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FAST ATOM BOMBARDMENT MASS SPECTROMETRY OF THE ANTHOCYANINS VIOLANIN AND PLATYCONIN

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Key Word Index—*Viola tricolor*; Violaceae; *Platycodon grandiflorum*; Campanulaceae; violanin; platyconin; fast atom bombardment mass spectrometry; flower pigment structures; anthocyanins.

Abstract—Fast atom bombardment mass spectrometry (FABMS) of the acylated anthocyanin violanin confirmed its molecular mass (919) and gave a fragmentation pattern in agreement with its known structure. FABMS of the more complex flower pigment platyconin gave its accurate molecular mass as 1421.3740 and a fragmentation pattern suggesting that platyconin is delphinidin 3-rutinoside-5-glucoside with two mols of glucosylcaffeic acid attached to the glucose moiety of rutinose in a branched-chain arrangement.

INTRODUCTION

Formerly, the application of mass spectrometry to the determination of MWs and structures of anthocyanins has been limited [1, 2]. Recently, the new technique of fast atom bombardment mass spectrometry (FABMS) has been applied successfully to studies of polar molecules and thermolabile and involatile compounds [3, 4]. We have now applied FABMS to anthocyanins and particularly to complex acylated anthocyanins to try to establish MWs and linkage modes between the sugar and phenolic acid residues. Our results with two flower pigments, viz. violanin and platyconin, are reported here.

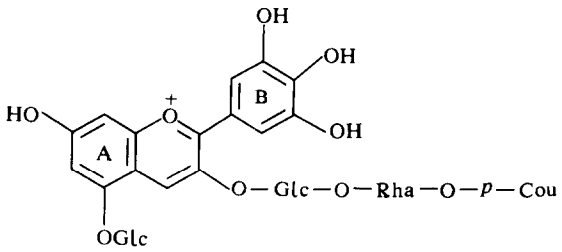
RESULTS AND DISCUSSION

In preliminary work, molecular ions were found for cyanidin (*m/z* 287) and malvidin 3-glucoside (*m/z* 493). The latter showed the fragment of its aglycone (malvidin, *m/z* 331). Attention was then turned to more complex anthocyanins.

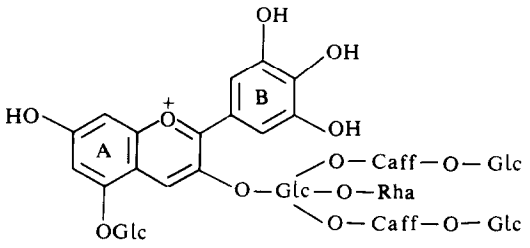
Violanin

Violanin was first isolated from *Viola tricolor* [5] and its stereostructure was established as delphinidin 3-[6-*O*-α-L-(*p*-coumaroyl)rhamnosyl-β-D-glucoside]-5-(β-D-glucoside) (**1**) by a detailed analysis of its ^{1}H NMR spectrum [6]. FABMS of violanin gave a well-defined molecular ion and a fragmentation route in which each residue or group of residues appeared to be lost by cleavage of the anomeric or ester bond with hydrogen replacement at the position of loss, e.g. R-O-glucose → ROH (glucose loss) (Table 1). The findings are in agreement with the known structure of violanin.

Other ions of low intensity were also observed and may have arisen from impurities: *m/z* (rel. int.) 903 (6) possibly a deoxy compound, 443 (5), 381 (4), 345 (2) and 325 (10).



1 (Violanin)



2 (Platyconin)

Platyconin

Platyconin was isolated from *Platycodon grandiflorum* and its structure was reported as delphinidin 3-(dicaffeoylrutinoside)-5-glucoside from spectroscopic and chemical analyses [7, 8]. However, recent ^{1}H NMR measurements have revealed the presence of two additional molecules of glucose in this pigment, probably conjugated to the anthocyanin 3-position [Saito, N. and Abe, K., unpublished result]. FABMS data on platyconin were as follows: *m/z* (rel. int.): 1421 $[M]^+$ (26), 1259 $[M-162]^+$ (1), 1097 $[M-2\ (162)]^+$ (1), 935 [M – 3

