Pergamon

Tetrahedron, Vol. 52, No. 28, pp. 9597-9608, 1996

0040-4020/96 $15.00 + 0.00

PII: S0040-4020(96)00497-8

Structure and Absolute Configuration of Triterpene Dimers from *Maytenus scutioides.*

Antonio G. González*, Nelson L. Alvarenga, Ana Estévez-Braun, Angel G. Ravelo, Isabel L. Bazzocchi and Laila Moujir[a]

Instituto Universitario de Bio-Orgánica Antonio González , Universidad de La Laguna, Avda. Astrofísico F° Sánchez 2, La Laguna, 38206, Tenerife. Canary Islands. Spain.

[a]Departamento de Microbiologia y Biología Celular, Universidad de La Laguna.

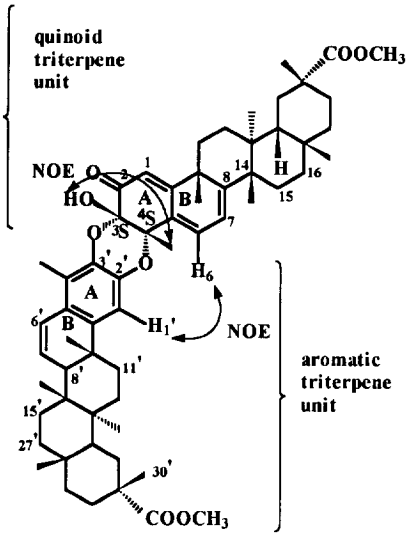
Abstract: Eight new triterpene dimers (**1-8**) were isolated from *Maytenus scutioides* (Celastraceae). Their structures were determined on the basis of spectroscopic evidence and their absolute configurations by means of CD studies. Their possible biogenetic route is discussed. One unnatural dimer was synthesized by a hetero Diels-Alder reaction.

As a part of our research on bioactive compounds from the South American medicinal plants[1,2], we studied the active metabolites of the genus *Maytenus* (Celastraceae), which have been widely used in folk medicine[3,4]. *Maytenus scutioides* Lourteig and O'Donnell[5] is a subtropical shrub, which is distributed in the Chaco (North of Argentina and South of Paraguay and Bolivia). The aerial part of this plant is used as cardiotonic and the roots as abortive by the inhabitants of these regions[6,7].

By antimicrobial and cytotoxicity-guided purification, a (n-hexane:Et_2O; 1:1) extract of the root bark of *M. scutioides* gave celastrol, pristimerin, tingenone, netzahualcoyene and scutione[8] and the new triterpene dimer (**8**) as its active principles. Seven dimer compounds (**1-7**), were also isolated. All dimer compounds were found to be composed of one quinoid-type triterpene, derived from pristimerin, 7,8-dihydro-pristimerin or netzahualcoyene and one aromatic triterpene, derived from pristimerin or 6-hydroxy-pristimerin, linked together by two ether linkages between the two A rings. Their structures were elucidated by means of ^1H and ^{13}C NMR spectroscopic studies, including ^1H-^{13}C heteronuclear correlation (HETCOR), long range correlation spectra with inverse detection (HMBC), and chemical evidence. Their CD curves allowed us to determine unequivocally their absolute configurations.

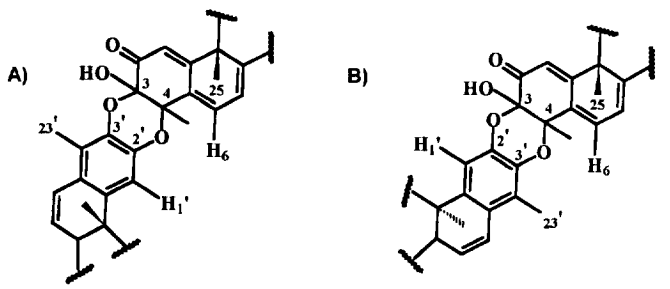
Diels Alder reactions have been postulated as key steps in a number of biosynthetic conversions[9]. In this paper we reinforce the tentative route proposed by Itokawa *et al.*[10] for the biosynthesis of these type of dimers, which involves hetero Diels-Alder reactions. We have synthesized a dimer compound (**10**) by reaction of an ortho-quinone (**9**)[11] with pristimerin under Diels-Alder reaction conditions, in a similar fashion to the postulated biogenetic pathway.

