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Absolute configuration of triterpene dimers from *Maytenus* species (Celastraceae)

Antonio G. González,[a] María L. Kennedy,[a] Félix M. Rodríguez,[a] Isabel L. Bazzocchi,[a,*] Ignacio A. Jiménez,[a] Angel G. Ravelo[a] and Laila Moujir[b]

[a]*Instituto Universitario de Bio-Orgánica "Antonio González", Universidad de La Laguna, Avda. Astrofísico Francisco Sánchez 2, La Laguna, 38206 Tenerife, Canary Islands, Spain*

[b]*Departamento de Microbiología y Biología Celular, Universidad de La Laguna, Avda. Astrofísico Francisco Sánchez 2, La Laguna, 38206 Tenerife, Canary Islands, Spain*

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Abstract—Three new triterpene dimers (**1**–**3**) based on two pristimerin units were isolated from *Maytenus blepharodes* and *Maytenus magellanica*. Their structures were elucidated on the basis of spectral analysis, including homonuclear and heteronuclear correlation NMR experiments (COSY, ROESY, HSQC and HMBC). Their absolute configurations were determined by CD studies. The compounds were assayed for antimicrobial and cytotoxic activities.

As a part of our studies of medicinal plants of the genus *Maytenus* (Celastraceae), which are widely used as folk medicines in South[1–3] and Central America,[4,5] we have reported previously from *Maytenus* species, phenolic[6] and quinone–methide[7] triterpenes, dimeric[8,9] and trimeric[10] triterpenes, and a large number of dihydro-β-agarofuran sesquiterpenes,[11,12] which have attracted a great deal of interest on account of their biological activities.

Triterpene dimers are composed of one quinoid form and one aromatic form of a nortriterpene derived from pristimerin, tingenone, netzahualcoyene and/or their congeners, joined by two ether linkages formed between the two A rings,[13–15] or between the A and the B rings,[16] and these types of compounds have only been studied by Itokawa and our group. On the other hand, triterpene trimers,[10] based on pristimerin units, were reported for the first time from *Maytenus scutioides* in our group.

In a continuation of our work on *Maytenus blepharodes* Lundell,[17] a species that grows in Panama and whose branches are purported to be antitumoral,[5] and *Maytenus magellanica* Lam.,[18] known locally as "black mayten" and found in the Antarctic Andean woodland covering parts of Argentina and Chile, we report herein on the isolation of three new triterpene dimers (**1**–**3**) from these species. Their structures were determined on the basis of spectroscopic data, including $^{1}H-^{13}C$ heteronuclear correlation (HSQC), long-range correlation with inverse detection (HMBC), and ROESY NMR experiments. Their CD curves allowed us to determine unequivocally their absolute configurations. Their antimicrobial activity was tested against Gram-positive and Gram-negative bacteria and the yeast *Candida albicans*, using a disk diffusion test;[19] the cytotoxic activity was assayed against HeLa (human cervix carcinoma) and Hep-2 (human larynx carcinoma) cell lines, using a colorimetric MTT reduction assay.[20]

Compound **1** was isolated as a pale yellow amorphous solid with $[\alpha]_D=+282.3°$ (*c* 1.58, $CHCl_3$). Its FABMS showed a molecular ion at *m/z* 929, and the molecular formula was determined to be $C_{60}H_{80}O_8$, based on HRFABMS analysis and its ^{13}C NMR spectrum. Its IR spectrum showed absorption bands for hydroxyl (3445 cm^{-1}), carbonyl of ester (1731 cm^{-1}), and carbonyl (1676 cm^{-1}) groups. In its ^{1}H NMR spectrum (Table 1) were observed a singlet at δ 6.49 assigned to an aromatic hydrogen (H-1′) and one conjugated double bond with the two vinyl protons as double doublets at δ 6.67 and 5.91, attributable to H-6′ and H-7′, respectively, together with an ABC system of three vinyl protons at δ 6.06 d (J=1.5 Hz), 6.21 dd (J=1.5, 6.5 Hz), and 5.94 d (J=6.7 Hz) attributable to H-1, H-6, and H-7, characteristic of a triterpenic quinoid system. Signals were also observed for 11 angular methyl groups, one methyl group on an aromatic ring at δ 2.30, and two methoxy groups at δ 3.61 and 3.62 as singlets. These data and the analysis of the ^{13}C NMR spectrum (Table 2) suggested that **1** was a triterpene dimer composed of two pristimerin-type triterpenes with one subunit in the quinoid form and the other in the aromatic form.[13]

Keywords: configuration; dimer; triterpenes.

