*J. Braz. Chem. Soc.*, Vol. 16, No. 3B, 643-646, 2005.
Printed in Brazil - ©2005 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Short Report

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

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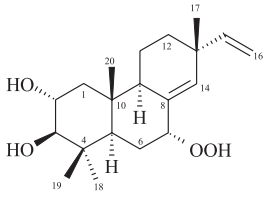
A fase em hexano do extrato etanólico das folhas de *Guarea macrophylla* (Meliaceae) foi submetida a fracionamento cromatográfico. Esses procedimentos permitiram o isolamento de um novo diterpenóide: 7$\alpha$-hidroperóxido-isopimara-8(14),15-dieno-2$\alpha$,3$\beta$-diol, um novo nor-diterpenóide: 19-*nor*-isopimara-7,15,4(18)-trien-3-ona além de sete diterpenóides de estruturas conhecidas. As estruturas dos compostos isolados foram definidas através de análise dos dados espectrométricos e comparação com aqueles descritos na literatura.

The hexane phase from the ethanol extract from leaves of *Guarea macrophylla* (Meliaceae) was submitted to chromatographic separation. These procedures allowed the isolation of one new diterpenoid: 7$\alpha$-hydroperoxy-isopimara-8(14),15-diene-2$\alpha$,3$\beta$-diol, one new nor-diterpenoid: 19-*nor*-isopimara-7,15,4(18)-trien-3-one besides seven known diterpenoids. Their structures were deduced by analysis of spectrometric data and comparison of data described in the literature.

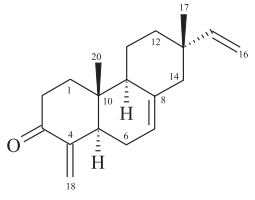
**Keywords:** *Guarea macrophylla*, diterpenoids, 7$\alpha$-hydroperoxy-isopimara-8(14),15-diene-2$\alpha$,3$\beta$-diol, 19-*nor*-isopimara-7,15,4(18)-trien-3-one

## Introduction

As part of ours 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 hold also in the Amazon region.[1] An earlier investigation of the dichloromethane extract of the leaves yielded one monoterpene, four sesquiterpenes, five diterpenes and one triterpene,[2] 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.[3] In the volatile oils from the leaves and stem bark were detected several sesquiterpene and diterpene derivatives as well as fatty acids.[4,5] In the present investigation we report the isolation and structural determination of two new minor diterpenoid derivatives: 7$\alpha$-hydroperoxy-isopimara-8(14),15-diene-2$\alpha$,3$\beta$-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.




**1**




**2**

## 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: 7$\alpha$-hydroperoxy-isopimara-8(14),15-diene-2$\alpha$,3$\beta$-diol (**1**), and one new

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*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-2$\alpha$-ol (**4**), isopimaradien-7,15-dien-3$\beta$-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 $C_{20}H_{32}O_3$ was deduced by analysis of the $^{13}C$ NMR spectra (BBD and DEPT 135°) and LREIMS. The $^1H$ NMR spectrum showed three dd at $\delta_H$ 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 $\delta_H$ 1.08, 1.04, 0.87, 0.86, suggested the presence of an isopimarane diterpene skeleton.[6] The $^{13}C$ NMR spectra (BBD and DEPT 135°) contained signals at $\delta_C$ 132.6 (C), 139.3 (CH), 147.7 (CH) and 111.3 ($CH_2$), characteristic of C-8/C-14 and C-15/C-16 double bounds (models **M1**[7] and **M2**[9]) as well as two oxygenated methines at $\delta_C$ 68.6 (CH) and 83.6 (CH), which were attributed, respectively, to C-2 and C-3.[10] These assignments were confirmed by analysis of the $^1H$- and DQ-COSY NMR spectra, which showed the correlated signals of H-2 and H-3 at $\delta_H$ 3.66 (ddd, *J* 11.7, 9.6 and 4.2 Hz, 1H) and 3.05 (d, *J* 9.6 Hz, 1H), respectively, and the comparison with $^{13}C$ NMR data reported to model compound **M3**.[11] The configuration of the hydroxyl groups were confirmed such as 2$\alpha$ and 3$\beta$ based on the value of the coupling constants, which were indicative of two trans-diaxial couplings [H-2$\beta$/H-3$\alpha$ (*J* 9.6 Hz) and H-2$\beta$/H-1$\alpha$ (*J* 11.7 Hz)] and one axial-equatorial coupling [H-2$\beta$/H-1$\beta$ (*J* 4.2 Hz)], in agreement with H-2$\beta$ and H-3$\alpha$ coupling constant values reported for the model **M5**.[10] The $^1H$ NMR spectrum showed also one dd at $\delta_H$ 4.34 (J 3.9 and 2.1 Hz, 1H) and one s at $\delta_H$ 7.41 (1H, exchangeable with $D_2O$), suggesting the presence of a hydroperoxy group at C-7, which could be confirmed by comparing the $^1H$ and $^{13}C$ NMR data of **1** with those reported to the diterpene methyl-7$\beta$-hydroperoxypimara-8(14),15-dien-19-oate (**M4**).[12] In the light of the above observations, the molecular formula of **1** was defined as $C_{20}H_{32}O_4$. However, the LREIMS spectrum did not show the molecular ion peak at *m/z* 336 Da but a fragment at *m/z* 320 Da instead, which is indicative of the lost of one oxygen atom, similar to the fragmentation observed to other hydroperoxyde derivatives.[13] Therefore, the structure of **1** was elucidated as 7$\alpha$-hydroperoxy-isopimara-8(14),15-diene-2$\alpha$,3$\beta$-diol.