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Table 1. FABMS of violanin

m/z	Rel. int.	Interpretation [M – fragment]$^+$
919	51	[M]$^+$ (for $C_{42}H_{47}O_{23}$)
773	2	[M – *p*-coumaroyl]$^+$
757	9	[M – glucosyl (pos. 5 in A ring)]$^+$
627	3	[M – *p*-coumaroylrhamnosyl]$^+$
611	1	[M – *p*-coumaroyl and glucosyl (A ring)]$^+$
465	18	[M – *p*-coumaroylrhamnosyl and glucosyl (A ring)]$^+$ and [M – *p*-coumaroylrutinosyl]$^+$
303	100	[M – *p*-coumaroylrutinosyl and glucosyl (A ring)]$^+$

(162)]$^+$(3), 773 [M – 4 (162)]$^+$ (2), 611 [M – 5 (162)]$^+$ (13), 465 (2) and 303 (100). Ions of mass 611, 465 and 303 were observed previously with violanin (Table 1) and represent delphinidin 3-rutinoside, delphinidin 3-glucoside and 5-glucoside, and delphinidin itself, respectively. Ions of larger mass originate by sequential loss of 162 mass units from the molecular ion as indicated. This suggests that glucosyl or caffeoyl residues are fragmented from platyconin according to the mechanism outlined for violanin. But with low resolution mass spectrometry it was not possible to distinguish between these residues since both possess the same nominal mass. To obtain accurate ion masses and so distinguish losses of glucosyl residues ($C_6H_{10}O_5$, 162.05282) from those of caffeoyl residues ($C_9H_6O_3$, 162.03169) spectra were obtained at intermediate resolution (7000 r.p.) in the range 600–1500 a.m.u. using internal lock references and averaged over 10 scans. Table 2 shows that the exact mass found for platyconin agreed with that expected with an error of only 6 ppm. Also, Table 2 lists the fragmentation possibilities (losses of glucosyl or caffeoyl residues) and compares the masses measured with those anticipated. The results suggest strongly that the ions of nominal *m/z* 1259, 1097 and 935 arise by sequential losses of up to three glucosyl residues, since any loss of a caffeoyl residue is attended by much greater errors. The ion of *m/z* 773 could be a composite measure of both the possibilities indicated in Table 2 but choice (g) is the more likely. The assignment of delphinidin 3-rutinoside to ion *m/z* 611 is supported by the low error (9 ppm) found for its exact mass.

Of interest is the loss of three glucosyl residues from platyconin prior to any loss of caffeoyl. A linear side-chain at position 3 would result in losses of only two glucosyl residues (one from the A ring and one from the side-chain). The further loss of an additional glucosyl residue rather than one of caffeoyl, suggests that the side-chain might be branched, with two mols of caffeoylglucose attached to two hydroxyl groups of the rhamnose moiety. However, additional ions were also observed in the spectrum of platyconin indicating fragmentation of a rhamnosyl residue ($C_6H_{10}O_4$, 146) from M^+ and the main fragments as follows: *m/z* (rel. int.): 1275 [M – 146]$^+$ (1), 1113 [1259 – 146]$^+$ (3), 951 [1097 – 146]$^+$ (1), 789 [935 – 146]$^+$ (< 1), 627 [773 – 146]$^+$ (< 1). Indeed, loss of a rhamnosyl residue from M^+ was reinforced by accurate mass measurement of the ion of nominal *m/z* 1275 (calculated for $C_{57}H_{63}O_{33}$ 1275.3245, found 1275.3380, deviation 16 ppm, error 11 ppm). Thus the most likely structure of platyconin based on its fragmentation pattern is **2**, with two mols of caffeoylglucose attached to two hydroxyl groups of the glucose component of rutinose. An alternative depside side-chain arrangement of diglucosylcaffeoylcaffeic acid seems less likely since a fragment corresponding to the depside (*m/z* 649) was not observed. In respect of its side-chain, platyconin thus appears to differ from the anthocyanin isolated from blue flowers of a Morning Glory *Ipomoea* which is thought to have a long linear side-chain of glucosylcaffeic acid residues attached to peonidin 3-sophoroside-5-glucoside [9].

