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New 3-Deoxyanthocyanidins from Leaves of *Arrabidaea chica*

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Two new 3-deoxyanthocyanidins, 6,7,3′,4′-tetrahydroxy-5-methoxyflavylium and 6,7,4′-trihydroxy-5-methoxyflavylium, and the pigment carajurin, which has been previously identified, were isolated from dried leaves of *Arrabidaea chica*, a creeper native to the American tropics. The structures of the components were elucidated by ^{1}H- and ^{13}C-NMR spectroscopy and HPLC-MS, including X-ray crystallographic analysis for carajurin. Copyright © 2002 John Wiley & Sons, Ltd.

Keywords: High speed countercurrent chromatography; HPLC-MS; NMR; X-ray; carajurin; 3-deoxyanthocyanidin; *Arrabidaea chica*.

INTRODUCTION

Arrabidaea chica [H.B.K] (Verlot), syn. *Bignonia chica* (Bignoniaceae) is a climbing plant widely distributed in the forests of tropical South America. Among 17 species of the genus *Arrabidaea* found in Colombia, *A. chica* is the best known (Torres, 1983). The leaves of this plant are traditionally used by the indigenous populations of the Orinoco and Amazon for body painting and for dyeing fibres black, red or yellow (Patiño, 1967). Moreover, leaves are also used as an anti-inflammatory agent and as a remedy for various diseases (intestinal colic, bleeding, diarrhoea, leucorrhoea, anaemia and leukaemia) and the dye is used to treat skin infections (Correa, 1984; Duke and Vasquez, 1998).

As a result of studies by Chapman *et al.* (1927) on carajura (an extract prepared from the leaves of *A. chica*) the physicochemical characteristics of the main constituent, carajurin (**3**), were described and a 3-desoxyanthocyanidin structure was proposed. In this work a second pigment, carajurone, was found but its structure was not fully elucidated. Although a synthesis of carajurin was published in 1953 by Ponniah and Seshadri, and its occurrence in leaves and flowers of *Bignonia chica* was confirmed by chromatographic and UV-visible spectral data more recently (Scogin, 1980), some authors have considered that the structure of this pigment was only partially described (Timberlake and Bridle, 1975; Mazza and Miniati, 1993). In the framework of our research on Colombian archaeological textiles, this dye plant has been re-investigated by HPLC-photodiode array (PAD) analysis (Devia, 1966; Devia and Wouters, 1996). In the present paper we report on the isolation and structural determination of two new 3-deoxyanthocyanidins as well as on the confirmation of the structure of carajurin which was isolated by high speed countercurrent chromatography (HSCCC).

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EXPERIMENTAL

Plant material. Leaves of *Arrabidaea chica* were collected in the neighbourhood of Honda, Tolima Department, Colombia in May 1997. The specimen was identified in the Colombia National Herbarium in Bogotá, and a voucher specimen of the plant is deposited in the herbarium of the Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá under the registration number 443777.

Extraction and isolation. Dried leaves (100 g) were macerated in an aqueous solution and kept at room temperature for 15 days in the dark. After this time, the solid material was separated by decantation, washed with petroleum spirit (40–60°C boiling point) and dried to give 36 g of a crude extract. A part of this extract (18 g) was submitted to partition with chloroform until the major soluble pigment was completely removed, and the chloroform extract was evaporated under reduced pressure and dried at 30°C in vacuum to obtain 3.6 g of a red solid. A fraction of this material was purified by HSCCC (see below) to give the major compound, carajurin (**3**).

A second part of the original crude extract (12 g) was extracted with methanol:1 M hydrochloric acid (95:5; 2 × 500 mL; 24 h) at 4°C, and filtered before concentration under reduced pressure and further washing with chloroform (which unexpectedly extracted **3**). Finally, the resulting acidic solution was fractionated by column chromatography to yield pigments **1** and **2**. For this last separation, a glass column (30 × 2.0 cm i.d.) was prepared with a mixture of Silica gel 60 for column

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chromatography (Merck, Darmstadt, Germany; 0.063–0.200 mm; 70%), Polyclar AT (20%), and Kieselgel 60 for TLC (Merck); (10%) according to the procedure of Bourzeix *et al.* (1982). Pigment separation was monitored by TLC and HPLC (see below).

