Alvaradoins A−D. Anthracenone C Arabinosides from *Alvaradoa jamaicensis*

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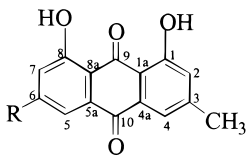
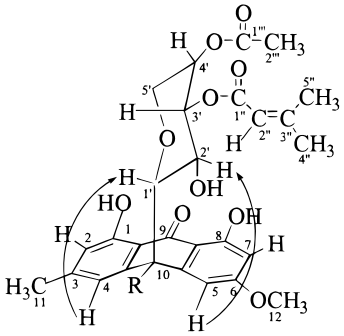
Received July 9, 1998

The aerial parts of *Alvaradoa jamaicensis* have yielded four new compounds. These are both C-10 epimers of 10-C-[4′-*O*-acetyl-3′-*O*-(3-methylbut-2-enoyl)]-*β*-D-arabinopyranosyl-1,8-dihydroxy-6-methoxy-3-methyl-anthracen-9(10H)-one (**3** and **4**) and of 10-C-[4′-*O*-acetyl-3′-*O*-(3-methylbut-2-enoyl)]-*β*-D-arabinopyranosyl-1,8,10-trihydroxy-6-methoxy-3-methyl-anthracen-9-one (**5** and **6**). The anthraquinones chrysophanol (**1**) and physcion (**2**) were also isolated.

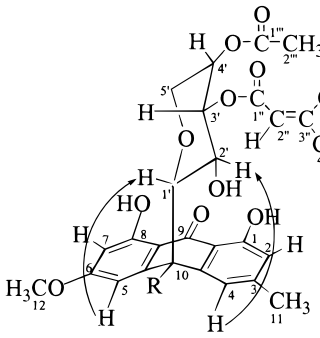
Six species are described for the simaroubaceous genus *Alvaradoa*, and these are distributed throughout the West Indies and Central and South America.[1] Previous phytochemical reports describe constituents of *A. amorphoides* seed oil and roots,[2,3] the latter being anthraquinones and a quassinoid typical of the family.[3] *Alvaradoa* is represented in Jamaica by two rare and local endemic species of which *A. jamaicensis* is the more accessible.[4]



1 R = H
2 R = OCH_3



3 R = H
5 R = OH



4 R = H
6 R = OH

Results and Discussion

Dried, ground leaves and twigs of *A. jamaicensis*, Benth. (Simaroubaceae) were defatted with hexanes and extracted with EtOH. The EtOH extract was partitioned between H_2O−EtOH (9:1) and CH_2Cl_2. Half of the residue from evaporation of the dichloromethane solution was subjected to VLC. Repeated Si gel column chromatography of selected fractions from the VLC yielded the anthraquinones chrysophanol (**1**) and physcion (**2**), identified from their spectral data and by comparison of these and physical data with literature values.[5−9]

Chromatography of the remaining portion of the crude dichloromethane extract gave a number of samples that appeared to be homogeneous by TLC but were revealed by NMR to be mixtures, each consisting of two isomers. Careful open-column chromatography (silica) with isocratic elution and monitoring of individual fractions by examination of the δ 12 region of the ^{1}H NMR spectra resulted in separation of two mixtures, providing alvaradoins A−D (**3**−**6**).

The HRMS of the mixture of alvaradoins A (**3**) and B (**4**) showed a molecular ion $[M]^{+\bullet}$ at *m*/*z* 526.1825, corresponding to a molecular formula $C_{28}H_{30}O_{10}$. The presence of one or more chelated hydroxyl groups, ester and cross-conjugated carbonyls, and at least one aromatic ring was indicated by the major IR absorption bands for **4** (3400, 1743, 1724, 1640, 1605, 1573, 1483 cm^{-1}). A bathochromic shift of the UV maxima after addition of potassium hydroxide suggested a phenolic structure.

