Triterpene Trimers from *Maytenus scutioides*: Cycloaddition Compounds?

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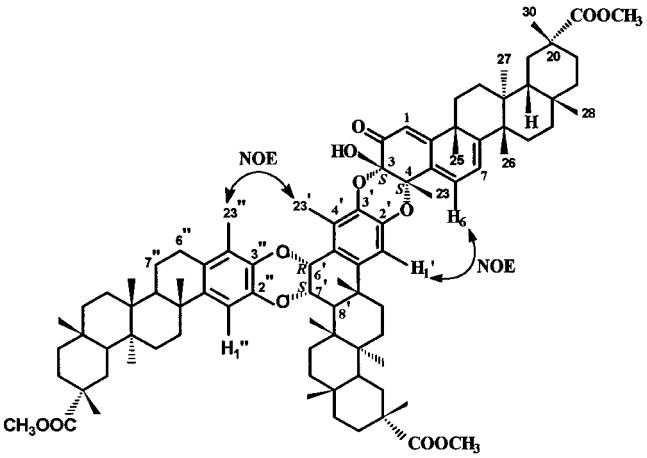
Two novel trimers, triscutins A and B (**1** and **2**), based on pristimerin triterpene units, were isolated and characterized from *Maytenus scutioides*. Their structures were determined on the basis of spectroscopic evidence, including $^{1}H-^{13}C$ heteronuclear correlation (HMQC), long-range correlation with inverse detection (HMBC), and ROESY NMR experiments; and their absolute configurations, by means of CD studies. Compounds **1** and **2** were assayed for antimicrobial and cytotoxic activities, and their possible biosynthetic route is proposed.

As part of an intensive study of the bioactive metabolites from species in the family Celastraceae used in folk medicine,[1] *Maytenus scutioides* Lourteig and O'Donnell[2] has been studied. *M. scutioides* is a subtropical shrub, distributed in the central region of South America, whose aerial parts are used as a cardiotonic and whose roots are employed as an abortifacient.[3] Antimicrobial and cytotoxic activities of quinone-methide[4] and dimeric triterpenes[5] have been reported in previous work on this species.

This paper reports, for the first time, the isolation of triterpene trimers that are based on pristimerin units, a quinone-methide triterpenoid common in plants of the Celastraceae. Their structures were determined on the basis of spectroscopic data, including $^{1}H-^{13}C$ heteronuclear correlation (HMQC), long-range correlation with inverse detection (HMBC), and ROESY NMR experiments; and their absolute configurations, by means of CD studies. The antimicrobial activity of **1** and **2** was tested against Gram-positive and Gram-negative bacteria and the yeast *Candida albicans*, using a disk diffusion test;[4] the cytotoxic activity was assayed against HeLa (human carcinoma of the cervix) and Hep-2 (human carcinoma of larynx) cell lines, using a colorimetric MTT reduction assay.[6] Their possible biosynthetic route involving hetero-Diels−Alder reactions is also discussed.

A crude (*n*-hexane−Et_2O 1:1) extract of the root bark of *M. scutioides* was repeatedly chromatographed on Sephadex LH-20 and Si gel to afford **1** (10 mg, triscutin A) and **2** (20 mg, triscutin B). Compound **1** was isolated as a crystalline solid. Its FABMS showed a molecular ion at *m*/*z* 1394 and, together with the ^{13}C NMR spectral data, indicated the molecular formula, $C_{90}H_{122}O_{12}$. In its ^{1}H NMR spectrum (Table 1) appeared signals for 16 angular methyl groups, two methyl groups on an aromatic ring at δ 2.02 and 2.17, and three methoxy groups. Signals were also observed for protons geminal to oxygen atoms at δ 4.84 (H-6′, d) and 4.95 (H-7′, dd); an ABC system of three vinyl protons at δ 6.03 (H-7), 6.08 (H-1), and 6.38 (H-6), characteristic of a methylenquinone system; and signals for two phenolic protons at δ 6.55 (H-1″) and 6.82 (H-1′). All these data suggested that **1** was a triterpene trimer composed of three pristimerin-type triterpenes, with one subunit in the quinoid form and the other two in the aromatic form.[5] The analysis of the ^{13}C NMR spectrum (Table 2) and HMBC (Table 3) and HMQC experiments showed, as in the case of some triterpene dimers,[5] the presence of four oxygenated adjacent quaternary carbons, with two in the quinoid triterpene unit at C-3 and C-4 and two in the aromatic triterpene central unit at C-2′ and C-3′. In addition, signals for two methine carbons at C-6′ and C-7′ on the B ring of the central unit at δ 71.6 and 73.8, respectively, and two oxygenated adjacent quaternary carbons at C-2″ (δ 140.3, s) and C-3″ (δ 138.8, s) on the A ring of the third aromatic triterpene unit, were observed; this type of linkage was recently reported in triterpene dimers.[7] All the above data indicated the presence of two diether bridges linking the three subunits in the molecule.



