Sesquiterpene Polyol Esters from the Leaves of *Maytenus macrocarpa*

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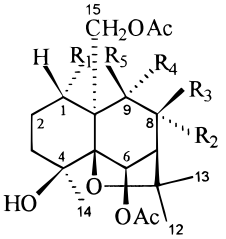
The aerial parts of *Maytenus macrocarpa* yielded three new β-dihydroagarofuran sesquiterpene polyol esters. Their structures were elucidated by spectroscopic analyses including 2D NMR techniques as 6β,8β,-15-triacetoxy-1α,9α-dibenzoyloxy-4β-hydroxy-β-dihydroagarofuran (**1**); 1α,6β,8β,15-tetraacetoxy-9α-benzoyloxy-4β-hydroxy-β-dihydroagarofuran (**2**) and (1*S*,4*S*,6*R*,7*R*,8*R*,9*R*)-1,6,15-triacetoxy-8,9-dibenzoyloxy-4-hydroxy-β-dihydroagarofuran (**3**). Compounds **1** and **2** showed marginal antitumor activity against four cell lines.

As part of our phytochemical studies on South American medicinal plants, we have analyzed the aerial parts of *Maytenus macrocarpa Briq.* (Celastraceae).[1,2] We previously described the isolation and structural elucidation of eight new dammarane triterpenes[3] and one new friedelane triterpenoid[4] from the stem bark exudate of *M. macrocarpa*. Here, we report the phytochemical study of the leaves of this species. The aerial parts of *M. macrocarpa* contained fewer sesquiterpenes than other *Maytenus* species, with triterpenes being the main secondary metabolites.

Repeated chromatography of an EtOH extract on Sephadex LH-20 and silica gel, yielded three new sesquiterpene polyol esters (**1**−**3**). The known triterpenes, lupeol,[5] friedeline,[6] and epifriedelinol,[7] together with the carotenoid lutein,[8] were also found.

Compound **1** was isolated as an amorphous white solid with molecular formula $C_{35}H_{40}O_{12}$ (HRMS). Its IR spectrum revealed absorptions for hydroxyl, ester, and aromatic groups. The ^{1}H NMR spectrum indicated the presence of three acetate esters and two benzoate esters. The ^{13}C NMR and DEPT spectra indicated that **1** contained a skeleton based on 15 carbons: three methyl carbons, three methylene carbons, five methine carbons and four quaternary carbons. These data suggested it to have a 1,4,6,8,9,15-hexasubstituted-β-dihydroagarofuran skeleton.[9] From the ^{1}H−^{1}H COSY spectrum of compound **1**, the double doublets at δ 5.61 and 5.64, and the doublets at δ 2.45 and 6.10 were assigned to H-1, H-8, H-7 and H-9, respectively. The singlet at δ 6.56 was assigned to H-6 because the dihedral angle of H-6 and H-7 was about 90°, and the two doublets at δ 4.64 and 5.03 were attributed to H-15. The locations of the hydroxy and ester functions were determined on the basis of ^{1}H−^{13}C long-range correlations, which indicated that two benzoate esters were located at C-1 and C-9, as well as three acetate esters at C-6, C-8, and C-15, and the hydroxyl group at C-4. The orientations of H-1, H-6, H-8, and H-9 were determined by analysis of the coupling constants and also by ROESY experiments. All of the above data led to 6β,8β,15-triacetoxy-1α,9α-dibenzoyloxy-4β-hydroxy-β-dihydroagarofuran as the structure for **1**.

Compound **2** was isolated as an amorphous powder with molecular formula $C_{30}H_{38}O_{12}$. It presented spectral data similar to those of **1**. The main differences were the presence of one more acetate and one less benzoate group than **1**. The location of these groups was established by HMBC experiments, in a way similar to that followed for **1**. This procedure allowed placement of the acetate groups at C-1, C-6, C-8, C-15, and the benzoate group at C-9. Five ^{1}H NMR signals of **2** were very close to those of **1** in terms of coupling patterns and coupling constants, which suggested that the stereochemistry of **2** was the same as that of **1**. The NOE effects obtained from the ROESY spectrum supported this assumption of the stereochemistry of **2**. Thus 1α,6β,8β,15-tetraacetoxy-9α-benzoyloxy-4β-hydroxy-β-dihydroagarofuran is the structure of **2**.