The low-field region of the ^{1}H NMR spectrum of **3** (Table 1) showed many similarities to that of **2**. There were two intramolecularly H-bonded phenolic groups with resonances at δ 12.47 and 12.15 assigned to groups at C-1 and C-8; two broad peaks at δ 6.74 and 6.83 were attributable to the aromatic C-2 and C-4 protons, *meta*-coupled and broadened by the C-11 methyl group; a second pair of *meta*-coupled aromatic signals at δ 6.42 (d, 2.5 Hz) and 6.58 (dd, 2.5, 0.9 Hz) were due to protons at C-7 and C-5. Further upfield, singlets at δ 3.87 and 2.38 were ascribed to the methoxyl and methyl groups attached to C-6 and C-3,

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10.1021/np980300i CCC: $18.00
Published on Web 12/01/1998

Table 1. NMR Data for Compounds 3 and 4 ($CDCl_3$, *J* Values Are Given in Hz in Parentheses)

	alvaradoin A (**3**)			alvaradoin B (**4**)	
position	^{13}C	^{1}H	HMBC[a]	^{13}C	^{1}H
1	161.8		1-OH	162.1	
1a	114.9		2, 4, 10	114.8	
2	116.4	6.74, br s	1-OH, 4, 11	116.5	6.72, br s
3	146.8		11	146.2	
4	119.8	6.83, br s	2, 10, 11	120.8	6.80, br s
4a	143.6		10	141.0	
5	108.5	6.58, *dd* (2.5, 0.9)	10	107.1	6.62, *dd* (2.5, 0.9)
5a	143.0		10	145.8	
6	164.9		5, 7, 12	164.6	
7	99.2	6.42, *d* (2.5)	8-OH	99.7	6.42, *d* (2.5)
8	164.8			165.4	
8a	111.6		5, 7, 8-OH, 10	111.3	
9	192.0			192.1	
10	44.4	4.54, *d* (2.4)	4, 5	44.7	4.50, *d* (2.0)
11	22.1	2.38, br s	2, 4	22.2	2.40, *s*
12	55.6	3.89, *s*		55.6	3.87, *s*
1′	86.7	3.47, *dd* (9.6, 2.2)	10, 5′	86.8	3.47, *dd* (9.6, 2.2)
2′	66.5	3.73, *td* (9.4, 4.3)	3′	66.7	3.64, *td* (9.3, 4.2)
3′	74.3	4.83, *dd* (9.5, 3.5)	1′, 4′, 5′	74.2	4.81, *dd* (10.4, 3.4)
4′	69.1	5.07, *quint* (1.6)	5′	69.2	5.06, *quint* (1.6)
5′	68.1	3.82, *dd* (13.5, 2.0)	1′, 4′	67.9	3.85, *dd* (13.5, 2.0)
		3.36, *dd* (13.5, 1.1)			3.38, *dd* (13.3, 1.0)
1″	166.4		3′	166.5	
2″	114.7	5.66, *sept* (1.3)	4″, 5″	114.8	5.66, *quint* (1.3)
3″	159.9		4″, 5″	160.0	
4″	20.5	2.16, *d* (1.2)	2″, 5″	20.5	2.15, *d* (1.2)
5″	27.6	1.91, *d* (1.2)	4″	27.6	1.91, *d* (1.3)
1‴	169.9		2′, 4′	170.1	
2‴	20.8	1.93, *s*		20.7	1.92 *s*
1-OH		12.15, *s*			12.15, *s*
8-OH		12.47, *s*			12.49, *s*
2′-OH		2.40, *d* (4.2)			2.28, *d* (4.3)

[a] Protons correlating with carbon shift.

respectively. Signals for the protonated aromatic carbons of the anthracenone moiety were assigned on the basis of HMQC spectra, while connectivities obtained from HMBC data enabled assignment of the quaternary carbon shifts. HMBC cross peaks from H-4 (δ 6.83) and H-5 (δ 6.58) established that position 10 was a methine, δ_C 44.4, δ_H 4.51 (broad doublet, 2.4 Hz).

^{1}H NMR and HMQC spectra showed that the residue attached to C-10 contained one oxymethylene and four oxymethine groups. The coupling constants suggested that these formed a pyran ring with the relative stereochemistry of the oxygen substituents being as for arabinose.

Characteristic signals were observed in the ^{1}H and ^{13}C NMR spectra for the acetyl and 3-methylbut-2-enoyl groups, the latter being formulated with the aid of HMBC data, which also established the esterified positions of the arabinopyranosyl moiety. Thus H-3′, the only proton on this ring to be axially and equatorially coupled (δ 4.83, dd, 9.5, 3.5 Hz) showed cross peaks to the carbonyl carbon of the 3-methylbut-2-enoyl group (δ_C 166.4), while the proton, which showed three small couplings, equatorial H-4′ (δ 5.07, ddd, 3.6, 1.6, 1.6 Hz), correlated with the acetyl carbonyl at δ 169.9.