* Corresponding author. Tel.: +34-922-318576; fax: +34-922-318571; e-mail: ilopez@ull.es

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Table 1. ^{1}H NMR (400 MHz) Data (δ, $CDCl_3$, J values in Hz) for compounds **1–3**

Position	**1**	**2**	**3**	Position	**1**	**2**	**3**
1	6.06 d (1.5)	6.05 d (1.4)	6.05 d (1.4)	1′	6.49 br s	6.57 s	6.55 s
3	4.97 s (OH)	4.92 s (OH)	4.80 s (OH)				
6	6.21 dd (1.5, 6.5)	6.20 dd (6.4, 1.5)	6.18 dd (1.2, 6.4)	6′	6.67 dd (2.9, 9.9)		4.28 br s
7	5.94 d (6.7)	5.92 d (6.5)	5.91 d (6.6)	7′	5.91 dd (2.4, 9.9)		
19α	2.38 m	2.40 m	2.38 m	19′			
23	1.55 s	1.53 s	1.53 s	23′	2.30 s	2.16 s	2.27 s
25	1.41 s	1.40 s	1.40 s	25′	0.99 s	1.14 s	1.09 s
26	1.17 s	1.16 s	1.16 s	26′	1.05 s	0.92 s	0.94 s
27	0.56 s	0.55 s	0.54 s	27′	0.80 s	0.74 s	0.80 s
28	1.07 s	1.06 s	1.06 s	28′	1.10 s	1.09 s	1.10 s
30	1.17 s	1.16 s	1.16 s	30′	1.17 s	1.16 s	1.25 s
COOMe	3.62[a] s	3.59[a] s	3.60[a] s	COOMe′	3.61[a] s	3.60[a] s	3.57[a] s
OMe (6′)							3.38 s

[a] Assignments may be interchangeable.

The analysis of its COSY, HSQC and HMBC spectra enabled the assignment of the signals of the quinoid and aromatic triterpene units, including the signals at C-3 (δ_C 91.8) and C-4 (δ_C 79.1) in the 3-hydroxy-4-methyl-3,4-dioxy part of the quinoid unit. The cited values[7,9] together with the chemical shifts of H-6 (δ_H 6.21) and C-23 (δ_C 22.0) suggested a *cis* orientation about the 3,4-dioxy bond. A ROESY experiment (Fig. 1) showing an NOE correlation between H-6 and Me-23′ revealed that the 3,4-dioxy bond in **1** consisted of C-3, C-2′ and C-4, C-3′ linkages.[7]

The absolute configuration of **1** was determined from its CD spectrum, showing a Davidoff-type split curve, with a first positive Cotton effect at 355 nm ($\Delta\varepsilon = +12.8$) and a second negative one at 230 nm ($\Delta\varepsilon = -9.7$), which corresponded to the interaction between the enone and the aromatic system, in accordance with a 3*S*,4*S* absolute configuration[9,13,21] (Fig. 1). The above data led to the conclusion that compound **1** is a regioisomer of scutionin αA,[9] for which we propose the name scutionin αB.