High speed countercurrent chromatography. An Ito Multi-layer Coil separator-extractor (PC Inc., Potomac, Maryland, USA) was equipped with a preparative coil of PTFE tubing (2.6 mm i.d.; 400 mL total volume). The separation was carried out at a speed of 800 rpm. The system solvent consisted of ethyl acetate:*n*-hexane:-methanol:water (2:1:1:1.5). The elution mode was tail-to-head with the heavy layer being the stationary phase with isocratic elution: the flow rate was 2.5 mL/min. Plant extracts were dissolved in the mobile phase and injected into the system by loop injection (10 mL loop volume). The amount of sample injected was 180 mg. Eighty-five fractions (10 mL each) were collected using a Tokyo Rikakiicai (Tokyo, Japan) collector. Crude carajurin (17 mg) was detected by TLC in fractions 19–25 (80% purity as determined from the relative peak areas detected at 254 nm following HPLC), and these bulked fractions were finally purified on a Sephadex LH-20 column using methanol as a solvent to obtain 10 mg of pure **3**. A sample of pure carajurin was re-dissolved in chloroform and orange-red crystals (needles) were obtained following slow evaporation.

Thin layer chromatography. TLC was carried out on plastic-backed microcrystalline cellulose layers (Macherey-Nagel, Düren, Germany) eluted with *n*-butanol:acetic acid:water (4:1:5; upper phase) and concentrated hydrochloric acid:formic acid:water (12.45:11.85:25.7). The dried plates were examined in visible light after spraying with ammonia, and in UV light at 365 nm after spraying with a solution containing 10 g/L of diphenylboric acid aminoethyl ester and 50 g/L of macrogol 400 in methanol.

Analytical high-performance liquid chromatography. Analytical HPLC was carried out using a Waters (Milford, MA, USA) model 625 solvent delivery system coupled to a Waters 996 PAD detector, operating in the range 254–800 nm. The column was a Lichrosorb RP-18 (Merck; 125 × 4.1 mm i.d.; 5 μm), and the mobile phases were: (A) methanol, (B) methanol:water (1:9), and (C) 5% phosphoric acid (all reagents were analytical grade). The elution profile consisted of an isocratic elution of A:B:C (23:67:10) for 3 min, followed by a linear gradient to A:B:C (90:0:10) in 26 min; the flow rate was 1.2 mL/min. Detection was performed at 254, 280, 330 and 470 nm using a photodiode array spectrophotometer, and UV–visible absorption spectra were measured on-line in the range 200–700 nm.

Spectroscopic measurements. UV and visible spectra of the isolated pigments were recorded in a Kontron Uvikon (Milan, Italy) 922 spectrophotometer and recorded in 0.1% hydrochloric acid in methanol and following the addition of two or three drops of 5% aluminium chloride.

NMR analyses were carried out with a Bruker (Wissembourg, France) model DRX 400 Avance spectrometer at 400.13 MHz (^{1}H) and 100.62 MHz (^{13}C) at room temperature. The chemical shifts were determined relative to tetramethylsilane at 0 ppm. The spectral assignments have been established from the signals in COSY, HMQC and HMBC spectra. All the programs used in performing the two-dimensional NMR experiments were from the Bruker library. One sample of crystals of **3** was dissolved in $CDCl_3$ and analysed, whilst another was dissolved in CD_3OD containing one drop of DCl and analysed after 1 h (as carajurin flavylium). The pigments **1** and **2** were dissolved in CD_3OD containing one drop of DCl.

Positive ESI-MS-MS spectra of carajurin were obtained using a VG (Manchester, UK) platform micromass 7070f, single quadruple instrument (30 eV).

