New Diterpenoids from Leaves of *Guarea macrophylla* (Meliaceae)

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Introduction

As part of our studies of *Guarea* species, we have investigated the chemical composition of the hexane phase of the ethanol extract from leaves of *G. macrophylla*. This species grows in Brazil from Rio Grande do Sul to Rio de Janeiro and Minas Gerais States extending to Mato Grosso and Brasilia, and is also found in the Amazon region. An earlier investigation of the dichloromethane extract of the leaves yielded one monoterpene, four sesquiterpenes, five diterpenes and one triterpene, indicating, for the first time, the co-occurrence of diterpenes and triterpenes in members of Meliaceae. In another study, nine related cycloartane triterpenoids were also detected in the leaves. In the volatile oils from the leaves and stem bark were detected several sesquiterpenes and diterpene derivatives as well as fatty acids. In the present investigation, we report the isolation and structural determination of two new minor diterpenoid derivatives: 7α-hydroperoxy-isopimara-8(14),15-diene-2α,3β-diol (1) and 19-nor-isopimara-7,15,4(18)-trien-3-one (2), in addition to seven known diterpenoids (3-9). All these compounds, except 1, 2, 7 and 8, have been described previously in this plant. Structures were elucidated by analysis of their spectrometric data and comparison with data described in the literature.

Results and Discussion

The crude EtOH extract from the leaves of *G. macrophylla* was partitioned between hexane and aqueous ethanol. The hexane phase was submitted to chromatographic separation on silica gel and Sephadex LH-20 to yield one new diterpenoid: 7α-hydroperoxy-isopimara-8(14),15-diene-2α,3β-diol (1), and one new
nor-diterpenoid: 19-nor-isopimara-7,15,4(18)-trien-3-one (2). In addition, seven known diterpenoids isopimara-7,15-dien-3-one (3), isopimara-7,15-dien-2α-ol (4), isopimaradien-7,15-dien-3β-ol (5), manoyl oxide (6), 19-hydroxy-manoyl oxide (7), labda-8,14-dien-13-ol (8), and phytol (9) were found.

Compound 1 was obtained as a white amorphous powder whose partial molecular formula C20H32O4 was deduced by analysis of the 13C NMR spectra (BBD and DEPT 135°) and LREIMS. The 1H NMR spectrum showed three dd at δH 4.94 (J 11.1 and 1.2 Hz, 1H), 4.97 (J 17.9 and 1.2 Hz, 1H) and 5.79 (J 17.9 and 11.1 Hz, 1H) and a broad singlet at 5.70 (1H). These signals associated with four methyl signals at δH 1.08, 1.04, 0.87, 0.86, suggested the presence of an isopimarane diterpene skeleton. The 13C NMR spectra (BBD and DEPT 135°) contained signals corresponding to six olefinic carbon atoms at δC 149.9 (CH), 143.6 (C), 135.5 (C), 124.3 (CH2), 121.7 (CH), and 109.6 (CH2), corresponding to three double bonds, and one signal at δC 200.7 (C), characteristic of an α,β-unsaturated carbonyl group. Comparison of the 13C and 1H NMR data of 2 with those reported for isopimara-7,15-dien-3-one (3) were characteristic of an isopimarane with two double bonds between C-7/C-8 and C-14/C-15. Based in these data, the third double bond could only be positioned at C-4/C-18 and the carbonyl group at C-3 to form an α,β-unsaturated system. This was confirmed by the presence of a dd at δH 2.26 (J 14.5 and 3.7 Hz) and a dd at δH 2.68 (J 14.5 and 5.3 Hz), assigned, respectively, to H-2α and H-2β, besides two singlets at δH 6.01 (1H) and 6.24 (1H), attributed to H-18a and H-18b. Therefore, the structure of compound 2 was elucidated as 19-nor-isopimara-7,15,4(18)-trien-3-one.

Compounds 3 to 9 were identified by analysis of their LREIMS, 13C and 1H NMR spectra and comparison with data reported in the literature.14,15 This is the first report of compounds 7 and 8 from the genus Guarea.

Several sesquiterpenes, diterpenes and triterpenes (cycloartane derivatives only) have been identified in G. macrophylla, while limonoids (meliacins), which are produced by the oxidative degradation of the side chain from tirucalane/euphane triterpenes, had so far not been detected. However, a biogenetic pathway proposed to the formation of 1 and 2 showed that the oxidative/degradative tendency, characteristic of Meliaceae, should be observed in G. macrophylla.

**Experimental**

**General experimental procedures**

NMR (Bruker DRX-500): 1H (500 MHz) and 13C (125 MHz) in CDCl3 (Aldrich) and TMS as internal standard; LREIMS were obtained at 70 eV (INCONS 50 Finnigan-Mat-quadrupole); IR spectra were obtained as a film in a Perkin Elmer Infrared Spectrometer model 1750; optical rotations were measured in CHCl3 in a digital polarimeter JASCO DIP-370 (Na filter, λ = 588 nm); Elemental analysis were obtained in a Perkin-Elmer Elemental Analyser model 2400 CHN; CC: silica gel 60 (Merck, 63-200 µm); Sephadex LH-20 (Sigma); TLC: silica gel plates PF254 (Merck).
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**Plant material**

The leaves of *G. macrophylla* were collected at Universidade de São Paulo on October 23th, 2001, in São Paulo city, São Paulo State, Brazil. The plant material was identified by Prof. Dr. José Rubens Pirani (Botanist, Instituto de Biociências at the Universidade de São Paulo, Brazil) and a voucher specimen has been deposited at Herbarium SFC (IB-USP).

