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ACYLATED ANTHOCYANINS FROM THE FLOWERS OF *IPOMOEA ASARIFOLIA*

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Key Word Index—*Ipomoea asarifolia*; Convolvulaceae; acylated anthocyanins; cyanidin; *p*-coumaric acid; caffeic acid.

Abstract—Among several anthocyanins detected in the flowers of *Ipomoea asarifolia*, two acylated anthocyanins were isolated and studied using chemical, GC, mass spectrometric and extensive NMR methods (TOCSY-1D, DQF-COSY and HMBC measurements). The first anthocyanin was identified as cyanidin 3-*O*-[2-*O*-(6-*O*-(*E*-caffeoyl)-β-D-glucopyranosyl)-6-*O*-(*E*-caffeoyl)-β-D-glucopyranoside]-5-*O*-[β-D-glucopyranoside], and the new structure as cyanidin 3-*O*-[2-*O*-(6-*O*-(*E*-*p*-coumaroyl)-β-D-glucopyranosyl)-6-*O*-(*E*-caffeoyl)-β-D-glucopyranoside]-5-*O*-[β-D-glucopyranoside].

INTRODUCTION

Ipomoea asarifolia [Desv.] Roem. and Schult. (Convolvulaceae) [1] is a tropical creeping plant in permanent flowering growing in West Africa. It is very abundant in area around rivers. To pursue our studies on the pigment of several plants growing in Burkina Faso and in connection with the increasing need of natural dyes, we investigated the anthocyanins content of the flowers. Previous works achieved on the tuber of *I. batatas* (sweet potato) [2–4] and on the flowers of *I. cairica* [5, 6] have led to the isolation of acylated anthocyanins based on cyanidin. Particularly, two partly characterized diacylated anthocyanins, cyanidin 3-*O*-(dicaffeoyl-sophoroside)-5-*O*-glucoside and cyanidin 3-*O*-(coumaroylcaffeoyl-sophoroside)-5-*O*-glucoside were isolated [5]. In addition, several acetylated pelargonidin glycosides and acylated cyanidin glycosides were more recently isolated from the flowers of *I. purpurea* [7, 8]. The present paper describes the structure elucidation of two major anthocyanins isolated form the flowers of *I. asarifolia*; one of them is new.

RESULTS AND DISCUSSION

The flower petals, freshly collected and freeze-dried, were ground and extracted by MeOH in acidic conditions. Anthocyanins screening in the concd crude extract was carried out by HPLC following the method described by Gao and Mazza [9]. The elution conditions were however modified according to the polarity of the pigments under investigation. Among eight pigments, the major anthocyanins **1** (R_t = 13.21 min) and **3** (R_t = 14.96 min) showing absorption at about 530 nm were selected for further purification and structure elucidation (Fig. 1). Successive CC on Amberlite XAD-7, LH-20, and RP-8 silica gel and prep. TLC allowed their isolation as TFA salts.

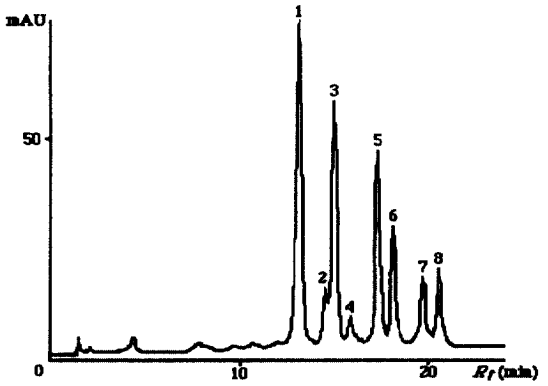

Fig. 1. HPLC chromatogram of a methanol crude extract of *I. asarifolia* flowers.

