New Dammarane Triterpenes from *Maytenus macrocarpa*

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Two new dammarane triterpenes have been isolated from the stem bark exudates of *Maytenus macrocarpa*. Their structures were determined by extensive 1D and 2D NMR spectroscopic studies as 24(*Z*)-3-oxodammara-20(21),24-dien-27-oic acid (1) and octa-*nor*-13-hydroxydammara-1-en-3,17-dione (2). These compounds were tested for antitumoral activity.

Key words *Maytenus macrocarpa*; Celastraceae; dammarane; triterpene

Plants of the family Celastraceae are well known for their medicinal use.[1)] Extracts derived from some species of this family have been shown to posses biological activities including cytostatic, antitumour, antileukaemic, antifeedant, and abortive.[2)] These results, together with the recent isolation of biologically active compounds, from its members have raised current interest in plants belonging to the family Celastraceae.[3)]

Maytenus macrocarpa BRIQ (Celastraceae) is a large tree found throughout the Amazonian region of Peru, and it is used in the treatment of rheumatism, influenza, gastrointestinal diseases, and as antitumoral agent for skin cancer.[4—6)] Several new terpenoids have been previously reported by us from the species *Maytenus macrocarpa*.[7—10)] We now report the isolation of two new dammarane triterpenes (**1**, **2**) from the stem bark exudates of *M. macrocarpa*. The structures of these two new triterpenes have been rigorously characterized by spectroscopic analysis including 2D NMR.

Results and Discussion

Compound **1** was isolated as a pale yellow oil with a molecular formula $C_{30}H_{46}O_3$. The main signals in its ^{1}H-NMR spectrum were a vinylic methyl (s, δ 1.89), five angular methyls (δ 0.88, 0.95, 1.01, 1.04, 1.08), and three olefinic protons, one of which was deshielded (δ 6.10, t, J=7.2 Hz), typical of the β position on α,β unsaturated carboxylic acid.[11)] The other two olefinic protons correspond to an unsaturated terminal methylene [δ 4.78 bs (1H), δ 4.73 bs (1H)]. Its ^{13}C-NMR spectral data, together with the MS fragmentation patterns, pointed out that **1** belong to the dammarane group.[7,12)] The spectral data of this compound were very similar to those of 24-(*Z*)-3-oxodammara-20(21),24-dien-27-al.[7)] The main differences were in the lateral chain, *i.e.*, in the values of Me-26 (δ 1.89 *vs.* 1.76) and H-24 (δ 6.53 *vs.* 6.10). The position of the α,β-unsaturated carboxylic acid through C-24, C-25 and C-27 was determined by the ^{1}H–^{13}C long-range correlations detected in the HMBC, which are shown in Fig. 1. A ROESY experiment showing the NOE effect between H-24 and Me-26 confirmed that the stereochemistry of the double bond Δ^{24} is *Z*. All of these data allow us to establish the structure of **1** as 24-(*Z*)-3-oxodammara-20(21),24-dien-27-oic acid.

Compound **2** was isolated as an amorphous white solid. It showed the molecular ion peak at *m*/*z* 344 in MS, and its molecular formula was determined to be $C_{22}H_{32}O_3$ by HR-MS. The fragment $[C_{14}H_{21}O]^+$ indicated a tetracyclic 3-oxotriterpene which shows typical fragmentation in ring C.[13)] The IR spectrum revealed the presence of hydroxyl (3400 cm^{-1}) and carbonyl groups (1730, 1662 cm^{-1}). Its ^{1}H-NMR spectrum showed the presence of five angular methyls at δ 0.82, 1.04, 1.09 and 1.15 (×2), and two vinyl hydrogens at δ 7.10 (d, J=10.2 Hz) and δ 5.82 (d, J=10.2 Hz). These signals are characteristic of a α,β-unsaturated carbonyl moiety. The main signals in its ^{13}C-NMR spectrum ($CDCl_3$) were two carbonyl carbons at δ 216.4 and δ 205.1, one quaternary carbon joined at oxygenated function at δ 78.2, two vinyl carbons conjugated to carbonyl group (δ 125.4, 159.3), and five methyl groups (δ 16.60, 18.29, 19.90, 21.40, 27.90). All the data mentioned above confirmed that compound **2** is an octanordammarane characterized by the lack of the usual C_8 side chain present at C-17.[13—17)] The locations of the different functional groups were established by the detected HMBC correlations. Thus, the position of the α,β-unsaturated carbonyl system through C-3, C-2, and C-1 was established by the HMBC correlations of Me-19 to C-1, Me-28 to C-3, and Me-29 to C-3. The position of the other carbonyl group at C-17, and also the hydroxyl group at C-13, were established by the following correlations: H-12/C-17; H-30/C-16; H-30/C-15 and H-15/C-17 (see Fig. 2).