The second $C_{28}H_{30}O_{10}$ isomer, alvaradoin B (**4**), isolated from the mixture of **3** and **4** had chemical shifts and coupling constants almost identical to those of **3** (Table 1) but differed in melting point and specific rotation. The possibility of regioisomerism within the arabinopyranosyl moiety was eliminated by the observation of HMBC cross peaks between the carbonyl of the acetoxy group (δ_C 170.1) and the equatorial acetoxymethine hydrogen H-4′ (δ 5.06, ddd, 3.4, 1.6, 1.6) as well as between the carbonyl of the 3-methylbut-2-enoyl group (δ_C 166.6) and the axially and equatorially coupled H-3′ (δ 4.81, 10.4, 3.4 Hz).

Alvaradoins A and B (**3** and **4**) could therefore only be stereoisomeric at C-10. In both isomers H-10 and H-1′ are in a gauche relationship, demonstrated by the small mutual couplings of 2.4 and 2.0 Hz. Absolute configurations at C-10 were assigned on the basis of NOE difference experiments and by analogy with aloins A and B,[10] the cascariosides,[11,12] and the anthracenone glycosides from *Picramnia antidesma* ssp. *fessonia*.[13]

It has been demonstrated that the energetically favorable conformation of the anthracenone C-10 C-glycosides in solution is as shown in **3**−**6**.[10] For the compound designated alvaradoin A (**3**) adoption of this conformation brings H-1′ into close proximity with H-4 and H-2′ with H-5. In fact, for compound **3** a 3.1% intensity enhancement was observed for H-1′ (δ 3.47) by irradiation of H-4 (δ 6.83), while irradiation of H-5 (δ 6.58) gave a 6.7% enhancement of H-2′ (δ 3.73). In the C-10 epimer, alvaradoin B (**4**), irradiation of H-4 (δ 6.80) and H-5 (δ 6.61) resulted in enhancements of 6.4% and 2.5% for H-2′ (δ 3.64) and H-1′ (δ 3.47), respectively. Compound **3**, therefore, has the 10*S* configuration and compound **4** is 10*R*.

Separation of the second mixture of isomers yielded alvaradoins C (**5**) and D (**6**). The $[M]^{+\bullet}$ in the HRMS was observed at *m*/*z* 542.1798, corresponding to a molecular formula of $C_{28}H_{30}O_{11}$, which is one oxygen more than **3** and **4**. The NMR spectra of **5** and **6** (Table 2) were very similar to those of **3** and **4** except for the replacement of the C-10 methine group (δ_C 44.4 and 44.7) by a quaternary oxygen-bearing carbon (δ 75.9 in both isomers) showing HMBC cross peaks to H-1′ and H-4 and a hydroxyl hydrogen at δ 5.16. These data and the molecular formula were easily accommodated by the placement of a hydroxyl group at C-10.

Table 2. NMR Data for Compounds **5** and **6** ($CDCl_3$, *J* Values Are Given in Hz in Parentheses)