1 Triscutin A

The absolute configuration of **1** was determined from its CD spectrum, showing a Davidoff-type split curve, with a first positive Cotton effect at 282.8 nm ($\Delta\epsilon$ +8.31) and a second negative one at 260.2 nm ($\Delta\epsilon$ −2.31), which corresponds to the interaction between the enone and the aromatic system, located on the central subunit, in accordance with a 3*S*,4*S* absolute configuration.[5] A third positive Cotton effect at 242.4 nm ($\Delta\epsilon$ +9.14) was also observed, whose second negative sign component we could not observe because it was destroyed by the strong positive absorption of the system; this second curve corresponds to the interaction of the two first chromophores, with the second aromatic system (the third subunit), and this agrees with an absolute configuration of 6′*R*,7′*S*. These data were

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Table 1. ^{1}H NMR (400 MHz) Data for **1** and **2** (δ, $CDCl_3$, *J* Values in Hz)

	H-1	H-6	H-7	Me-23	H-1′	H-6′	H-7′	Me-23′	H-1″	Me-23″
1	6.08 d (1.5)	6.38 dd (1.4, 6.4)	6.03 d (6.6)	1.54 s	6.82 s	4.84 d (3.0)	4.95 dd (3.0, 11.7)	2.17 s	6.55 s	2.02 s
2	6.07 d (1.3)	6.19 dd (1.4, 6.4)	5.93 d (6.6)	1.57 s	6.60 s	4.93 d (3.0)	4.95 dd (2.6, 14.0)	2.40 s	6.55 s	2.05 s

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

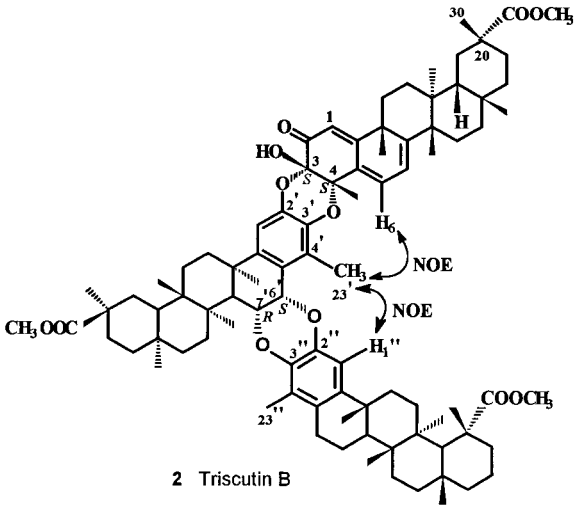
position	**1**	**2**	position	**1**	**2**	position	**1**	**2**
1	115.4 d	115.4 d	1′	109.8 d	110.4 d	1″	109.3 d	109.4 d
2	190.8 s	190.7 s	2′	142.0 s	142.0 s	2″	140.3 s	140.0 s
3	91.7 s	91.8 s	3′	138.2 s	137.2 s	3″	138.8 s	138.8 s
4	78.9 s	79.2 s	4′	127.6 s	126.1 s	4″	125.5 s	123.6 s
5	130.6 s	130.4 s	5′	123.2 s	124.5 s	5″	125.5 s	125.5 s
6	127.0 d	126.1 d	6′	71.6 d	71.2 d	10″	145.0 s	144.8 s
7	116.6 d	116.0 d	7′	73.8 d	73.6 d	23″	11.0 q	10.8[b] q
8	160.6 s	161.0 s	8′	44.9 d	44.4 d	−OMe	51.3 q	51.3 q
9	41.7 s	41.8 s	9′	40.6 s	40.5 s		51.5 q	51.5 q
10	173.9 s	174.7 s	10′	145.2 s	143.9 s		51.6 q	51.6 q
23	22.3 q	22.3 q	23′	11.0 q	10.7[b] q			

[a] Data are based on HMQC, HMBC and DEPT experiments. [b] Interchangeable values.