The $^1H$ NMR spectrum of **2** showed three dd at $\delta_H$ 5.81 (*J* 17.5 and 10.5 Hz, 1H), 4.95 (*J* 17.5 and 1.5 Hz, 1H) and 4.91 (*J* 10.5 and 1.5 Hz, 1H), suggesting the presence of a vinylic group. The $^{13}C$ NMR spectra (BBD and DEPT 135°) contained 19 signals corresponding to two methyl, eight methylenes, four methine and four quaternary and one carbonyl carbons. These data together with the elemental analysis and LREIMS (70 eV) spectrum, which showed the $[M]^+$ at *m/z* 270 Da, indicated the molecular formula $C_{19}H_{26}O$. The $^{13}C$ NMR spectra (BBD and DEPT 135°) contained signals corresponding to six olefinic carbon atoms at $\delta_C$ 149.9 (CH), 143.6 (C), 135.5 (C), 124.3 ($CH_2$), 121.7 (CH), and 109.6 ($CH_2$), corresponding to three double bonds, and one signal at $\delta_C$ 200.7 (C), characteristic of an $\alpha,\beta$-unsaturated carbonyl group (IR $\nu_{max}$/cm$^{-1}$: 1666). Comparison of the $^{13}C$ and $^1H$ NMR data of **2** with those reported for isopimara-7,15-dien-3-one[2] (**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 $\alpha,\beta$-unsaturated system. This was confirmed by the presence of a dt at $\delta_H$ 2.26 (*J* 14.5 and 3.7 Hz) and a td at $\delta_H$ 2.68 (*J* 14.5 and 5.3 Hz), assigned, respectively, to H-2$\alpha$ and H-2$\beta$, besides two singlets at $\delta_H$ 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, $^{13}C$ 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 $^{13}C$ (125 MHz) in $CDCl_3$ (Aldrich) and TMS as internal standard; LREIMS were obtained at 70 eV (INCOS 50 Finnigan-Mat-quadrupole); IR spectra were obtained as a film in a Perkin Elmer Infrared Spectrometer model 1750; optical rotations were measured in $CHCl_3$ in a digital polarimeter JASCO DIP-370 (Na filter, $\lambda$ = 588 nm); Elemental analysis were obtained in a Perkin-Elmer Elemental Analyser model 2400 CHN; CC: silica gel 60 (Merck, 63-200 $\mu$m); Sephadex LH-20 (Sigma); TLC: silica gel plates $PF_{254}$ (Merck).

