Aylthonic acid, a new tetranorneoclerodane from *Aylthonia macrantha* (Velloziaceae)

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1. Introduction

The Velloziaceae comprise a family of tropical monocotyledons with most of its 200 species occurring in Brazil. These plants grow in a characteristic ecosystem fully exposed on mountain sides in rocky or sandy soils. Although plants in the family Velloziaceae live under conditions of high solar irradiation and low water availability, they show a surprising longevity. Several species from different genera of Velloziaceae have been chemically studied, and diterpenoids, triterpenoids and flavonoids have been isolated (Branco et al., 2001, 2002; Salatino et al., 2000). The Brazilian species studied have been shown to produce diterpenoids of the iso-pimarane (Dantas et al., 2003), friedolabdane (Pinto et al., 1995a, 1996), clerodane (Pinto et al., 1994), cleisthantane (Da Silva et al., 2001), barbacenane (Pinto et al., 1985), kaurane (Pinto et al., 1981), rosane (Pinto et al., 1983), velloziolane (Pinto et al., 1982), totarane (Pinto et al., 1995b), eunicellane and bis-diterpenoid (Pinto et al., 1997) classes.

As part of a continuing study of the secondary metabolites from plants of the Brazilian Velloziaceae, we report here the results of the first chemical study of

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Aylthonia macrantha, a species collected at the National Park of Serra do Cipó, Minas Gerais State, Brazil.

2. Materials and methods

2.1. General procedures

NMR spectra were recorded in CDCl₃ solution on Varian Unity 500 MHz and Bruker 200 MHz spectrometers. IR spectra were recorded on a Perkin-Elmer model 1600 (FTIR) spectrometer. Mass measurements were obtained using a HP 5989A mass spectrometer.

2.2. Plant material

Aylthonia macrantha (Lem.) N. L. Menezes was collected in June 1992 at the National Park of Serra do Cipó, Minas Gerais State, Brazil. A voucher specimen (SPF, N. Menezes 491) is deposited in the São Paulo University Herbarium, Brazil.

2.3. Extraction and isolation of the diterpenes

The whole plant (10 g) was washed with n-hexane in order to remove epicuticular waxes. Part of dried and powdered resulting material (1 g) was exhaustively extracted with n-hexane. Evaporation of the solvent under reduced pressure resulted in a brownish gum (700 mg) that was chromatographed in a silica gel (70–230 mesh) column using a solvent gradient of increasing polarity (pure n-hexane, n-hexane-ethyl acetate and pure ethyl acetate) yielding pure compounds 1 (5 mg) and 2 (8 mg).

2.4. 6α-hydroxyannonene (1)

Colourless oil. IR, ¹H and ¹³C NMR data obtained are identical to those reported previously (Silveira and McChesney, 1994); HRMS: [M + H] m/z 303.2319 (calcld 303.2324 for C₂₀H₃₁O₂).

2.5. 6α-hydroxy-13,14,15,16-tetranor-neo-clerodan-3-en-12-oic acid (aylthonic acid) (2)

Colourless oil. IR (film) νmax cm⁻¹: 3500, 1245 and 830; ¹H and ¹³C NMR see Table 1; EIMS m/z (rel. int.): 266 (3), 248 (10), 233 (7), 206 (17), 189 (100), 188 (71), 173 (35), 152 (27), 133 (29), 109 (47), 95 (36), 81 (59).

3. Results and discussion

Silica gel chromatography of the crude hexane extracts of roots, stems and leaves of Aylthonia macrantha, using a solvent gradient of increasing polarity (from hexane to ethyl acetate), furnished two pure compounds.
Compound 1 showed an [M + H]+ ion at m/z 303.2319 in HRMS, matching the molecular formula C_{20}H_{31}O_{2} + H + (calcd 303.2324). 1H and 13C NMR spectroscopic analysis of 1 revealed that it was the 6α-hydroxyannonene (Fig. 1) previously reported from Croton sonderianus (Silveira and McChesney, 1994).

The molecular formula of 2 C_{16}H_{26}O_{3} was deduced from the MS spectra ion [M]+ 266 combined with 13C NMR data. A strong IR absorption between 3500 and 3000 cm\(^{-1}\) and the fragments at m/z 248 [M-H_2O] and 189 [M-H_2O and CH_2CO_2H], observed in the mass spectrum, indicated the presence of hydroxyl and

\[ \text{Table 1} \]

