

Article

## 3-Epiabruslactone A, a New Triterpene Lactone Isolated from *Austroplenckia populnea*

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Um triterpeno lactônico inédito isolado do cerne de *Austroplenckia populnea* (Celastraceae) foi caracterizado como 3 $\alpha$ -hidroxiolean-12-en-29,22 $\alpha$ -olídeo (a  $\gamma$ -lactona do ácido 3 $\alpha$ ,22 $\alpha$ -diidroxiolean-12-en-29 $\alpha$ -óico), o epímero em C-3 da abruslactona A, com base em dados espectrais, oxidação e análise cristalográfica.

A new lactonic triterpene isolated from the heartwood of *Austroplenckia populnea* (Celastraceae) was characterized as 3 $\alpha$ -hydroxyolean-12-en-29,22 $\alpha$ -olide (the  $\gamma$ -lactone of the 3 $\alpha$ ,22 $\alpha$ -dihydroxyolean-12-en-29 $\alpha$ -oic acid), the 3-epimer of the abruslactone A, on the basis of its spectral data, chemical transformations, and single crystal X-ray analysis.

**Keywords:** *Austroplenckia populnea*, *Celastraceae*, 3-epiabruslactone A, structure elucidation

### Introduction

The investigation of chemical constituents of plants of the Celastraceae family has aroused considerable attention on account of the antitumoral and anti-leukemic activities found in maytansinoids and quinonemethide triterpenes isolated from plants of that family<sup>1</sup>.

In previous papers we described the chemical constitution of the root bark<sup>2</sup>, bark wood<sup>3</sup> and root wood<sup>4</sup> of *Austroplenckia populnea* (Reiss) Lundell, specimen of the Celastraceae family. The plant is popularly known in the state of Minas Gerais as "mangabeira-brava", and it is a source of material for the industry of vimen furniture. The decoction of its branches is used as a traditional antidiarrheal medicine<sup>5</sup>.

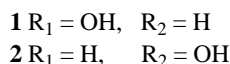
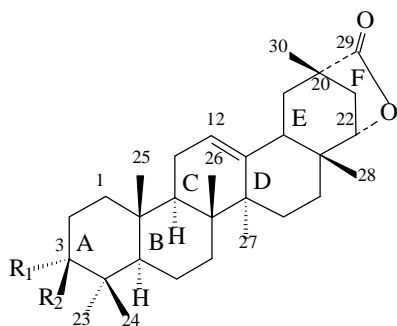
During our continued investigation of this species, one new oleanane derivative (**1**) has been isolated along with an extensive range of triterpenes including the known lactonic triterpene abruslactone A (**2**). The compound **1** showed spectral characteristics of an abruslactone A de-

rivative. Thus, in this paper we present the identification of abruslactone A and structural elucidation of a new triterpene lactone, the epimer in C-3 (**1**) of abruslactone A, whose chemical structures are shown in Fig. 1. Both triterpenes were isolated from the ethanolic extract of the heartwood of *Austroplenckia populnea*, from which were also isolated the following triterpenes previously found in other parts of the same plant: 3-oxofriedelan-29 $\alpha$ -oic acid (populnonic acid)<sup>1,2,3</sup>, 3-hydroxy-2-oxofriedelan-3-en-29 $\alpha$ -oic acid, 3 $\beta$ -hydroxyolean-12-en-29 $\alpha$ -oic acid (epikatonic acid)<sup>2</sup>, and 3-oxoolean-12-en-29 $\alpha$ -oic acid (katononic acid)<sup>2</sup>.