Table 3. Long-Range Heteronuclear Correlations (HMBC) for **1** and **2**

^{1}H position	**1**	**2**
1	C-3, C-5	C-3, C-5
6	C-4, C-8, C-10	C-4, C-8, C-10
7	C-9	C-5, C-9
23	C-3	C-3, C-4[a], C-5
1′	C-3′, C-5′	C-2′ [a], C-3′, C-5′
6′	C-5′ [a]	C-5′ [a], C-10′
7′	C-6′ [a]	C-5′
23′	C-4′ [a], C-5′	C-3′, C-4′ [a], C-5′
1″	C-3′, C-5′	C-2″ [a], C-3′, C-5′
23″	C-3′, C-4′ [a], C-5″	C-3′, C-4′ [a], C-5″

[a] Two-bond coupling enhancement observed.



2 Triscutin B

supported by a positive $[\alpha]^{20}_D$ and a UV spectrum with bands of absorption at 383, 282, and 261 nm. The regiosubstitution was determined by a ROESY experiment, showing NOE effects between H-6 and H-1′ and between Me-23′ and Me-23″, which correspond to the linkages between the units as 3−O−3′, 4−O−2′ and 6′−O−3″, 7′−O−2″.

Compound **2** was isolated as a yellow-orange crystalline solid. Its FABMS and NMR data (Tables 1, 2, and 3) indicated that **2** was an isomer of **1** with different regiosubstitution of the subunits. Its absolute configuration was determined by its CD curve. In the spectrum was observed a Davidoff-type split curve, with a first positive Cotton effect at 285.4 nm ($\Delta\epsilon$ +9.30) and a second negative one at 259.8 nm ($\Delta\epsilon$ −8.15), which is in accordance with a 3*S*,4*S* configuration;[5] a third Cotton effect was also observed, with a positive sign at 240.0 nm ($\Delta\epsilon$ +10.9), whose negative component was obscured by the strong positive absorption of the system, in agreement with a 6′*S*,7′*R* configuration. These data were supported by a positive $[\alpha]^{20}_D$ and the UV spectrum, showing bands of absorption at 382, 282, and 262 nm. The regiosubstitution of **2** was determined by a ROESY experiment, showing NOE effects between Me-23′ and H-6 and H-1″, corresponding to a linkage between the units as 3−O−2′, 4−O−3′ and 6′−O−2″, 7′−O−3″.

Compounds **1** and **2** were deemed inactive (up to 40 μg/mL) in all antimicrobial[4] and cytotoxicity[6] bioassays in which they were evaluated.

Diels−Alder reactions have been postulated as key steps in a number of biosynthetic conversions.[8,9] Itokawa *et al.*[10] proposed a route for the biosynthesis of the triterpene dimers involving hetero-Diels−Alder reactions, which was supported by the synthesis of one dimer carried out in our group.[5] In the same way, the biosynthesis of the triterpene trimers **1** and **2** could take place by reaction between a dimer precursor with a double bond at C-6′, C-7′, as in the case of scutionin α A,[5] and an *ortho*-quinone triterpene to yield the corresponded adducts (Figure 1). As a hypothesis, the triterpenes could be stored in the plant as polymers, which could release biologically active units[4,5] as *ortho* or methylene quinoid forms, via a retro-Diels−Alder process, depending on the need of the plant.

Experimental Section

General Experimental Procedures. Melting points were determined on a Kofler apparatus and are uncorrected. Optical rotations were measured on a Perkin−Elmer 241 automatic polarimeter and $[\alpha]^{20}_D$ are given in 10^{-1} deg cm^{-1} g^{-1}. CD spectra were run on a JASCO J-600 spectropolarimeter. UV spectra were collected on a Perkin−Elmer model 550-SE spectrophotometer. IR spectra were taken on a PE 681 spectrophotometer. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker WP-400 SY instrument in $CDCl_3$ at 400 and 100 MHz, respectively, with TMS as internal reference. The HMBC and HMQC spectra were run at 400 MHz. FABMS were recorded on a VG Autospec mass 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. *Maytenus scutiodes* was collected in Chaco húmedo boreal, Paraguay, in December 1993, and a voucher specimen (R. Degen 3117) is on file with the Herbarium of the Departamento de Botánica, Facultad de Ciencias Químicas, Universidad Nacional de Asunción, Asunción, Paraguay. The

