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Anthracene derivatives from *Auxemma oncocalyx*

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Abstract

Three anthracene derivatives, auxenone, oncocalyxonol and auxemim, were isolated from *Auxemma oncocalyx*. The structures of these compounds as 1,4,8-trihydroxy-2-methoxy-5-methyl-9,10-anthraquinone, *rel*-9α,11α-epoxy-1,4,8α,11α-tetrahydroxy-2-methoxy-8aβ-methyl-5,6,7,8,8a,9, 10,10aβ-octahydro-10-anthracenone and *rel*-8α,11β-epoxy-2,11-dimethoxy-8aβ-methyl-5,6,7,8,8a,9-hexahydro-1,4-anthracenedione were determined by analysis of spectral data (1D and 2D NMR, IR, HREIMS and UV).

Keywords: *Auxemma oncocalyx*; Boraginaceae; Anthraquinone; Anthracene derivatives

1. Introduction

Auxemma oncocalyx (Boraginaceae) is a common tree found in the state of Ceará in Brazil (Braga, 1960). Previous investigations of *Auxemma* species resulted in isolation of eight compounds classified as cordiacromes and hydroquinones (Pessoa et al., 1993, 1995; Costa et al., 1999). In a continuation of our phytochemical investigation of plants of this genus, we now report the isolation and structural elucidation of three anthracene derivatives auxenone (**1**), oncocalyxonol (**2**)) and auxemim (**3**)). Their structures were elucidated based on spectral analysis, including 2D NMR experiments, and by comparison with related compounds reported in the literature (Moir et al., 1972; Moir and Thomson, 1973; Manners and Jurd, 1977). Terpenoids, benzoquinones and hydroquinones are commonly found in *Auxemma*; however, this is the first example of anthraquinones from this genus.

2. Results and discussion

The structures were determined by the use of ^{1}H, ^{13}C NMR spectroscopy, including 2D NMR experiments (COSY, HMQC, HMBC), IR, and EIMS. Relative configurations were obtained by analysis of ROESY and NOE difference spectra.

The HBBD-^{13}C NMR spectrum of **1** revealed signals corresponding to 16 carbon atoms (Table 1). Chemical shifts and comparative analyses of HBBD and DEPT ^{13}C NMR spectra were used to elucidate the carbon signals as two carbonyl groups, 12 aromatics (nine quaternary and three methine carbons) and two sp^3 carbon atoms (one methyl group and one methoxyl group). The ^{1}H NMR, HBBD and DEPT ^{13}C NMR spectra of **1** were consistent with the molecular formula $C_{16}H_{12}O_6$, as confirmed by the parent ion at m/z 300 ($[M]^+$). The IR spectrum showed absorption bands for a hydroxyl group (ν 3446 cm^{-1}), a conjugated carbonyl (ν 1595 cm^{-1}) and an aromatic ring (ν 1595 and 1478 cm^{-1}). In addition, the ^{1}H NMR spectrum showed characteristic deshielded signals for three chelated hydroxyl groups at δ_H 13.92 (1H, *s*), 12.71 (1H, *s*) and 12.66 (1H, *s*). The ^{1}H NMR spectrum revealed the presence of one set of *ortho* coupled doublets at δ_H 7.47 (*d*, $J=8{,}6$ Hz, H-6) and 7.16 (*d*, $J=8{,}6$ Hz, H-7) attributable to two aromatic hydrogens. The location of this *para*-system, the chelated hydroxyl (δ_H 12.66) and the methyl (δ_H 2.75) groups in ring C, were determined by analysis of the HMQC and HMBC spectra. The hydroxyl proton at δ_H 12.66 exhibited heteronuclear interactions with the carbon atoms at δ_C 162.3 (C-8,

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Table 1
^{13}C NMR spectral data for auxenone **1** ($CDCl_3$), oncocalyxonol **2** (DMSO-d_6) and auxemim **3** ($CDCl_3$)[a]

