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3-DEOXYANTHOCYANINS FROM THE FERN *BLECHNUM PROCERUM*

R. K. CROWDEN and S. J. JARMAN

Botany Department, University of Tasmania, Box 252C G.P.O., Hobart, Tasmania, 7001, Australia

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Abstract—Identification of 6 apigeninidin and 6 luteolinidin anthocyanins from the fern *Blechnum procerum* (Forst. f) Schwartz is reported.

3-DEOCYANTHOCYANINS have been identified in mosses,[1] ferns[2] and in a few angiosperm species.[3] The pigments so far described have been relatively simple derivatives of apigeninidin (5-glucoside), luteolinidin (5-glucoside and 5-diglucoside) and tricetinidin (5-glucoside).

We have recently isolated a number of deoxyanthocyanins from young fronds of the fern *Blechnum procerum* and the probable identification of twelve pigments is reported here. These represent two parallel series based on apigenidin and luteolinidin. Glycosylation patterns present include not only the 5-mono- and di-glucosides reported previously,[1–3] but also 7-mono and 7-diglucosides, 5,7-diglucoside and a 5-substituted mixed disaccharide containing glucose and rhamnose. The pigments are listed in Table 1. At least 3 minor compounds, additional to those reported, have yet to be identified.

On acid hydrolysis, all pigments yielded either apigeninidin or luteolinidin as the sole aglycone. Assignment of glycosylation to position C-5 (pigments A4, A7, L2, L6 and L7), was made largely on the basis of spectral examination using a low value of the ratio A_{440}/A_{max} as indicative of 5-substitution.[2,4] A high value of this ratio was taken as evidence that position C-5 was unsubstituted (pigments A1, A3, A5, L1, L3 and L5). Glycosylation at position C-7 (pigments L3 and L5) was then deduced after showing that the B-ring hydroxyls were free (bathochromic shift of approximately 30 nm after addition of $AlCl_3$). By analogy, pigments A3 and A5 are also considered to be 7-substituted. The minor pigments A2, A6 and L4 were not present in sufficient quantity to permit rigorous purification and consequently did not yield reliable spectral ratios.

Additional evidence relating to structural determinations of the twelve pigments was obtained from a chromatographic and spectral comparison between all pigments and all intermediate products obtained from their partial hydrolyses. The nature of the linkage present in the disaccharide substituents, viz. rhamnosylglucoside and glucosylglucoside was not determined.

[1] BENZ, G., MARTENSSON, O. and TERENIUS, L. (1962) *Acta Chem. Scand.* **16**, 1183.
[2] HARBORNE, J. B. (1962) *Phytochemistry* **2**, 85.
[3] HARBORNE, J. B. (1960) *Phytochemistry* **5**, 589.
[4] HARBORNE, J. B. (1966) *Comparative Biochemistry of Flavonoids*, Academic Press, New York.

TABLE 1. CHARACTERISTICS OF 3-DEOXYANTHOCYANINS FROM *Blechnum procerum*

Pigment no.	Identification*	R_f's in solvent†			Spectral data	
		BAW	3% HCl	WAH	Max. nm	A_{440}/A_{max}
A1	Apigeninidin				480	45
A2	Ap-5-G	0·39	0·33	0·40	480	42
A3	Ap-7-G	0·39	0·34	0·43	480	44
A4	Ap-5-GG	0·27	0·36	0·45	482	38
A5	Ap-7-GG	0·27	0·36	0·45	480	44
A6	Ap-5-RG	0·39	0·67	0·69	480	42
A7	Ap-5G-7G	0·28	0·34	0·42	480	41
L1	Luteolinidin				498	33
L2	Lu-5-G	0·26	0·19	0·28	496	20
L3	Lu-7-G	0·25	0·19	0·29	497‡	29
L4	Lu-5-GG	0·15	0·20	0·30	497	25
L5	Lu-7-GG	0·16	0·20	0·29	497‡	28
L6	Lu-5-RG	0·29	0·59	0·61	497	19
L7	Lu-5G-7G	0·16	0·22	0·31	497	20

* Key: Ap = apigeninidin; Lu = luteolinidin; G = glucose; R = rhamnose.
† Solvents: BAW = BuOH–HOAc–H_2O (5:1:4); WAH = H_2O–HOAc–conc. HCl (82:15:3).
‡ Give a bathochromic shift of ~30 nm with $AlCl_3$.

Methods. Isolation and purification was achieved using a combination of procedures involving column chromatography on Bio-Rex 70 H^+ (Bio-Rad Laboratories, Richmond, California) and polyamide, and repetitive PC using Whatman 3MM paper as described previously.[5] Purified pigments were identified using standard techniques of spectroscopy, partial and complete hydrolysis with 2N HCl, and by PC with a variety of solvent systems.[4,5] Relevant chromatographic and spectral data are shown in Table 1.

[5] JARMAN, S. J. and CROWDEN, R. K. (1973) *Phytochemistry* **12**, 171.