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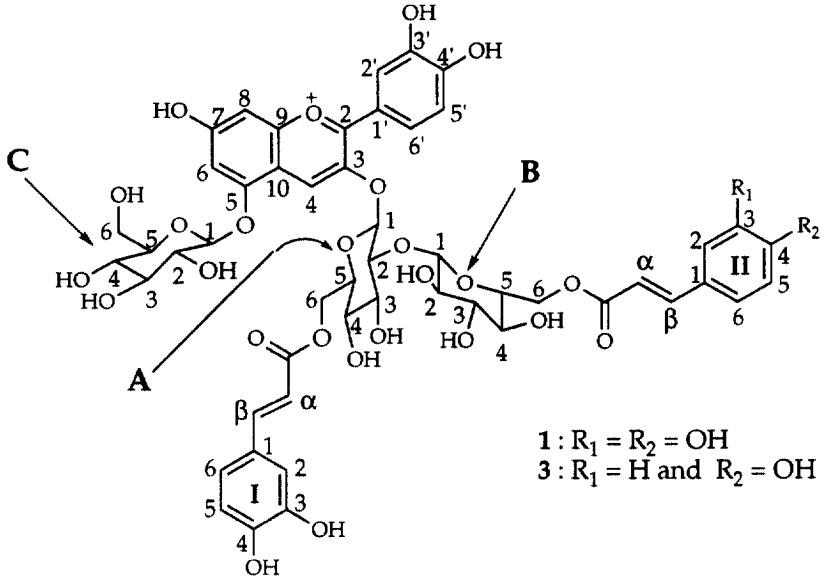

Pigment **1** was identified as 3-*O*-[2-*O*-(6-*O*-(*E*-caffeoyl)-*β*-D-glucopyranosyl)-6-*O*-(*E*-caffeoyl)-*β*-D-glucopyranoside]-5-*O*-[*β*-D-glucopyranoside] by direct comparison of its UV (Table 1), with those earlier published for the identical pigment isolated from *I. pupurea* [8].

As observed for **1**, the UV-visible spectrum of **3** showed two characteristic absorptions at 295 and 319 nm corresponding to phenolic acid residues in addition to the absorption at 528 nm (Table 1). The $E_{acyl}/E_{vis.}$ ratio (Table 1) indicated the presence of two phenolic acid moieties. The $E_{440}/E_{vis.}$ ratio (Table 1) compared to that of cyanidin suggested that the C-5 was substituted [10]; the bathochromic shift on the maximum visible wavelength, obtained by addition of $AlCl_3$, indicated the presence of vicinal free hydroxyl groups in the anthocyanidin. On acidic hydrolysis, **3** afforded only glucose that was identified by GC as the TMSSi ether. The TLC analysis of the products obtained by alkaline hydrolysis allowed the identification of caffeic and *p*-coumaric acids. This result indicated that at least one molecule of each of these acids esterified a glucosyl moiety and was not included in the chain [11]. The ESI MS showed a molecular ion at 1081 (100%) $[M^+]$ consistent with $C_{51}H_{53}O_{26}$ molecular formula, 919 $[M-caffeoyl]^+$, 773 [M−(coumaroyl-caffeoyl) or −(coumaroyl-glucosyl)]$^+$, 611 [M-coumaroyl−caffeoyl−glucosyl]$^+$, $[M-coumaroyl-caffeoyl-(glucosyl)\times 2]^+$, 287 $[M-coumaroyl-caffeoyl-(glucosyl)\times 3]^+$. In the 91H NMR spectra, the ring protons signals of cyanidin appeared at *δ* 8.85 (H-4), 8.22 (H-6′), 7.92 (H-2′), 7.00 (H-5′), 6.86 (H-6) and 6.84 (H-8). Two pairs of doublet signals at *δ* (5.96 and 7.20) and *δ* (5.81 and 7.10) with large coupling constants (J = 16 Hz) were observed, indicating the *E*-configuration of the olefinic protons of the two phenolic acid residues, caffeic and coumaric acids. Most of the sugars protons appeared between *δ* 3.0–5.5 as overlapping signals. However, the three characteristic doublets corresponding to the anomeric protons of Glc A, B and C were clearly sepd at respectively *δ* 5.32, 4.79, and 5.12. Their *β*-D-pyranoside configurations were confirmed by their large coupling constants (J = 7.2–8.4