Table 2. ^{13}C NMR (100 MHz) Data (δ, $CDCl_3$, J values in Hz) for compounds **1–3**

C	**1**	**2**	**3**	C′	**1**	**2**	**3**
1	115.4 d	115.4 d	115.5 d	1	108.8 d	110.4 d	110.4 d
2	191.0 s	191.2 s	191.0 s	2	140.8 s	139.1 s	141.3 s
3	91.8 s	91.7 s	91.8 s	3	136.5 s	136.2 s	137.0 s
4	79.1 s	79.9 s	79.0 s	4	121.3 s	122.9 s	125.5 s
5	130.6 s	130.8	130.8 s	5	126.0s	127.9 s	127.2 s
6	126.1 d	126.1 d	125.9 d	6	124.0 d	26.38 t	75.2 d
7	116.2 d	116.2 d	116.1 d	7	129.4 d	18.5 t	21.8 t
8	160.8 s	160.6 s	160.6 s	8	45.5 d	43.9 d	38.5 d
9	41.8 s	41.7 s	41.7 s	9	37.4 s	36.8 s	37.6 s
10	173.8 s	173.8 s	173.5 s	10	142.8 s	144.4 s	144.7 s
11	32.8 t	32.7 t	32.7 t	11	30.6 t	33.9 t	33.8 s
12	29.5 t	29.5 t	29.5 t	12	30.0 t	30.0 t	29.8 t
13	38.2 s	38.1 s	38.1 s	13	38.9 s	38.9 t	38.9 s
14	44.5 s	44.5 s	44.5 s	14	39.0 s	39.4 s	39.1 s
15	28.3 t	28.4 t	28.4 t	15	28.4 t	28.9 t	29.0 t
16	36.4 t	36.5 t	36.4 t	16	36.0 t	36.4 t	36.1 t
17	30.4 t	30.5[a] t	30.2 s	17	30.6 s	30.6[a] t	29.3 s
18	44.2 d	44.6 d	44.1 d	18	44.5 d	44.1 d	44.4 d
19	30.9 t	30.9 t	30.9 t	19	31.0 t	30.4 t	30.5 t
20	40.5 s	40.5 s	40.5 s	20	40.4 s	40.4 s	40.4 s
21	29.8 t	29.87 t	29.9 t	21	29.7 t	30.3 t	30.3 t
22	34.8 t	34.8 t	34.7 t	22	36.4 t	36.3 t	36.6 t
23	22.0 q	22.4 q	22.5 q	23	10.8 q	10.9 q	10.6 q
25	34.8 q	34.8 q	34.8 q	25	22.5 q	27.2 q	31.5 q
26	22.3 q	22.5 q	22.5 q	26	16.9 q	15.8 q	16.3 q
27	18.7 q	18.6 q	18.6 q	27	17.4 q	17.2 q	17.4 q
28	31.6 q	31.6 q	31.7 q	28	31.8 q	31.8 q	26.4 q
29	178.7 s	179.1[a] s	179.0[a] s	29	178.7 s	178.7[a] s	178.7[a] s
30	32.7 q	32.9[a] q	31.9 q	30	32.1 q	32.7[a] q	32.7 q
OMe (29)	51.7 s	51.7[a] s	51.5[a] s	OMe (29)	51.6 s	51.5[a] s	51.7[a] q
				OMe (6)			55.4 s

Data are based on DEPT and HSQC experiments.
[a] Assignments may be interchangeable.

