Artigo

CLERODANE DITERPENES FROM LEAVES OF *Casearia sylvestris* SWARTZ

André G. dos Santos, Carla C. Perez, Aristeu G. Tininis, Vanderlan da S. Bolzani and Alberto J. Cavalheiro*
Instituto de Química de Araraquara, Universidade Estadual Paulista "Júlio de Mesquita Filho", R. Francisco Degni, s/n, 14800-900 Araraquara - SP, Brasil

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Ethanolic extracts of the leaves of *Casearia sylvestris* yielded a novel clerodane diterpene, 15-hydroxy-3-cleroden-2-one, together with the known diterpenes (-)-hardwickiic acid, reported for the first time from this species, and casearins B and G, previously isolated from *C. sylvestris*. The structures of all four compounds were determined by spectrometric analysis. The new clerodane diterpene and (-)-hardwickiic acid contain structural features that are completely different from the highly oxygenated casearins and casearvestrins isolated from *C. sylvestris*.

Keywords: *Casearia sylvestris*; clerodane diterpenes; 15-hydroxy-3-cleroden-2-one.

INTRODUCTION

Casearia sylvestris Swartz (Flacourtiaceae) is a tree that is widely distributed within various ecosystems of South America, such as the Cerrado and the Atlantic and Amazon forests[1]. In the popular medicine of Brazil, the use of the plant[1] is correlated with its pharmacological properties including anti-inflammatory[2], anti-ophidian[2,3] and anti-ulcer[1,4] activities. A number of phytochemical investigations of species of *Casearia*[5-9] have revealed the occurrence within the genus of oxygenated tricyclic clerodane diterpenes exhibiting a *cis* configuration between rings A and B and a characteristic diacetal system in ring C at positions C-18 and C-19. Several compounds of this type have been isolated from *C. sylvestris*, specifically casearins and casearvestrins, and they exhibited cytotoxic activity[5,7-9].

This paper describes the isolation and structure elucidation of a novel clerodane diterpene, 15-hydroxy-3-cleroden-2-one (**1**), from the leaves of *C. sylvestris,* together with (-)-hardiwickiic acid (**2**), reported for the first time from this species. In addition, casearin B (**3**) and G (**4**) were isolated, diterpenes that have been previously detected in the species[5,7,8]. The structures of compounds **1-4** (Figure 1) were deduced on the basis of their spectral data and comparison with appropriate values reported in the literature.

RESULTS AND DISCUSSION

Ethanol extract of the leaves of *C. sylvestris* was submitted to chromatographic fractionation and yielded compounds **1**-**4**. In the positive-ion mode, the HRTOF-ESIMS of **1** exhibited an $[M+Na]^+$ ion at *m/z* 329.2481 that was compatible with a molecular formula of $C_{20}H_{34}O_2$. Analysis of the PND and DEPT 135º ^{13}C-NMR spectra of **1** revealed twenty signals similar to those of the clerodane diterpene skeleton[6-11], being five of them attributed to methyl groups: C-16 (δ 19.7), C-17 (δ 16.0), C-18 (δ 20.5), C-19 (δ 32.2), C-20 (δ 19.3). The IR and UV absorptions at 1653 cm^{-1} and at 218 nm (λ_{max}) and the NMR signals at δ 199.3 (C-2), 128.6 (C-3), 168.5 (C-4) and 5.84 *br s* (H-3) indicated the presence of an α,β-unsaturated ketone group. In addition, the IR absorption at 3448 cm^{-1} and the NMR signals observed at δ 61.2 (C-15) and 3.68 *m* (H-15) are characteristic of a hydroxymethine group. The ^{1}H NMR and ^{13}C assignments (Table 1) together with the results of HMQC, COSY and HMBC (Figure 2) experiments led to structure of compound **1**. The relative configuration assignment at C-5, C-8, C-9 and C-10 was firstly proposed based on comparison of chemical shifts of methyl groups C-17, C-19 and C-20 of compound **1** with literature data, as follow. The *cis* stereochemistry at the junction of rings A and B in compound **1** could be deduced from the chemical shift of C-19 (δ 32.2) when compared with C-19 chemical shifts of *cis*-clerodane diterpenes, for example floridiolic acid[12] and 13-hydroxy-*cis*-*ent*-cleroda-3,14-diene[13] (δ 33.6 and 33.0, respectively). In contrast, the *trans*-clerodane diterpenes 2-oxokolavenic acid[10] and eremone[11] presented chemical shifts for this methyl carbon at δ 19.5 and 18.8, respectively. In clerodane diterpenes with a *cis* configuration between methyl groups C-17 and C-20, such as hautriwaic acid[11], 2-oxokolavenic acid[10] and floridiolic acid[12], the chemical shift of C-20 was observed at approximately δ 18.0, whilst clerodane diterpenes with a *trans* configuration between these methyl groups, as in the casearins and casearvestrins[5-9], show chemical shift for C-20 at approximately δ 26.0. So, the chemical shifts attributed to C-20 in compound **1** (19.3) is indicative of a *cis*

