Notes

Friedelane Triterpenoids from *Maytenus macrocarpa*

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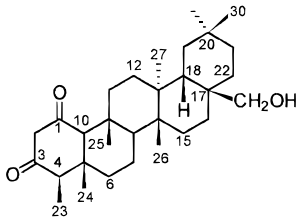
A set of friedelane triterpenoids has been isolated from the stem bark exudates of *Maytenus macrocarpa*. It includes a new friedelan triterpene **(1)**, together with the known compounds friedelin, 3-oxo-29-hydroxyfriedelane, 3-oxofriedelan-25-al, and canophyllol. The structures of these compounds were elucidated by spectroscopic and chemical evidence. Complete ^{1}H and ^{13}C assignments were achieved by 2D NMR spectroscopy. The new compound showed weak activity against aldose reductase. It did not display antitumor activity against P-388 lymphoid neoplasm, A-549 human lung carcinoma, HT-29 human colon carcinoma, or MEL-28 human melanoma cell lines.

Maytenus macrocarpa (R. & P.) Briquet (Celastraceae)[1,2] is endemic to the Amazonian region of Peru, and it is frequently used in traditional medicine for the treatment of rheumatism, influenza, gastrointestinal diseases, and as an antitumor agent for skin cancer. We described in a previous paper[3] the isolation and structure elucidation of eight new dammaranes from the stem bark exudates of *Maytenus macrocarpa*, which was the first record of this type of triterpenes in the Celastraceae. The present study reports the isolation of five friedelane triterpenes, one of which (**1**) is new to the literature. The friedelanes constitute an important class of triterpenes, members of which have shown interesting biological activities.[4−6]



1

The ^{1}H- and ^{13}C-NMR spectral data, together with the MS fragmentation patterns, revealed that isolate **1** and the other triterpenes belong to the friedelane group. The known compounds were identified as friedelin,[7] 3-oxo-29-hydroxyfriedelane,[7] 3-oxofriedelan-25-al,[8] and canophyllol,[7] respectively, from their physical constants and spectral data.

Compound **1**, a new friedelane triterpene, was obtained as an amorphous white solid. Its IR spectrum exhibited bands for carbonyl (1720, 1700 cm^{-1}) and hydroxyl (3520 cm^{-1}) groups. Signals for 30 carbon atoms appeared in its ^{13}C-NMR spectrum. No molecular ion showed up in the EIMS, although a fragment was detected at *m/z* 425, which was assigned by HREIMS to $C_{29}H_{45}O_2$ ($M^+ - CH_2OH$). These data and the ^{1}H-NMR spectrum permitted us to assign the molecular formula $C_{30}H_{48}O_3$ to **1**.

The ^{1}H-NMR spectrum (Table 1) showed six tertiary methyl singlets and a secondary methyl doublet. The presence of a primary hydroxyl group in **1** was evident from the ^{1}H-NMR spectrum (Table 1), which shows a broad singlet (2H) at δ 3.68. The existence of this group was also supported by the signal at δ 67.98 in the ^{13}C-NMR spectrum. An additional feature of the ^{1}H-NMR was the presence of two doublets at δ 3.46 (1H, J = 16 Hz) and 3.25 (1H, J = 16 Hz), typical of a 1,3-diketone. The position of the OH group on C-28 was established by the analysis of the fragments in the MS spectra and also by HMBC correlations. The MS spectra yielded fragment ion peaks at *m/z* 315, 287, 220, and 123. The HMBC experiments showed three-bond coupling between the broad singlet at δ 3.68 (2H), two methylene carbons at δ 29.02 (C-16) and 34.35 (C-22), and a methine carbon at δ 39.32 (C-18). From HMBC, HMQC, and DEPT experiments, all carbons and protons were unambiguously assigned (Table 1). Under acetylation conditions, **1** formed a major compound (**1a**), together with traces of **1b** and **1c** (Figure 1).

The main differences between the ^{1}H-NMR spectra (Table 1) of the diacetate **1a** and **1** were a doublet at δ 5.68 (1H, J = 1.4 Hz) characteristic of vinylic protons, and two doublets at δ 4.27 (1H, J = 11 Hz) and 4.00 (1H, J = 11 Hz), which we assigned to H-28 by HMQC experiments. The ^{1}H-NMR spectrum also presented two singlets at δ 2.06 and 2.21, corresponding to the methyls of acetate groups. The structure of the enol acetate **1a** was confirmed by the three-bond coupling between the