C	1	2	3
1	149.1	137.2	181.9
2	156.8	157.3	159.4
3	107.6	99.5	105.9
4	160.7	156.6	186.1
4a	106.7	110.2	137.6
5	134.9	39.2	41.1
6	142.3	23.8	19.9
7	123.6	30.5	23.3
8	162.3	74.8	74.6
8a	116.6	38.6	38.3
9	191.8	67.8	29.5
9a	112.4	123.6	135.1
10	185.9	204.6	114.7
10a	130.5	55.2	152.5
11	23.6	89.8	101.5
12	–	22.2	18.6
MeO-2	56.6	56.6	56.2
MeO-11	–	–	55.2

[a] Multiplicity of signals of carbon atoms deduced by comparative analysis of HBBD- and DEPT-^{13}C NMR spectra. Heteronuclear 2D ^1H–^{13}C-COSY-$^nJ_{CH}$ (Table 2) and homonuclear 2D ^1H–^1H-COSY NMR spectra were also used in these assignments.

$^2J_{CH}$), 116.6 (C-8a, $^3J_{CH}$) and 123.6 (CH-7, δ_H 7.16, $^3J_{CH}$). The methyl hydrogens at δ_H 2.75 (*s*) showed ^2J correlation with the carbon at δ_C 134.9 (CH-5) and ^3J carbon at δ_C 142.3 (CH-6, δ_H 7.47). In addition, H-6 (δ_H 7.47, *d*, $J=8.6$ Hz) revealed a correlation with the carbon atoms at δ_C 162.3 (C-8, $^3J_{CH}$), 134.9 (C-5, $^2J_{CH}$) and 130.5 (C-10a, $^3J_{CH}$). These data indicated that the location of the hydroxyl and methyl groups were at carbon atoms C-8 and C-5, respectively, in accordance with all previous cordiachromes and hydroquinones isolated from *A. oncocalyx*. The hydrogen corresponding to a singlet at δ_H 6.70 (*s*) could be located at C-2 or C-3, which appears shielded relative to H-6 and H-7 because of the electron releasing effects of the OH and OMe groups at *ortho* positions. The presence of a hydrogen at C-3 (δ_H 6.70) was confirmed by the HMBC spectrum via interactions of both non-hydrogenated carbon atoms at δ_C 149.1 (C-1, $^2J_{CH}$) and 112.4 (C-9a, $^3J_{CH}$) with HO-1 (δ_H 12.71) and H-3 (δ_H 6.70) δ_C 149.1 (C-1, $^2J_{CH}$), as well as a correlation of the quaternary carbon atoms at δ_C 160.7 (C-4) at 106.7 (C-4a) with HO-4 (δ_H 13.92) and H-3 (δ_H 6.70). These data were also important for the determination of the assignment of the hydrogen signal of the HO-4 group. The location of the methoxyl group at carbon C-2, and not C-3, was also deduced by analysis of cross-peaks corresponding to heteronuclear long-range coupling of C-2 (δ_C 156.8) and hydrogen atoms of both MeO (δ_H 3.98, $^3J_{CH}$) and HO-1 (δ_H 12.71, $^3J_{CH}$) groups observed in the HMBC spectrum (Table 2). The *para*-mesomeric effect of MeO-2 can be used to justify the shielding of the carbon atom C-4a (δ_C 106.7) when compared with C-9a (δ_C 112.4). The assigned position of the methyl group to C-5 was strengthened by analysis of the NOE difference spectra: irradiation at δ_H 2.75 (3H-11) resulted in a 2.0% NOE at δ_H 7.47 (H-6) and an 0.2% NOE at δ_H 13.92 (HO-4); irradiation at δ_H 6.70 (H-3) showed a 7.0% NOE at δ_H 3.98 (MeO-2) and an 0.5% NOE at δ_H 13.92 (HO-4). Therefore, the structure of compound **1** was determined as 1,4,8-trihydroxy-2-methoxy-5-methyl-9,10-anthraquinone, trivially named auxenone.