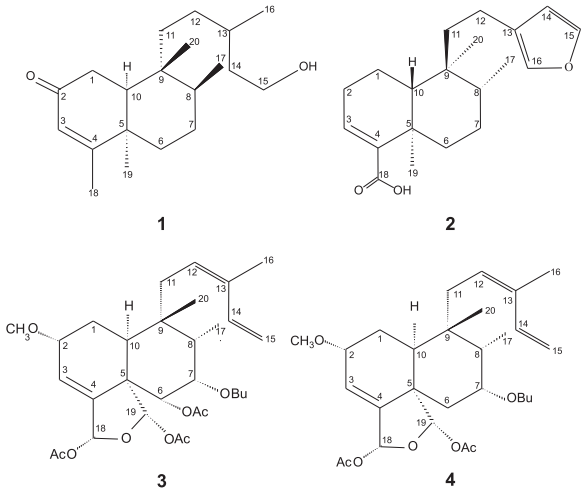

Figure 1. *Structures of 15-hydroxy-3-cleroden-2-one (**1**), (-)-hardiwickiic acid (**2**), casearin B (**3**) and casearin G (**4**): AcO-acetate, BuO-butanoate*

*e-mail: albjcava@iq.unesp.br

relationship between these methyl groups. These propositions were confirmed by the NOE enhancements arising from the dipolar interactions between H-19 (1.22 *s*) and H-10 (1.87 *br d*; 6.5 Hz) and between H-17 (0.77 *d*; 7.0 Hz) and H-20 (0.57 *s*). In addition, correlations observed in the NOE experiments (Figure 3) between H-1α (δ 2.71) and H-19 and between H-1β and H-20, revealed the *trans* relationship between C-17/C-20 and C-19 methyl groups. A diastereomer of compound **1** was previously isolated from *Cistus populifolius* L. (Cistaceae)[14], but the ^{13}C NMR data of this compound were not included in the article to a complete comparison.

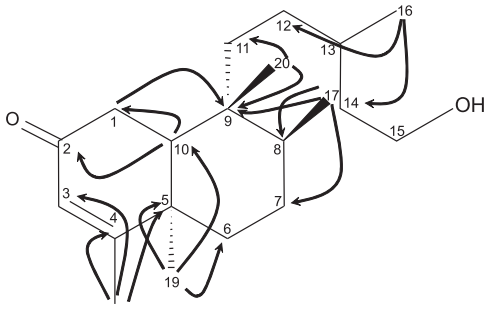

Figure 2. *Selected correlations observed in the HMBC spectra of 15-hydroxy-3-cleroden-2-one (**1**): (H→C)*

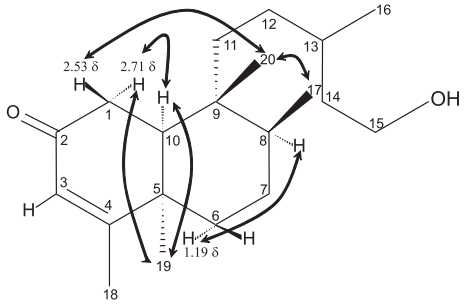

Figure 3. *Selected correlations observed by NOE difference and NOESY spectroscopy of 15-hydroxy-3-cleroden-2-one (**1**)*