The (*n*-hexane:Et_2O; 1:1) extract of the root bark of *Maytenus scutioides* was repeatedly chromatographed on Sephadex LH-20 and silica gel to give compounds **1-8**.



1 Scutionin α A

Compound **1** was isolated as a yellow lacquer. Its IR spectrum showed absorption bands for hydroxyl group (3436 cm^{-1}), carboxyl group (1731 cm^{-1}) and carbonyl group (1678 cm^{-1}). The molecular formula was shown to be $C_{60}H_{80}O_8$ by FAB-MS and ^{13}CNMR data. Its ^{1}H NMR spectrum (Table 1) showed signals for 11 angular methyl groups, one methyl group on aromatic ring at δ 2.05 and two methoxy groups at δ 3.58 and δ 3.67; also it was observed an ABC system of three vinyl protons, at δ 6.32 as a double doublet and two doublets at δ 6.06 and 5.92, attributable to H-6, H-1 and H-7, characteristic of a triterpenic quinoid system. All these data suggested that **1** was a triterpene dimer composed of two pristimerin type triterpenes, one in quinoid form, and the other in aromatic form. The analysis of the ^{13}C NMR spectrum (Table 2), HMBC and HMQC experiments showed that the quinoid triterpene unit contained two oxygenated adjacent quaternary carbons at C-3 and C-4 of the ring A at δ 91.81 and δ 78.73, respectively. As regards, the aromatic triterpene unit, the signals at δ 108.03 (C-1'), 140.04 (C-2'), 137.61 (C-3'), 122.42 (C-4'), 125.02 (C-5'), 124.01 (C-6'), 129.12 (C-7'), 45.51 (C-8'), 38.23 (C-9') and 143.72 (C-10'), showed that it contained an aromatic ring for A ring, one conjugated double bond at C-6',7' on B ring and oxygenated carbons at C-2', C-3' on A ring. The hydroxyl group was located on C-3 by an HMBC experiment, showing three bond couplings of the hydrogen of the OH group with C-2 and C-4. A NOE effect between the OH group on C-3 and the Me on C-4 indicated that the two units are joined by two ether linkages in *cis* disposition. There may be two different regioisomers (A or B) (Figure 1) depending on the

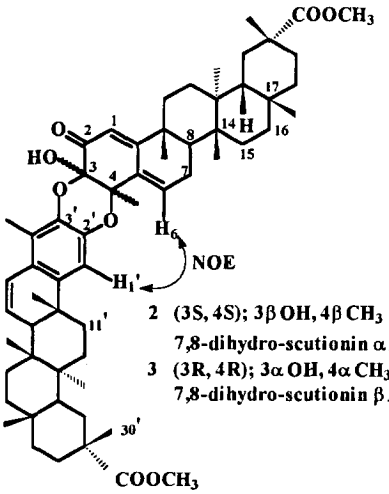


[3-O-3] [4-O-2] [3-O-2] [4-O-3]

Figure 1

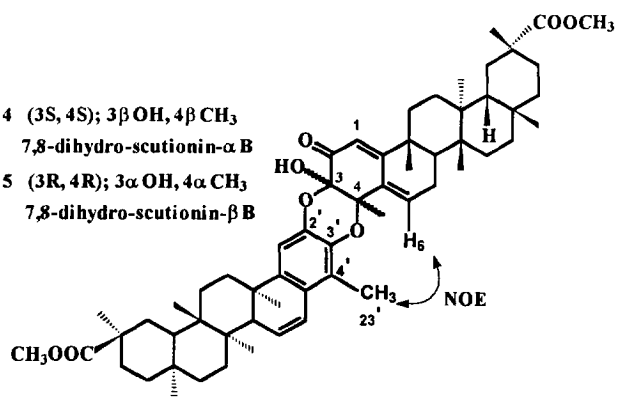
linkages between the two units. A ROESY experiment showing NOE effect between H-1' and H-6 revealed that the option A was the correct isomer for **1**. This is the first time that a dimer compound with linkages between the units [3-O-3] [4-O-2] is reported.

