* × *alba* cv. Russelliana. All these willows, despite their reputed dissimilar identities are morphologically similar, which, with the chemical evidence reported above, suggests that these important European basket willows are hybrids of *S. triandra* and *S. purpurea*.

EXPERIMENTAL

The outer layer of bark was stripped from shoots, extracted with methanolic 0·1% HCl, filtered and concentrated. After removal of chlorophyll by Et_2O extraction the concentrate was streaked on thick paper (Whatman No. 3 or 3 MM) and chromatographed in BAW (*n*-BuOH–HOAc–H_2O, 4:1:5 or 6:1:2). Most samples yielded two coloured bands, a red major band (cyanidin 3-glucoside) accompanied by a violet minor component (delphinidin 3-glucoside) of smaller R_f value. The colour of the cyanidin 3-glucoside band of some *purpurea* cultivars was modified by the presence of an additional orange component of about the same R_f. This band was then cut out and the anthocyanins were eluted with MAW (MeOH–HOAc–H_2O, 94:3:3), the extract was concentrated, re-streaked on paper and chromatographed in solvent HOAc–HCl (HOAc–HCl–H_2O, 15:3:82). Because of its high R_f, the new anthocyanin separated well from cyanidin 3-glucoside and could be clearly detected. Semi-quantitative results were obtained by cutting a vertical section from the dried chromatogram, soaking the cut out anthocyanin bands in MeOH containing 0·01% conc. HCl, and measuring their colour intensities. Small amounts of the new anthocyanin were estimated by eye. Whether a compound is detectable on a chromatogram naturally depends upon the amount of material applied to the paper. Therefore, papers were consistently loaded with sufficiently large amounts of anthocyanin, i.e. cyanidin 3-glucoside plus the unknown component, so that the latter was just detectable visibly at a level of about 1% of that of cyanidin 3-glucoside. Traces of other components appeared in extracts of some cultivars in both BAW and HOAc–HCl, but the amounts were insufficient for examination.

Pure anthocyanin samples in amounts sufficient for analysis were obtained by successive chromatography using solvents BAW and HOAc (15% HOAc), and were identified by standard techniques, with additional confirmation of fructose by Dr. A. A. Williams using GLC.