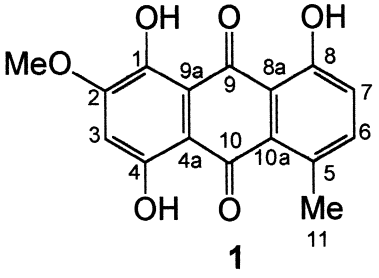
Compound **2**, $[\alpha]_D^{25}$ −48.6° (DMSO; *c* 0.001), was obtained as yellowish–green needles. The molecular formula of $C_{17}H_{20}O_7$ was determined from its HBBD ^{13}C NMR and DEPT spectral data in combination with 1 and 2D-^1H NMR. The ^1H NMR and ^{13}C NMR spectra of **2** were similar to those of other compounds isolated from *A. oncocalyx*, especially *rel*-9α,11-epoxy-1,4,8α-trihydroxy-2-methoxy-8aβ-methyl-5,6,7,8,8a,9,10,10a,10aβ-octahydro-10-anthracenone (Pessoa et al., 1995). The characteristic ^{13}C NMR signal at δ 89.8 (methine carbon) suggested a hemiacetal carbon, which was correlated with the hydrogen signal at δ_H 4.76 (*m*) in the HMQC spectrum. The ^1H NMR spectrum revealed signals associated with four hydroxyl groups δ_H 4.89 (*s*, HO-8), 6.44 (*s*, HO-11), 8.61 (*s*, HO-1) and 12.27 (*s*, HO-4), one of them hydrogen bonded (HO-4). These attributes were established unambiguously by analysis of the ^2J and ^3J heteronuclear couplings as visualized through a HMBC experiment (Table 2). The relative stereochemistry of **2** was assigned based on ROESY correlations, as shown in Fig. 1. The cross peaks indicated dipolar–dipolar interactions between 3H-12 (δ_H 0.74) and H-8 (δ_H 3.5–3.3), H-9 (δ_H 5.39) and H-10a (δ_H 2.42); H-10a (δ_H 2.42) and H-5 (δ_H 1.79), H-6β (δ_H 1.50) and H-8 (δ_H 3.5–3.3); H-11 (δ_H 4.76) and H-5 (δ_H 1.79), respectively. The absence of cross peaks corresponding to dipolar–dipolar coupling of the H-11 (δ_H 4.76) with 2H-6 (δ_H 2.10 and 1.50) and 2H-7 (δ_H 2.30 and 1.67) was used to established the stereochemistry of the carbon CH-11, as shown in Fig. 1. Thus, the relative configuration of this new natural product was established as *rel*-9α,11α-epoxy-1,4,8α,11α-tetrahydroxy-2-methoxy-8 αβ-methyl-5,6,7,8,8a,9,10,10aβ-octa-hydro-10-anthracenone (**2**), which we have named oncocalyxonol.

Compound **3**, $[\alpha]_D^{25}$ +81.3° ($CHCl_3$; *c* 0.0003), was obtained as orange needles. This compound was deduced to have the same skeleton as the cordiachromes, terpenoid quinones, isolated from *Auxemma* and *Cordia* species. The molecular formula of $C_{18}H_{20}O_5$ was determined from a combination of IR, NMR spectra data (Table 1). The presence of a 2-methoxy-*para*-quinone moiety, one methyl at C-8a, one trisubstituted double bond between C-10 and C-10a, and a heterocyclic ring, was well established by a detailed analysis of the ^1H and ^{13}C spectral data involving a comparison with literature values of analogous compounds (Pessoa et al., 1995).