The absolute configuration of **1** was determined as 3S,4S[10,12] by analysis of the CD spectrum, showing a split CD curve type Davidoff, with a first positive Cotton effect at 282.0 nm ($\Delta\varepsilon$=+9.20) and a second negative one at 253.6 nm ($\Delta\varepsilon$=-7.48); also it was observed a positive Cotton effect at 354.0 nm ($\Delta\varepsilon$=+7.02) which corresponds to a n-π^* transition, characteristic of enone systems without analytical value. All these data indicated **1** was (3S,4S) quinoid pristimerin [3-O-3] [4-O-2] aromatic pristimerin. We propose the name scutionin α A for this compound.



2 (3S, 4S); 3β OH, 4β CH_3
7,8-dihydro-scutionin α A
3 (3R, 4R); 3α OH, 4α CH_3
7,8-dihydro-scutionin β A

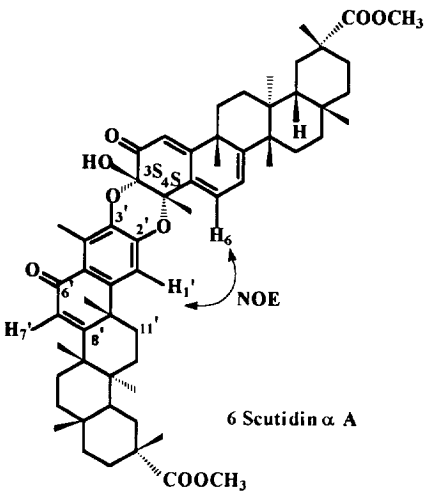
The compounds **2** and **3** presented the same molecular formula, $C_{60}H_{82}O_8$. In their ^1H NMR spectra, the main differences with respect to **1** were the absence of the signal at δ 5.92 (H-7) and the presence of H-6 as a broad singlet at δ 6.34 for **2** and at δ 6.47 for **3** (Table 1). These data indicated that **2** and **3** do not present the double bond C-7,8, which was confirmed by an HMBC experiment of **2**, showing two bond coupling between C-6 and H-7 and three bond coupling between C-6 and H-8. A ROESY experiment of **2**, confirmed that the linkages between the units were [3-O-3] and [4-O-2]. Their DC spectra revealed that both compounds were diastereoisomers. The CD curve of **2** showed a first positive Cotton effect at 280.4 nm, but we could not observe the second negative effect at 244.8 nm because of the presence of strong positive absorption of the styrenic system, which destroyed the second component of the split curve. The CD curve of **3** showed a split curve with a first negative Cotton effect at 268.2 nm and a second positive effect at 229.6 nm. The absolute configurations of **2** and **3** were established as 3S,4S and 3R,4R, respectively.



4 (3S, 4S); 3β OH, 4β CH_3
7,8-dihydro-scutionin-α B
5 (3R, 4R); 3α OH, 4α CH_3
7,8-dihydro-scutionin-β B

The compounds **4** and **5** were diastereoisomers, showing the molecular formula, $C_{60}H_{82}O_8$. Their NMR spectral data were similar to those of **2** and **3**, the only

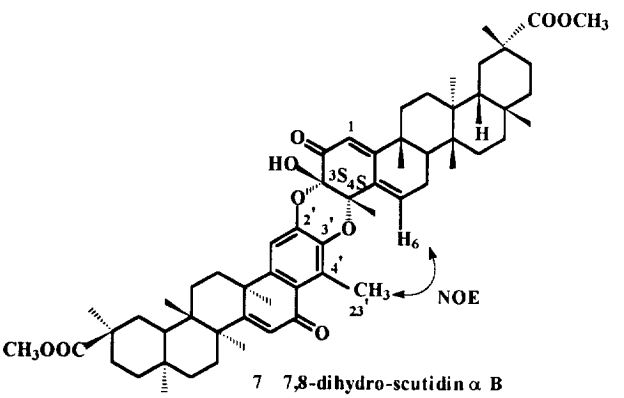
difference being that the linkages between the units were [3-O-2] [4-O-3], instead of [3-O-3] [4-O-2] as in **1-3**. This was determined by a ROESY experiment showing NOE effect between Me-23' and H-6. The CD spectra of **4** and **5** established their absolute configurations as 3S,4S and 3R,4R, respectively.