<table>
<thead>
<tr>
<th>#C/H</th>
<th>δ(^{13})C (CH(_n))(^b)</th>
<th>δ(^{1})H (nH; m; J in Hz)</th>
<th>(^{1})H–(^{1})H COSY(^c)</th>
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<tr>
<td>1eq</td>
<td>18.6 (CH(_2))</td>
<td>1.90 (1H; m)</td>
<td>1ax, 2, 10</td>
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<tr>
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<td>1.62 (1H; m)</td>
<td>1eq, 2, 10</td>
</tr>
<tr>
<td>2</td>
<td>26.4 (CH(_2))</td>
<td>2.06 (2H; m)</td>
<td>1eq, 1ax, 3</td>
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<tr>
<td>3</td>
<td>122.2 (CH)</td>
<td>5.20 (1H; br s)</td>
<td>2, 18</td>
</tr>
<tr>
<td>4</td>
<td>143.3 (C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>44.0 (C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>74.7 (CH)</td>
<td>3.61 (1H; dd; 11.2, 4.4)</td>
<td>7eq, 7ax</td>
</tr>
<tr>
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<td>37.6 (CH(_2))</td>
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<tr>
<td>7ax</td>
<td></td>
<td>1.50 (1H; ddd; 12.0, 12.0, 11.2)</td>
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<tr>
<td>8</td>
<td>35.1 (CH)</td>
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<td>9</td>
<td>40.0 (CH)</td>
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<td>10</td>
<td>46.5 (CH)</td>
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<td>2.34 (1H; d; 14.0)</td>
<td>11b</td>
</tr>
<tr>
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<td></td>
<td>2.43 (1H; d; 14.0)</td>
<td>11a</td>
</tr>
<tr>
<td>12</td>
<td>174.4 (C)</td>
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<tr>
<td>17</td>
<td>15.7 (CH(_3))</td>
<td>0.95 (3H; d; 7.0)</td>
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<td>18</td>
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</tr>
<tr>
<td>19</td>
<td>14.8 (CH(_3))</td>
<td>1.04 (3H; s)</td>
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<td>20</td>
<td>16.7 (CH(_3))</td>
<td>0.78 (3H; s)</td>
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</table>

\(^a\) \(^{1}\)H NMR spectrum was recorded at 500 MHz and \(^{13}\)C NMR spectra (PND and DEPT) at 50 MHz in CDCl\(_3\).

\(^b\) Determined by DEPT experiments.

\(^c\) \(^{1}\)H–\(^{1}\)H COSY and HETCOR experiments were performed at 200 MHz.

![Fig. 1. Clerodanes from Aylthonia macrantha.](image)
carboxylic acid functions. Its $^1$H NMR spectrum (Table 1) showed a vinyl hydrogen at $\delta$ 5.20 (1H, br s, H-3); a double doublet centered at $\delta$ 3.61 (1H, dd, $J = 11.2$ and 4.4 Hz, H-6); an AB spin system at $\delta$ 2.34 (1H, d, $J = 14.0$ Hz, H-11a) and 2.43 (1H, d, $J = 14.0$, H-11b); a vinyl methyl at $\delta$ 1.83 (3H, br s, H-18); two quaternary methyls, as singlets at $\delta$ 0.78 (H-20) and 1.04 (H-19); and a tertiary methyl, as a doublet, at $\delta$ 0.95 ($J = 7.0$ Hz, H-17). Analysis of the PND and DEPT $^{13}$C NMR spectra confirmed the presence of two sp$^2$ carbons at $\delta$ 122.2 (CH-3) and 143.3 (C-4); a carboxylic acid at $\delta$ 174.4 (C-12); a deshielded sp$^3$ methine carbon at $\delta$ 74.7 (CH-6); and four methyl carbons at $\delta$ 14.8 (CH$_3$-19), 15.7 (CH$_3$-17), 16.7 (CH$_3$-20) and 22.2 (CH$_3$-18). Direct 2D $^1$H–$^1$H and $^1$H–$^{13}$C correlation spectra allowed the unambiguous assignment of all hydrogen and carbon chemical shifts (Table 1). The axial position of H-6 was indicated by the double doublet coupling constants of 11.4 and 4.4 Hz. (Fig. 1)

In order to establish the relative stereochemistry of all five chiral C atoms, several nOe difference spectra were obtained. As illustrated by the conformation of 2 in Fig. 2, the axial hydrogen at C-6 ($\delta$ 3.61) showed strong nOe correlation with H-8 ($\delta$ 2.10) and H-10 ($\delta$ 1.60), but showed no correlation to methyl groups CH$_3$-17 ($\delta$ 0.95), CH$_3$-19 ($\delta$ 1.04) and CH$_3$-20 ($\delta$ 0.78). Strong correlations were also observed between CH$_3$-19 ($\delta$ 1.04) and those hydrogens at $\delta$ 1.83 (CH$_3$-18), 1.62 (H-1ax), 0.78 (CH$_3$-20) and 1.50 (H-7ax). These nOe observations can only be explained by the overall molecular geometry as defined in Fig. 2.

The proposed structures for clerodanes previously isolated from *Vellozia bicolor* (Pinto et al., 1994), are being revised suggesting that all the Velloziaceae clerodanes should have the same relative stereochemistry, as should other biosynthetic related diterpenes (Pinto et al., in preparation). In fact, methyl groups linked to C-8 and C-9 in the tetranorditerpene halimanoic acid (3) isolated from *V. flavicans* have the same spatial relationship as in compounds (1) and (2) (Miranda et al., 2001; Pinto et al., 1995b).

Although Velloziaceae family is characterised by an oxidative system that breaks down the diterpenes yielding nor, bisnor and tetranorditerpenes, aylthonic acid (2) is the first tetranorneoclerodane reported. These compounds are probably involved
in plant herbivore defence and their insecticidal activity has been recently reviewed (Viegas, 2003).

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References


