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Diterpenoids from *Humirianthera ampla*

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Abstract

Two diterpenoids, humirianthol and acrenol, as well as the known annonalide, were isolated from *Humirianthera ampla*. Humirianthol and acrenol were determined by 1D and 2D NMR spectroscopic techniques to be 3β,20:14β,16-diepoxy-3α,15α-dihydroxy-7-pimaren-19,6β-olide and 3β,20-epoxy-3α,15,16-trihydroxy-7-pimaren-19,6β-olide, respectively.

Keywords: *Humirianthera ampla*; Icacinaceae; Diterpenoids; Humirianthol; Acrenol

1. Introduction

Previous chemical studies on species of the Icacinaceae have revealed this family to be a rich source of terpenoids of uniformly high oxidation level (Kaplan, Ribeiro & Gottlieb, 1991; Zogbi, Roque & Gottlieb, 1981). In the present study, the known diterpenoid annonalide (**1**) and two new diterpenoids, humirianthol (**2**) and acrenol (**3**), were isolated from the tubers of *Humirianthera ampla* Miers, collected from Rio Branco, Acre (Amazons), Brazil. The tuber is used in traditional medicine as a snake bite treatment.

2. Results and discussion

The ethanol extract of dried tubers of *H. ampla* was successively partitioned against hexane, dichloromethane and *N*-butanol. The dichloromethane fraction was subjected to repeated silica gel column chromatography to afford pure annonalide (**1**), humirianthol (**2**) and acrenol (**3**).

Compound **1** was obtained as a crystalline solid and its IR, NMR and FABMS spectra and elemental analysis data agreed with those published previously for annonalide (Mussini, Orsini & Pellizone, 1973; Orsini, Pellizoni, McPhail, Onan & Wenkert, 1977). Additional proof for structure **1** was provided by single crystal X-ray analysis (Burrow, Farrar, Graenber, Lough & Morel, 1999).

Humirianthol (**2**), $[\alpha]_D^{20}$ −140.7° ($CHCl_3$:MeOH, 5:1; *c* 0.85), was obtained as a colourless crystalline material. Its FABMS displayed a prominent $[M + H]^+$ at *m*/*z* 363, in combination with the ^{13}C-NMR spectroscopic and elemental analysis data, which suggested that **2** had the molecular formula $C_{20}H_{26}O_6$. The IR band at 1740 cm^{-1} and the peak at δ 178.17 ppm in the ^{13}C-NMR spectrum gave evidence for the presence of a γ-lactone ring.

The ^{1}H NMR spectrum (DMSO-d_6, 400 MHz) of **2** displayed two methyl singlets at δ 1.25 and δ 0.82, assigned to the C-18 and C-17 methyl protons, respectively. The C-5, C-6, C-7, C-9, C-14 and C-15 methine protons appear at δ 2.27 (*dd*, $J_{5,20} = 2.5$; $J_{5,6} = 7$ Hz), 4.92 (*dd*, $J_{6,5} = 7$; $J_{6,7} = 5$ Hz), 5.84 (*d*, $J_{7,6} = 5$ Hz),

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1.80 (*dd*, $J_{9,\,11\beta} = 12.3$; $J_{9,\,11\alpha} = 2.8$ Hz), 3.83 (*s*) and 3.65 (*dd*, $J_{15,\,16} = 4$ Hz; $J_{15,\,15\text{-OH}} = 4$ Hz), respectively. The C-1, C-2, C-11, C-12, C-20 and C-16 methylene protons appear at δ 1.60–1.65 (*m*), 1.70–1.98 (*m*), 1.52 (*m*) and 1.15 (*m*), 1.28 (*m*), 3.41 (*dd*, $J_{20,\,1\alpha} = 2$; $J_{20',\,20} = 9$ Hz) and 3.73 (*dd*, $J_{20',\,5\alpha} = 2.5$; $J_{20',\,20} = 9$ Hz) and 3.52 (*d*, $J_{16,\,16'} = 9.5$ Hz) and 4.23 (*dd*, $J_{16,\,15} = 4$; $J_{16,\,16'} = 9.5$ Hz), respectively. The NMR spectrum also allows the assignment of the two hydroxylic protons at δ 5.35 (3-OH, *s*) and 4.99 (15-OH, *d*, $J_{\text{OH},\,15} = 4$ Hz). COSY spectra revealed the presence of four spin systems in **2**. The first spin system shows connectivity between C-1 methylene protons (δ 1.60 and 1.65) and C-2 methylene protons (δ 1.70 and 1.98). The second spin system starts with C-5 methine proton (δ 2.27) which exhibits vicinal coupling with C-6 methine proton (δ 4.92) and which, in turn, shows coupling with the C-7 olefinic proton (δ 5.84). The linkage between spin systems clearly establishes the existence of a double bond between atoms C-7 and C-8, as in compound **1**. In the third spin system, we observed vicinal coupling between the C-9α methine proton (δ 1.80) and the C-11 methylene protons (δ 1.15 and 1.52). The latter exhibits cross-peaks with the C-12 methylene protons (δ 1.28). The fourth spin system shows the presence of a tetrahydrofuran (THF) moiety in **2**. It starts with C-15 hydroxyl proton (**δ** 4.99) which exhibits a cross-peak with the C-15 methine proton (**δ** 3.65), which in turn shows coupling with C-16 methylene protons (**δ** 3.52 and 4.23). C-16 also couples with the C-3 hydroxyl proton (**δ** 4.99). The location of the THF moiety in structure **2** was confirmed from the 2D NOESY spectrum which shows cross-peaks from the C-14 methine proton to both the C-7 olefinic proton and C-13 methyl protons. No NOESY correlations were present between C-13 methyl protons and the C-15 methine proton, suggesting that they are in an *anti* position. Assignments of all protons in **2**, which were made by a series of 2D NMR experiments (COSY and NOESY) are reported in Table 1. Fig. 1 shows the NOE correlations for **2**.