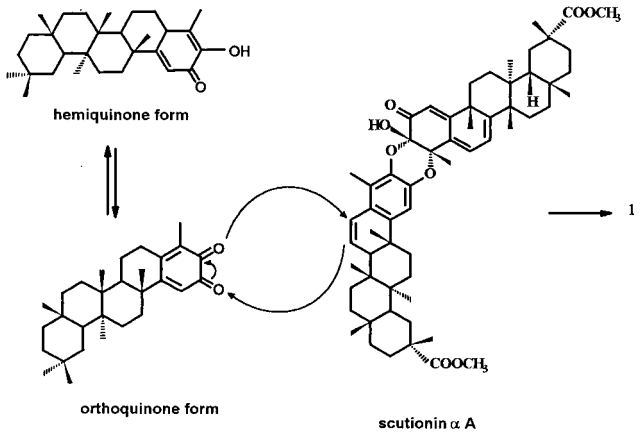


Figure 1.

root bark (3 kg) of the plant was extracted with *n*-hexane−Et_2O (1:1) (3 L) in a Soxhlet apparatus. The extract (115 g) was subjected to chromatography on Sephadex LH-20, using as solvent mixtures of *n*-hexane−$CHCl_3$−MeOH (2:1:1). The fraction containing **1** and **2** (fraction A) was repeatedly chromatographed on Si gel, using as eluent 35% EtOAc in *n*-hexane, and the largest subfraction (55 mg) was further separated by preparative HPTLC (HPTLC−Platten Nano-SIL 20 UV_{254}) to yield **1** (10 mg) and **2** (20 mg).

Triscutin A (1): obtained as a yellow crystalline solid: mp 241−242 °C; $[\alpha]^{20}_D$ +247.9° (*c* 1.25, $CHCl_3$); CD λ_{max} (MeOH) nm 282.8 ($\Delta\epsilon$ +8.31), 260.2 ($\Delta\epsilon$ −2.31), 242.4 ($\Delta\epsilon$ +9.14); UV (EtOH) λ_{max} (log ϵ) 383 (6.63), 282 (6.71), 261 (6.61) nm; IR ν_{max} ($CHCl_3$) 3500, 3024, 2949, 2872, 1723, 1667, 1481, 1464, 1240, 1143, 1095 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.60 (3H, s), 0.80 (3H, s), 0.83 (3H, s), 0.95 (3H, s), 1.09 (3H, s), 1.11 (3H, s), 1.14 (3H, s), 1.19 (3H, s), 1.21 (12H, s), 1.33 (3H, s), 1.34 (3H, s), 1.44 (3H, s), 2.35 (1H, d, J = 11.5 Hz, H-8′), 3.60 (6H, s, 2 × OMe), 3.62 (3H, s, OMe), 4.97 (1H, s, int. with D_2O, H−OH), for other signals, see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 16.0 (q), 17.3 (q), 17.5 (q), 18.4 (q), 18.6 (q), 22.6 (q), 27.3 (q), 28.0 (t), 28.3 (t), 29.1 (t), 29.5 (t), 29.8 (t), 30.0 (t), 30.2 (t), 30.2 (t), 30.3 (t), 30.5 (t), 30.7 (t), 30.8 (q), 31.0 (t), 31.2 (t), 31.6 (q), 31.8 (q), 31.8 (q), 31.9 (q), 32.8 (q), 32.9 (t), 34.2 (t), 34.7 (t), 34.8 (t), 35.0 (q), 36.1 (t), 36.3 (t), 36.3 (t), 36.6 (t), 36.8 (t), 37.0 (t), 38.2 (s), 39.0 (s), 39.4 (s), 39.6 (s), 40.0 (s), 40.3 (s), 40.4 (s), 40.5 (s), 40.5 (s), 40.5 (s), 40.6 (s), 40.6 (s), 43.5 (d), 44.0 (d), 44.2 (d), 44.4 (d), 44.6 (s), 178.9 (s), 179.3 (s), 179.5 (s), for other signals, see Table 2; FABMS *m*/*z* 1394 [M^+] (12).