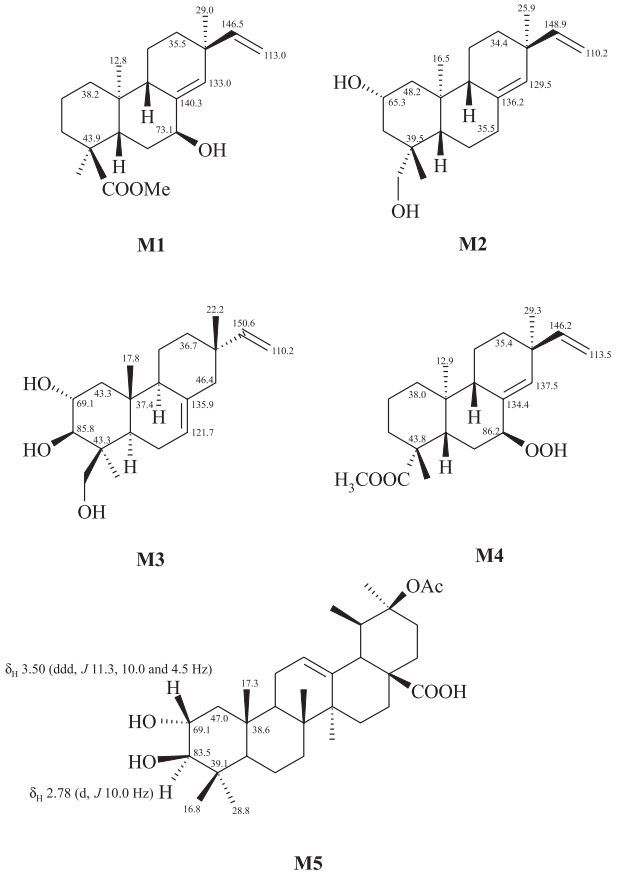


**Figure 1.** Model compounds used in the structure elucidation of compounds **1** and **2** with some of their $^{13}C$ and $^{1}H$ NMR data.[7,9-12]

*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-$CH_2Cl_2$-EtOAc to yield 535 mg of **3** and 286 mg of **6**. Fraction 3 was separated by after CC on silica gel and elution with a gradient mixture (hexane-$CH_2Cl_2$-EtOAc) to yield **8** (14 mg). Fraction 4 was submitted to chromatography on silica gel, eluted with mixtures of hexane-$CH_2Cl_2$-EtOAc, affording 158 mg of **9** and 23 mg of **5**. Fraction 5 was submitted to CC on Sephadex LH-20 using a solvent gradient elution[16] to yield three sub-fractions (I-III). Sub-fraction I was submitted to CC on silica gel, eluted with mixtures of $CH_2Cl_2$-EtOAc, to afford 24 mg of **7**. Sub-fraction III was submitted to CC on silica gel, eluted with mixtures of $CH_2Cl_2$-EtOAc, to yield 44 mg of **4**. Fraction 6 was submitted to preparative TLC on silica gel eluted with $CH_2Cl_2$, 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, $[\alpha]_D$ + 63.8 ($CHCl_3$, c0.05); IR $\nu_{max}$/cm$^{-1}$ (film): 3403, 2921, 2854, 1708, 1646, 1462, 1377, 1156, 1054, 910, 872, 773, 721; $^{1}H$ NMR spectral data (500 MHz, $CDCl_3$): 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, $J_{2\beta,1\alpha}$ 11.7 Hz, $J_{2\beta,3\alpha}$ 9.6 Hz, $J_{2\beta,1\alpha}$ 4.2 Hz, H-2β), 4.34 (1H, dd, $J_{7\beta,6\beta}$ 3.9 Hz, $J_{7\beta,6\alpha}$ 2.1 Hz, H-7β), 4.94 (1H, dd, $J_{16a,15}$ 11.1 Hz, $J_{16a,16b}$ 1.2 Hz, H-16a), 4.97 (1H, dd, $J_{16b,15}$ 17.9 Hz, $J_{16b,16a}$ 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, OO<u>H</u>); $^{13}C$ NMR spectral data (BBD and DEPT 135° spectra, 125 MHz, $CDCl_3$): 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), 38.0 (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, $[\alpha]_D$ + 32.8 ($CHCl_3$, c0.06); IR $\nu_{max}$/cm$^{-1}$ (film): 3442, 2925, 2854, 1716, 1666, 1459, 1416, 1378, 1276, 1228, 1051, 1005, 910; Elemental analysis: Found C, 85.03; H 9.87. $C_{19}H_{26}O$ requires C 84.39; H 9.69; $^{1}H$ NMR spectral data (500 MHz, $CDCl_3$): 1.10 (3H, s, H-20), 1.17 (3H, s, H-17), 2.26 (1H, dt, $J_{2\alpha,2\beta}$ 14.5 Hz, $J_{2\alpha,1\alpha}$ 3.7 Hz, $J_{2\alpha,1\beta}$ 3.7 Hz, H-2α), 2.68 (1H, td, $J_{2\beta,2\alpha}$ 14.5 Hz, $J_{2\beta,1\alpha}$ 14.5 Hz, $J_{2\beta,1\beta}$ 5.3 Hz, H-2β), 4.91 (1H, dd, $J_{16a,15}$ 10.5 Hz, $J_{16b,16a}$ 1.5 Hz, H-16a), 4.95 (1H, dd, $J_{16b,15}$ 17.5 Hz, $J_{16b,16a}$ 1.5 Hz, H-16b), 5.47 (1H, br s, H-7), 5.81 (1H, dd, $J_{15,16b}$ 17.5 Hz, $J_{15,16a}$ 10.5 Hz, H-15), 6.01 (1H, s, H-18a), 6.24 (1H, s, H-18b); $^{13}C$ NMR spectral data (BBD and DEPT 135° spectra, 125 MHz, $CDCl_3$): 36.9 (t, C-1), 36.0 (t, C-2),