6 Scutidin α A

Compound **6** was isolated as a yellow amorphous solid and had the molecular formula, $C_{60}H_{78}O_9$. In its ^1H NMR spectrum two singlets at δ 6.21 and δ 6.99 are considered as the most significant signals in the aromatic triterpene unit. The first of these signals is assigned to an hydrogen in α–position to an α–β-unsaturated ketone system and the second to an aromatic hydrogen (H-1'). The ^{13}C NMR spectrum confirmed that the aromatic triterpene unit presents an aromatic ring system for A ring, one carbonyl group at C-6' and one conjugated double bond at C7'-C8' on B ring. The regiosubstitution of the linkages between the two units was established as [3-O-3] [4-O-2] by a ROESY experiment, showing NOE effect between H-1' and H-6. Its absolute configuration was established as 3S, 4S by analysis of its CD spectrum. **6** was named scutidin α A.

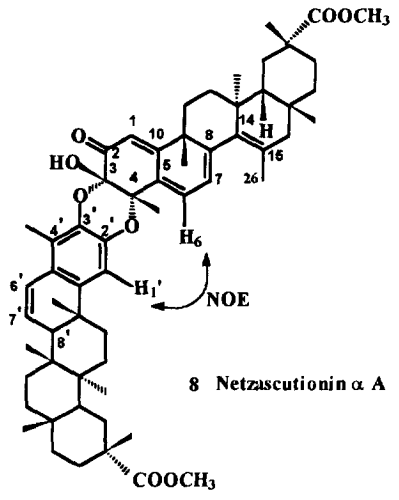
Compound **7** showed in its FAB-MS a molecular ion at m/z 930 and its NMR spectral data were similar to that of **6**. The main difference of the ^1H NMR spectra was the absence of the doublet at δ 5.94 corresponding to H-7; this was supported by a ^1H-^1H COSY experiment showing coupling between H-6 and other signal assigned to two hydrogens at δ 2.14. A NOE effect between Me-23' and H-6 pointed out that the two units are linked together by two ether bonding [3-O-2] [4-O-3]. Its absolute configuration was established by the CD spectrum as 3S,4S. All these data indicated **7** was 7,8-dihydro-scutidin α B.



7 7,8-dihydro-scutidin α B

Compound **8** showed in its NMR data an aromatic triterpene unit similar to that of **1**, while the other quinoid triterpene unit had an additional conjugated double bond on C_{14}-C_{15} and one migrated methyl group on double bond. This was confirmed by an HMBC experiment, that showed three and two bond couplings between the Me-26 and C-14 and C-15, respectively. These data are agree with a triterpene quinoid unit derived from netzahualcoyene skeleton[1], instead

of pristimerin. A ROESY experiment determined its regiosubstitution. The CD spectrum showed a split curve with a first positive Cotton effect at 276.0 nm and a second negative one at 251.0 nm which established an absolute configuration 3S,4S; **8** was named netza-scutionin. This is the first time that a dimer compound with a netzahualcoyene unit is reported.



8 Netzascutionin α A

All dimers were assayed for antibiotic and cytotoxic activities. Compound **8** was the only dimer that showed antibiotic activity against Gram positive bacteria; its CMIs on *Bacillus subtilis* and *B. pumilus* were 1-2 and 20 μg/ml, respectively. None of the dimers showed cytotoxic activity against the cell lines used at least at 20 μg/ml. These results support that the additional double bond C-14,15 plays an important role for the antibiotic activity[8].

Table 1: ^{1}H NMR ($CDCl_3$) (400 MHz) of **1-8** (J are given in Hz in the brackets).