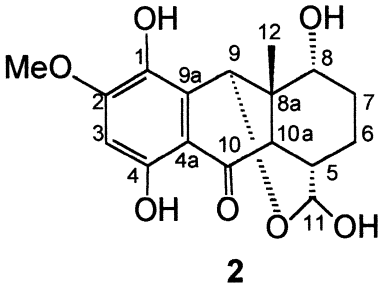
Table 2
Long range couplings observed in the HMBC spectra of auxenone **1** ($CDCl_3$), oncocalyxonol **2** (DMSO-d_6) and auxemim **3** ($CDCl_3$)[a]

C	**1**		**2**		**3**	
	$^2J_{CH}$	$^3J_{CH}$	$^2J_{CH}$	$^3J_{CH}$	$^2J_{CH}$	$^3J_{CH}$
1	HO-1	H-3		H-3		H-3, 2H-9
2		HO-1, MeO-2		MeO-2	H-3	MeO-2
3		HO-4				
4	HO-4, H-3		H-3		H-3	H-10
4a		HO-4, H-3		H-3, H-9		H-3, 2H-9
5	3H-11, H-6	H-7			H-11	H-10
6		3H-11			H-5, H-7	H-8, H-11
7		HO-8			H-6	
8	HO-8	H-6				3H-12
8a		HO-8	H-10a, 3H-12		2H-9, 3H-12	H-10
9				3H-12		H-8, 3H-12
9a		HO-1	H-9		2H-9	H-10
10			H-10			H-8, 3H-12
10a		H-6			H-5	2H-9, H-11
11				H-9, H-10a		
12						2H-9

[a] Multiplicity of signals of carbon atoms deduced by comparative analysis of HBBD- and DEPT-^{13}C NMR spectra. Heteronuclear 2D ^1H–^{13}C-COSY-$^1J_{CH}$ (Table 1) and homonuclear 2D ^1H–^1H-COSY NMR spectra were also used.



1



2

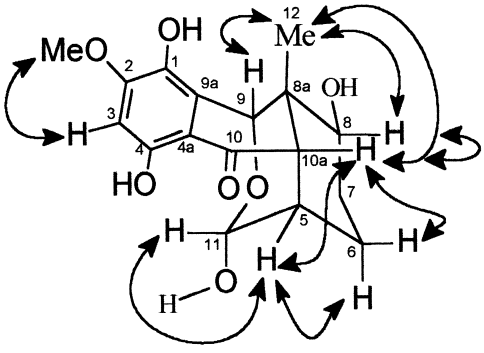


Fig. 1. Important ^1H–^1H-NOE, ROESY (double arrows) correlations of compound **2**.

The linkage of this ring through an ether bridge from C-8 to C-11 in **3** was established unambiguously by analysis of the ^2J and the ^3J heteronuclear couplings as visualized through a HMBC experiment (Table 2). The relative stereochemistry of **3** was established on the basis of ROESY correlations (Fig. 2), including comparison with NMR spectral data of related compounds (Pessoa et al., 1995). The cross-peaks indicated dipolar–dipolar

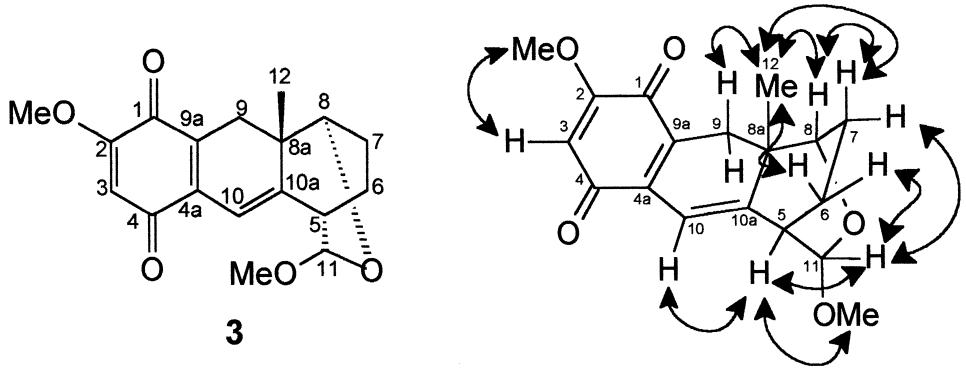


Fig. 2. Important ^{1}H–^{1}H-NOE, ROESY (double arrows) correlations of compound **3**.