Table 1. Chromatographic and UV-vis data of the anthocyanins **1** and **3** from *Ipomoea asarifolia* flowers

Anthocyanin	TLC ($R_f \times 100$) BAW*	TLC ($R_f \times 100$) EAFW†	HPLC R_t (min)	UV–vis λ_{max} (nm) (log ε)	$\frac{E_{acyl}}{E_{vis.max}}$	$\frac{E_{440}}{E_{vis.max}}$	$+AlCl_3$‡ Δ (nm)
1	24	35	13.21	283 (4.39), 296 (4.41) 330 (4.44), 528 (4.44)	0.95	12	+12
3	30	43	14.96	283 (4.51), 295 (4.54) 319 (4.54), 528 (4.48)	1.10	12	+11

Adsorbents: * microcrystalline cellulose F and † silica gel 60 (Merck). For HPLC and TLC conditions: see Experimental.
‡ 2–3 drops 5% $AlCl_3$ in MeOH were added.

Table 2. ^{13}C and ^{1}H NMR spectral data for anthocyanins **1** and **3** from *Ipomoea asarifolia* in (CD_3OD–TFA-d_1 (5: 1))*

		1		**3**	
Assignment		δ_H (ppm) J (Hz)	δ_C (ppm)	δ_H (ppm) J (Hz)	δ_C (ppm)
Aglycone	2		165.0		164.8
	3		145.0		145.0
	4	8.4, *s*	138.7	8.85, *s*	139.0
	5		156.9		156.7
	6	6.85, *d* (1.8)	105.6	6.86, *d* (1.8)	105.6
	7		170.0		170.0
	8	6.83, *d* (1.8)	97.6	6.84 *d* (1.8)	97.6
	9		156.6		157.1
	10		113.0		113.0
	1′		120.0		120.7
	2′	7.92, *d* (1.8)	118.6	7.92, *d* (1.8)	118.5
	3′		147.3		147.4
	4′		156.3		156.4
	5′	7.00, *d* (8.4)	117.5	7.00, *d* (8.4)	117.5
	6′	8.19, *dd* (8.4, 1.8)	129.1	8.22, *dd* (8.4, 1.8)	129.2
Glucose A	1	5.36, *d* (7.4)	102.9	5.32, *d* (7.2)	102.9
	2	4.00, *dd* (7.4, 9.0)	83.7	3.98, *dd* (7.2, 9.0)	83.9
	3	3.81, *t* (9.0)	76.0	3.78, *t* (9.0)	75.9
	4	3.60, *dd* (9.0, 9.6)	71.1	3.59, *dd* (9.0, 9.6)	71.2
	5	6.76, *m* (9.6, 5.5)	77.7	3.74, *m* (9.6, 5.4)	77.7
	6a	4.45, *d* (5.5, 12)	64.1	4.43, *dd* (5.4, 12.0)	64.1
	6b	4.11, *d* (4.4)	64.1	4.10, *d* (12.0)	64.1
Glucose B	1	4.81, *d* (7.0)	106.0	4.79, *d* (8.4)	106.1
	2	3.52, *dd* (7.0, 9.0)	75.3	3.50, *dd* (8.4, 9.6)	75.4
	3	3.50, *dd* (9.0, 6.8)	77.3	3.44, *dd* (9.6, 7.2)	77.4
	4	3.46, *dd* (6.8, 6.0)	71.2	3.46, *dd* (7.2, 6.6)	71.1
	5	3.48, *dd* (6.0, 2.4)	78.6	3.52, *dd* (6.6, 3.4)	78.7
	6	4.32, *dd* (2.4, 12.0)	63.9	4.24, *dd* (3.4, 12.0)	64.0
		4.28, *d* (12.0)	63.9	4.24, *d* (12.0)	64.0
Glucose C	1	5.16, *d* (7.6)	102.4	5.12, *d* (7.8)	106.2
	2	3.80, *dd* (7.6, 9.0)	74.5	3.76, *dd* (7.8, 9.0)	75.5
	3	3.65, *t* (9.0)	76.0	3.61, *t* (9.0)	76.4
	4	3.53, *dd* (9.0, 9.6)	71.4	3.50, *dd* (9.0, 9.6)	71.4
	5	3.70, *m* (9.6, 2.4)	77.8	3.57, *m* (9.6, 2.4)	77.8
	6a	3.73, *dd* (2.4, 12.0)	62.4	3.79, *dd* (2.4, 12.0)	62.5
	6b	4.02, *d* (12.0)	62.4	4.03, *d* (12.0)	62.5
Acyl I		*E*-Caffeoyl		*E*-Caffeoyl	
	1		127.3		127.3
	2	6.82, *d* (1.8)	115.2	6.63, *d* (1.8)	115.2
	3		146.4		146.4
	4		149.5		149.6
	5	6.72, *d* (8.4)	116.5	6.75, *d* (8.4)	116.5
	6	6.64, *dd* (8.4, 1.8)	123.4	6.68, *dd* (1.8, 8.4)	123.3
	α	5.94, *d* (16.0)	114.4	5.96, *d* (16.0)	114.4
	β	7.18, *d* (16.0)	147.1	7.20, *d* (16.0)	147.1
	C=O		169.0		169.0
Acyl II		*E*-Caffeoyl		*E*-Coumaroyl	
	1		127.0		126.4
	2	6.60, *d* (1.8.0)	115.0	7.03, *d* (8.4)	130.9
	3		146.4	6.67, *d* (8.4)	116.8
	4		149.3		161.0
	5	6.61, *d* (8.4)	116.3	6.67, *d* (8.4)	116.8
	6	6.50, *dd* (8.4, 1.8)	122.8	7.03, *d* (8.4)	130.9
	α	5.75, *d* (16.0)	114.3	5.81, *d* (16.0)	114.3
	β	7.00, *d* (16.0)	146.6	7.10, *d* (16.0)	146.3
	C=O		168.6		168.7