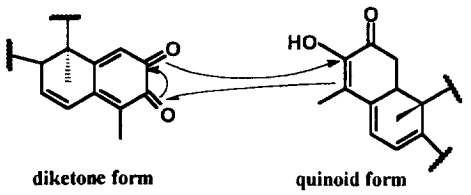
H	1	2	3	4	5	6	7	8
H-1	6.06 d (1.2)	5.94 s	5.98 s	5.99 s	5.95 s	6.09 d (1.4)	5.98 s	6.03 d (1.4)
H-6	6.32 dd (1.2, 6.3)	6.34 br s	6.47 br s	6.55 br s	6.23 br s	6.30 dd (1.4, 6.5)	6.33 br s	6.54 dd (1.4, 6.4)
H-7	5.92 d (6.3)	2.15 m	2.13 m	2.15 m	2.16 m	5.94 d (6.6)	2.14 m	5.75 d (6.4)
Me-23	1.53 s	1.44 s	1.51 s	1.49 s	1.45 s	1.56 s	1.48 s	1.56 s
H-1'	6.70 s	6.67 s	6.43 s	6.63 s	6.50 s	6.99 s	6.97 s	6.71 s
H-6'	6.63 dd (2.7, 10.2)	6.64 dd (2.8, 9.9)	6.68 dd (2.2, 9.6)	6.58 dd (1.6, 10.0)	6.66 dd (2.7, 10.0)	--------	--------	6.64 dd (2.8, 10.0)
H-7'	5.90 dd (2.5, 10.2)	5.91 dd (2.6, 9.9)	5.91 dd (2.2, 9.6)	5.88 dd (1.7, 10.0)	5.91 dd (2.2, 10.0)	6.21 s	6.23 s	5.91 dd (2.6, 10.0)
Me-23'	2.05 s	2.09 s	2.29 s	2.01 s	2.28 s	2.48 s	2.53 s	2.08 s
OMe	3.58 s 3.67 s	3.65 s 3.66 s	3.62 s 3.64 s	3.63 s 3.65 s	3.63 s 3.64 s	3.57 s 3.57 s	3.55 s 3.65 s	3.67 s 3.68 s

Table 2: ^{13}C NMR ($CDCl_3$) (100 MHz) of **1-2** and **4-8**.

C	1	2	4	5	6	7	8
1	115.60 d	112.83 d	112.76 d	112.73 d	115.72 d	112.70 d	115.37 d
2	191.12 s	192.27 s	192.14 s	191.17 s	190.32 s	191.20 s	190.55 s
3	91.81 s	91.37 s	91.02 s	91.28 s	91.68 s	90.95 s	91.39 s
4	78.73 s	78.86 s	77.20 s	79.22 s	79.25 s	79.15 s	78.36 s
5	130.70 s	134.12 s	135.51 s	133.91 s	130.39 s	133.72 s	131.53 s
6	126.34 d	134.01 d	136.84 d	133.76 d	126.24 d	133.72 d	127.56 d
7	116.30 d	29.68 t	30.04 t	30.56 t	116.01 d	29.09 t	120.29 d
8	160.51 s	41.65 d	43.13 d	41.80 d	161.16 s	41.29 d	151.03 s
9	41.62 s	37.36 s	38.76 s	37.39 s	41.70 s	39.81 s	44.14 s
10	173.71 s	170.42 s	170.23 s	170.38 s	173.39 s	169.81 s	170.18 s
1'	108.03 d	108.10 d	108.13 d	108.81 d	110.42 d	110.14 d	108.15 d
2'	140.04 s	140.10 s	139.78 s	140.31 s	144.35 s	144.17 s	140.07 s
3'	137.61 s	137.50 s	137.39 s	136.53 s	138.24 s	137.94 s	137.79 s
4'	122.42 s	122.49 s	122.14 s	121.29 s	129.20 s	129.03 s	122.54 s
5'	125.02 s	124.96 s	125.26 s	125.97 s	123.22 s	122.94 s	125.07 s
6'	124.01 d	124.01 d	123.99 d	124.01 d	187.20 s	186.85 s	124.02 d
7'	129.12 d	129.20 d	129.17 d	129.40 d	126.15 d	125.95 d	129.19 d
8'	45.51 d	45.55 d	45.39 d	45.46 d	170.95 s	170.58 s	45.53 d
9'	38.23 s	37.49 s	37.10 t	37.39 s	40.01 s	39.75 s	37.50 s
10'	143.72 s	143.71 s	143.72 s	142.72 s	151.66 s	151.41 s	143.68 s

Data based on HMBC, HMQC and DEPT experiments.