1 R_1=R_4=OBz; R_2=R_5=H; R_3=OAc
2 R_1=R_3=OAc; R_4=OBz; R_2=R_5=H;
3 R_1=OAc; R_2=R_5=OBz; R_3=R_4=H;

Compound **3** had the same molecular formula ($C_{35}H_{40}O_{12}$) as compound **1**. Its ^{1}H NMR spectrum contained signals for three acetate and two benzoate groups. The HMBC spectrum placed the acetate groups on C-1, C-6 and C-15, and the benzoate groups on C-8 and C-9, respectively. The signals for H-1 and H-6 were similar to those of compounds **1** and **2**, except for H-8 and H-9. Two doublets at δ 5.59 (J = 3.0 Hz) and δ 2.49 (J = 3.0 Hz) and a singlet at δ 5.86, were assigned to H-8, H-7, and H-9, respectively, using the ^{1}H−^{1}H COSY experiments. This information provided the relative stereochemistry at H-8 and H-9 (H-8eq and H-9eq). The NOE effects observed in the ROESY spectrum were in good agreement with this stereochemical assigment for **3**. The absolute stereochemistry for compound **3** was determined by application of the CD exciton chirality method.[10] The CD spectrum showed a split curve with a first negative Cotton effect at 237.0 nm ($\Delta\epsilon$ = −17.9) and a second positive Cotton effect at 218.0 nm ($\Delta\epsilon$ = +2.3), typical of benzoate groups at 8α and 9β.[11] Therefore, the absolute configuration of **3** was determined as (1*S*,4*S*,6*R*,-7*R*,8*R*,9*R*)-1,6,15-triacetoxy-8,9-dibenzoyloxy-4-hydroxy-β-dihydroagarofuran. Note that the absolute stereochemistry of **1** and **2** cannot be determined by this method since the two cromophores of **1** are coplanar, and compound **2** has only one chromophore.

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Compounds **1** and **2** were tested for antitumor activity[12] against P-388 lymphoid neoplasm, A-549 human lung carcinoma, HT-29 human colon carcinoma, and MEL-28 human melanoma cell lines. They showed marginal activity. None of these compounds showed significant inhibitory activity in the aldose reductase assay[13] ($IC_{50} > 25\ \mu g/mL$). Compound **2** also showed low MDR reversing activity on the parasite protozoan *Leishmania tropica* line.[14]

Experimental Section

General Experimental Procedures. IR spectra were taken on a PE 681 spectrophotometer. ^1H and ^{13}C NMR, HMBC, HMQC, and ROESY spectra were obtained using a Bruker AM-400 NMR spectrometer, with TMS as internal reference and $CDCl_3$ as solvent. Optical rotations were measured with a Perkin-Elmer 241 automatic polarimeter; $[\alpha]_D^{20}$ are given in 10^{-1} deg cm^2 g^{-1}. UV spectra were collected with a Jasco V-560. MS were recorded on a VG Micromass ZAB-2F and a Hewlett-Packard 5995. HRMS were recorded on a VG Autospec spectrometer. CD spectra were run on a Jasco J-600 spectropolarimeter. Schleicher-Schüll F-100/LS 254 and preparative TLC 1510/LS 254 foils were used for TLC, while silica gel (0.2−0.63 mm) and Sephadex LH-20 were used for column chromatography.

Plant Material. The plant was collected in Loreto Region (Perú), in November 1996, and it was identified by the botanist J. Ruiz. A voucher specimen is on file with the Herbarium of the Departamento de Botánica, Universidad Nacional de la Amazonía (Iquitos, Peru).