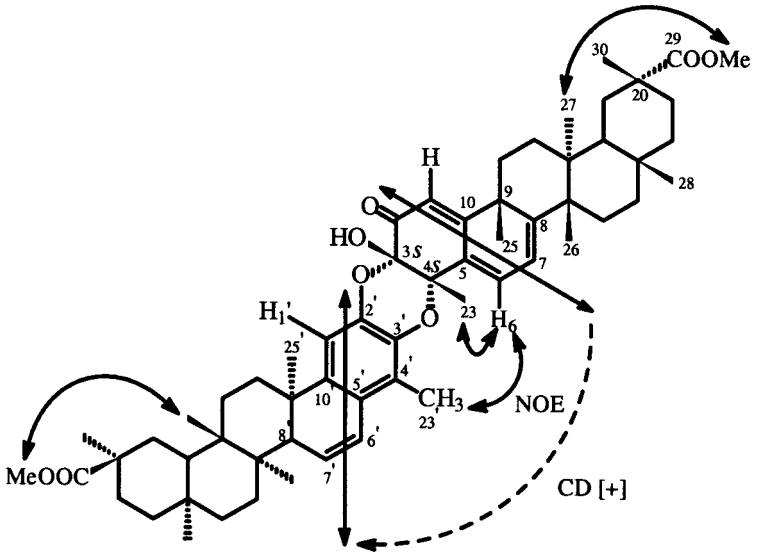
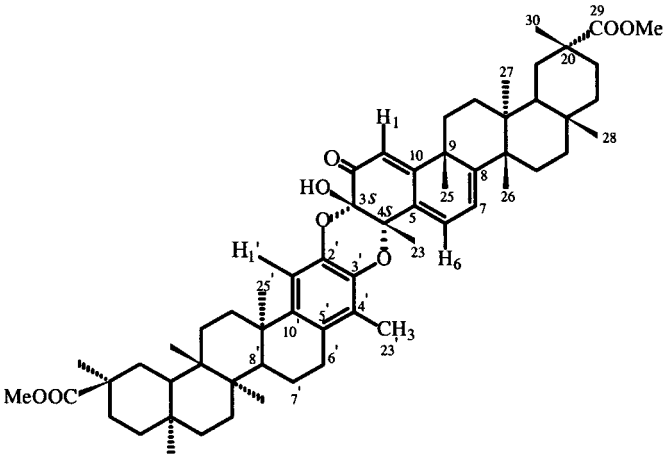


Figure 1. NOE effects (solid line) and CD exciton coupling (dashed line) for **1**.



2 6',7'-Dihidroscutionin αB

Compound **2** was isolated as a pale yellow amorphous solid and showed a molecular formula $C_{60}H_{82}O_8$ by HRFABMS and its ^{13}C NMR spectrum. In its ^{1}H and ^{13}C NMR spectra (Tables 1 and 2), the main differences with respect to **1** were the absence of the signals at δ_H 6.67 dd and 5.91 dd, and at δ_C 124.0 d and 129.4 d, respectively, assigned to the conjugated double bond at C-6′,C-7′. Analysis of its HSQC and HMBC spectra enabled assignments of the signals of the quinoid and aromatic triterpene units. A ROESY experiment, showing correlations between the H-23′ methyl protons and H-6, and the CD spectrum, which showed a positive first Cotton effect at 356 nm similar to that of **1**, confirmed an α orientation about the *cis* 3,4-dioxy bonds. Therefore, the structure **2** was assigned as 6′,7′-dihydroscutionin αB.

Compound **3** was a yellow amorphous solid with $[\alpha]_D^{25}=+276.2°$ (*c* 0.21, $CHCl_3$) and showed to be a dimeric triterpene based on its spectral data, and showed a molecular formula, $C_{61}H_{84}O_9$, based on its HRFABMS and ^{13}C NMR

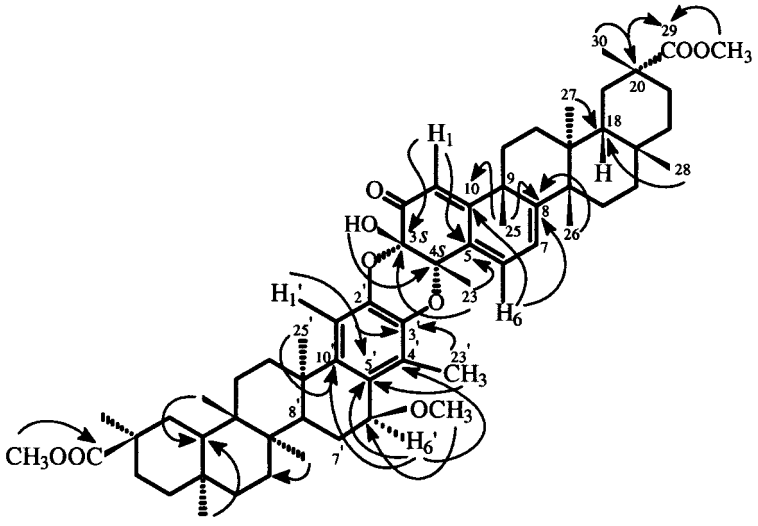


Figure 2. ^{1}H–^{13}C (HMBC) coupling for **3**.