* Assignments determined by COSY, HMBC, and TOCSY 1D.

Hz). ^{1}H NMR, ^{13}C NMR, TOCSY 1D spectra, DIFFNOE and HMBC experiments (Table 2) confirmed that the structural features of **3** were identical to those of **1** except for caffeic acid (acyl II) which was replaced by coumaric acid. This modification was confirmed by the ^{13}C NMR spectra which showed clearly signals at δ 130.9 and 116.8 with double intensities corresponding to (C-2, C-6) and (C-3, C-5) of *p*-coumaric acid. Finally, pigment **3** was identified as cyanidin 3-*O*-[2-*O*-(6-(*E*-*p*-coumaroyl)-β-D-glucopyranosyl)-6-*O*-(*E*-caffeoyl)-β-D-glucopyranoside]-5-*O*-[β-D-glucopyranoside].

EXPERIMENTAL

General procedures

TLC was carried out on plates of microcrystalline cellulose F (Merck) and silica gel (Merck) using three mobile phases: EAFW (EtOAc–HOAc–HCO_2H–H_2O, 100:11:11:26) and BAW (1-BuOH–HOAc–H_2O, 4:1:5 upper phase) for anthocyanins and toluene–HOAc–H_2O (40:10:5) for the phenolic acids. These latest were detected under UV light. HPLC was performed on a Waters Associates apparatus using a Nova Pak C_{18} column (160 × 4 mm, 4 μm) at 25° with a flow rate of 0.8 ml min^{-1} and monitoring with a photo diode array detector operating at 520 nm. A linear gradient solvent system was applied for 20 min from 40 to 75% of B (MeOH–H_2O–HCO_2H, 75:24.5:0.5) in A (H_2O–HCO_2H, 60:1); an isocratic elution was then maintained for 10 min. UV–vis spectra were recorded on a UV-265 FS spectrophotometer (Shimadzu) in 0.01 N HCl–MeOH (from 250 to 600 nm). Mass spectra were measured in MeOH with an electrospray LC-mass spectrometer (ESI) from Micromass. ^{13}C NMR (150 MHz) and ^{1}H NMR (600 MHz) spectral data were obtained on a Varian spectrometer in CD_3OD–TFA-d_1 (0.5:0.1 ml). When the signals of the anomeric protons of the investigated anthocyanins were partially overlapped by the signal of the dissolved H_2O, an additional shift to lower magnetic field of this signal was produced by adding 30 μl of TFA-d_1.