High performance liquid chromatography–mass spectrometry. A Hewlett-Packard (Waldbronn, Germany) model 1100 chromatograph was used, provided with a quaternary pump and a photodiode array detector. The column was a Phenomenex (Hosbach, Germany) LUNA C18 (15.0 × 0.46 cm i.d.; 5 μm) and the solvents were: (A) 1% trifluoroacetic acid, (B) methanol, and (C) water. The elution profile consisted of an isocratic elution of A:B:C (10:40:50) for 3 min, followed by a linear gradient to A:B:C (10:90:0) in 25 min, isocratic elution for 7 min, and a linear gradient to A:B:C (10:40:50) in 10 min, all at a flow rate of 0.5 mL/min. The injection volume was 50 μL. A first detection was made by PAD at an analytical wavelength of 470 nm, followed by a second detection in the MS. Mass spectrometry was performed on a Finnigan (San Jose, CA, USA) model LCQ equipped with an API source, using an electrospray ionisation (ESI) interface. Data treatment was carried out with a Finnigan Navigator 1.1. The HPLC system was connected to the probe of the MS via the UV cell outlet using PEEK tubing. Both the auxiliary and the sheath gas were a mixture of nitrogen and helium. ESI conditions were optimised using compounds **1, 2** and **3**; the capillary temperature was 220°C and the capillary voltage 3V. Spectra were recorded in the positive ion mode and the MS was programmed to perform a series of three scans—a full mass, a zoom scan of the most abundant ion in the first scan, and an MS-MS of the most abundant ion using a collision energy of 30eV.

X-ray crystallography. Diffraction data were measured at room temperature using a Stoe-Siemens (Darmstadt, Germany) model AED four-circle diffractometer. Intensity data were corrected for Lorentz polarisation effects, and for absorption (semi-empirical method, scan). The structure was solved by direct methods (SHELXS-97a; Sheldrick, 1997a). The least-squares refinement (SHELXS-97b; Sheldrick, 1997b) included independent position parameters and anisotropic thermal coefficients for all non-hydrogen atoms and two global isotropic thermal parameters for hydrogen atoms (one for non-methyl and one for methyl atoms). During refinement, H atoms were included as riding atoms at calculated positions. The final difference Fourier map had no significant features. Atomic scattering factors were taken from *International Tables for X-ray Crystallography* (1992).

RESULTS AND DISCUSSION

The HPLC chromatogram of a crude extract of the leaves

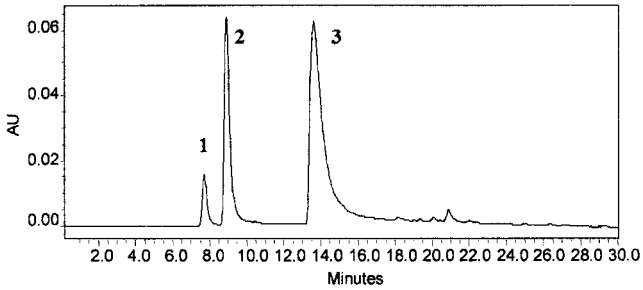

Figure 1. HPLC (analytical system) chromatogram of an extract of *Arrabidaea chica* detected at 470 nm. Key to peak identity: **1**, pigment **1**; **2**, pigment **2**; and **3**, carajurin (**3**). (For chromatographic protocol see Experimental section.)

of *Arrabidaea chica* showed the presence of three major anthocyanins (Fig. 1).

Purification of carajurin (3)

HSCCC is a support-free all-liquid chromatographic technique that is widely used in natural product analysis by virtue of its gentle operating conditions. Separation of **3** was achieved with a solvent system of ethyl acetate:*n*-hexane:methanol:water (2:1:1:1.5) with the less dense layer acting as the stationary phase. The sample load was 180 mg of dried chloroform extract, re-dissolved in the mobile phase, and this yielded 10 mg of **3**. Extraction with petrol prior to HSCCC separation enabled the separation of relatively pure **3**. Minor impurities were further eliminated by purification on Sephadex LH-20. HSCCC offers several advantages compared to preparative HPLC. As no solid stationary phase is used, no irreversible adsorption on active surfaces can occur (Degenhardt *et al.*, 2000). In the case of **3**, the solvent system achieved the separation of its quinonoidal form. This structure was elucidated by positive ESI-MS-MS spectrometry, ^{1}H-NMR and ^{13}C-NMR spectroscopy and X-ray crystallography.