In the positive-ion mode, the HRTOF-ESIMS of compound **2** exhibited an $[M+H]^+$ ion at *m/z* 317.2117, compatible with a molecular formula of $C_{20}H_{28}O_3$, which was confirmed by the finding of $[M+Na]^+$ ion at *m/z* 339.1963. Analysis of the PND ^{13}C-NMR spectra revealed twenty signals similar to those of the clerodane diterpene skeleton[6-11] and showed the presence of three methyl groups: C-17 (δ 16.0), C-19 (δ 20.6) and C-20 (δ 18.3). IR absorptions presented at 3397 cm^{-1} and 1684 cm^{-1}, UV absorption (λ_{max}) at 210 nm and the signals observed in the ^{13}C-NMR spectra at δ 172.5 (C-18), 140.2 (C-3) and 141.5 (C-4) were indicative of an α,β-unsaturated carboxylic group. In addition, the ^{1}H and ^{13}C NMR spectra showed signals that could be ascribed to a β-mono-substituted furan ring (C-13, C-14, C-15 and C-16) in the lateral chain of the diterpene. The complete attribution of the NMR signals (**table 1**) was based on the correlations observed in the HMQC, HMBC and COSY contour plots and this attribution is in agreement with the NMR data from literature for hardiwickiic acid[15]. The negative value of $[\alpha]_D^{25}$ confirmed the absolute configuration as (-)-hardiwickiic acid[16]. Compound **2** or its enantiomer were previously isolated from other species of different families, as *Copaifera duckei*, *C. guianensis*, *Croton californicus*, *C. aromaticus*, *Hardwickia pinnata*, *Salvia divinorum* and *Sindora sumatrana*[15,17], and now we found it for the first time in a *Casearia* species.

The ^{1}H- and ^{13}C-NMR data of **3** ($C_{31}H_{44}O_{10}$) and **4** ($C_{29}H_{42}O_8$) showed signals identical of the compounds previously reported in *C. sylvestris*[5,7,8], casearins B and G, respectively.

EXPERIMENTAL

General

Column chromatography (CC) and solid phase extraction (SPE) were performed over activated charcoal (Synth), silica gel G (40-63 μm; Merck) or RP-C18 (40-63 μm; Merck) and the resulting fractions were monitored by TLC, HPLC-UV and ^{1}H-NMR. Comparative TLC was carried out on silica gel G60 layers (0.25 mm thickness; Merck). HPLC-UV analyses were conducted using a Supelcosil LC-18 column (250 x 4.6 mm; i.d. 5 μm) connected to a Varian chromatographic system consisting of a ProStar 240 solvent delivery module, a ProStar 330 photodiode array detector, a ProStar 410 autosampler and a Star chromatography workstation. For preparative HPLC, a Supelcosil PLC-18 column (250 x 21.2 mm; i.d. 12 μm) was coupled to a Varian system consisting of Dynamax model Sd-1 solvent delivery system and a ProStar 320 detector with Star integrator software.

IR spectra (KBr disc) were obtained using a Perkin Elmer FTIR 1600 spectrometer, UV spectra were measured on a Cary 13E instrument and a Perkin Elmer 341 polarimeter was employed to determine optical activities. NMR spectra of **1** and **2** were recorded at 500 and 125 MHz for ^{1}H- and ^{13}C-NMR, respectively, using a Varian INOVA 500 spectrometer with $CHCl_3$ as internal standard. The NMR spectra of **3** and **4** were measured at 200 and 50 MHz for ^{1}H- and ^{13}C-NMR, respectively, on a Bruker AC-200 instrument using the same internal standard. Accurate-mass measurements were performed on an ESI-quadrupole-time of flight instrument (UltrOTOF$_Q$, Bruker Daltonics, Billerica, MA).

Plant material

Leaves of *Casearia sylvestris* Swartz (Flacourtiaceae) were collected at the Carlos Botelho State Park (São Paulo State, Brazil) and in Araraquara (São Paulo State, Brazil) between August and November 1999. Voucher specimens (AGS13 and AGS45) are deposited at the State Herbarium "Maria Eneida P. Kaufmann" of the Botanic Institute (São Paulo State, Brazil).

