A New Antibiotic Nortriterpene Quinone Methide from *Maytenus catingarum*

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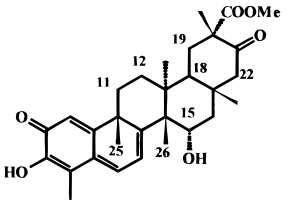
15α-Hydroxy-21-keto-pristimerine (**1**), a new nortriterpene quinone methide was isolated from the root bark of *Maytenus catingarum* along with other well-known related compounds, including pristimerine (**2**), tingenone, and 20α-hydroxy-tingenone. The structure of **1** was determined by means of ^{1}H and ^{13}C NMR spectroscopy, including homonuclear and heteronuclear correlations. Compound **1** showed antibiotic activity against Gram-positive bacteria.

The use of different species of plants of the Celastraceae family in folk medicine of various countries is well known.[1] Among the secondary metabolites known from that family are sesquiterpenes with the agarofuran skeleton, which show antifeedant activity,[2,3] and nortriterpene quinone methides with the friedo-oleane skeleton, which show antitumor and antibiotic activities.[4] The nortriterpene quinone methides are isolated exclusively from the Celastraceae and Hippocrataceae and are considered chemotaxonomic indicators.[1] This paper reports the isolation and identification of a new nortriterpene quinone methide, 15α-hydroxy-21-keto-pristimerine (**1**), and three known nortriterpene quinone methides, pristimerine (**2**), tingenone,[5,6] and 20α-hydroxy-tingenone,[5,6] from *Maytenus catingarum* Reiss (Celastraceae),



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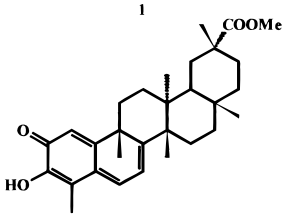

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Compound **1**, a minor component of root bark, was isolated as an amorphous orange-red solid with the molecular formula $C_{30}H_{38}O_6$ (HREIMS). Its IR spectrum showed absorption bands for hydroxy and carbonyl groups. These data, in conjunction with its ^{1}H NMR spectrum (Table 1), indicate that **1** has a structure related to the nortriterpene quinone methide pristimerine (**2**).

The main differences between the ^{1}H MNR spectra of **1** and **2** were the presence of a broad singlet at δ 4.36, assigned to a geminal proton of a hydroxyl group,[7] which was located at C-15; two doublets at δ 2.86 and δ 2.01, assigned to H-19 protons; and two others at δ 3.70 and δ 2.07, assigned to H-22 protons, respectively. The other difference was the shift of the signal due to proton H-7 from δ 6.34 to 6.49. The ^{13}C NMR spectra (Table 2) show an additional carbonyl group, located at C-21 and a carbon bearing a hydroxyl group, located at C-15 in **1**. The assignments are based on ^{1}H−^{13}C 2D experiments (Table 3). The HMBC experiment shows correlations between the H-26 methyl group and an aromatic carbon, located at C-8, and the carbon bearing the hydroxyl group, whereas the H-25 methyl group shows two correlations with aromatic carbons (C-8 and C-10). These findings eliminate the C-11 and C-12 positions for carrying the hydroxyl. In an ^{1}H−^{1}H COSY experiment, the secondary OH group could not be located at C-16, C-19, or C-22 positions due to the lack of connectivity between H-18 and the proton geminal to the OH. This proton was determined to be β-equatorial on the basis of molecular mechanics calculations and coupling constants of the preferred conformer.[7,8] Therefore, compound **1** is 15α-hydroxy-21-keto-pristimerine.

Table 1. ^{1}H NMR (400 MHz) Data (δ, $CDCl_3$) of Compounds **1** and of **2** (200 MHz)[a]

	compound	
proton	**1**	**2**
H-1	6.53 s	6.52 s
H-6	6.98 d (7.04)	7.00 d (7.10)
H-7	6.49 d (7.04)	6.34 d (7.10)
H-15	4.36 s	
H-16	1.72 d−2.15 d (15.7)	
H-18	1.85 d (8.64)	
H-19	2.01 d−2.86 d (15.96)	
H-22	2.07 d−3.70 d (14.80)	
Me-23	2.21 s	2.20 s
Me-25	1.52 s	1.44 s
Me-26	1.30 s	1.17 s
Me-27	0.88[b] s	0.53 s
Me-28	0.90[b] s	1.09 s
Me-30	1.36 s	1.26 s
COOMe	3.58 s	3.55 s

[a] *J* values are given in parentheses. [b] Vertical interchangeable values.

Compound **1** was tested against several Gram-positive and Gram-negative bacteria and the yeast *Candida albicans*. It showed antibiotic activity against *Bacillus subtilis*,

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Table 2. ^{13}C NMR (100 MHz) Data (δ, $CDCl_3$) of Compounds **1** and **2** (50 MHz)

	compound	
carbon	**1**	**2**
1	118.5 d	119.5 d
2	178.3 s	178.4 s
3	146.1 s	117.1 s
4	117.5 s	127.2 s
5	128.4 s	146.0 s
6	131.9 d	133.8 d
7	119.9 d	118.0 d
8	165.8 s	164.0 s
9	43.1 s	38.4 s
10	164.1 s	169.8 s
11	32.3 t	28.6 t
12	31.0 t	29.6 t
13	38.1 s	39.3 s
14	49.5 s	40.3 s
15	72.7 d	29.8 t
16	41.9 t	30.4 t
17	36.7 s	44.9 s
18	44.1 d	44.2 d
19	53.2 t	33.5 t
20	34.2 s	30.5 s
21	210.0 s	34.8 t
22	53.5 t	36.3 t
23	10.3 q	10.2 q
25	40.8 q	38.2 q
26	23.6 q	21.5 q
27	21.3 q	18.3 q
28	32.7 q	31.5 q
30	25.5 q	30.8 q
COOMe	175.2 s	178.1 s