Compound **2** did not form the corresponding acetyl deriv-

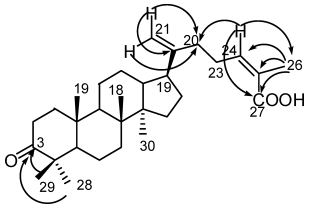

Fig. 1. Key HMBC Correlations for Compound **1**

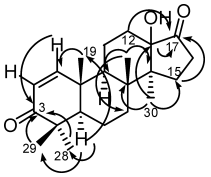

Fig. 2. Key HMBC Correlations for Compound **2**

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ative when it was treated under different acetylation conditions, including some specific conditions for tertiary alcohols.[18,19] However, because of this difficulty to form the acetyl derivative, the β-stereochemistry for the hydroxyl group is the most plausible one, it would naturally explain the difficulty due to 1,3-steric interactions of the bulky Ac_2O with Me-18.

This type of compound is unusual, since there are no many examples in the literature.[13—17] Probably, these octanordammarane triterpenes loose the side chain in a way similar to the cholesterol when it looses its chain to yield products like testosterone.[20]

Because of the existence of dammarane triterpenes exhibiting cytotoxic activities, we decide to test the new compounds.[21—23]

A set of isogenic yeast strains defective for the G1/S and G2/M DNA damage checkpoints was used in order to detect the potential cytotoxicity specific for these genetic backgrounds of compounds **1** and **2**.[24] Neither **1** nor **2** exhibited cytotoxicity for any of the eight yeast strains tested using concentrations at least at 100 μg/ml. As these strains are hypersensitive to DNA-damaging agents, we can therefore conclude that both compounds are not genotoxic. The viability of the wild-type strain was not affected by the treatment, suggesting that compounds **1** and **2** did not exhibit antifungal activity.

Experimental

General Experimental Procedures UV spectra were collected in absolute EtOH on a JASCO V-560 spectrophotometer. IR spectra were taken on a Bruker IFS28/55 spectrophotometer. ^{1}H and ^{13}C spectra were recorded in $CDCl_3$ at 300 and 75 MHz, respectively, with TMS as internal reference. The 2D-NMR experiments were conducted on a Bruker WP-400 SY NMR spectrometer in $CDCl_3$ at 400 MHz. High- and low-resolution mass spectra were obtained on a VG Autospec spectrometer. Macherey-Nagel polygram Sil G/UV_{254} and preparative TLC Sil G-100UV254 foils were used for TLC. Silica gel (0.2—0.63 mm) and Sephadex LH-20 were used for column chromatography. Semipreparative scale HPLC on-Kromasil 100 Si 5 μ was also used for final purification of some products.

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

Extraction and Isolation The stem bark exudates of *Maytenus macrocarpa* (0.18 kg) was extracted with *n*-hexanes–Et_2O (1 : 1) (2 l) in a Soxhlet apparatus. The extract (40 g) was chromatographed on Sephadex LH-20 using as eluent mixtures of *n*-hexanes–$CHCl_3$–MeOH (2 : 1 : 1). Five fractions, A—E, were separated, studied and chromathographed on silica gel using mixtures of *n*-hexanes : AcOEt of increasing polarity. Further purification based on TLC preparative and HPLC yielded a set of products. Fraction A provided friedelin,[25] 29-hydroxyfriedelane-3-one,[26] and olean-12-ene-3β,6β-diol.[27] Fraction B yielded tingenone,[28] pristimerin,[28] glut-5-ene-3β,29-diol,[29] celastrol,[30] 24-(*Z*)-3-oxodammara-20(21),24-dien-26-al,[7] and 24-(*E*)-3-oxodammara-20(21),24-dien-26-al.[7] Fraction C afforded 24-(*E*)-6β,26-dihydroxydammara-20(21),24-dien-3-one,[7] 28-hydroxyfriedelane-1,3-dione[8] and 25-oxo-friedelane-3-one,[32] netzahualcoyene,[33] glut-5-ene-3β,29-diol,[27] 24(*E*)-26-hydroxydammara-20(21),24-dien-3-one,[7] 24(*E*)-23α-hydroxy-26-oxodammara-20(21),24-dien-3-one,[7] 24(*E*)-23β-hydroxy-26-oxodammara-20(21),24-dien-3-one,[7] 24(*E*)-6β-hydroxy-26-oxodammara-20(21),24-dien-3-one,[7] 24(*E*)-6β,26-dihydroxydammara-20(21),24-dien-3-one,[7] 24(*E*)-26-hydroxydammara-20(21),24-dien-3-one,[7] and 23(*Z*)-25-*nor*-dammara-20(21),24-dien-3,25-dione.[7] From the last fraction E, we isolated **1** (11.2 mg), **2** (10.6 mg), and ilicifoline.[31]