	alvaradoin C(**5**)			alvaradoin D (**6**)	
position	^{13}C	^{1}H	HMBC[a]	13C	^{1}H
1	161.6		1-OH	162.3	
1a	114.0		1-OH	114.2	
2	117.7	6.76, br s	1-OH, 11	117.7	6.78, br s
3	146.4			146.4	
4	118.5	7.26, *s*	2, 11	118.5	7.10, *d* (1.6)
4a	147.3			148.1	
5	105.2	6.88, *d* (2.4)	7	105.2	7.01, *d* (2.4)
5a	146.2		10-OH, 1′	148.8	
6	165.3		5, 12	165.1	
7	100.0	6.44, *d* (2.4)	5, 8-OH	100.5	6.42, *d* (2.4)
8	164.9		8-OH	164.5	
8a	110.5		5, 7, 8-OH	110.3	
9	191.0			191.0	
10	75.9		4,10-OH, 1′	75.9	
11	22.5	2.40, *s*	2	22.5	2.44, *s*
12	55.8	3.90, *s*		55.6	3.88, *s*
1′	85.2	3.26, *d* (10)	10-OH, 2′-OH, 5′	85.4	3.31, *d* (9.4)
2′	67.6	3.76, (obscured)	2′-OH, 3′, 4′	67.6	3.71, *ddd* (9.4,9.4, 3.1)
3′	73.7	4.84, *dd* (10.4, 2.7)	2′-OH, 4′, 5′	73.7	4.84, *dd* (10, 3.4)
4′	68.9	4.98, (obscured)	3′, 5′	68.9	4.98, (obscured)
5′	67.8	3.27, *dd* (13.0,1.6)	3′	67.8	3.31, *dd* (13.5,1.1)
		3.77, (obscured)			3.78, *dd* (12.9, 2.1)
1″	166.8		3′, 2″	166.8	
2″	114.8	5.65, *m* (1.4)	4″, 5″	114.8	5.64, *m* (1.2)
3″	160.5		4″, 5″	160.5	
4″	20.6	2.15, *d* (1.3)	2″, 5″	20.6	2.15, *d* (1.0)
5″	27.5	1.91, *d* (1.3)	2″, 4″	27.5	1.91, *d* (1.0)
1‴	170.1		2‴, 4′	170.1	
2‴	20.7	1.86, *s*		20.7	1.86, *s*
1-OH		12.02, *s*			12.11, *s*
8-OH		12.40, *s*			12.32, *s*
10-OH		5.16, *s*			5.03, *s*
2′-OH		3.57, *d* (3.2)			3.54, *d* (3.3)

[a] Protons correlating with carbon shift.

NOE associations for alvaradoins C (**5**) and D (**6**) led to the stereochemical assignments shown, 10*R* to **5** and 10*S* to **6**. For compound **5**, selective irradiation of H-4 (δ 7.26) and H-5 (δ 6.88) led to enhancements of H-1′ (2.8%) and H-2′ (3.2%), respectively. Irradiation of H-4 (δ 7.10) and H-5 (δ 7.01) of compound **6** gave enhancements of 5.6% and 2.4% for H-2′ and H-1′, respectively.

Chrysophanol (**1**), physcion (**2**), and alvaradoins A−D (**3**−**6**) were tested for activity against *Mycobacterium tuberculosis* and were found to be inactive at concentrations of 12.5 μg/mL.[14]

Experimental Section

General Experimental Procedures. Melting points were determined on a Thomas−Hoover capillary melting point apparatus. Optical rotations were measured on a Perkin−Elmer 241MC polarimeter. EIMS were obtained at 70 eV on VG 70−250S mass spectrometer. IR spectra were recorded on a FT-IR SPECTRUM 1000 spectrometer with KBr pellets. UV spectra were recorded in EtOH on a Perkin-Elmer UV/vis/NIR Lambda 19 spectrometer. NMR spectra were obtained on Varian GEMINI-200 and UNITY-500 spectrometers with $CDCl_3$ as solvent and TMS as internal standard. Adsorption column chromatography was performed with Si gel 60 (230−400 mesh) and VLC with Si gel (40−63 μm). TLC analyses were performed with Whatman precoated Si gel 60 F_{254} plates. Spots were visualized under UV and by spraying with 4% phosphomolybdic acid in 5% H_2SO_4 followed by heating.

Plant Material. Aerial parts of *A. jamaicensis* were collected at Ramgoat Cave, Trelawny, Jamaica, in March 1995. A voucher specimen is deposited in the Herbarium, Department of Life Sciences, University of the West Indies, Mona.

Extraction and Isolation. Dried, ground leaves and twigs (660 g) were extracted by cold percolation with hexanes followed by EtOH. Evaporation of the EtOH in vacuo gave a gum (60 g) that was dissolved in EtOH−H_2O (1:9). The EtOH−H_2O solution was extracted with CH_2Cl_2, and the residue (11.0 g) from evaporation of the CH_2Cl_2 solution was chromatographed in two portions. One portion (5.8 g) was subjected to VLC in Me_2CO−hexane mixtures (1−20% Me_2CO). The fractions eluted with 10% Me_2CO−hexane were rechromatographed by column chromatography in 10% diisopropyl ether−hexane to yield chrysophanol (**1**) and physcion (**2**). The second portion of the CH_2Cl_2 residue (5.2 g) was separated by column chromatography in 35%-50% Me_2CO−hexane.