spectra. The most notable differences in its ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) with respect to those of **2** were the presence of a signal at δ_H 4.28 br s and a methoxyl group at δ_H 3.38 s and their corresponding carbons at δ_C 75.2 d and 55.4 s, respectively. An HMBC (Fig. 2) experiment showing three-bond correlations between the signal at δ_H 4.28 (H-6′) and C-4′ (δ_C 125.5) and C-10′ (δ_C 144.7), and two-bond coupling between H-6′ and C-5′ (δ_C 127.2), sited the methoxyl group on C-6′. A ROESY experiment showing NOE correlations between the Me-23′ and H-6 revealed that the linkages between the units were [3–O–2] and [4–O–3], while an NOE effect between the methoxy methyl protons at C-6′ and H-8′β enabled the relative stereochemistry of the methoxyl group at C-6′ to be established as β. The CD curve of **3** showed a first positive Cotton effect at 355 nm and a second negative effect at 260 nm, establishing the absolute configuration of **3** as 3*S*,4*S*. Thus, compound **3** was 6′β-methoxy-dihydro-scutionin αB.

Diels–Alder reactions have been postulated as key steps in biosynthetic pathways.[22] The isolation of dimeric compounds, implying different stereo- and regio-isomeric relationships and derived by hypothetical hetero-Diels–Alder reactions, leads to the possibility of studying potential enzymatic systems with Diels–Alder-ase activity,[23] as has been put forward by the present authors.[9,10]

Compounds **1**–**3** were deemed inactive in all antimicrobial[19] (up to 40 μg/mL) and cytotoxicity[20] (up to 40 and 20 μg/mL for HeLa and Hep-2 cell lines, respectively) bioassays in which they were evaluated. These results reinforce our hypothesis that size in this type of compound plays an important role in their activity and that they could be stored in the plants as polymers, which could release biologically active units[9,19] as *ortho* or methylene quinoid forms, via a retro-Diels–Alder process, depending on the need of the plant.

1. Experimental

1.1. General experimental procedures

Optical rotations were measured on a Perkin–Elmer 241 automatic polarimeter and $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. CD spectra were run on a JASCO J-600 spectropolarimeter. IR spectra were recorded in $CHCl_3$ on a Bruker IFS 55 spectrophotometer and UV spectra were collected in absolute EtOH on a Jasco V-560. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 NMR spectrometer at 400 MHz and 100 MHz, respectively. FABMS and HRFABMS were recorded on a VG Autospec mass spectrometer. TLC 1500/LS 25 Schleicher and Schuell foils were used for thin-layer chromatography, while silica gel (0.2–0.63 mm) and Sephadex LH-20 were used for column chromatography.

1.2. Plant material

Maytenus blepharodes was collected at Varu volcano, Chirique, Panama, in August 1991, and *Maytenus magellanica* was gathered in the Novena region, Province of Temuca, on the slopes of the Osorno volcano, Chile, in January 1987. Voucher specimens are on file in the Department of Medicinal Chemistry and Pharmacognosy, University of Panama and with the Facultad de Ciencias, Universidad de Chile, Santiago, respectively.