24(*Z*)-3-oxodammara-20(21),24-dien-27-oic Acid (1) Pale yellow oil; $[\alpha]_D^{20}$+0.14° (*c*=0.7, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3325, 2931, 1704, 1456, 1384, 1216, 756 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, s, H_3-18), 0.95 (3H, s, H_3-19), 1.01 (3H, s, H_3-28), 1.04 (3H, s, H_3-29), 1.08 (3H, s, H_3-30), 1.89 (3H, s, H_3-26), 4.72 (1H, s, H-21a), 4.78 (1H, s, H-21b), 6.10 (1H, t, *J*=7.2 Hz, H-24); ^{13}C-NMR ($CDCl_3$) δ: 15.3 (CH_3, C-19), 15.8 (CH_3, C-30), 16.0 (CH_3, C-18), 19.7 (CH_2, C-6), 20.5 (CH_3, C-26), 21.0 (CH_3, C-29), 21.9 (CH_2, C-11), 24.9 (CH_2, C-12), 26.7 (CH_3, C-28), 28.4 (CH_3, C-16), 28.9 (CH_2, C-23), 31.3 (CH_2, C-15), 33.7 (CH_2, C-22), 34.1 (CH_2, C-2), 34.7 (CH_2, C-7), 36.9 (C, C-10), 39.3 (CH_2, C-1), 40.4 (C, C-8), 45.5 (CH, C-13), 47.4 (CH, C-17), 47.5 (C, C-4), 49.4 (C, C-14), 50.3 (CH, C-9), 55.3 (CH, C-5), 111.8 (CH_2, C-21), 137.8 (CH_3, C-25), 146.2 (CH, C-24), 151.6 (C, C-20), 172.2 (C, C-27), 218.3 (C, C-3); EI-MS *m*/*z* 454 [M^+] (31), 436 (50), 245 (50), 205 (100), 121 (65), 95 (90); HR-EI-MS *m*/*z* 454.3510 (Calcd for $C_{30}H_{46}O_3$ 454.3545).

Octa-*nor*-13-hydroxydammara-1-en-3,17-dione (2) Amorphous white solid. $[\alpha]_D^{20}$+2.8° (*c*=0.2, $CHCl_3$); UV: λ_{max}^{EtOH} nm (log ε) 227 (3.85); IR: ν_{max} (neat) 3400, 2924, 1736, 1662, 1460, 1384, 1250, 1158, 1107, 1033, 1001, 971, 816 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.10 (1H, d, *J*=10.2 Hz, H-1), 5.82 (1H, d, *J*=10.2 Hz, H-2), 2.22 (1H, dt, *J*=10.9, 1 Hz, H-12b), 2.60 (m, H-16a), 2.10 (m, H-16b), 1.90 (1H, m, H-15a), 1.70 (1H, m, H-15b), 1.45 (1H, m, H-12a), 1.15 (6H, s, H_3-28+H_3-30), 1.09 (3H, s, H_3-29), 1.04 (3H, s, H_3-19), 0.82 (3H, s, H_3-18); ^{13}C-NMR ($CDCl_3$) δ: 216.4 (C, C-17), 205.1 (C, C-3), 159.3 (CH, C-1), 125.4 (CH, C-2), 78.2 (C, C-13), 53.6 (CH, C-5), 49.3 (C, C-14), 45.2 (CH, C-9), 44.7 (C, C-4), 40.9 (C, C-8), 39.3 (C, C-10), 34.5 (CH_2, C-7), 34.11 (CH_2, C-16), 28.29 (CH_2, C-12), 27.90 (CH_3, C-28), 27.22 (CH_2, C-15), 21.40 (CH_3, C-29), 19.90 (CH_3, C-19), 18.74 (CH_2, C-11), 18.39 (CH_3, C-18), 18.29 (CH_2, C-6), 16.60 (CH_3, C-30); EI-MS: *m*/*z* (rel.int) 344 [M^+] (33), 326 (16), 316 (100), 288 (16), 273 (40), 203 (65), 137 (72), 123 (55); HR-EI-MS *m*/*z* 344.2335 (Calcd for $C_{22}H_{32}O_3$ 344.2351).

Biological Assays. Yeast Strains All the strains used in this study have the W3031A genetic background (MATa, ade2-1, can1-100, his3-11, leu2-3, trp11-1, ura3.1) The rad9, rad17, rad24, mec3 and tel1 strains, harboring disruptions of the respective genes have been described elsewhere.[34] The mec1-1 and rad53-11 yeast mutants have also been described previously.[34]

Yeast Growth Assays Standard methods for yeast culture and manipulations were used.[35] To assess cell growth in the presence of increasing concentrations of each compound tested, mid-log cultures of each strain growing on liquid YPD medium were 10-fold serially diluted and volumes of around 3 μl were applied with a stainless-steel replicator (SIGMA) on solid plates containing 2% Bacto-Agar (Difco) and YPD medium with 10-fold increasing doses of each product. Growth was recorded after 2—3 d in all cases.

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