interactions between 3H-12 (δ_H 0.93) and H-6β (δ_H 1.85–1.75), H-7β (δ_H 2.01–1.90), H-8 (δ_H 3.67) and H-9β (δ_H 2.52); H-5 (δ_H 2.58) and H-10 (δ_H 6.30) and H-11 (δ_H 4.78); H-11 (δ_H 4.78) and H-6α (δ_H 1.85–1.75) and H-7α (δ_H 2.01–1.90). Based on all these results, the structure of compound **3** was assigned as *rel*-8α,11β-epoxy-2,11-dimethoxy-8aβ-methyl-5,6,7, 8,8a,9-hexahydro-1,4-anthracenedione, and was named auxemim.

3. Experimental

3.1. General experimental procedures

Melting points were determined using a melting point apparatus and are uncorrected. IR spectra were obtained on a Perkin–Elmer 1000 FT–IR instrument using KBr pellets. Optical rotations were measured on a Perkin–Elmer 341 digital polarimeter. UV spectra were run on a VARIAN CARY 50 CONC spectrometer and MS were obtained on a VG-Auto Spec Fisons instrument. NMR spectra were recorded on a Bruker DRX 500 [500 MHz (^{1}H) and 125 MHz (^{13}C)] spectrometer. Chemical shifts were recorded in δ (ppm) relative to TMS, $CDCl_3$ (δ 7.24 and 77.0 ppm), and DMSO-d_6 (δ 2.49 and 39.5 ppm). TLC (Silica gel 60, Carlo Erba) was visualized first by UV (254 and 366 nm) and then by spraying with a mixture of vanillin–perchloric acid–EtOH.

3.2. Plant material

A. oncocalyx Taub was collected in Pentecoste, State of Ceará, Brazil, and identified by Professor A.G. Fernandes, a botanist of the Universidade Federal do Ceará, where a voucher specimen is deposited (Herbarium Prisco Bezerra, number 18459).

3.3. Extraction and isolation

The air-dried and pulverized heartwood (1.70 kg) was extracted exhaustively with EtOH at room temperature. The EtOH extract was concentrated under reduced pressure, and the residue was subjected to (silica gel) chromatography using hexane as eluent, $CHCl_3$, $CHCl_3$–EtOAc (1:1), EtOAc and MeOH. The $CHCl_3$ fraction was subjected to additional Silica gel column chromatography to give compound **1** (8 mg). The air-dried and pulverized roots (5.5 kg), extracted with MeOH, were subjected to a treatment similar to the heartwood. The $CHCl_3$–EtOAc fraction was subjected to column chromatography (Silica gel) to give compounds **2** (18 mg) and **3** (35 mg).

3.4. 1,4,8-Trihydroxy-2-methoxy-5-methyl-9,10-anthraquinone (*1*)

Red needles, mp 260–261°; 8 mg (0.0005% whole plant); UV $\lambda^{CHCl_3}_{max}$ nm (log ε) 286.97 (3.34), 296.99 (3.25); ν^{KBr}_{max} 3446, 2922, 1595, 1478, 1288–1215, 997–787 cm^{-1}; EIMS (probe) 70 eV, *m/z* (rel. int.): 300 $[M]^+$ (100), 282 $[M\text{-}H_2O]^+$ (12), 257 $[M\text{-Me-CO}]^+$ (32), 239 $[M\text{-}H_2O\text{-Me-CO}]^+$ (13); ^{1}H NMR spectral data ($CDCl_3$, 500 MHz): δ_H 2.75 (3H, *s*, H-11), 3.98 (3H, *s*, MeO-2), 6.70 (1H, *s*, H-3), 7.16 (1H, *d*, J = 8.6 Hz, H-7), 7.47 (1H, *d*, J = 8.6 Hz, H-6), 12.66 (1H, *s*, HO-8), 12.71 (1H, *s*, HO-1), 13.92 (1H, *s*, HO-4); ^{13}C NMR spectral data ($CDCl_3$): Table 1; HMBC data ($CDCl_3$): Table 2.

