CLERODANE DITERPENES FROM LEAVES OF *Casearia sylvestris* SWARTZ

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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. In the popular medicine of Brazil, the use of the plant is correlated with its pharmacological properties including anti-inflammatory, anti-ophidiain and anti-ulcer activities. A number of phytochemical investigations of species of *Casearia* 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.

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 (-)-hardwickiic 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. 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 13C-NMR spectra of 1 revealed twenty signals similar to those of the clerodane diterpene skeleton, being five of them attributed to methyl groups: C-16 (δ 19.9), 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 1H NMR and 13C 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 and 13-hydroxy-cis-ent-cleroda-3,14-diene (δ 33.6 and 33.0, respectively). In contrast, the trans-clerodane diterpenes 2-oxokolavenic acid and eremone 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 hauatrivic acid, 2-oxokolavenic acid and floridiolic acid, 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, show chemical shift to 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 configuration between these methyl groups.

![Figure 1. Structures of 15-hydroxy-3-cleroden-2-one (1), (-)-hardwickiic acid (2), casearin B (3) and casearin G (4): AcO-acetate, BuO-butanoate.](image-url)
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 C₁₅H₂₀O₃, which was confirmed by the finding of [M+Na]⁺ ion at m/z 339.1963. Analysis of the PND ¹³C-NMR spectra revealed twenty signals similar to those of the clerodane diterpene skeleton and showed signals that could be ascribed to an α,β-unsaturated carboxylic group. In addition, the ¹H and ¹³C NMR data of this compound was previously isolated from other species of different families, as shown in Table 1. The complete attribution of the NMR signals showed signals identical of the compounds previously reported in C. sylvestris, 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 ¹H-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 2.1 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 I and 2 were recorded at 500 and 125 MHz for ¹H- and ¹³C-NMR, respectively, using a Varian INOVA 500 spectrometer with CHCl₃ as internal standard. The NMR spectra of 3 and 4 were measured at 200 and 50 MHz for ¹H- and ¹³C-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 (UltrOTOFQ, 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 CH₂Cl₂ (1.5 L) and the CH₂Cl₂ 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 CH₂Cl₂, CH₂Cl₂:MeOH (9:1) and MeOH, yielding 8 fractions. SPE-fraction 5 (13.5 g) eluted with CH₂Cl₂: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 CH₂Cl₂. 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 1.0 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, 4:6)
Table 1. $^{13}$C- and $^1$H-NMR data for 15-hydroxy-3-cleroden-2-one (1) and (-)-hardwickiic acid (2)

<table>
<thead>
<tr>
<th>Position</th>
<th>15-Hydroxy-3-cleroden-2-one (1)</th>
<th>(-)-Hardwickiic acid (2)</th>
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<tr>
<td></td>
<td>$^1$C-NMR $^a$</td>
<td>$^1$H-NMR $^b$</td>
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<tr>
<td>1</td>
<td>35.5 $t$</td>
<td>2.53 $d$ (19.0)</td>
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<td>2</td>
<td>199.3 $s$</td>
<td>273 $t$</td>
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<td>3</td>
<td>128.6 $d$</td>
<td>5.84 br $s$</td>
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<td>141.5 $s$</td>
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<td>1.19 $m$</td>
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<td>47.2 $d$</td>
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<td>13</td>
<td>30.2 $d$</td>
<td>$n^r$</td>
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<td>14</td>
<td>40.0 $t$</td>
<td>1.38 $m$</td>
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<tr>
<td>15</td>
<td>61.2 $t$</td>
<td>3.68 $m$</td>
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<td>1.94 $d$ (1.5)</td>
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<td>19</td>
<td>32.2 $q$</td>
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<tr>
<td>20</td>
<td>19.3 $q$</td>
<td>0.57 $s$</td>
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</table>

$^a$ Measured in CDCl$_3$, at 125 MHz; $^b$ Measured in CDCl$_3$, at 500 MHz; $^n^r$ not resolved due to overlapping of signals

isocratic mode, flow rate 10.0 mL/min, $\lambda$ 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, 6:4, 1:1, 4:6 and 3:7) and EtOAc, yielding 3:7 (28.4 g) was submitted to CC over silica gel:activated charcoal (1:1) eluted sequentially with hexane:EtOAc (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, and elutro, 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 $\lambda_{max}$ nm (MeOH) 218 ($\epsilon$ = 10.5 x 10$^3$ cm$^{-1}$ mol$^{-1}$ cm$^{-1}$). IR $\nu_{max}$ cm$^{-1}$ 3448, 2958-2583, 1653, 1037 (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$_{29}$O$_3$Na$^+$, 339.1930).

(-)-Hardwickiic acid (2)

White powder (29.0 mg). $[\alpha]_D^{20}$ -35º ($c$ 1.0; MeOH). UV $\lambda_{max}$ nm (MeOH) 210 ($\epsilon$ = 7.2 x 10$^3$ cm$^{-1}$ mol$^{-1}$ cm$^{-1}$). IR $\nu_{max}$ 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$_{29}$O$_3$Na$^+$, 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$^{3,5}$. However, whilst the newly described (+)-15-hydroxy-3-cleroden-2-one (1) is a typical cis-clerodane, (-)-hardwickiic 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 biosynthesizing 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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