Extraction and Isolation. Dried leaves of *M. macrocarpa* (0.93 kg) were extracted with EtOH at room temperature. The dried extract (0.23 kg) was treated with EtOAc to afford a dark residue (soluble in EtOAc). This residue (45 g) was chromatographed on Sephadex LH-20 and silica gel using mixtures of *n*-hexane−$CHCl_3$−MeOH (2:1:1) and of *n*-hexanes−EtOAc, respectively, yielding **1** (10 mg), **2** (3.3 mg), and **3** (2.5 mg).

6*β*,8*β*,15-Triacetoxy-1α,9α-dibenzoyloxi-4*β*-hydroxy-*β*-dihydroagarofuran (1): amorphous white solid; $[\alpha]_D^{20}$ −7.9° (*c* 0.8, $CHCl_3$); UV (EtOH) λ_{max} 227.4, 276.3, 283.8 nm; IR ($CHCl_3$) ν_{max} 3528, 2924, 1730, 1601, 1472, 1450, 1372, 1339, 1234, 1093 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 7.57 (2H, d, J = 7.6 Hz); 7.38 (1H, t, J = 7.4 Hz); 7.36 (2H, d, J = 7.4 Hz); 7.19 (1H, t, J = 7.5 Hz); 7.14 (2H, t, J = 7.8 Hz); 6.89 (2H, t, J = 7.8 Hz); 6.56 (1H, s, H-6), 6.10 (1H, d, J = 9.8 Hz, H-9), 5.64 (1H, dd, J = 9.8, 3.4 Hz, H-8), 5.61 (1H, dd, J = 13.5, 4.3 Hz, H-1), 5.03 (1H, d, J = 12.7 Hz, H-15a), 4.64 (1H, d, J = 12.7 Hz, H-15b), 2.45 (1H, d, J = 3.3 Hz, H-7), 2.41 (3H, s, OAc-15), 2.12 (3H, s, OAc-6), 1.97 td (1H, J = 13.8, 4.4 Hz, H-3b), 1.90 (1H, m, H-2b), 1.78 (3H, s, OAc-8), 1.74 (1H, dt, J = 10.0, 3.5 Hz, H-3a), 1.70 (3H, s, Me-13), 1.55 (3H, s, Me-12), 1.45 (1H, dd, J = 13.5, 3.5 Hz, H-2a), 1.36 (3H, s, Me-14); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 92.4 (s, C-5), 84.1 (s, C-11), 76.9 (d, C-1), 75.3 (d, C-6), 75.1 (d, C-9), 73.8 (d, C-8), 70.2 (s, C-4), 61.1 (t, C-15), 52.1 (d, C-7), 50.8 (s, C-10), 37.9 (t, C-3), 29.6 (q, Me-12), 25.6 (q, Me-13), 24.8 (t, C-2), 23.3 (q, Me-14), OAc [20.7 (q, C8−OCO*C*H$_3$), 21.2 (q, C15−OCO*C*H$_3$), 21.4 (q, C6−OCO*C*H$_3$), 169.7 (s, C8−O*C*OCH$_3$), 169.8 (s, C6−O*C*OCH$_3$), 170.5 (s, C15−O*C*OCH$_3$)], OBz [165.1 (C1−O*C*OPh), 165.0 (C9−O*C*OPh), 132.7 (C), 132.4 (C), 129.2 (C), 129.1 (2 × CH), 129.1 (C), 128.0 (2 × CH), 127.6 (2 × CH)]; EIMS m/z 652 (M^+) (1), 592 (10), 550 (4), 288 (11), 164 (8), 105 (100), 77 (16); HREIMS m/z 652.2511 (calcd for $C_{35}H_{40}O_{12}$, 652.2519).