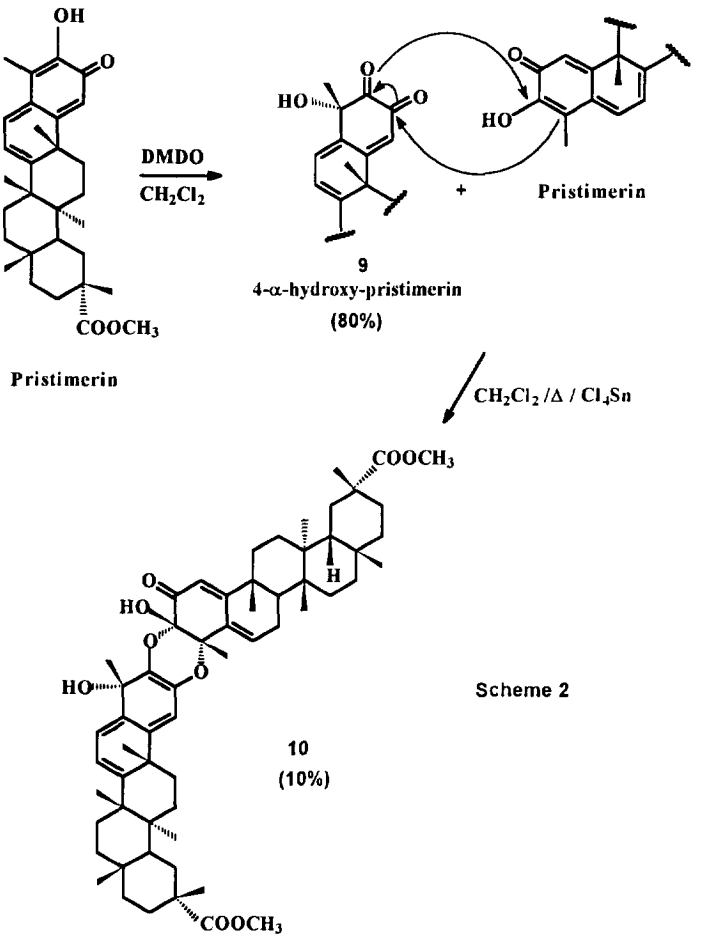
Recently Itokawa *et al* [10] have postulated a route for the biosynthesis of similar triterpene dimers, based on Diels-Alder reactions, which have been postulated as key steps in a number of biosynthetic conversions[9]. They suggest that the quinoid triterpene-type is in an equilibrium state with its 2,3-diketon type, and both could react to give the corresponding triterpene dimer (Scheme 1).



Scheme 1

Compounds with orthoquinone system have never been isolated from the Celastraceae species, although triterpenes with catechol moiety have been reported[13]. These catechols derivatives could be precursors of the 2,3-diketone type triterpenes under enzymatic oxidation in plants. To test whether dimer compounds are formed by a

Diels-Alder reaction we prepared a 2,3-diketone triterpene-type. When pristimerin was treated with DMDO gave 4α-hydroxy-pristimerin[11] derivative (**9**). Reaction of **9** with pristimerin under Diels-Alder conditions yielded a dimer compound (**10**) (Scheme 2). These results and the isolation of the different possible regio and stereoisomers (A or B and α or β) of the dimer compounds reinforces the biogenetic route proposed by Itokawa *et al.*, and open new perspectives related to the synthesis, hemisynthesis and reactivity of these compounds.