### Experimental

#### General experimental procedure

IR, NMR, mass spectroscopy, and melting point. IR spectra were obtained with a Galaxy FTIR 3000-Mattson in KBr pellets. A Bruker AC-200 (<sup>1</sup>H: 200 MHz; <sup>13</sup>C: 50,3



**Figure 1.** Chemical Structure for C<sub>30</sub>H<sub>46</sub>O<sub>3</sub>.

MHz) was used for the measurement of the <sup>13</sup>C-NMR and <sup>1</sup>H-NMR, using CDCl<sub>3</sub> as solvent and TMS as internal standard. The multiplicities of the <sup>13</sup>C signals were determined by means of the DEPT method. Mass spectra were obtained using an HP mass spectrometer. Melting points were determined using a Leitz thermic microscope without corrections.

### 3-Epiabruslactone A (**1**)

Mp. 295-296 °C (CHCl<sub>3</sub>/MeOH), [α]<sub>D</sub><sup>20</sup> +40.1° (c = 1.0, CHCl<sub>3</sub>), MS: m/z 454 [M]<sup>+</sup>, 246 (100) and 207 [RDA C ring]<sup>+</sup>, IR max: 3550 and 1755 cm<sup>-1</sup>, <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 200 MHz): δ (m) 5.30 (t, 1H, J = 4.4 Hz), 4.14 (d, 1H, J = 5.6 Hz), 3.40 (t, 1H, J = 2.9 Hz), 1.20, 1.09, 0.95, 0.95, 0.92, 0.86, 0.84 (s, 3H each), <sup>13</sup>C-NMR: Table 1.

### Abruslactone A (**2**)

Mp. 319.5-322.0 °C (CHCl<sub>3</sub>/MeOH), [α]<sub>D</sub><sup>20</sup> -34.2° (c = 1.0, CHCl<sub>3</sub>), <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 200 MHz): δ (m) 5.30 (t, 1H, J = 3.0 Hz), 4.17 (d, 1H, J = 5.0 Hz), 3.2 (1H, dd, J = 6.0, 9.0 Hz), 1.21, 1.07, 0.99, 0.94, 0.93, 0.87, 0.79 (s, 3H each) in good agreement with previously reported data by Budzikiewicz, Wilson and Djerassi<sup>10</sup>, Chang, Chiang and Mak<sup>11</sup> and Nozaki *et al.*<sup>12</sup>

### 3-oxoolean-12-en-29,22-olide (Oxidation of **1** or **2**)

10.0 mg of **1** (or **2**) and 200.0 mg of BaMnO<sub>4</sub> in 10.0 mL of CH<sub>2</sub>Cl<sub>2</sub> were refluxed for 120 h. Filtration and evaporation of the solvent furnished a residue that was submitted to SiO<sub>2</sub> gel column chromatography. The product of the reaction was obtained in 40% yield by elution with CHCl<sub>3</sub>/MeOH (19:1). Mp. 289-290 °C, IR max: 1770 and 1700 cm<sup>-1</sup>.

**Table 1.** <sup>13</sup>C-NMR data of compound **1** in CDCl<sub>3</sub> (δ in ppm).

Carbon	δ (m)	Carbon	δ (m)
1	33.8 (t)	16	24.2 (t)
2	25.2 (t)	17	35.2 (s)
3	76.0 (d)	18	43.4 (d)
4	37.3 (s)	19	39.8 (t)
5	48.9 (d)	20	42.6 (s)
6	18.2 (t)	21	33.1 (t)
7	33.0 (t)	22	83.1 (d)
8	39.4 (s)	23	28.2 (q)
9	47.3 (d)	24	16.9 (q)
10	37.1 (s)	25	15.4 (q)
11	23.4 (t)	26	21.0 (q)
12	124.6 (d)	27	24.9 (q)
13	140.2 (s)	28	24.2 (q)
14	39.5 (s)	29	182.4 (s)
15	25.2 (t)	30	22.3 (q)

### X-ray crystallography

**Data collection and processing.** The X-ray diffraction data were obtained at *ca.* 293 K on a CAD-4 Enraf Nonius diffractometer using Mo Kα radiation monochromated by graphite. Intensity data for both crystals were collected in the 2θ range from 1 to 21°, using ω-2θ scans with the scan width given by (0.8 + 0.35tanθ) and corrected for the Lp factors using the Enraf Nonius Structure Determination Package<sup>6</sup>, *hkl* range: 0 < *h* < 11, 0 < *k* < 14, and -2 < *l* < 16. Single crystal size 0.53 x 0.45 x 0.35 mm. Total number of reflections measured: 1849 of which 1798 independent and 1677 had I > 2σ, and two standard reflections were monitored every hour which however did not present any significant changes in intensities.