Purification of pigments 1 and 2

Once the carajurin, and other substances, had been eliminated by chloroform from the dried acid–methanol extract, separation by column chromatography on a mixed stationary phase was carried out. In the first fractions to be eluted, the principal constituent was compound **2** whilst the final fractions contained compound **1**. Both compounds were purified by repeated fractionation and then dried under reduce pressure and lyophilised.

Determination of the structure of carajurin

Crystal data. C_{17} H_{14} O_5, molecular weight = 298.28, $T = 293$ (2). Carajurin crystallised in the triclinic system, space group P-1 with $Z = 2$ molecules in a unit cell of dimensions (a) 8.150 (1), (b) 8.195 (1), (c) 10.912 (1) Å, (α) 96.736 (8), (β) 100.780 (9) and (γ) 99.235 (8)°; crystal size 0.53 × 0.11 × 0.11 mm. The refinement (Sheldrick, 1997b) was made in F^2. The final conventional r value, calculated for the $I > 2\sigma$ (I) observed reflections, was 0.041. The complete results are available as a CIF format file which can be obtained from the authors. An ORTEP view (Burnett and Johnson, 1996; displacement ellipsoidal at 50% probability levels) is given in Fig. 2.

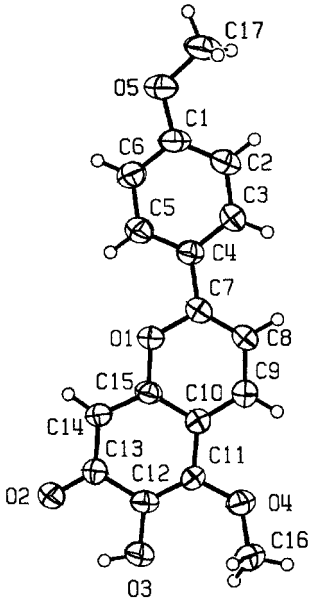

Figure 2. Crystal structure of carajurin (**3**; ORTEP view).

Positive ESI-MS-MS showed a molecular ion $[M]^+$ at *m/z* 299.3, supporting the molecular formula $C_{17}H_{14}O_5$ (Ponniah and Seshadri, 1953), with fragments at *m/z* 284.2 and 269.5, suggesting the presence of two methyl groups (Table 1). Carajurin showed λ_{max} at 294.7 and 468.1 nm in the UV–visible spectrum measured directly from the HPLC column, 295 and 469 nm measured in 0.1% hydrochloric acid in methanol, and 295 and 469 nm following the addition of three drops of 5% aluminium chloride. The visible wavelength maximum at 460 nm for **3** (1×10^{-5} M) in 0.1 M hydrochloric acid was similar to the value previously reported by Ponniah and Seshadri (1953) for the diluted solution of synthetic and natural carajuridin in 1% hydrochloric acid. In a solution of acidic methanol (1% hydrochloric acid), **3** exhibited a λ_{max} at 469 nm, lower than the previously reported value of 473 nm (Scogin, 1980) for the same pigment in acidic methanol. The PAD spectrum obtained in the HPLC solvent (methanol:water:phosphoric acid) showed a similar trend.