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Table 1

	compound 1			compound 1a		
no.	δ_H, coupling (Hz)	δ_C	$^1H-^{13}C$ corr.	δ_H, coupling (Hz)	δ_C	$^1H-^{13}C$ corr.
1		202.73 C			199.90 C	
2	3.25 d, 16 3.46 d, 16	60.60 CH_2	C_1, C_3, C_4, C_{10}	5.68 d, 1.4	118.74 CH_2	C_3, C_4
3		204.06 C			167.06 C	
4	2.58 q, 6.7	59.05 CH	C_2, C_{10}, C_{24}	2.81 dq, 1.4, 7.2	48.78 CH	C_2, C_6, C_{10}, C_{23}
5		37.23 C			42.32 C	
6	1.84−1.91 m	40.57 CH_2		1.91 m	40.99 CH_2	C_4, C_8, C_{10}
7	1.50 m	18.04 CH_2		1.40 m	17.62 CH_2	
8	1.20 m	51.51 CH		1.25 m	51.50 CH	
9		37.79 C			36.99 C	
10	2.39 s	71.88 CH	C_2, C_4, C_6, C_8	2.20 s	68.80 CH	C_2, C_4, C_{24}
11	2.15 t, 3.4 2.17 t, 3.4	33.36 CH_2	C_8, C_{10}, C_{13}, C_{25}	2.42 t, 3.3 2.39 t, 3.3	34.51 CH_2	C_8, C_{10}, C_{13}
12	1.50−1.30 m	29.71 CH_2		1.20−1.40 m	29.92 CH_2	
13		39.16 C			39.07 C	
14		39.08 C			39.04 C	
15	1.50−1.30 m	31.24 CH_2		1.20−1.40 m	28.01 CH_2	
16	1.84−1.91 m	29.02 CH_2		1.70 m	29.51 CH_2	
17		35.13 C			33.74 C	
18	1.40 m	39.32 CH		1.35 m	38.08 CH	
19	1.30 m	34.45 CH_2		1.20−1.40 m	33.06 CH_2	
20		28.12 C			29.51 C	
21	1.50−1.30 m	31.39 CH_2		1.20−1.40 m	31.46 CH_2	
22	1.40 m	34.35 CH_2		1.20−1.40 m	32.24 CH_2	
23	1.06 d, 6.7	7.27 CH_3	C_3, C_4, C_5	0.97 d, 6.7	8.95 CH_3	C_3, C_4, C_5
24	0.69 s	15.95 CH_3	C_4, C_6, C_{10}	0.92 s	15.12 CH_3	C_4, C_6, C_{10}
25	1.26 s	18.10 CH_3	C_8, C_{10}, C_{11}	1.17 s	18.21 CH_3	C_8, C_{10}
26	0.94 s	19.08 CH_3	C_8, C_{13}, C_{14}, C_{15}	0.98 s	19.35 CH_3	C_8, C_{13}, C_{15}
27	1.10 s	19.24 CH_3	C_{12}, C_{13}, C_{18}	1.08 s	19.23 CH_3	C_{12}, C_{13}
28	3.68 br s	67.98 CH_2	C_{17}, C_{18}, C_{22}	4.00 d, 11.0 4.27 d, 11.0	69.04 CH_2	C_{17}, C_{18}, C_{22}
29	1.00 s	34.20 CH_3	C_{19}, C_{20}, C_{21}	0.98 s	34.38 CH_3	C_{19}, C_{20}, C_{21}
30	0.98 s	32.82 CH_3	C_{19}, C_{20}, C_{21}	0.96 s	32.53 CH_3	C_{19}, C_{20}, C_{21}

quaternary vinyl carbon at δ 167.06 and the doublet at δ 0.97 due to Me-23.

The MS spectrum of the enol acetate **1b** showed a fragment at *m/z* 466 [$M^+ - CH_2OAc$], which corresponds to the formula $C_{31}H_{46}O_3$. Its ^{1}H-NMR spectrum showed neither signals of vinylic protons nor singlets attributable to H-10. The other minor diacetate compound **1c** presented the molecular formula $C_{34}H_{52}O_5$. Its ^{1}H-NMR spectrum had a singlet at δ 2.58 due to H-10, no vinylic protons, and a singlet at δ 1.57 (3H) attributable to Me group on double bond.

The enol acetate (C_2-C_1-OAc) was not formed, in agreement with the acetylation of other 1,3-diketone triterpenes.[9] This result could be explained by the energetic differences between the enol acetate (C_2-C_1-OAc) and the rest of the enol acetates, which we modeled using Molecular Mechanics Calculations.[10]

The compounds **1** and **1a** were tested for antitumor activity. Neither of them showed significant inhibitory activity (IC_{50} > 10 mg/mL) against the following cell lines: P-388, suspension culture of a lymphoid neoplasm from a DBA/2 mouse; A-549, monolayer culture of a human lung carcinoma; HT-29, monolayer culture of a human colon carcinoma; MEL-28, monolayer culture of a human melanoma. The new compound **(1)** was also assayed for aldose reductase (AR) inhibitory activity (this enzyme appears to initiate the cataractous process in diabetic persons[11]) and showed an IC_{50}= 12 mg/mL.