1α,6*β*,8*β*,15-Tetraacetoxy-9α-(benzoyloxy)-4*β*-hydroxy-*β*-dihydroagarofuran (2): amorphous white solid; $[\alpha]_D^{20}$ −5.8° (*c* 0.7, $CHCl_3$); UV (EtOH) λ_{max} 230.0, 273.8 282.5 nm; IR ($CHCl_3$) ν_{max} 3464, 2922, 1747, 1450, 1370, 1278, 1225, 1092, 1039 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ 7.82 [2H, dd, J = 8.2, 1.1 Hz (H-2′ + H-6′, OBz)], 7.48 [1H, t, J = 7.5 Hz, (H-4′, OBz)], 7.35 [2H, t, J = 8.0 Hz, (H-3′ + H-5′, OBz)], 6.54 (1H, s, H-6), 6.01 (1H, d, J = 9.8 Hz, H-9), 5.59 (1H, dd, J = 9.8, 3.4 Hz, H-8), 5.26 (1H, dd, J = 12.3, 4.3 Hz, H-1), 4.83 (1H, d, J = 12.7 Hz, H-15a), 4.47 (1H, d, J = 12.7 Hz, H-15b), 2.46 (1H, d, J = 3.4 Hz, H-7), 2.37 (3H, s, OAc−C15), 2.12 (3H, s, OAc−C8), 1.88 (3H, s, OAc−C1), 1.55 (3H, s, OAc−C6), 1.69 (3H, Me-13), 1.54 (3H, Me-12), 1.32 (3H, Me-14); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 92.3 (s, C-4), 83.9 (s, C-11), 76.9 (d, C-1), 75.4 (d, C-9), 75.2 (d, C-6), 73.9 (d, C-8), 70.1 (s, C-4), 61.0 (t, C-15), 52.0 (d, C-7), 50.4 (s, C-10), 37.9 (t, C-3), 29.6 (q, Me-12), 25.6 (q, Me-13), 24.4 (t, C-2), 23.3 (q, Me-14), OAc [21.3 (q, C8−OCO*C*H$_3$), 21.1 (q, C15−OCO*C*H3), 20.8 (q, C1−OCO*C*H$_3$), 20.7 (q, C6−OCO*C*H$_3$), 170.4 (s, C15−O*C*OCH$_3$), 169.8 (s, C8−O*C*OCH$_3$), 169.7 (s, C6−O*C*OCH$_3$ + C1−O*C*OCH$_3$)], OBz [165.5 (s, C-9), 133.3 (d, C-4′), 128.6 (d, C-3′+C-5′), 129.5 (d, C-2′+C-6′), 129.4 (s, C-1′)]; EIMS m/z 590 (M^+) (1), 530 (15), 488 (12), 246 (10), 164 (19), 149 (11), 105 (100), 77 (13); HREIMS m/z 590.2404 (calcd for $C_{30}H_{38}O_{12}$, 590.2463).