### Results and Discussion

Compounds **1** and **2** analyzed as C<sub>30</sub>H<sub>46</sub>O<sub>3</sub> ([M]<sup>+</sup>, m/z 454), and presented a positive Liebermann-Burchard test for a pentacyclic triterpene. The presence of hydroxyl and lactonic carbonyl groups for **1** or **2** was shown by the bands at 3550 and 1755 cm<sup>-1</sup> in the IR spectra. The <sup>1</sup>H-NMR spectrum of **1** presented singlet signals corresponding to the seven tertiary methyl groups (δ 1.20, 1.09, 0.95, 0.95, 0.92, 0.86 and 0.84), a signal attributed to a vinylic proton at δ 5.30 (1H, t, J 4.4 Hz, H-12) and two signals assigned to protons of carbinol type: δ 4.14 (1H, d, J 5.6 Hz, H-22) and δ 3.4 (1H, t, J 2.9 Hz, H-3). The spectral analysis IR, <sup>1</sup>H-NMR and <sup>13</sup>C-NMR, which revealed the presence of thirty signals, including δ 182.4 (C-29); 140.2 (C-13);

124.6 (C-12); 76.0 (C-3) and 83.1 (C-22), was used to establish that compound **1** contains the backbone of  $\beta$ -amirine. Both compounds exhibited similar NMR spectra, but the doublet of doublets observed at  $\delta$  3.22 (J 6.0 and 10.5 Hz) for H-3 in **2** was replaced by a triplet at  $\delta$  3.4 (J 2.9 Hz) for H-3 in **1**, indicating an axial position for the hydroxyl group at C-3 for **1**. Both triterpenes on oxidation with barium manganate in dichloromethane<sup>13</sup>, gave 3-oxoolean-12-en-29,22 $\alpha$ -olide, confirming that they are epimeric at C-3.

However, compounds **1** and **2** differ significantly in their melting points and optical rotations [295-296 °C, CHCl<sub>3</sub>/MeOH;  $[\alpha]^{20}$  +40.1° (c. 1.0 CHCl<sub>3</sub>)] and [329-330 °C; 317-318 °C;  $[\alpha]^{20}$  -33.4° (c. 0.2 CHCl<sub>3</sub>)]<sup>10,11</sup>.

The MS spectrum of compound **1** presented a retro-Diels Alder standard fragmentation<sup>10</sup> [m/z 246 (base peak) and 207], indicating that the lactonic ring was localized at rings D and/or E.

The above data strongly suggested that **2** could be identical with abruslactone A isolated by Chang *et al.*<sup>11</sup> from *Abrusprecatorius* (Leguminosae).