**Extraction and isolation of the constituents**

The air-dried plant material (550 g) was extracted with ethanol four times. The crude extract (50.1 g) was partitioned between hexane and aqueous ethanol. The hexane phase (21.8 g) was submitted to chromatography on silica gel and eluted with a gradient mixture (hexane-EtOAc-MeOH) to yield seventeen fractions. Fraction 2 was submitted to CC on silica gel and eluted with gradient mixtures of hexane-CH₂Cl₂-EtOAc to yield 535 mg of **1** and 286 mg of **2**. Fraction 3 was separated by after CC on silica gel and elution with a gradient mixture (hexane-CH₂Cl₂-EtOAc) to yield 8 (14 mg). Fraction 4 was submitted to preparative TLC on silica gel eluted with CH₂Cl₂, to yield 5 mg of **2**. Column chromatography on silica-gel of fraction 8, eluted with gradient mixture of hexane-EtOAc, to yield 5 mg of **1**.

**7α-hydroperoxy-isopimara-8(14),15-diene-2α,3β-diol (1)**

White amorphous powder, [α]D + 63.8 (CHCl₃, c0.05); IR ν̇ cm⁻¹ (film): 3403, 2921, 2854, 1708, 1646, 1462, 1377, 1156, 1054, 910, 872, 773, 721; 1H NMR spectral data (500 MHz, CDCl₃): 0.86 (3H, s, Me-19), 0.87 (3H, s, Me-20), 1.04 (3H, s, Me-17), 1.08 (3H, s, Me-18), 3.05 (1H, d, J 9.6 Hz, H-3α), 3.66 (1H, ddd, J 2β,1α 11.7 Hz, J 2β,3α 9.6 Hz, J 2β,1α 4.2 Hz, H-2β), 4.34 (1H, dd, J 7β,6β 3.9 Hz, J 7β,6α 2.1 Hz, H-7β), 4.94 (1H, dd, J 16α,15 11.1 Hz, J 16β,16α 1.2 Hz, H-16a), 4.97 (1H, dd, J 16b,16a 1.2 Hz, H-16b), 5.70 (1H, br s, H-14), 5.79 (1H, dd, J 15,16b 17.9 Hz, J 15,16a 11.1 Hz, H-15), 7.41 (1H, s, OH); 13C NMR spectral data (BBD and DEPT 135° spectra, 125 MHz, CDCl₃): 44.9 (t, C-1), 68.6 (d, C-2), 83.6 (d, C-3), 38.9 (s, C-4), 46.5 (d, C-5), 29.7 (t, C-6), 86.1 (d, C-7), 132.6 (s, C-8), 47.1 (d, C-9), 34.2 (s, C-10), 18.5 (t, C-11), 34.2 (t, C-12), 81.0 (s, C-13), 139.3 (d, C-14), 147.7 (d, C-15), 111.3 (t, C-16), 25.9 (q, C-17), 28.8 (q, C-18), 15.3 (q, C-19), 16.5 (q, C-20); LREIMS m/z (relative intensity %): 320 (20), 291 (13), 203 (14), 185 (29), 161 (30), 149 (53), 109 (100), 105 (95), 95 (93), 91 (75), 81 (84), 69 (70), 55 (65).

**19-nor-isopimara-7,15,4(18)-trien-3-one (2)**

White amorphous powder, [α]D + 32.8 (CHCl₃, c0.06); IR ν̇ cm⁻¹ (film): 3403, 2921, 2854, 1708, 1646, 1462, 1377, 1156, 1054, 910, 872, 773, 721; 1H NMR spectral data (500 MHz, CDCl₃): 1.10 (3H, s, H-20), 1.17 (3H, s, H-17), 2.26 (1H, dt, J 2α,2β 14.5 Hz, J 2α,1α 3.7 Hz, J 2β,2β 3.7 Hz, H-2α), 2.68 (1H, td, J 2β,2β 14.5 Hz, J 2β,1β 3.7 Hz, J 2β,1α 3.7 Hz, J 2β,1β 14.5 Hz, J 2β,1α 3.7 Hz, J 2β,1α 3.7 Hz, H-2β), 4.91 (1H, dd, J 16a,15 10.5 Hz, J 16b,15 10.5 Hz, J 16,16a 1.5 Hz, H-16a), 4.95 (1H, dd, J 16b,15 17.5 Hz, J 16,16b 1.5 Hz, H-16b), 5.47 (1H, br s, H-7), 5.81 (1H, dd, J 15,16 17.5 Hz, J 15,16 10.5 Hz, H-15), 6.01 (1H, s, H-18a), 6.24 (1H, s, H-18b); 13C NMR spectral data (BBD and DEPT 135° spectra, 125 MHz, CDCl₃): 36.9 (t, C-1), 36.0 (t, C-2), 28.0 (t, C-3), 81.0 (d, C-4), 47.1 (d, C-5), 29.7 (t, C-6), 86.1 (d, C-7), 132.6 (s, C-8), 47.1 (d, C-9), 34.2 (s, C-10), 18.5 (t, C-11), 34.2 (t, C-12), 81.0 (s, C-13), 139.3 (d, C-14), 147.7 (d, C-15), 111.3 (t, C-16), 25.9 (q, C-17), 28.8 (q, C-18), 15.3 (q, C-19), 16.5 (q, C-20); LREIMS m/z (relative intensity %): 320 (20), 291 (13), 203 (14), 185 (29), 161 (30), 149 (53), 109 (100), 105 (95), 95 (93), 91 (75), 81 (84), 69 (70), 55 (65).

**Figure 1.** Model compounds used in the structure elucidation of compounds 1 and 2 with some of their 13C and 1H NMR data.

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