Table 1
^{1}H- and ^{13}C-NMR spectral data for compound **2** (in DMSO-d_6, 400/100 MHz)[a]

H/C	δ ^{1}H (*J*, Hz)	δ ^{13}C (ppm)	HMBC correlations	
			$^2J_{CH}$	$^3J_{CH}$
1	1.60 *m*; 1.65 *m*	28.30		H-5, H-20
2	1.70 *m*; 1.98 *m*	27.96		3-OH
3	–	96.16	3-OH	H-18
4	–	49.80	H-18, H-5	3-OH, H-2
5	2.27 *dd* (2.5, 7.0)	43.80		H-9, H-18
6	4.92 *dd* (5.0, 7.0)	71.05	H-7	
7	5.84 *d* (5.0)	116.38	H-6	H-14
8	–	145.58		H-6
9	1.80 *dd* (12.3, 2.8)	36.21	H-7 5	H-14, H-7, H-
10	–	29.80	H-5, H-9 1	H-20, H-1, H-6
11	1.15 *m*; 1.52 *m*	24.90	H-9	
12	1.28 *m*	31.91		H-11, H-14, H-17
13	–	48.10	H-14, H-17	H-11, 15-OH
14	3.83 *s*	85.26		H-17, H-7, H-15
15	3.65 *dd* (4.0, 4.0)	77.41	15-OH, H-16	H-14, H-17
16	3.52 *d* (9.5)	75.08		15-OH
17	0.82 *s*	15.04		
18	1.25 *s*	18.30		H-5
19		178.17		
20	3.41 *dd* (2.0, 9.0)	71.04		H-5
3-OH	5.35 *s*			
15-OH	4.99 *d* (4.0)			

[a] Assignments were obtained by ^{1}H–^{1}H COSY, NOESY, DEPT 135°, HMQC and HMBC experiments.

The ^{13}C-NMR spectrum (Table 1) of **2** shows the presence of 20 carbons atoms. The assignments of the carbon chemical shifts were made by the analysis of the proton noise decoupled ^{13}C spectroscopy, DEPT 135°, two-dimensional heteronuclear correlated spectroscopy (HMQC and HMBC) and by comparison with the δ values of the corresponding carbon atoms in structurally similar compounds (Mussini et al., 1973; Orsini et al., 1977; Piozzi, Paternostro & Passannanti, 1985; Kato et al., 1997). In the HMBC spectrum of **2** (Table 1), long range H/C-correlations were observed: δ_H 2.27 (H-5)-δ_C 36.21 (C-9), δ_C 28.30 (C-1) and δ_C 18.30 (C-18); δ_H 3.83 (H-14)-δ_C 31.91 (C-12) and δ_C 48.10 (C-13); δ_H 0.82 (H-17)-δ_C 31.91 (C-12), δ_C 48.10 (C-13) and δ_C 85.26 (C-14); δ_H 5.35 (3-OH)-δ_C 27.96 (C-2), δ_C 96.16 (C-3) and δ_C 49.80 (C-4); δ_H 4.99 (15-OH)-δ_C 48.10 (C-13), δ_C 77.41 (C-15), δ_C 15.04 (C-17) and δ_C 75.08 (C-16). The structure **2** was thus proposed based on the data described.