Experimental Section

General Experimental Procedures. IR spectra were taken on a PE 681 spectrophotometer and ^{1}H and ^{13}C NMR on a Bruker W-200SY at 200 and 50 MHz, respectively, with TMS as internal reference. The HMBC, HMQC, and ROESY were run on a Bruker at 400 MHz. Optical rotations were measured with a Perkin-Elmer 241 automatic polarimeter and $[\alpha]^{20}_D$ are given in 10^{-1} deg cm^2 g^{-1}. UV spectra were collected with a Perkin-Elmer model 550-SE. MS were recorded on VG Micromass ZAB-2F and Hewlett-Packard 5995 spectrometers. HRMS were recorded on a VG Autospec spectrometer. Schleicher-Schüll F-100/LS 254 and preparative TLC 1510/LS 254 foils were used for TLC, while Si gel (0.2−0.63 mm) and Sephadex LH-20 were used for column chromatography.

Plant Material. The plant was gathered in Peru, in November 1995, and 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.

Extraction and Isolation. The stem bark exudates of *M. macrocarpa* (0.18 kg) was extracted with *n*-hexanes−Et_2O (1:1) (2 L) in a Soxhlet apparatus. The extract (40 g) was repeatedly chromatographed on Sephadex LH-20 and Si gel using as solvents, mixtures of *n*-hexanes−$CHCl_3$−MeOH (2:1:1) and of *n*-hexane−EtOAc, respectively. The chromatographed extract yielded **1** (25 mg), friedelin (30 mg), 3-oxo-29-hydroxyfriedelane (100 mg), 3-oxofriedelan-25-al (15 mg), and canophyllol (50 mg).

28-Hydroxyfriedelane-1,3-dione (1): amorphous white solid; $[\alpha]^{20}_D$ +3.0 (*c* 0.5, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3520, 2900, 2890, 1720, 1700, 1460, 1390, 1250, 1180, 1100, 900, EIMS *m/z* (rel int) 425 [$M^+ - CH_2OH$] (27), 315 (21), 287 (65), 220 (7), 123 (6); HREIMS calculated for $C_{29}H_{45}O_2$ 425.341 956, found 425.341 917. ^{1}H NMR

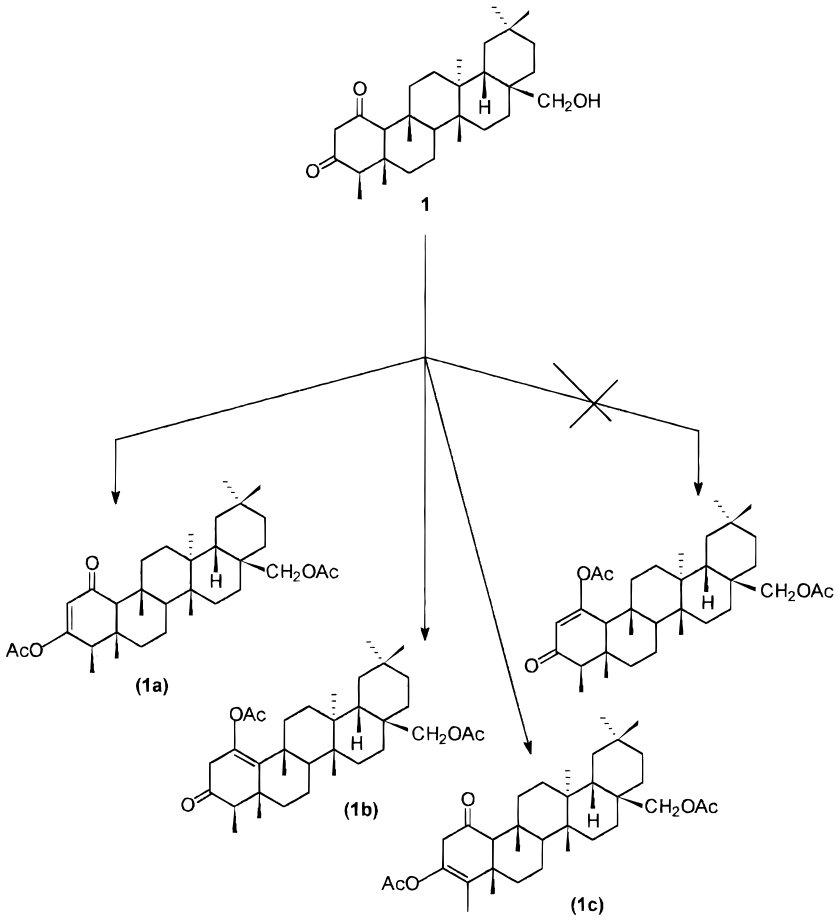

Figure 1.

