Pergamon 0031-9422(95)00073-9 *Phytochemistry*, Vol. 40, No. 1, pp. 275–277, 1995
Elsevier Science Ltd
Printed in Great Britain
0031-9422/95 $9.50 + 0.00

ACYLATED ANTHOCYANINS FROM FLOWERS OF *BEGONIA*

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(*Received in revised form* 19 *December* 1994)

Key Word Index—*Begonia*; acylated anthocyanin; coumaric acid; caffeic acid; cyanidin 3-*O*-(2″-xylosyl,6″-*trans* caffeoyl)-glucoside; ^{1}H NMR; ^{13}C NMR; FAB-MS.

Abstract—Six anthocyanins were isolated by classical methods from flowers of different horticultural *Begonia* and have been identified by chemical and/or physical analysis. One is defined unambiguously as cyanidin 3-*O*-(2″-xylosyl,6″-*trans* caffeoyl)-glucoside (^{13}C and ^{1}H NMR), and the other five have been determined, by degradative methods and comparison with standards, as the *cis* form of the previous compound, and as *cis* and *trans* isomers of cyanidin 3-*O*-(2″-xylosyl,6″-*p*-coumaroyl)-glucoside and cyanidin 3-*O*-(2″-glucosyl,6″-*p*-coumaroyl)-glucoside. These pigments have also been found in different horticultural types of begonias by HPLC analysis.

INTRODUCTION

The only results of analysis of *Begonia* anthocyanins were reported by Harborne and Hall in 1964 in terms of biosides and triosides branched at the 3-position of flavylium nuclei: cyanidin 3-*O*-glucoside, cyanidin and pelargonidin 3-*O*-diglucoside, cyanidin 3-*O*-xylosyl-glucoside, cyanidin 3-*O*-rhamnosyldiglucoside and cyanidin 3-*O*-(xylosyl,rhamnosylglucoside) [1]. From a wide investigation of *Begonia* flowers by HPLC analysis, several anthocyanins were isolated. Some have been completely identified by standard procedure as pelargonidin 3-*O*-glucoside, pelargonidin 3-*O*-xylosyl-glucoside, pelargonidin 3-*O*-(xylosyl,rhamnosyl)-glucoside and pelargonidin 3-*O*-rhamnosyl-glucoside; these compounds are known anthocyanins and will not be discussed further. Three anthocyanins were not entirely elucidated but they presented the following partial structures: cyanidin 3-*O*-xylosylglucoside, cyanidin 3-*O*-dixyloside and pelargonidin 3-*O*-xylosylglucoside; each of them showing a lipophilic residue not yet elucidated [2]. Here, six anthocyanins are characterized as new natural compounds.

RESULTS AND DISCUSSION

HPLC analysis of a neutral extract from flowers of *Begonia* showed, in the lipophilic part of the profile, three major compounds **4** (R_t, 39 min), **5** (R_t, 42 min) and **6** (R_t, 44 min) and three minor compounds **1** (R_t, 34 min), **2** (R_t, 36 min) and **3** (R_t, 37 min). Classical analysis of **4**, which is a 3-*O*-substituted cyanidin, indicated the presence of aromatic acid, glucose and xylose residues [3]. A partial acid hydrolysis gave rise to 3-*O*-xylosylglucoside and 3-*O*-glucoside of cyanidin (see Experimental).

The FAB mass spectrum showed the $[M]^+$ *m/z* at 743. The ^{1}H NMR of **4** provided, at least, three major pieces of information: the aromatic region, 6–8 ppm, confirmed the presence of an aromatic acid such as caffeoyl, the coupling constants indicated a *trans* isomer (J_{H-H_4} = 16 Hz) and in the sugar region, the anomeric proton (*ca d*, 5, 67) of the 3-glucose had a large coupling constant (J = 7.26 Hz) indicating the glucose residue to be the β-anomer (Table 1) [4, 5]. The ^{13}C NMR of **4** confirmed the placement of the glucose moiety at the 3-position of the aglycone cyanidin (*d*, 144, 4); the 2″ and 6″ carbons of the glucose were linked to a xylose residue at the 2″ position (*d*, 81, 7) and with a caffeoyl part at the 6″ position (*d*, 64, 4) [Table 2] [6, 7]. Compound **4** is identified as a cyanidin 3-*O*-(2″-xylosyl,6″-*trans* caffeoyl)-glucoside; it is the first mention of such an anthocyanin in the flavonoid literature.