Acrenol (**3**), with the same ring A and B pattern as compound **2**, had a structure closely related to **1**, but differed by the presence of a hydroxyl group attached

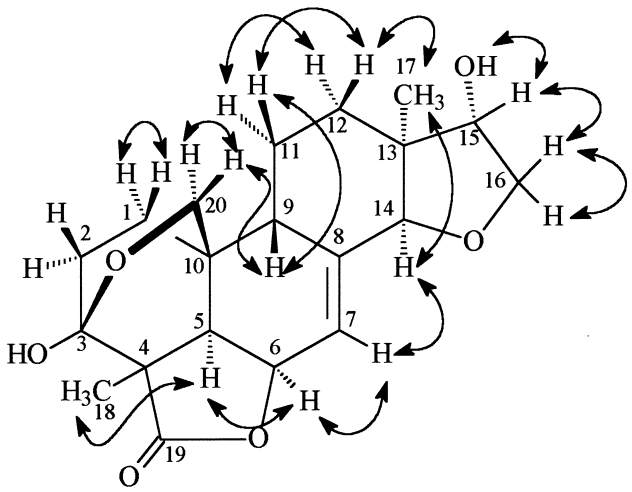


Fig. 1. Significant NOESY correlations spectra of humirianthol (**2**).

to C-15. Compound **3** was obtained as a colourless crystalline material, $[\alpha]_D^{25} = -140.7^\circ$ ($CHCl_3$:MeOH, 5:1). The molecular formula $C_{20}H_{28}O_6$ was deduced from the FABMS (*m/z* 365 $[M + 1]^+$), ^{13}C-NMR and elemental analysis.

The ^{1}H-NMR spectrum of **3** (DMSO-d_6) is similar to that of **1**, but differs by the presence of additional signals for the carbinolic and hydroxylic protons at δ_H 3.12 (H-15) and 4.52 (15-OH). In the 2D ^{1}H–^{1}H COSY spectrum, the signals at δ 4.35, which correspond to the 16-OH, have a cross-peak with the signal at δ 3.12, which correspond to H-15. The latter shows a cross-peak with H-16 at δ 3.50 and 3.25; H-16 also has a second coupling with the 16-OH at δ 4.35. This spin system indicates the presence of a diol moiety as a side chain in **3**. The assignments, along with coupling constants of different protons, is presented in Table 2, and were further confirmed by NOESY experiments, which conclusively shows cross-peaks at the expected positions. Fig. 2 shows NOE correlations for **3**.

The ^{13}C-NMR spectrum of **3** revealed the presence of 20 carbons. Determination of the multiplicity obtained by DEPT 135°, DEPT 90° and HMQC spectra, indicated that its carbon skeleton was composed of two methyls, seven methylenes, four methines, five quaternary and two carbonyl carbons. Unambiguous assignments of the carbon resonances of **3** and the observed long-range correlations (HMBC) are summarised in Table 2.

Table 2
^{1}H- and ^{13}C-NMR spectral data for compound **3** (in DMSO-d_6, 400/100 MHz)[a]

H/C	δ ^{1}H (*J*, Hz)	δ ^{13}C (ppm)	HMBC correlations	
			$^2J_{CH}$	$^3J_{CH}$
1	1.55 *m*; 1.60 *m*	28.20	H-2	H-5, H-20
2	2.0 *m*; 1.70 *m*	27.96	H-1	3-OH
3	–	96.19	3-OH	H-20, H-18
4	–	50.02	H-5, H-18	3-OH
5	2.23 *dd* (1.5, 7.0)	43.38	H-6	H-7, H-20
6	4.98 *dd* (5.0, 7.0)	72.17	H-7, H-5	
7	5.54 *d* (5.0)	113.66	H-6	H-14
8	–	146.47	H-14	H-6
9	1.40 *dd* (2.9, 12.3)	42.38		H-14, H-7, H-5
10	–	29.91	H-5, H-20	H-6
11	1.58 *m*; 1.60 *m*	24.30	H-9	
12	1.35 *m*; 1.54 *m*	34.03		H-14, H-17
13	–	39.52	H-14, H-17	15-OH
14	2.0 *s*	43.89		H-17, H-7
15	3.12 *m*	79.41	15-OH	16-OH, H-17
16	3.25 *m*; 3.50 *m*	62.00	H-15	15-OH
17	0.66 *s*	17.82		H-14, H-15
18	1.26 *s*	18.30		H-5
19		178.43		H-5, H-18
20	3.44 *dd* (1.5, 8.0)	71.82		
3-OH	5.30 *s*			
15-OH	4.52 *d* (4.0)			
16-OH	4.35 *t* (5.5)			

[a] Assignments were obtained by ^{1}H–^{1}H COSY, NOESY, DEPT 135°, HMQC and HMBC experiments.