1.3. Extraction and isolation

The root bark (500 g) of *Maytenus blepharodes* and the roots (570 g) of *Maytenus magellanica* were extracted with *n*-hexane–Et_2O (1:1) (4 L) in a Soxhlet apparatus, yielding 10.0 g and 13.5 g of extract, respectively. Both extracts were chromatographed on Sephadex LH-20, using *n*-hexane–$CHCl_3$–MeOH (2:1:1) as eluent, followed by repeated chromatography on silica gel (*n*-hexane–EtOAc mixtures of increasing polarity), and preparative HPTLC (HPTLC–Platten–SIL 20 UV_{254}) (*n*-hexane–$CHCl_3$–acetone, 6:3:1). In addition to the known compounds scutidin αA, 7,8-dihydroscutidin αB, scutionin αA, 7,8-dihydroscutionin αA, cangorosin A, 6′,7′-dihydroisocangorosin A and xuxuarine Eβ, we have isolated compound **1** (15.0 mg) from *M. blepharodes*, and compounds **1** (35.0 mg), **2** (2.6 mg) and **3** (2.6 mg) from *M. magellanica*, not previously described. The known compounds were identified by spectroscopic methods and by comparison with authentic samples or reported data.[9,14,16]

1.4. Bioassays

1.4.1. Antimicrobial activity. Activity was tested against Gram-positive (*Staphylococcus aureus* ATCC 6538, *S. epidermidis* CECT 232, *S. saprophyticus* CECT 235, *Enterococcus faecalis* CECT 481, *Bacillus subtilis* CECT 39, *B. cereus* CECT 496, *Mycobacterium smegmati* CECT 3032) and Gram-negative (*Escherichia coli* CECT 99, *Proteus mirabilis* CECT 170, *Salmonella* sp. CECT 456, 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 (dimethyl sulfoxide) were estimated in liquid medium following the method of Buttiaux et al.[24]

1.4.2. 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% new-born 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 [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] reduction assay.[20]

1.4.3. Scutionin αB (1). Pale yellow amorphous solid; $[\alpha]_D^{25}=+282.3°$ (*c* 1.58, $CHCl_3$); CD λ_{max} (MeOH) nm 355 ($\Delta\varepsilon=+12.8$), 248 ($\Delta\varepsilon=+9.1$), 230 ($\Delta\varepsilon=-9.7$); UV (EtOH) λ_{max} (log ε) 381 (3.86), 265 (3.99), 252 (3.95) nm; IR ν_{max} 3445, 2943, 2870, 1731, 1676, 1463, 1377, 1312, 1201, 1143, 772, 736 cm^{-1}; for ^{1}H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m/z* 929 $(M+1)^+$ (2); HRFABMS *m/z* (M^+) 928.582730 (calcd for $C_{60}H_{80}O_8$, 928.585320).

1.4.4. 6′,7′-Dihydro-scutionin αB (2). Pale yellow amorphous solid; $[\alpha]_D^{25}=+311.4°$ (*c* 0.22, $CHCl_3$); CD λ_{max} (MeOH) nm 356 ($\Delta\varepsilon=+2.8$), 261 ($\Delta\varepsilon=-4.3$); UV (EtOH) λ_{max} (log ε) 242 (4.41) nm; IR ν_{max} 3439, 2927, 2870, 1731, 1675, 1463, 1377, 1305, 1201, 1142, 756 cm^{-1}; for ^1H and ^{13}C NMR data, see Table 1; FABMS *m/z* 931 $(M+1)^+$ (5), 464 (3); HRFABMS *m/z* $(M)^+$ 930.601153 (calcd for $C_{60}H_{82}O_8$, 930.600970).

1.4.5. 6′β-Methoxy-6′,7′-dihydro-scutionin αB (3). Pale yellow amorphous solid; $[\alpha]_D^{25}=+276.2°$ (*c* 0.21, $CHCl_3$); CD λ_{max} (MeOH) nm 355 ($\Delta\varepsilon=+9.6$), 286 ($\Delta\varepsilon=+4.9$), 260 ($\Delta\varepsilon=-4.6$); UV (EtOH) λ_{max} (log ε) 382 (3.65), 282 (3.44), 269 (3.37) nm; IR ν_{max} 3441, 2924, 2854, 1731, 1681, 1463, 1377, 1201, 1143, 756 cm^{-1}; for ^1H and ^{13}C NMR data, see Table 1; FABMS *m/z* 961 $(M+1)^+$ (2); HRFABMS *m/z* $(M)^+$ 960.614145 (calcd for $C_{61}H_{84}O_9$, 960.611535).

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