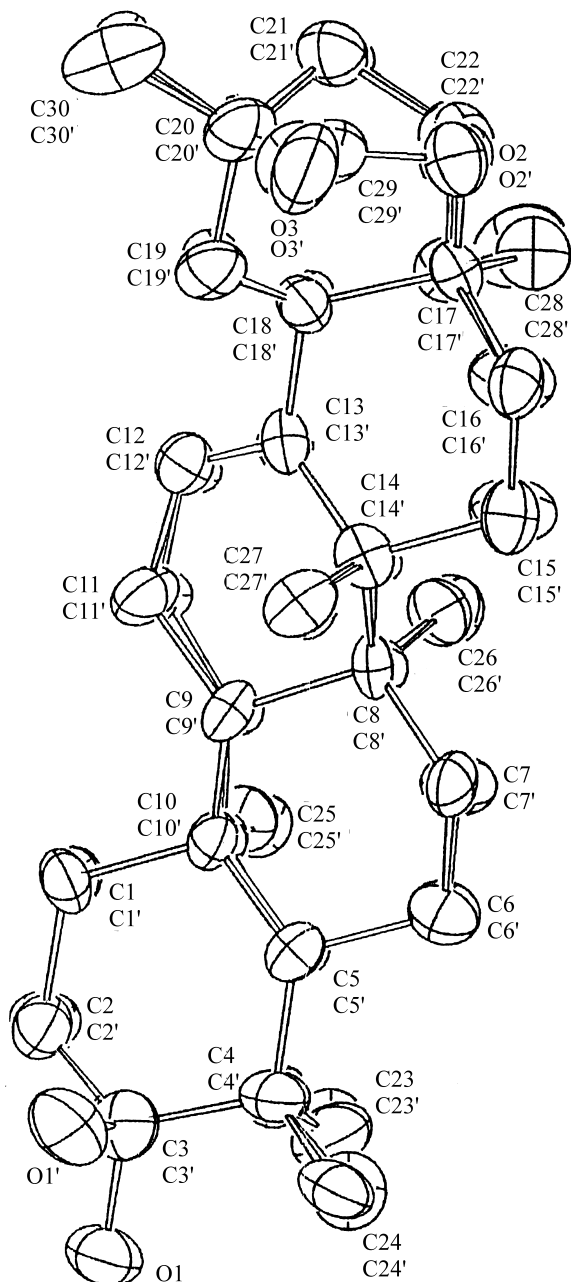
As a further confirmation of the structure and stereochemistry of **1** and **2**, an X-ray analysis was carried out for both. As the structure abruslactone was fully confirmed, the X-ray crystal data will be given only for 3-Epiabruslactone. *Crystal data.* C<sub>30</sub>H<sub>46</sub>O<sub>3</sub>. M = 454.7 u., orthorhombic system, space group P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub>, Z = 4, D<sub>x</sub> = 1.202 g cm<sup>-3</sup>,  $\mu$  = 0.7 cm<sup>-1</sup>, F(000) = 1,000. Cell dimensions  $a$  = 11.205(1),  $b$  = 14.164(6),  $c$  = 15.894(3) Å, V = 2522(1) Å<sup>3</sup>.

*Structure analysis and refinement.* The structures were solved by direct methods using the program SHELXS-86<sup>7</sup> and refined by full matrix least-squares method using the program SHELX-93<sup>8</sup> considering anisotropic temperature factors for all atoms except for the hydrogen atoms that had their positional parameters fixed as determined from Fourier difference maps and a refined single isotropic temperature factor common for all hydrogens. Atomic scattering factors from International Tables for X-ray Crystallography<sup>9</sup>. Final R, ( $R = \sum (|F_{\text{obs}} - F_{\text{calc}}|) / \sum |F_{\text{obs}}|$ ), for all observed reflections with  $I > 2\sigma(I)$  and 300 refined parameters (atomic parameters, scale, extinction coefficient) was 5.3%. Final positional and equivalent isotropic temperature factors for all non-hydrogen atoms with estimated standard deviation in parentheses are given in Table 2. All calculated interatomic distances and angles were in agreement with the expected values for the respective bond types. Tables of hydrogen parameters, anisotropic temperature factors and observed and calculated structure factors are available from one of the authors (YPM). Similar conformation of the rings A, B, C, D, E and F were found for compounds **1** and **2** in the crystal and are exactly the same as for the structure found for abruslactone A<sup>3</sup> with the only difference residing, as expected, in the relative posi-

tion of O(1) of compound **1**, the epimer of **2**. In both crystal structures an intermolecular hydrogen bond is established in a head and tail fashion between O(1) and O(3) of molecules related, for epi-abruslactone by the symmetry operations (x, y, z) and (0.5+x, 0.5-y, -z) with O(1)-O(3), O1-H1, and O3-H1 distances of 2.919(8), 1.157(8) and 1.902(8) Å