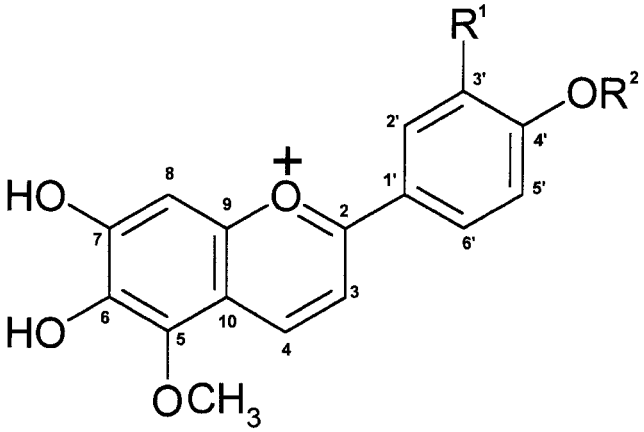

Figure 3. Structures of anthocyanidins from *Arrabidaea chica*. Pigment **1**, R_1 = OH, R_2 = H; pigment **2**, R_1 = H, R_2 = H; carajurin (**3**), R_1 = H, R_2 = CH_3.

Table 1. Chromatographic and spectral data for the anthocyanidins in *Arrabidaea chica*

Compound	TLC[a] R_f values		HPLC[a] t_R (min)		λ_{max} (nm)		Shift with aluminium chloride	ESI-MS (*m/z*)	Mass fragments
	Hydrochloric acid:formic acid:water	*n*-butanol:acetic acid:water	Analytical HPLC	HPLC-MS	Analytical HPLC	0.1% hydrochloric acid in methanol			
1	0.22	0.23	8.15	13.90	481.6	492	+	301.2	286.2
2	0.38	0.39	9.51	15.49	468.3	474		285.3	270.2
Carajurin **3**	0.47	0.45	14.23	19.40	468.1	469		299.3	284.2 269.5

[a] For chromatographic protocols see Experimental section.

No UV absorbance maximum was found in the 310 nm range, indicating no acylation with hydroxy aromatic acids. No bathochromic shifts were observed after the addition of aluminium chloride, which indicates the absence of a free ortho-dihydroxyl group in **3**. Signals at 1648 and 1576 cm^{-1} in the IR spectrum, assigned to 7-ceto and 6-hydroxy in the A ring, are compatible with the quinonoidal structure (Fig. 3).

The proton signals of **3** (in $CDCl_3$) were assigned using information regarding coupling constants (COSY) and chemical shifts. The ^1H spectrum showed the presence of seven aromatic protons and two methoxy groups. The protonated carbons were assigned by their direct correlations observed in HMQC. The HMBC spectrum confirmed these attributions and allowed the assignment of the quaternary carbons, with a 7-ceto, 6-hydroxy and 5-methoxy in the A ring (Table 2).

Determination of the structure of carajurin flavylium

The presence of two signals, corresponding to three-proton singlets, at δ 3.98 and 4.14 in the ^1H-NMR (Table 3) suggested the presence of two methoxy groups; one of them (δ 3.98) showed correlation with an aromatic carbon at δ 168.4 (Table 4) assigned to C-4′, and the other showed correlation with an aromatic carbon at δ 144.1 attributed to C-5. Two doublets in the ^1H-NMR spectrum at δ 7.25 and 8.40, each integrating for two protons, had the same coupling constant ($J = 9.0$ Hz). The HMBC spectrum showed a correlation between these two signals and C-1′ (δ 123.6) and C-4′ (δ 168.4). The protons could be attributed to the aromatic protons of B ring H-2′/H-6′ and H-3′/H-5′. Protons at δ 8.40 were assigned to H-2′/H-6′ because of a correlation with C-2 in the HMBC. Carbons C-2′, C-3′, C-5′ and C-6′ were readily located through HMQC correlations.

Two doublets at δ 9.13 and 8.29 with the same coupling constant ($J = 8.8$) were attributed to H-3 and H-4, respectively. Both protons showed a correlation with C-2 (δ 171.6), one of them (H-3) with a carbon at δ 155.1 assigned to C-10, and the other (H-4) with a carbon at δ 118.3 attributed to C-9. Carbons C-3 and C-4 were located from HMQC correlations with H-3 and H-4. An aromatic proton at δ 7.41 was assigned to H-8. The position was elucidated because of a correlation between this proton and the four aromatic carbons (142.4; 163.9; 118.3; and 155.1) in the HMBC through ^2J and ^3J. The signal at δ 100.2 was attributed to C-8 because of an HMQC correlation with H-8.