3.5. Rel-9α,11α-Epoxy-1,4,8α,11α-tetrahydroxy-2-methoxy-8aβ-methyl-5,6,7,8,8a,9,10,10aβ-octahydro-10-anthracenone (*2*)

Yellowish–green needles, mp 193.6°; 18 mg (0.0003% whole plant); $[\alpha]^{25}_D$ −48.6° (DMSO; *c* 0.001); UVλ^{DMSO}_{max} nm (log ε) 288.99 (3.89), 314.05 (3.22); ν^{KBr}_{max} 3408, 2929, 1727, 1624, 1487, 1365, 1270–868 cm^{-1}; EIMS (probe) 70 eV, *m/z* (rel. int.): 336 $[M]^+$ (42), 318 $[M\text{-}H_2O]^+$ (90), 272 $[M\text{-}H_2O\text{-}HCO_2H]^+$ (15), 257 $(M\text{-}H_2O\text{-}HCO_2H\text{-Me}]^+$ (23), 219 $[M\text{-}CH_3CHO\text{-}CH_2CHCo_2H\text{-H}]$ (100); HREIMS *m/z* 336.1277 (calcd for $C_{17}H_{20}O_7$, 336.1209); ^{1}H NMR spectral data (DMSO, 500 MHz): δ_H 0.74 (3H, *s*, Me-12), 1.79 (1H, *d*, J = 2.8 Hz, H-5), 1.50 (1H,

m, H-6β), 2.10 (1H, *m*, H-6α), 1.67 (1H, *m*, H-7β), 2.30 (H, *m*, H-7α), 2.42 (1H, H-10a), 3.5–3.3 (1H, *m*, H-8), 3.89 (3H, *s*, MeO-2), 4.76 (1H, *s*, H-11), 4.89 (1H, *d*, $J=5.2$ Hz, HO-8), 5.39 (1H, *d*, $J=2.0$ Hz, H-9), 6.44 (1H, *d*, $J=5.1$ Hz, HO-11), 6.53 (1H, *s*, H-3), 8.61 (1H, *s*, HO-1), 12.27 (1H, *s*, HO-4); ^{13}C NMR spectral data (DMSO-d_6): Table 1; HMBC data (DMSO-d_6): Table 2.

3.6. *Rel-8α,11β-Epoxy-2,11-dimethoxy-8aβ-methyl-5,6,7, 8,8a,9-hexahydro-1,4-anthracenedione* (**3**)

Orange needles, mp 199–201°; 35 mg (0.0006% whole plant); $[\alpha]_D^{25}$ +81.3° ($CHCl_3$; *c* 0.0003); UV $\lambda_{max}^{CHCl_3}$ nm (log ε) 291.97 (3.51), 267.00 (3.81); ν_{max}^{Kbr} 2961, 1640, 1566, 1467, 1371, 1238–1029 cm^{-1}; EIMS (probe) 70 eV, *m*/*z* (rel. int.): 316 $[M]^+$ (5), 256 $[M\text{-}HCO_2Me]^+$ (100), 241 $[M\text{-}HCO_2Me\text{-}Me]^+$ (47), 227 $[M\text{-}HCO_2Me\text{-}C_2H_5]^+$ (28); ^{1}H NMR spectral data ($CDCl_3$, 500 MHz: δ_H 0.93 (3H, *s*, Me-12), 1.85–1.75 (2H, *m*, H-6), 2.01–1.90 (2H, *m*, H-7), 2.52 (1H, *d*, $J=17.8$ Hz, H-9α), 2.95 (1H, *d*, $J=17.8$ Hz, H-9α), 2.58 (1H, *s*, H-5), 3.67 (1H, *s*, H-8), 3.35 (3H, *s*, MeO-11), 3.79 (3H, *s*, MeO-2), 4.78 (1H, *s*, H-11), 5.80 (1H, *s*, H-3), 6.30 (1H, *s*, H-10); ^{13}C NMR spectral data ($CDCl_3$): Table 1; HMBC data ($CDCl_3$): Table 2.

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