200.7 (s, C-3), 143.6 (s, C-4), 48.4 (d, C-5), 23.8 (t, C-6), 121.7 (d, C-7), 135.5 (s, C-8), 49.0 (d, C-9), 39.7 (s, C-10), 20.1 (t, C-11), 36.0 (t, C-12), 36.9 (s, C-13), 46.3 (t, C-14), 149.9 (d, C-15), 109.6 (t, C-16), 21.6 (q, C-17), 124.3 (t, C-18), 16.0 (q, C-20); LREIMS *m/z* (relative intensity): 270 (45), 255 (100), 199 (22), 187 (33), 185 (69), 171 (33), 123 (61), 117 (61), 105 (89), 95 (46), 91 (98), 81 (56), 79 (55), 69 (63), 55 (73).

## Acknowledgements

The authors are grateful to FAPESP and CNPq for the financial support and to Prof. José Rubens Pirani for the plant material identification.

## References

1. Correa, M.P.; *Dicionário de Plantas Úteis e das Exóticas Cultivadas*, Imprensa Nacional, Rio de Janeiro, 1984, vol.1.
2. Lago, J.H.G.; Brochini, C.B.; Roque, N.F.; *Phytochemistry* **2000**, *55*, 727.
3. Lago, J.H.G.; Roque, N.F.; *Phytochemistry* **2002**, *60*, 329.
4. Lago, J.H.G.; Roque, N.F.; *J. Essent. Oil Res.* **2002**, *14*, 12.
5. Lago, J.H.G.; Reis, A.A.; Roque, N.F.; *Flavour Fragr. J.* **2002**, *17*, 255.
6. Knudsen, F.S.; Vilegas, W.; Oliveira, F.; Roque, N.F.; *Phytochemistry* **1986**, *25*, 1240.
7. De Kimpe, N.; Schamp, N.; Puyvelde, L.N.; Dubé, S.; Changon-Dubé, M.; Borremans, F.; Anteunis, M.J.O.; Declercq, J.P.; Germain, G.; Meerssche, M.V.; *J. Org. Chem.* **1982**, *47*, 3628.
8. Esquivel, B.; Martínez, N.S.; Cárdenas, J.; Ramamoorthy, T.P.; Rodriguez-Hahn, L.; *Planta Med.* **1989**, *55*, 62.
9. Puyvelde, L.V.; Kimpe, N.D.; Borremans, F.; Zhang, W.; Schamp, N.; *Phytochemistry* **1987**, *26*, 493.
10. Begum, S.; Hassan, S.I.; Siddiqui, B.S.; Shaheen, F.; Ghayur, M.N.; Gilani, A.H.; *Phytochemistry* **2002**, *61*, 399.
11. Polonski, J.; Baskevitch, Z.; Bellavita, N.C.; Ceccherelli, P.; Buckwalter, B.L.; Wenkert, E.; *J. Am. Chem. Soc*. **1972**, *94*, 4369.
12. Cruz, F. G.; Cerqueira, M.D.; Roque, N.F.; *J. Braz. Chem. Soc*. **2003**, *14*, 456.
13. Vilegas, W.; Alvarenga, M.A.; Roque, N.F.; *Rev. Latinoamer. Quim.* **1989**, *20*, 98.
14. Bastard, J.; Duc, D.K.; Fetizon, M.; Francis, M.J.; Grant, P.K.; Weavers, R.T.; Kaneko, C.; Baddeley, G.V.; Bernassau, J.M.; Burfitt, I.R.; Wovkulich, P.M.; Wenkert, E.; *J. Nat. Prod.* **1984**, *47*, 592.
15. Stierle, D.B.; Stierle A.A.; Larsen, R.D.; *Phytochemistry* **1988**, *27*, 517.
16. Cardellina II, J. H.; *J. Nat. Prod.* **1987**, *50*, 196.

*Received: November 3, 2003*
*Published on the web: March 9, 2005*

**FAPESP helped in meeting the publication costs of this article.**