Table 3. Three-bond $^1H-^{13}C$ Couplings (HMBC) in Compound **1**

protons	correlations
H-1	C-3, C-5, C-9
H-6	C-4, C-8, C-10
H-7	C-5, C-9, C-14
H-16	C-14, C-15[a], C-18, C-22
H-19	C-17, C-20[a], C-21, C-29
H-22	C-17[a], C-21[a], C-28
H-25	C-9[a], C-8, C-10, C-11
H-26	C-8, C-13, C-14[a], C-15
H-30	C-19, C-20[a], C-21, C-29

[a] Two-bond coupling enhancement observed.

with a MIC (minimal inhibitory concentration) of 1.25 to 2.5 μg mL^{-1}. It was also active against *Staphylococcus aureus*, with a MIC of 5.0 μg mL^{-1}. The compound was inactive against the Gram-negative bacteria *Escherichia coli* and *Salmonella typhimurium* and the yeast.

Experimental Section

General Experimental Procedures. IR spectra were taken on a Perkin−Elmer 681 spectrophotometer and ^1H and ^{13}C NMR spectra on a Bruker WP-200 SY in $CDCl_3$ (at 200 and 50 MHz, respectively). The HMQC, HMBC, and COSY NMR spectra were obtained on a Bruker AMX-400 spectrometer at 400 and 100 MHz. MS were recorded on a Micromass LTD -ZAB-2F and/or on a HP 5930 A spectrometer at 70 eV. Optical rotations were measured on a Perkin−Elmer 241 polarimeter, and the UV spectra were obtained on a Perkin−Elmer model 550-SE. Schleicher-Schüell F-1500/LS 254 Si gel was used for TLC, and Si gel (<0.063 mm) and Sephadex LH-20 employed for column chromatography.

Plant Material. *Maytenus catingarum* Reiss was gathered at Parque Nacional Amambay, Paraguay, in August 1995, and a voucher specimen (Soria 5598) is on file with the Herbarium of the Departamento de Botánica, Facultad de Ciencias Químicas, Universidad Nacional de Asunción, Paraguay.

Extraction and Isolation. The root-bark (3 kg) of the plant was ground and then extracted with *n*-hexane−Et_2O in a Soxhlet apparatus. The extract (42 g) was repeatedly chromatographed on Sephadex LH-20 and Si gel using mixtures of *n*-hexane−$CHCl_3$−MeOH (2:1:1) and of *n*-hexane−EtOAc, respectively, to afford **1** (2 mg), **2** (500 mg), tingenone (200 mg), and 20α-hydroxy-tingenone (20 mg).

Antibiotic Activity. *Bacillus subtilis* CECT 39, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* CECT 99, *Salmonella typhimurium* UBC 1, and the yeast *Candida albicans* UBC2 were used. The strains were maintained on nutrient agar (Oxoid) or Sabouraud (Oxoid). The bacterial cultures were grown in nutrient broth (Oxoid) and the yeast culture in YEPD medium. The MICs were determined in liquid medium. The compound was added in a solution of DMSO, and tubes with the same proportion of DMSO were used as controls. The cultures were incubated at 37° C in a rotary shaker, and growth was measured by viable counting on nutrient agar or Sabouraud plates.

15α-Hydroxy-21-keto-pristimerine (1): compound **1** obtained as an amorphous orange-red solid; $[\alpha]^{20}_D$ −31.3° (*c* 0.08 $CHCl_3$); UV λ_{max} (EtOH) 428, 209 nm; IR ν_{max} ($CHCl_3$) 3700, 2900, 2800, 1725, 1705, 1590, 1510, 1430, 1370, 1075, 860, 750 cm^{-1}; ^1H NMR (400 MHz), Table 1; ^{13}C NMR (100 MHz), Table 2; EIMS *m*/*z* 494 $[M]^+$ (63), 241 (26), 201 (23), 149 (6), 121 (8), 107 (7), 95 (20), 69 (49), 55 (57), 43 (100); HREIMS *m*/*z* 494.27023 (calcd for $C_{30}H_{38}O_6$ 494.26684).

Pristimerine (2): amorphous orange-red solid; UV λ_{max} (EtOH) 420, 205 nm; IR ν_{max} ($CHCl_3$) 3280, 2930, 2860, 1722, 1630, 1585, 1546, 1509, 1430, 1370, 1280, 1213, 1200, 1182, 1151, 1135, 1092, 1083, 878 cm^{-1}; ^1H NMR (200 MHz), Table 1; ^{13}C NMR (50 MHz), Table 2; EIMS *m*/*z* 464 $[M]^+$ (77), 450 (12), 241 (100), 203 (31), 202 (27), 201 (42), 200 (10).

Tingenone: amorphous reddish-brown solid; UV λ_{max} (EtOH) 422, 204 nm; IR ν_{max} ($CHCl_3$) 3312, 3245, 2985, 2940, 2860, 1696, 1630, 1584, 1545, 1506, 1430, 1370, 1306, 1280, 1222, 1183, 1080, 1037, 1001, 870 cm^{-1}; ^1H and ^{13}C NMR and MS identical to literature.[5,6]

20α-Hydroxy-tingenone: amorphous orange-red solid; UV λ_{max} (EtOH) 424, 205 nm; IR ν_{max} ($CHCl_3$) 3395, 3020, 3004, 2950, 2925, 2864, 1708, 1590, 1512, 1440, 1377, 1286, 1185, 1083 cm^{-1}; ^1H and ^{13}C NMR, and MS identical to literature.[5,6]

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