Extraction and isolation of 1, 3 and 4

Dried and powdered leaves of *C. sylvestris* (2.1 kg) were extracted by sonication with EtOH (3 x 6.0 L; 20 min per extraction) and concentrated under reduced pressure to yield 139.0 g of residue. The crude extract was partitioned between MeOH:water (7:3, v/v, 0.8 L) and CH_2Cl_2 (1.5 L) and the CH_2Cl_2 layer separated, dried and concentrated under reduced pressure to yield 71.0 g of a residue that was subjected to SPE over silica gel eluted sequentially with CH_2Cl_2, CH_2Cl_2:MeOH (9:1) and MeOH, yielding 8 fractions. SPE-fraction 5 (13.5 g) eluted with CH_2Cl_2:MeOH (9:1) was submitted to CC over silica gel eluted sequentially with hexane:EtOAc (8:2, 6:4 and 4:6), EtOAc, EtOAc:MeOH (9:1) and MeOH, yielding 12 CC-fractions. CC-fraction 8 (1.45 g) eluted with hexane:EtOAc 4:6 was submitted to RP-CC over octadecyl silane eluted sequentially with MeOH:water (6:4, 8:2 and 9.5:0.5), MeOH and CH_2Cl_2. Sub-fraction 3 (120.0 mg) eluted with MeOH:water 8:2 was submitted to preparative RP-HPLC (C-18, 65% MeOH, isocratic mode, flow rate 13.0 mL/min, λ 235 nm) to yield **1** (5.0 mg). Further, CC-fraction 7 (2.4 g) eluted with hexane:EtOAc 6:4 was submitted to preparative RP-HPLC (C-18, 75% MeOH,

Table 1. ^{13}C- and ^{1}H-NMR data for 15-hydroxy-3-cleroden-2-one (1) and (-)-hardwickiic acid (2)

Position	15-Hydroxy-3-cleroden-2-one (**1**)		(-)-Hardwickiic acid (**2**)	
	^{13}C-NMR [a] δ_C (ppm)	^{1}H-NMR [b] δ_H (ppm) *J* (Hz)	^{13}C-NMR [a] δ_C (ppm)	^{1}H-NMR [b] δ_H (ppm) *J* (Hz)
1	35.5 *t*	2.53 *d* (19.0) 2.71 *dd* (19.0, 6.5)	17.5 *t*	1.70 *m*
2	199.3 *s*	——-	27.3 *t*	1.42 *m*
3	128.6 *d*	5.84 *br s*	140.2 *d*	6.82 *dd* (4.5, 3.0)
4	168.5 *s*	——-	141.5 *s*	——-
5	39.3 *s*	——-	37.6 *s*	——-
6	36.9 *t*	1.19 *m* 2.09 *m*	35.9 *t*	2.40 *dt* (13.0, 3.0) 1.17 *td* (13.0, 4.0)
7	28.4 *t*	1.34 *m*	27.5 *t*	*nr**
8	36.6 *d*	1.48 *m*	36.3 *d*	*nr**
9	40.1 *s*	¾	38.9 *s*	——-
10	47.2 *d*	1.87 *br d* (6.5)	46.7 *d*	1.36 *dd* (11.0, 1.0)
11	33.8 *t*	1.18 *m* 1.42 *m*	38.7 *t*	1.52 *m* 1.64 *m*
12	29.7 *t*	0.94 *m* 1.24 *m*	18.2 *t*	*nr*[c] *nr*[c]
13	30.2 *d*	*nr*[c]	125.6 *s*	——-
14	40.0 *t*	1.38 *m* 1.61 *m*	111.0 *d*	6.23 *dd* (2.0, 1.0)
15	61.2 *t*	3.68 *m*	142.7 *d*	7.32 *t* (2.0)
16	19.7 *q*	0.91 *d* (7.0)	138.4 *d*	7.18 *m*
17	16.0 *q*	0.77 *d* (7.0)	16.0 *q*	0.81 *d* (6.5)
18	20.5 *q*	1.94 *d* (1.5)	172.5 *s*	——-
19	32.2 *q*	1.22 *s*	20.6 *q*	1.24 *s*
20	19.3 *q*	0.57 *s*	18.3 *q*	0.74 s

[a] Measured in $CDCl_3$ at 125 MHz; [b] Measured in $CDCl_3$ at 500 MHz; [c] *nr* – not resolved due to overlapping of signals

isocratic mode, flow rate 10.0 mL/min, λ 235 nm), yielding compounds **3** (86.2 mg) and **4** (30.9 mg).