Determination of the structure of pigment 1 (6,7,3′,4′-tetrahydroxy-5-methoxyflavylium)

The UV-visible spectrum of **1** (Fig. 4) exhibited a bathochromic shift on addition of aluminium chloride (5% in methanol) which indicated the presence of two free oxygen functions in the B-ring (Ribérau-Gayon, 1968), a pattern confirmed by NMR analysis (Tables 3 and 4). Compound **1** showed λ_{max} at 492 and 304 nm in 0.1% hydrochloric acid in methanol, and a lower R_f than compounds **2** and **3** in *n*-butanol:acetic acid:water and hydrochloric acid:formic acid:water (Table 1). The electrospray MS (positive mode) showed a molecular ion at *m/z* 301.2 corresponding to a $C_{16}H_{13}O_6$ and a signal

Table 2. $^{1}H-$ and $^{13}C-$NMR data[a] for carajurin (3)

Position	^{1}H[b,c]	Homonuclear correlation (H/H)	^{13}C[b]	HMBC (H/C correlation)[d]
2	—	—	158.9	—
3	6.97 (d, 7.7)	4	102.6	C10, C2
4	7.99 (d, 7.7)	3, 8	133.9	C5, C9, C2
5	—	—	135.0	—
6	—	—	139.9	—
7	—	—	176.7	—
8	6.53 (d, 0.8)	4	98.6	C10, C6, C9, C7
9	—	—	156.8	—
10	—	—	118.1	—
1′	—	—	123.3	—
2′–6′	7.86 (d, 8.8)	(3′–5′)	127.7	C2, C4′, (C2′–6′)
3′–5′	7.02 (d, 8.8)	(2′–6′)	114.7	C1′, C4′, (C3′–5′)
4′	—	—	162.6	—
5-OCH_3	4.10 (s)	—	60.4	C5
4′- OCH_3	3.89 (s)	—	55.6	C4′

[a] Data recorded at 400/100 MHz in $CDCl_3$.
[b] Chemical shift (δ) in ppm from TMS.
[c] Multiplicities and coupling constants in Hz are shown in parentheses.
[d] Correlation from H to the indicated carbons.

Table 3. ^{1}H-NMR data[a] for compounds 1 and 2 and carajurin (3)

Position	Compound 1[b]	Compound 2[b]	Carajurin (3)[b]
3	9.03 (d, 8.6)	9.07 (d, 8.8)	9.13 (d, 8.8)
4	8.18 (d, 8.6)	8.23 (d, 8.8)	8.29 (d, 8.8)
8	7.37 (s)	7.38 (s)	7.41 (s)
2	7.78 (d, 2)	8.32 (d, 8.8)	8.40 (d, 9)
3		7.08 (d, 8.8)	7.25 (d, 9)
5	7.60 (d, 7.6)	7.08 (d, 8.8)	7.25 (d, 9)
6	7.89 (dd, 7.6 – 2)	8.32 (d, 8.8)	8.40 (d, 9)
5-OCH_3	4.11 (s)	4.12 (s)	4.14 (s)
4′-OCH_3			3.98 (s)

[a] Data recorded at 400 MHz in CD_3OD: DCl.
[b] Chemical shift (δ) in ppm from TMS; multiplicities and coupling constants in Hz are shown in parentheses.

at 286.2 that supported the presence of one methyl group in the structure.