These findings illustrate the ability and potential of FABMS not only to measure molecular masses of complex plant pigments but also to be of structural aid in elucidating the sequences of sugars and cinnamic acids. But it cannot decide the mode of bonding between sugar and cinnamic acid such as 4-glucosylcaffeic acid [9, 10]

Table 2. FABMS of platyconin—molecular weight and fragmentation pattern

Fragment lost (possibilities)	Formula	Calculated mass	Measured		
			Mass	Deviation (ppm)	Error (ppm)
	$C_{63}H_{73}O_{37}$	1421.3830	1421.3740	8	6
(a) 1 glucosyl	$C_{57}H_{63}O_{32}$	1259.3302	1259.3280	17	2
(b) 1 caffeoyl	$C_{54}H_{67}O_{34}$	1259.3513	—	—	19
(c) 2 glucosyl	$C_{51}H_{53}O_{27}$	1097.2774	1097.2650	16	11
(d) 1 glucosyl and 1 caffeoyl	$C_{48}H_{57}O_{29}$	1097.2985	—	—	31
(e) 3 glucosyl	$C_{45}H_{43}O_{22}$	935.2246	935.2212	15	4
(f) 2 glucosyl and 1 caffeoyl	$C_{42}H_{47}O_{24}$	935.2457	—	—	26
(g) 3 glucosyl and 1 caffeoyl	$C_{36}H_{37}O_{19}$	773.1929	773.2009	10	10
(h) 2 glucosyl and 2 caffeoyl	$C_{33}H_{41}O_{21}$	773.2140			17
(i) 3 glucosyl and 2 caffeoyl	$C_{27}H_{31}O_{16}$	611.1612	611.1558	7	9

and 3-glucosylcaffeic acid [9] nor the positions of attachment on the sugar molecules. Other ions of low intensity were also observed in the spectrum of platyconin, as follows: *m/z* (rel. int.): 1405 (4), 773 (2), 771 (1), 638 (2), 409 (2), 401 (2), 345 (6) and 331 (17). In particular the ion of *m/z* 1405 was consistent with the presence of a deoxy compound (composition $C_{63}H_{73}O_{36}$, calculated 1405.3881, measured 1405.3910, error 2 ppm, deviation 16 ppm), similar to that found with violanin. It is considered that this and the other unexplained ions observed with platyconin may possibly have arisen from small impurities in the sample of platyconin used and that they do not detract from the main fragmentation pattern proposed.

EXPERIMENTAL

Mass spectrometry. Spectra were recorded, after FAB ionization, using a MM ZAB2F (HF) combined with a VG 2035 data system. The collision gas used was Xe (ion gun conditions: 8 kV and 1 mA) and the solvent used was thioglycerol ($C_3H_8O_2S$). The primary calibration was carried out using a mixture of NaI–CsI–RbI (3:1:1) and accurate mass determination carried out by data processing the relative times of arrival of ions in the spectrum of platyconin to those in the spectrum of two internal references. The internal references used were *N*-{*N*,*N*′-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-2,4,6-triiodo-3,5-phenyl-carboxamide}-5-[(2-hydroxy-1-oxopropyl)amino] *N*′-[2-hydroxy-1-(hydroxymethyl)ethyl]-2,4,6-triiodo-1,3-benzene-carboxamide and *N*-(2,3-dihydroxypropyl)-*N*′-[2-hydroxy-1-(hydroxymethyl)ethyl]-5-[2-hydroxy-1-oxopropyl) amino]-2,4,6-triiodo-1,3-benzenecarboxamide.

Materials. Violanin was isolated from *Viola tricolor* by the reported method [5]. Platyconin was isolated from *Platycodon grandiflorum* as in the previous reports [7, 8]. Internal reference materials were supplied by Dr. P. Traldi.

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REFERENCES

1. Nilsson, E. (1973) *Chem. Scr.* **4**, 49.
2. Bombardelli, E., Bonati, A., Gambetta, B., Martinelli, E. M. and Mustich, G. (1977) *J. Chromatogr.* **139**, 111.
3. Barber, M., Bordoli, R. S., Sedgwick, R. D. and Tyler, A. N. (1981) *Nature (London)* **293**, 270.
4. Williams, D. H., Bradley, C., Bojesen, G., Santikarn, S. and Taylor, L. C. E. (1981) *J. Am. Chem. Soc.* **103**, 5700.
5. Takeda, K. and Hayashi, K. (1963) *Bot. Mag.* **76**, 206.
6. Goto, T., Takase, S. and Kondo, T. (1978) *Tetrahedron Letters* 2413.
7. Saito, N., Osawa, Y. and Hayashi, K. (1971) *Phytochemistry* **10**, 445.
8. Saito, N., Osawa, Y. and Hayashi, K. (1972) *Bot. Mag.* **85**, 105.
9. Goto, T., Imagawa, H., Kondo, T. and Miura, I. (1982) *Heterocycles* **17**, 355.
10. Yoshitama, K. (1981) *Phytochemistry* **20**, 186.