Early fractions consisted of an approximately 1:1 mixture of **3** and **4** (60 mg, 0.20% of crude extract); separation was achieved by column chromatography in 3% Me_2CO−$CHCl_3$. More polar fractions contained **5** and **6** in a 1:1 ratio (20 mg, 0.07% of crude extract); these were separated by column chromatography in 90% diisopropyl ether−$CHCl_3$.

Chrysophanol (1): yellow plates (EtOAc−hexane), mp 197−199 °C, [lit.[5] 194−196 °C]; IR, UV, NMR, and MS data were in agreement with lit. values.[5,6]

Physcion (2): yellow plates (EtOAc−hexanes), mp 205−207 °C, [lit.[7] 210 °C]; NMR data was in agreement with lit. values.[8,9]

Alvaradoin A (3): orange plates, mp 194−198 °C; $[\alpha]_D$ +12.7° (*c* 0.0036, EtOH); IR ν_{max} 3442, 1741, 1719, 1641, 1621, 1602, 1487 cm^{-1}; UV λ_{max} nm (log ϵ) 258 sh (4.00), 274 (4.09), 360 (3.93) + KOH (log ϵ) 270 (4.48), 364 (3.72), 384 (3.93); EIMS for mixture of **3** and **4**, *m*/*z* (rel int) $[M]^{+\bullet}$ 526 (10), 270 (100), 257 (40), 241 (19), 197 (8), 97 (54), 83 (91). HREIMS for a mixture of **3** and **4** *m*/*z* 526.1825 (calcd for $C_{28}H_{30}O_{10}$, 526.1839); ^{1}H (500 MHz) and ^{13}C NMR, see Table 1.

Alvaradoin B (4): yellow plates, mp 199−202 °C; $[\alpha]_D$ −26.9° (*c* 0.0056, EtOH); IR ν_{max} 3481, 3017, 1743, 1726, 1641, 1621, 1602, 1569, 1487 cm^{-1}; UV λ_{max} nm (log ϵ) 258 sh (1.10),

274 (1.12), 364 (0.92) + KOH (log ϵ) 270 (4.45), 364 (3.72), 384 (3.86); ^{1}H (500 MHz) and ^{13}C NMR, see Table 1.

Alvaradoin C (5): yellow plates, mp 201−204 °C; $[\alpha]_D$ +43.8° (*c* 0.0036, EtOH); IR ν_{max} 3426, 2960, 1742, 1721, 1640, 1618, 1605 cm^{-1}; UV λ_{max} nm (log ϵ) 258 sh (1.38), 276 (1.43), 364 (1.28) + KOH (log ϵ) 268 (3.99), 368 (3.65); EIMS for mixture of **5** and **6** *m*/*z* (rel int) $[M]^{+\bullet}$ 542 (5), 286 (97), 257 (40), 241 (4), 197 (11), 97 (50), 83 (100); HREIMS for a mixture of **5** and **6** *m*/*z* 542.1798 (calcd for $C_{28}H_{30}O_{11}$, 542.1788); ^{1}H (500 MHz) and ^{13}C NMR, see Table 2.

Alvaradoin D (6): yellow plates mp 199−203 °C; $[\alpha]_D$ −10.6° (*c* 0.0096, EtOH); IR ν_{max} 3400, 3016, 1743, 1724, 1640, 1618, 1605, 1573, 1483 cm^{-1}; UV λ_{max} nm (log ϵ) 258 sh (1.15), 276 (1.21), 364 (1.28) + KOH (log ϵ) 268 (4.11), 388 (3.82); ^{1}H (500 MHz) and ^{13}C NMR, see Table 2.

Acknowledgment. We thank Mrs. Nella Stewart of the Conservation Data Center, UWI, for assistance with plant collection. Financial support from the UWI Research and Publications Fund Committee, the Natural Sciences and Engineering Council of Canada and the Canada−UWI Institutional Strengthening Project is gratefully acknowledged.

Supporting Information Available: Experimental data for compounds **1** and **2** (2 pages). Ordering information is given on any current masthead page.

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NP980300I