The presence of a three-proton singlet at δ 4.11 in the ^{1}H-NMR and of a signal at δ 62.6 in the ^{13}C-NMR was in agreement with a methoxy group. These protons showed correlation with an aromatic carbon at δ 143.2 assigned to C-5. The presence of two doublets at δ 7.78 and 7.60 with coupling constants $J = 2.0$ and 7.6, respectively, and a double doublet at δ 7.89 with coupling constants $J = 7.6$ and 2.0 indicated a substitution of the B-ring at C-3′ and C-4′. The signal at δ 7.78 attributed to H-2′ showed a correlation with C-2 (δ 171.0) and C-4′ (δ 155.7). The signal attributed to H-5′ (δ 7.60) showed correlation with C-3′ (δ 121.8) and the signal assigned to H-6′ (δ 7.89) showed correlation with C-4′ (δ 155.7). Carbons C-2′, C-5′ and C-6′ were readily assigned from HMQC correlations. Two doublets at δ 9.03 and 8.18 with the same

Table 4. ^{13}C-NMR data[a] for compounds 1 and 2 and carajurin (3)

Position	Compound 1[b]	HMBCc	Compound 2[b]	HMBCc	Carajurin (3)[b]	HMBCc
2	171.0	2′,4,3	171.3	3,4,2′6′	171.6	3′5′,3,4,6′2
3	147.8		148.2		149.4	
4	112.7		112.4		113.4	
5	143.2	4,3, 5-OCH_3	143.4	5-OCH_3	144.1	5-OCH_3
6	141.2	8	141.4	8	142.4	8
7	162.1	8	162.5	8	163.9	8
8	99.4		99.4		100.2	
9	116.7	8,4	117.0	4,8	118.3	8,4
10	153.6	8,3	154.0	3,8	155.1	8,3
1	108.8		121.5	3′5′	123.6	3′5
2	115.7		133.0		133.4	
3	121.8	5	118.4		117.7	
4	155.7	2′ 6′	167.0	2′ 6′	168.4	2′6, 4′-OCH_3
5	117.9		118.4		117.7	
6	125.0		133.0		133.4	
5-OCH_3	62.6		62.5		63.4	
4′-OCH_3					57.6	

[a] Data recorded at 100 MHz in CD_3OD: DCl.
[b] Chemical shift (δ) in ppm from TMS.
[c] Correlation from H to the indicated carbons.

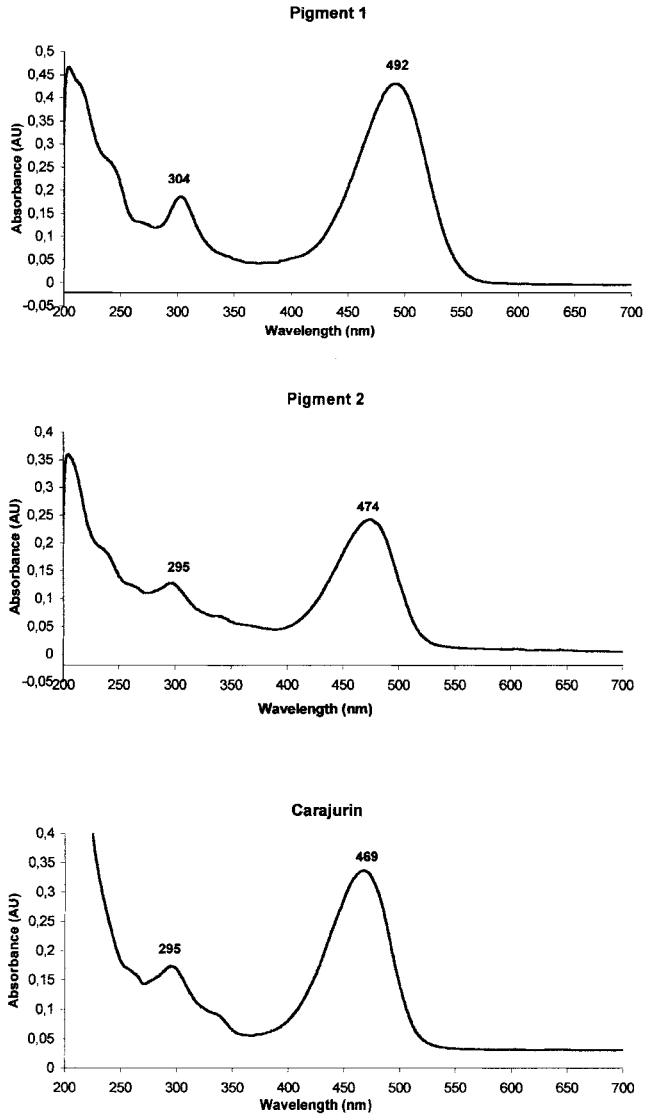

Figure 4. UV and visible spectra of pigments **1** and **2** and carajurin (**3**) recorded in 0.1% hydrochloric acid in methanol.