Plant material

The flowers of *Ipomoea asarifolia* [Desv.] Roem. and Schult. (Convolvulaceae) [1] were freshly harvested in August 1995 in the station of the University of Ouagadougou where a voucher specimen has been deposited. They were immediately freeze-dried and ground; they were stored in the dark and kept in a dry place.

Extraction and isolation of anthocyanins

10 g of sample were extracted with 200 ml of 1% TFA–MeOH at 5° overnight. The filtration residue was washed with 100 ml of solvent. The filtrates were pooled and concd under vacuum to dryness under 35°. 10 ml of (0.5% TFA–H_2O)/MeOH (7:3) was added. The soln was filtered and concd to 5 ml. The mixt. was transferred on an Amberlite XAD-7 column that was first washed with 0.5% TFA–H_2O. Anthocyanins were eluted with (0.5% TFA–H_2O)/MeOH (3:7). The resulting soln was concd and subjected to a Sephadex LH-20 CC using [(0.5% TFA–H_2O)–MeOH, 7:3)]. Each fr. was fractionated twice on a LiChroPrep RP-8 (440–37, 40–60 μm, Merck) column using (0.5% TFA–H_2O)/MeOH (6:4) at a flow rate of 3 ml min^{-1}. Fractions (about 10 ml) were collected and controlled by TLC on a silica gel with EAFW as solvent (Table 1). The major frs were concd and the purification was achieved by prep. TLC using EAFW as mobile phase. The isolated bands were eluted with 0.5% TFA–MeOH, concd and rechromatographed on a RP-18 silica gel using (0.5% TFA–H_2O)/MeOH (6:4). The eluates were concd and freeze-dried to give **1**, **3** and **5** as TFA salts.

Hydrolysis procedures and identification of the acyl and the sugar moieties

Alkaline hydrolysis was performed under nitrogen according to the method described by Terahara *et al.* [12]. Prep. TLC on silica gel was carried out to purify the acyl moieties before the final identification by TLC in comparison with standards. Acidic hydrolysis were performed by dissolving 2 mg of each pigment in 4 ml of 2 N TFA–H_2O in a sealed vial and heating at 110° for 45 min. The anthocyanidins were extracted twice by 0.5 ml of 3-methyl-2-butanol. The resulting aq. frs were evaporated to dryness. The residues were taken for derivatization in pyridine/*N*-(trimethylsilyl) imidazole (2:1) and heated at 60° for 15 min. The TMSSi of sugar residues were identified by GC on a CP-Sil-8-CB Chromapack column (25 m × 0.32 mm I.D.) in comparison with authentic sugar standards.

Anthocyanin 1. 1097 (100), 935 [M − caffeoyl]$^+$ (35), 773 [M − (caffeoyl) × 2 or caffeoylglycosyl)] (1), 611 [M − (caffeoyl) × 2 − glucosyl]$^+$ (3), 449 [M − (caffeoyl-glycosyl) × 2]$^+$ (25), 287 [M − (caffeoyl) × 2 − (glucosyl) × 3]$^+$ (54).

Anthocyanin 3. 1081 [M$^+$] (100), 919 [M − caffeoyl]$^+$ (41), 773 [M − (coumaroyl-caffeoyl) or −(coumaroyl-glucosyl)]$^+$ (1), 611 [M − coumaroyl − caffeoyl − glucosyl]$^+$ (3), 449 [M − coumaroyl − caffeoyl − (glucosyl) × 2]$^+$ (37), 287 [M − coumaroyl − caffeoyl − (glucosyl) × 3]$^+$ (52).

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