($CDCl_3$, 400 MHz), see Table 1;[13]C NMR ($CDCl_3$,100 MHz), see Table 1.

Acetylation of 1. Compound **1** (10 mg) was treated with Ac_2O in pyridine at room temperature for 24 h. The reaction mixture was purified by preparative TLC using *n*-hexanes−EtOAc (9:1) as eluent to obtain **1a** (6 mg), **1b** (2 mg), and **1c** (1 mg).

Compound 1a: amorphous white solid; $[\alpha]^{20}_D$ +22.2 (*c* 0.5, $CHCl_3$); IR ($CHCl_3$) ν_{max} 2900, 2890, 1720, 1680, 1460, 1390, 1380, 1250, 1180, 1130, 1100, 900; EIMS *m/z* (rel int) 498 [$M^+ - CH_2OH$] (2), 287 (31), 247 (20), 237 (1), 177 (20), 163 (14), 139 (72), 123 (41); HREIMS calculated for $C_{32}H_{50}O_4$ 498.370 911, found 498.370 531, ^{1}H NMR ($CDCl_3$, 400 MHz) 2.06 s (3H, OAc), 2.21 s (3H, OAc), for the rest of the signals, see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz) 171.44 s ($C_{28}OCOCH_3$), 167.80 s (C_3OCOCH_3), 21.05 q (C_3OCOCH_3),20.83 q ($C_{28}OCOCH_3$), for the rest of the signals see Table 1.

Compound 1b: amorphous white solid; $[\alpha]^{20}_D$ +9.3 (*c* 0.2, $CHCl_3$); IR ($CHCl_3$) ν_{max} 2900, 2890, 1720, 1660, 1460, 1100, 900; EIMS *m/z* (rel int) 466 [$M^+ - CH_2OAc$] (7), 424 (21), 273 (20), 257 (10), 247 (10), 229 (25), 208 (10), 203 (100); HREIMS calculated for $C_{31}H_{46}O_3$ 466.344 696, found 466.344 452; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.27 d (1H, J = 10.4 Hz), 4.00 d (1H, J = 10.4 Hz), 2.29q (1H, J = 7.3 Hz), 2.23 s (3H), 2.07 s (3H), 1.29 d (3H, J = 6.3 Hz), 1.18s (3H), 1.11s (3H), 1.04s (3H), 0.99 s (6H).

Compound 1c: amorphous white solid; $[\alpha]^{20}_D$ +5.5 (*c* 0.2, $CHCl_3$); IR ν_{max} ($CHCl_3$) 2900, 2890, 1720, 1660, 1550, 1460, 1250, 1100, 900; MS *m/z* (rel int) 540 (M^+) (1), 467 (13), 329 (43), 303 (18), 235 (10), 203 (14) 198 (18), 181 (100); HREIMS calculated for $C_{34}H_{52}O_5$ 540.381 475, found 540.380 176; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.27 d (1H, J = 10.8 Hz), 4.00 d (1H, J = 10.8 Hz), 2.58 s (1H), 2.56 s (3H), 2.06 s (3H), 1.57s (3H), 1.22 s (3H), 1.09s (3H), 0.98s (6H), 0.80 s (3H).

Cytotoxic Assays. Compounds **1** and **1a** were tested for cytotoxic activity[12] against the following cell lines: P-388 (ATCC CCL-46), suspension culture of a lymphoid neoplasm from a DBA/2 mouse; A-549 (ATCC CCL-185), monolayer culture of a human lung carcinoma; HT-29 (ATCC HTB-38), monolayer culture of a human colon carcinoma; MEL-28 (ATCC HTB-72), monolayer culture of a human melanoma. Cells were maintained, in logarithmic growth in EMEM/neaa, supplemented with 5% fetal calf serum (FCS), 10−2 M sodium bicarbonate, and 0.1 g/L penicillin G + 0.1 g/L streptomycin sulfate. The compounds assayed were dissolved in DMSO−MeOH (1:9) and tested following the method described previously.[12]

Assay of Aldose Reductase Activity. The purification of the recombinant human aldose reductase used in the bioassay is based on the method described by Nishimura et al.[13] The aldose reductase inhibitory activity in vitro was determined following a modification of the method reported above.

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