Treatment of **3** with acetone yielded an acetal derivative **4**. The isopropylidene moiety in the structure is shown by the proton signal at δ 1.41 ppm (6H, *s*) and by the carbon signals at δ 26.25 and δ 26.42 ppm.

Comparison of the ^{1}H- and ^{13}C-NMR spectra of **2** and **3** with those of **1**, shows that **2** and **3** are also diterpenoids of the same pimarane framework, with very similar A/B-ring systems. Compound **2** differs structurally from **1** by the presence of a THF moiety, while the only difference between **3** and **1** is a hydroxyl group in place of a carbonyl group at C-15.

The ^{1}H-NMR spectrum, with unambiguously established couplings, was fundamental for the determination of the stereochemical features of **2** and **3** (Fig. 3). For compounds **2** and **3**, the two diastereotopic protons on C-20 show $^4J_{HH}$ values of relatively large magnitudes indicating a *w*-type arrangement. The high field peak of the two signals was split by H-5 (1.5 Hz), indicating a *trans* junction between rings A and B and that the proton giving rise to these signals was *cisoid* to C-1 (Zogbi, Roque & Gottlieb, 1981). The other proton of the oxymethylene was located over ring B and shows a larger $^4J_{HH}$ coupling (3.0 Hz), with H-1α (*w*-arrangements) (Fig. 3). The stereochemistry of C-3, C-4, C-5, C-6 and C-10 was confirmed by the strong similarity between the ^{1}H and ^{13}C spectral data of **2** and **3** and those of annonalide (**1**). In addition, there was a correlation between H-9, which occupies an axial position (H-β) in the bridgehead between rings B and C, and H-20, in the NOESY spectra of **2** and **3**. This suggests the oxymethylene to be on the same face of the molecule as H-9 (C-20 and H-9 in a *cis* relationship). The relative stereochemistry of C-13 in compound **3**, which is not established directly, is assumed to be the same as in compound **1**, whereas

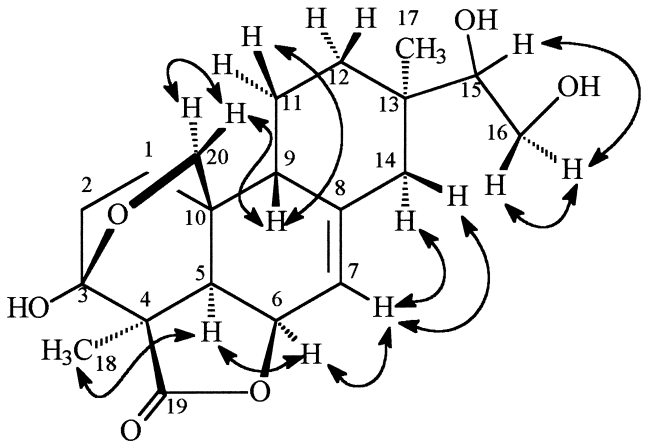


Fig. 2. Significant NOESY correlations of acrenol (**3**).

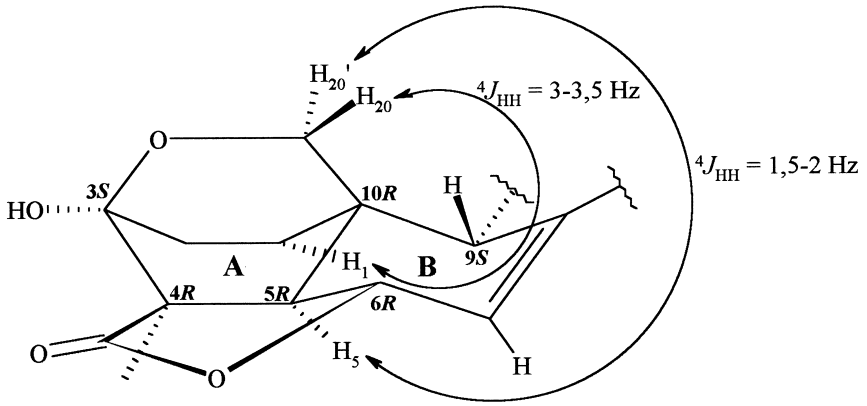
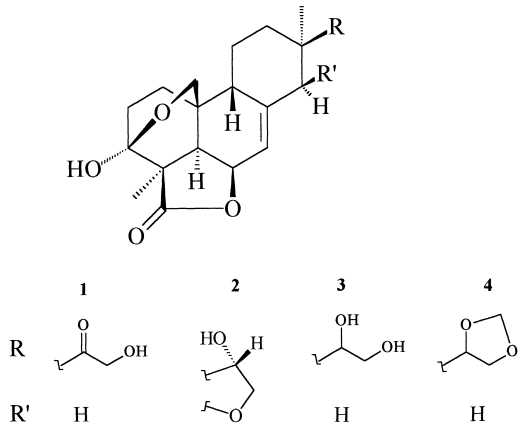