Extraction and isolation of 2

Dried and powdered leaves of *C. sylvestris* (2.0 kg) were extracted by maceration under agitation with EtOH (3 x 3.0 L; 2 h per extraction) and concentrated under reduced pressure to yield 190.0 g of residue. The crude extract was partitioned between EtOH/Water (6:4, v/v, 1.5 L) and hexane (1.5 L), and the hexane layer separated, dried and concentrated under reduced pressure to yield 120.2 g of a residue that was subjected to SPE over silica gel:activated charcoal (1:1) eluted sequentially with hexane:EtOAc (7:3 and 3:7) and MeOH. The fraction eluted with hexane/EtOAc 3:7 (28.4 g) was submitted to CC over silica gel eluted sequentially with hexane:EtOAc (7:3, 6:4, 1:1, 4:6 and 3:7) and EtOAc, yielding 24 fractions. Fraction 11 (34.0 mg) eluted with hexane:EtOAc 1:1 was submitted to SPE over RP-C18 eluted with MeOH:water (98:2) to yield **2** (29.0 mg).

*15-hydroxy-3-cleroden-2-one (**1**)*

White powder (5.0 mg). $[\alpha]_D^{20}$ +42º (*c* 0.14; MeOH). UV λ_{max} nm (MeOH) 218 (ε = 10.5 x 10^3 cm^3 mol^{-1} cm^{-1}). IR ν_{max} cm^{-1} 3448, 2958-2853, 1653, 1037 (KBr). ^{1}H- and ^{13}C-NMR see Table 1. HRTOF-ESIMS *m/z* 329.2481 $[M+Na]^+$ (calcd for $C_{20}H_{34}O_2Na^+$, 329.2451).

*(-)-Hardiwickiic acid (**2**)*

White powder (29.0 mg). $[\alpha]_D^{25}$ -35º (*c* 1.0; MeOH). UV λ_{max} nm (MeOH) 210 (ε = 7.2 x 10^3 cm^3 mol^{-1} cm^{-1}). IR ν_{max} cm^{-1} 3397, 2958-2864, 1684, 1640, 1257, 1078 (KBr). ^{1}H- and ^{13}C-NMR see Table 1. HRTOF-ESIMS *m/z* 317.2117 $[M+H]^+$ (calcd for $C_{20}H_{29}O_3$, 317.2117) and *m/z* 339.1963 $[M+Na]^+$ (calcd for $C_{20}H_{28}O_3Na^+$, 339.1930).

CONCLUSIONS

The new compound (+)-15-hydroxy-3-cleroden-2-one (**1**) and (-)-hardwickiic acid (**2**) are the first diterpenes reported in *C. sylvestris* without the typical highly oxygenated backbone. Casearins and casearvestrins, previously isolated from this species, have common stereochemical features of a *cis*-clerodane diterpene, notably a *cis* configuration at the junction of rings A and B and, in addition, *trans* configuration between the methyl groups at C-17 and C-20[5,7-9]. However, whilst the newly described (+)-15-hydroxy-3-cleroden-2-one (**1**) is a typical *cis*-cledorane, (-)-hardiwickiic acid (**2**) possesses a *trans*-clerodane configuration. In conclusion, the occurrence of compounds **1**, **2**, **3** and **4** in *C. sylvestris* reveals the ability of this species in biosynthesize both *cis* and *trans*-clerodanes as it is common in other plant species as *Adelanthus lindenbergianus* (Adelhantaceae)[13] and *Grangea maderaspatana* (Asteraceae)[18].

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