(1*S*,4*S*,6*R*,7*S*,8*S*,9*R*)-1,6,15-Triacetoxy-8α,9*β*-dibenzoyloxy)-4*β*-hydroxy-*β*-dihydroagarofuran (3): white gum; $[\alpha]_D^{20}$ −7.9° (*c* 0.8, $CHCl_3$); UV (EtOH) λ_{max} 227.4, 276.3, 283.8 nm; IR ($CHCl_3$) ν_{max} 3528, 2924, 1730, 1601, 1472, 1450, 1372, 1339, 1234, 1093 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.18 (2H, m); 8.07 (2H, m); 7.61 (2H, m); 7.49 (4H, m); 6.65 (1H, s, H-6), 5.86 (1H, s, H-9), 5.59 (1H, d, J = 3 Hz, H-8), 5.48 (1H, dd, J = 11.8, 4.2 Hz, H-1), 4.78 (1H, d, J = 12.8 Hz, H-15a), 4.63 (1H, d, J = 12.8 Hz, H-15b), 2.49 (1H, d, J = 3 Hz, H-7), 2.12 (3H, s, OAc-6), 2.09 (3H, s, OAc-15), 1.74 (3H, s, Me-13), 1.62 (3H, s, Me-12), 1.51 (3H, s, OAc-1), 1.34 (3H, s, Me-14); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 91.6 (s, C-5), 83.0 (s, C-11), 76.8 (d, C-8), 75.4 (d, C-6), 72.8 (d, C-9), 72.7 (d, C-1), 70.4 (s, C-4), 65.2 (t, C-15), 53.8 (s, C-10), 53.5 (d, C-7), 37.8 (t, C-3), 29.5 (q, Me-12), 25.6 (q, Me-13), 23.3 (t, C-2), 22.7 (q, Me-14), OAc [21.4 (q, C1−OCO*C*H$_3$), 21.1 (q, C15−OCO*C*H$_3$), 21.0, (q, C6−OCO*C*H$_3$), 169.4 (s, C1−O*C*OCH$_3$), 169.8 (s, C6−O*C*OCH$_3$), 170.5 (C15−O*C*OCH$_3$)], OBz [165.2 (C8−O*C*OPh), 164.3 (C9−O*C*OPh), 133.6 (CH), 133.3 (CH), 130.0 (4×CH), 129.7 (CH), 129.7 (C), 129.4 (C)]; EIMS m/z 652 (M^+) (1), 592 (10), 550 (4), 288 (11), 164 (8), 105 (100), 77 (16); HREIMS m/z 652.2511 (calcd for $C_{35}H_{40}O_{12}$, 652.2519).

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References and Notes

(1) Soukup, J. *Nombres Vulgares de la Flora Peruana*; Editorial Salesianos: Lima, Peru, 1997.
(2) Gupta, M. P. *270 Plantas Medicinales Iberoamericanas*; Convenio Andrés Bello; Colombia, Bogotá, 1995.
(3) Chávez, H.; Estévez-Braun, A.; Ravelo, A. G.; González A. G. *Tetrahedron* **1997**, *53*, 6465−6472.
(4) Chávez, H.; Estévez-Braun, A.; Ravelo, A. G.; González, A. G. *J. Nat. Prod.* **1998**, *61*, 82−85.
(5) Reynolds, W. F.; McLean S.; Poplawski J.; Enriquez, R. G.; Escobar L. I.; Leon I. *Tetrahedron* **1986**, *42*, 3419−3428.
(6) Tanaka, R.; Matsunaga, S. *Phytochemistry* **1988**, *27*, 3579−3584.
(7) Betancor, C.; Freire, R.; González, A. G.; Salazar, J. A.; Pascard, C.; Prangge, T. *Phytochemistry* **1980**, *19*, 1989−1993.
(8) Lützow, M.; Haaf, F.; Beyer, P.; Kleinig, H. *Phytochemistry* **1996**, *41*, 729−734.
(9) González, A. G.; Jiménez I. A., Ravelo, A. G. Sazatornil J. G.; Bazzocchi I. L. *Tetrahedron* **1993**, *49*, 697−702.
(10) Harada, N. and Nakanishi, K. *Circular Dichroism Spectroscopy Exciton Coupling in Organic Stereochemistry*; University Science Book, Mill Valley, CA, 1983.
(11) Tincusi, B. M.; Jiménez, I. A.; Ravelo, A. G.; Missico, R. *J. Nat. Prod.* **1998**, *61*, 1520−1523.
(12) Bergeron, R. J.; Cavanaugh, P. F., Kline, S. J.; Hughes, R. G.; Elliot, G. T.; Porter, C. W. *Biochem. Biophys. Res. Commun.* **1984**, *121*, 848−854.
(13) Nihismura, C.; Yamaoka, T.; Mizutani, M.; Yamashita, K.; Akera, T.; Tanimoto, T. *Biochim. Biophys. Acta* **1991**, *1078*, 171.
(14) Pérez-Victoria, J. M.; Castanys, S.; Gamarro, F.; Tincusi, B. M.; Jiménez, I. A.; Bazzocchi, I.; Ravelo, A. G.; *J. Med. Chem.* **1999**, submitted.

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