coupling constant ($J = 8.6$) were attributed to H-3 and H-4, respectively. The correlation of these protons confirmed the chemical shift of C-2 (δ 171.0) and allowed the assignments of C-10 (δ 153.6) and C-9 (δ 116.7). Carbons C-3 and C-4 were assigned from HMQC correlations with H-3 and H-4. A one proton singlet at δ 7.37 was finally attributed to proton H-8 (ring A). The position was elucidated from correlation between this proton and C-6, C-7, C-9 and C-10 in the HMBC through ^2J and ^3J. Moreover, the signal at δ 99.4 was assigned to C-8 from a HMQC correlation with H-8.

Determination of the structure of pigment 2 (6,7,4′-trihydroxy-5-methoxyflavylium)

The UV-visible spectrum of **2** (Fig. 4) revealed λ_{max} at 295 and 475 nm in 0.1% hydrochloric acid in methanol with no bathochromic shift on the addition of aluminium chloride. The electrospray MS (positive mode) showed a molecular ion at *m/z* 285.3 [M+] corresponding to a $C_{16}H_{13}O_5$, whilst a signal at 270.3 represented a fragment of the form [M^+-15] and supporting the presence of one methyl group in the structure. The molecular ion at *m/z* 285.3 [M+] supported the molecular formula ($C_{15}H_9O_5.OCH_3$) proposed for the second component of the pigment extracted from *A. chica* and named carajurone by Chapman *et al.* (1927).

The presence of signals (3H, s) at δ 4.12 in the ^{1}H-NMR and at δ 62.5 in the ^{13}C-NMR (Tables 3 and 4) suggested the presence of a methoxy group. These protons showed correlation with an aromatic carbon at δ 143.4 assigned to C-5. Two doublets in the ^{1}H-NMR spectrum at δ 7.08 and 8.32, each integrating for two protons, had the same coupling constant ($J = 8.8$ Hz). The HMBC spectrum showed a correlation between these two signals and C-1′ (δ 121.5) and C-4′ (δ 167.0), and allowed the assignments of the aromatic protons of the B ring H-2′/H-6′ and H-3′/H-5′. Protons at δ 8.32 were assigned to H-2′/H-6′ from a correlation with C-2 in the HMBC. Protonated aromatic carbons C-2′, C-3′, C-5′ and C-6′ were readily assigned from their direct correlations observed in the HMQC spectrum. Two doublets at δ 9.07 and 8.23 with the same coupling constant ($J = 8.8$) were attributed to H-3 and H-4, respectively. Both protons showed a correlation with C-2 (δ 171.3), whilst one of them (H-3) correlated with a carbon at δ 154.0, assigned to C-10, and the one (H-4) correlated with a carbon at δ 117.0 assigned to C-9. Carbons C-3 and C-4 were assigned from HMQC correlations to H-3 and H-4. An aromatic proton at δ 7.38 was assigned to H-8. The position was elucidated through correlations between this proton and C-6, C-7, C-9 and C-10 in the HMBC via ^2J and ^3J values. The signal at δ 99.4 corresponding to C-8 was deduced from the HMQC spectrum.

The novel pigments **1, 2** and carajurin (**3**; Fig. 3), in addition to the carajuflavone described by Takemura *et al.* (1995), support the hypothesis of Harborne (1967) that the 6-hydroxylation is a common structural pattern for the flavonoids, *sensu lato*, present in the Bignoniaceae.

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