Triscutin B (2): obtained as a yellow crystalline solid: mp 220−221 °C; $[\alpha]^{20}_D$ +309.7° (*c* 2.4, $CHCl_3$); CD λ_{max} (MeOH) 285.4 ($\Delta\epsilon$ +9.30), 259.8 ($\Delta\epsilon$ −8.15), 240.0 ($\Delta\epsilon$ +10.9) nm; UV (EtOH) λ_{max} (log ϵ) 382 (6.95), 282 (7.01), 262 (6.91) nm; IR ν_{max} ($CHCl_3$) 3457, 3022, 2949, 1724, 1666, 1481, 1377, 1304, 1226, 1143, 1094 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.56 (3H, s), 0.74 (3H, s), 0.79 (3H, s), 0.95 (3H, s), 1.06 (3H, s), 1.10 (3H, s), 1.17 (12H, s), 1.20 (3H, s), 1.22 (3H, s), 1.27 (3H, s), 1.31 (3H, s), 1.42 (3H, s), 2.34 (1H, d, J = 13.5 Hz, H-8′), 3.57 (3H, s), 3.58 (3H, s), 3.61 (3H, s), 5.03 (1H, s, int. with D_2O), for other signals, see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 16.0 (q), 17.2 (q), 17.4 (q), 18.2 (q), 18.4 (t), 18.6 (q), 22.4 (q), 27.2 (q), 27.9 (q), 28.0 (t), 28.4 (t), 29.0 (t), 29.5 (t), 29.5 (t), 29.7 (s), 29.8 (t), 29.9 (t), 30.2 (s), 30.2 (t), 30.5 (s), 30.5 (t), 30.6 (t), 30.7 (q), 30.8 (t), 31.2 (t), 31.5 (q), 31.8 (q), 31.8 (q), 31.9 (q), 32.7 (q), 32.7 (t), 34.3 (t), 34.5 (t), 34.7 (t), 34.8 (q), 36.1 (t), 36.2 (t), 36.3 (t), 36.5 (t), 36.8 (s), 36.9 (t), 38.1 (s), 38.9 (s), 39.3 (s), 39.5 (s), 39.9 (s), 40.2 (s), 40.3 (s), 40.4 (s), 43.6 (d), 44.0 (d), 44.1 (d), 44.3 (d), 44.6 (s), 178.7 (s), 179.2 (s), 179.2 (s), for other signals, see Table 2; FABMS *m*/*z* 1394 [M^+] (8).

Antimicrobial Activity. Activity was tested against Gram-positive (*Staphylococcus aureus* ATCC 6538, *S. albus* SAB C1, *S. epidermidis* CECT 232, *S. saprophyticus* CECT 235, *S. warnieri* CECT 236, *Bacillus subtilis* CECT 39, *B. pumilus* CECT 29, *B. alvei* CECT 2, *B. megaterium* CECT 44, *B. cereus* CECT 496, *Micrococus luteus* CECT 241) and Gram-negative (*Escherichia coli* CECT 99, *Proteus mirabilis* CECT 170, *Salmonella tiphymurium* UBC 2, *Salmonella* sp. CECT 456, *Klebsiella pneumoniae* CECT 367, *Enterobacter hafniae* CECT 400 and *Pseudomonas aeruginosa* AK 958) bacteria and a yeast (*Candida albicans* UBC 1). The bacteria were maintained on Nutrient Agar (Oxoid) and the yeast on Sabouraud Agar (Oxoid) at 37 °C. The minimal inhibitory concentrations (MIC) of compounds previously dissolved in DMSO were estimated in liquid medium following the method of Buttiaux et al.[11]

Cytotoxic Activity. HeLa (human carcinoma of the cervix) and Hep-2 (human carcinoma of the larynx) cell lines were each grown as a monolayer in Dulbecco's modified Eagle's medium (DMEM, Gibco), supplemented with 10% newborn calf serum (Gibco), and 1% of penicillin-streptomycin mixture (10 000 UI/mL). The cells were maintained at 37 °C in 5% CO_2 and 90% humidity. Cytotoxicity was assessed using the colorimetric MTT reduction assay.[6]

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