Fig. 3. $^4J_{HH}$ values of A/B-ring systems of compounds **1**, **2** and **3**.

the stereochemistry of C-15 remains unsolved. The absolute stereochemistry of the secondary alcohol at C-15 in compound **2** was determined by the Horeau method, including enantioselective gas chromatography (König, Gehrcke & Weseloh, 1994) which permitted us to establish that C-15 has an (*S*)-configuration.



3. Experimental

Mps are uncorrected. ^{1}H and ^{13}C spectra were obtained on a Bruker DPX-400 operating at 400 and 100.6 MHz, respectively. Chemical shifts are given in δ (ppm) using TMS as internal standard. FABMS were recorded with a VG analytical 70-150-S mass spectrometer equipped with a FAB ion source from 3-nitrobenzylalcohol matrix. Thin layer chromatography (TLC) was performed on precoated TLC plates (Merck, silica 60 F-254).

3.1. Plant material

Humirianthera ampla was collected in May 1997 around Rio Branco, in the state of Acre, Brazil. A voucher specimen (No. 8285) is deposited in the herbarium at the University of Acre, Brazil.

3.2. Extraction and isolation

Air dried, powdered tubers (0.960 kg) of *H. ampla* were extracted exhaustively with ethanol at room temperature. Dry ethanol extract was dissolved in H_2O and the solution was successively partitioned between hexane, CH_2Cl_2, EtOAc and *n*-BuOH. The CH_2Cl_2 fraction was subjected to silica gel CC with $CHCl_3$–MeOH mixtures to yield **1** (120 mg), **2** (80 mg) and **3** (90mg).

Annonalide (**1**). Mp 260°C; $[\alpha]_D^{20} = -142°$ ($CHCl_3$:MeOH, 1:1; *c* 1.40).

Humirianthol (**2**). Mp 268°C; $[\alpha]_D^{20} = -156.9°$ ($CHCl_3$:MeOH, 5:1; *c* 0.85); IR ν_{max} cm^{-1}: 3470 (OH), 2930 (=CH), 1740 (C=O), 1700 (C=C); FABMS (positive) *m/z*: 363 [$C_{20}H_{26}O_6$]; (Found: C, 66.35; H, 7.13; Calc. for $C_{20}H_{26}O_6$: C, 66.28; H, 7.23%.); ^{1}H- and ^{13}C-NMR spectral data: see Table 1.

Acrenol (**3**). Mp 155°C; $[\alpha]_D^{20} = -140.7°$ ($CHCl_3$:MeOH, 5:1; *c* 0.92). IR ν_{max} cm^{-1}: 3550 (OH), 2940 (=CH), 1739 (C=O), 1660 (C=C); FABMS (positive) *m/z* 365 [$C_{20}H_{28}O_6$]; (Found: C, 65.12; H, 7.63; Calc. for $C_{20}H_{28}O_6$: C, 65.92; H, 7.74%.); ^{1}H- and ^{13}C-NMR spectral data: see Table 2.

3.3. Horeau method (König et al., 1994)

To 5 μmol of **2**, ca. 6 μmol of (±) α-phenylbutyric anhydride and 30 μl dry pyridine was added and kept for 1 h at room temperature. After standing with 10 μl of water for 30 min; usual work-up gave the enantiomeric excess of (+) 2-phenylbutyric acid. A solution of a diazomethane in diethyl ether (50 μl) is added until a permanent yellow colour formed. The solution was concentrated under a stream of N_2 to ca. half of

its volume and the remaining solution used for gas chromatographic analysis.

3.4. Gas chromatography

A 25 m fused silica capillary column with heptakis (2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin (König, Icheln, Runge, Pforr & Krebs, 1990) diluted with polysiloxane OV 1701 (1:1 w/w) at 85°C column temperature was used in a Varian 3800 gas chromatograph, equipped with FID, for the separation of the enantiomers excess of α-phenylbutyric acid methyl ester.

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