As concerns the elucidation of two other major compounds **5** and **6**, only FAB-MS analysis and classical methods were used. The FAB mass spectrum gave respectively the $[M]^+$ *m/z* at 757 and at 727 for **5** and **6**. The spectrometric analysis indicated, as for **4**, the presence of a substitution in the 3-position and an aromatic acid (Table 3). Partial acid hydrolysis of **5** and **6** gave rise, respectively, to cyanidin 3-*O*-diglucoside and cyanidin 3-*O*-xylosylglucoside and in both cases to the 3-*O*-monoglucoside of cyanidin. Hence, the following structures can be proposed: **5**: cyanidin 3-*O*-(2″-glucosyl,6″-*p*-coumaroyl)-glucoside and **6**: cyanidin 3-*O*-(2″-xylosyl,6″-*p*-coumaroyl)-glucoside. They represent two interesting anthocyanins; indeed though NMR analysis could not be

Table 1. ^{1}H NMR data of **4**: cyanidin 3-*O*-(2″-xylosyl,6″-*trans* caffeoyl)-glucoside (δ ppm, J Hz in DMSO-d_6–TFA)

	Cyanidin	3-*O*-glucoside	2″-*O*-xylosyl	6″-caffeoyl
CH				6.18 *d* 16
CH				7.36 *d* 16
H-1		5.67 *d* 7.26	4.66 *d* 7.70	
H-2		3.93 *m*	2.99 *t* 8	6.95 *d* 1.7
H-4	8.79 *s*			
H-5				6.74 *d* 8.12
H-6	6.66 *d* 2.14			6.84 *d* 8.9
H-8	6.82 *d* 2.14			
H-2′	7.94 *d* 2.57			
H-5′	6.99 *d* 8.97			
H-6′	8.29 *dd* 8.6 2.5			

Table 2. ^{13}C NMR data of **4**: cyanidin 3-*O*-(2″-xylosyl,6″-*trans* caffeoyl)-glucoside (δ ppm in DMSO-d_6 + TFA)

C	Cyanidin	3-*O*-glucoside	2″-*O*-xylosyl	6″-caffeoyl
2	162.6	1″ 99.6	1‴ 103.5	CO 167.5
3	144.4	2″ 81.7	2‴ 75.1	CH 126.5
4	134.6	3″ 77.6	3‴ 77.2	CH 146.6
5	158.4 (OH)	4″ 70.4	4‴ 70.6	1⁗ 126.5
6	105.6	5″ 75.3	5A‴ 67.02	2⁗ 112.5
7	169.3 (OH)	6A″ 64.4		3⁗ 146.5
8	95			4⁗ 149.5
9	156.7			5⁗ 116.4
10	114.5			6⁗ 117.3
1′	122			
2′	118.4			
3′	147.2 (OH)			
4′	155.5 (OH)			
5′	116.8			
6′	128.5			

realized, we think nevertheless that sufficient proof exists to warrant the novelty of such anthocyanins.

For the other three minor compounds **1** (R_f, 34 min), **2** (R_f, 36 min) and **3** (R_f, 37 min), the structural elucidation was obtained by co-chromatographic procedures, with previously described glycosides and their derivatives. The main experimental procedure consisted of the 20 min irradiation under UV light of **4**, **5** and **6** which gave rise to *cis* isomeric forms from the natural aromatic acid residue in the *trans* configuration; these isomerized anthocyanins were identical to the natural anthocyanins **1**–**3**, respectively [8]. Finally these six acylated cyanidins constituted useful markers of the *Begonia* genus and their occurrence will be reported in a study of over 200 begonia cultivars in a future paper.

EXPERIMENTAL

Plant material. The extraction of the anthocyanins was carried out on flowers of different horticultural groups of *Begonia*.

Isolation and purification. The phenolic pool was extracted, under reflux, by boiling in a neutral alcoholic mixt. (MeOH–EtOH, 1/1). The anthocyanins were sepd by PC with 2 successive eluting systems (BAW and AcOH 10%). Final purification was carried out on Sephadex LH-20 with elution solvent MAW. Pigment purity was checked by HPLC (Reverse phase nucleosil C-18 column 5 μm; solvent A: HCOOH 5%–H_2O, solvent B: MeOH; gradient from 20% to 60% solvent B, for 55 min) coupled to a photodiode-array detector between 250 and 600 nm.

Table 3. Characteristics of four of the new acylated anthocyanins of *Begonia*

Pigments	Photodiode array	Hydrolysis		In 0, 1% HCl–MeOH				R_f values × 100	
	λ_{max} (nm)	acid	alkaline	λ_{max} (nm)	EUV max / Evis max	Eacyl max / Evis max	E440 / Evis max	HCl 1%	BAW
5 (**2**)	285, 315, 525 285, 525	G	*p*-co.	284, 310, 529	106	73	24	62	21
6 (**3**)	285, 315, 525 285, 525	G, X	*p*-co.	284, 314, 530	106	70	21	66	15

Abbreviations: G, glucose; X, xylose; *p*-co., *p*-coumaroyl; BAW, BuOH–AcOH–H_2O (4:1:5); HPLC solvents: MeOH in AcOH 5%–H_2O.

Spectrophotometric analysis of **4**. Photodiode array: 285, 330 and 525 nm; 284, 332 and 530 nm in 0.1% HCl–MeOH; Euv max/Evis max = 115; Eacyl max/Evis max = 65; E440/Evis max = 26.

Physical analysis. ^{1}H NMR (399, 65 Hz; 1D) and ^{13}C (100.4 Hz; 1D), ^{1}H NMR was recorded in DMSO-d_6 at 25°; ^{13}C NMR in DMSO-d_6–TFA at 25°.

Acknowledgements—We thank Dr Favre-Bonvin, "Centre de Spectrométrie de Masse de Lyon", for the physical analysis and for his help in the structural elucidation.

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