**Table 2.** Atomic coordinates ( $\times 10^4$ ) and equivalent isotropic displacement parameters ( $\text{\AA}^2 \times 10^3$ ) for Epiabruslactone. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

	x	y	z	U(eq)
O(01)	-1216(4)	10753(4)	6927(3)	83(2)
O(02)	-1507(4)	7827(4)	467(3)	66(1)
O(03)	-2824(5)	8961(5)	234(4)	91(2)
C(01)	-81(6)	10313(6)	7135(4)	65(2)
C(02)	-94(6)	9235(5)	7003(4)	51(2)
C(03)	-64(6)	9044(4)	6027(4)	46(2)
C(04)	916(5)	9533(4)	5507(4)	39(2)
C(05)	859(5)	10609(4)	5716(4)	45(2)
C(06)	899(6)	10823(5)	6658(4)	53(2)
C(07)	-90(6)	7992(5)	5807(4)	56(2)
C(08)	-501(6)	7882(5)	4882(4)	55(2)
C(09)	297(6)	8399(4)	4250(4)	42(2)
C(10)	1482(6)	7847(5)	4138(5)	57(2)
C(11)	-358(5)	8454(4)	3369(4)	38(2)
C(12)	-1569(5)	8945(6)	3439(4)	58(2)
C(13)	388(5)	9023(4)	2753(4)	35(2)
C(14)	1123(6)	9684(4)	3036(4)	44(2)
C(15)	1362(6)	9921(4)	3949(4)	46(2)
C(16)	543(5)	9418(4)	4561(4)	35(2)
C(17)	-606(7)	7456(5)	3030(4)	62(2)
C(18)	-897(7)	7441(5)	2094(4)	63(2)
C(19)	92(6)	7846(4)	1561(4)	46(2)
C(20)	1264(7)	7284(5)	1668(5)	76(3)
C(21)	309(5)	8876(4)	1808(4)	40(2)
C(22)	-556(6)	9559(4)	1375(4)	49(2)
C(23)	-719(6)	9343(5)	414(4)	52(2)
C(24)	221(7)	8631(5)	122(4)	54(2)
C(25)	-220(6)	7792(5)	605(5)	54(2)
C(26)	-1804(7)	8741(6)	343(5)	63(2)
C(27)	-810(8)	10234(5)	-104(5)	78(3)
C(28)	2195(6)	9212(5)	5696(4)	51(2)
C(29)	936(7)	8806(6)	7491(5)	75(2)
C(30)	-1260(7)	8856(7)	7374(4)	72(2)



**Figure 2.** ORTEP representation of the superposition of the relative molecular structures of compounds **1** and **2** (primed = epiabruslactone) showing the different orientation of the hydroxyl group at C-3.

O(1)-H(1)-O(3) angle of  $144.1(6)^\circ$ . Figure 2 is an ORTEP<sup>14</sup> representation of the superposition of the relative molecular structures of compounds **1** and **2** including only the non-hydrogen atoms.

## Conclusion

All the experimental findings in this work are conclusive of the existence of the two proposed configurations of the herein studied compound in the plant, compound **1** is  $3\alpha$ -hydroxyolean-12-en-29,22 $\alpha$ -olide and compound **2** is

$3\beta$ -hydroxyolean-12-en-29,22 $\alpha$ -olide. The isolation of the epimeric pair gives rise to doubts on abruslactone precursor. Hitherto the maytenfolic acid ( $3\beta$ -22 $\alpha$ -dihydroxyolean-12-en-29 $\alpha$ -oic acid) has been considered as abruslactone A precursor. But what could be the 3-epi-abruslactone A precursor? In the course of our future chemical investigations we will attempt to resolve this question. It would be very interesting to further investigate if the epimers present different biological activities.

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