Scheme 2

EXPERIMENTAL

General experimental procedures

Melting points are uncorrected. IR spectra were taken on a PE 681 spectrophotometer. ^{1}H and ^{13}C NMR spectra were taken on a Bruker WP-400 SY in $CDCl_3$ at 400 and 100 MHz, respectively, with TMS as internal

reference. The HMBC and HMQC were run on a Bruker at 400 MHz. Optical rotations were measured on a Perkin-Elmer 241 automatic polarimeter and $[\alpha]_D^{20}$ are given in 10^{-1} deg cm^2 g^{-1}. FAB-MS were recorded on a VG Autospec spectrometer. CD spectra were run on a Jasco J-600 spectropolarimeter. UV spectra were collected on a Perkin-Elmer model 550-SE. Schleicher-Schüll F-100/LS 254 and prep. TLC 1510/LS 254 foils were used for TLC, while silica gel (0.2-0.63 mm) and Sephadex LH-20 were used for CC.

Plant material

The plant was gathered in Paraguay, in December 1993, and a voucher specimen (n° R. Degen 3117) is on file with the Herbarium of the Departamento de Botánica, Facultad de Ciencias Químicas, Universidad Nacional de Asunción.

Extraction and isolation

The root bark (3Kg) of the plant was extracted with n-hexane-Et_2O (1:1) (3 liters) in a Soxhlet apparatus. The extract (115 g) was repeatedly chromatographed on Sephadex LH-20 and Si gel using as solvents mixtures of n-hexane-$CHCl_3$-MeOH (2:1:1) and of n-hexane-AcOEt, respectively to afford **1** (10 mg), **2** (16 mg), **3** (1.5 mg), **4** (20 mg), **5** (13 mg), **6** (40 mg), **7** (20 mg) and **8** (9.8 mg).

Bioassay

Antimicrobial activity. *Staphylococcus aureus* ATCC 6538, *S. epidermidis* CECT 232, *S. saprophyticus* CECT 235, *Bacillus subtilis* CECT 39, *B. pumilus* CECT 29, *Escherichia coli* CECT 99, *Proteus mirabilis* CECT 170, *Salmonella tiphymurium* UBC 2 and *Pseudomonas aeruginosa* AK 958, were used. The bacteria were grown and maintained in Nutrient Agar (Oxoid) and cultured in YP medium [composition per liter: yeast extract (Oxoid) 10 g, peptone (Oxoid) 10 g]. The inocula were prepared by diluting overnight cultures with a sterile saline solution. The different fractions and compounds were tested for antibiotic activity by the disk diffusion test[14]. The minimal inhibitory concentrations[2] were determined only for those compounds that had previously shown an inhibition zone in the disk diffusion test.

Cytotoxy activity. HeLa (human carcinoma of the cervix) and Hep-2 (human carcinoma of larynx) cell lines wre 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. The cytotoxic activity was assessed using colorimetric MTT reduction assay[15].

Scutionin a A (1). Yellow lacquer; $[\alpha]_D^{20}$ -338.8° (c 0.4, $CHCl_3$); CD λ_{max}(MeOH) nm ($\Delta\varepsilon$): 354.0 (+7.02), 282.0 (+9.20), 253.6 (-7.48); IR ν_{max} ($CHCl_3$) cm^{-1}: 3436, 2931, 2860, 1731, 1678, 1460, 1378, 1313, 1237, 1202, 1143, 1096, 1061, 1026; UV λ_{max}(MeOH) nm (log ε): 377, 281; ^{1}H NMR ($CDCl_3$): 0.53 (3H, s), 0.87

(3H, s), 0.99 (3H, s), 1.06 (3H, s), 1.07 (3H, s), 1.11 (3H, s), 1.17 (3H, s), 1.21 (3H, s), 1.41 (3H, s), for other signals, see Table 1; ^{13}C NMR ($CDCl_3$): 10.91 (q), 17.03 (q), 17.50 (q), 18.31 (q), 22.24 (q), 22.24 (q), 22.50 (q), 28.21 (t), 28.32 (t), 29.51 (t), 29.70 (t), 29.80 (t), 30.02 (t), 30.41 (s), 30.52 (t), 30.60 (s), 31.02 (t), 31.22 (t), 31.5° (q), 31.81 (q), 32.20 (q), 32.84 (q), 32.91 (t), 34.81 (t), 34.90 (q), 36.01 (t), 36.33 (t), 36.41 (t), 38.93 (s), 39.01 (s), 40.41 (s), 40.52 (s), 44.24 (d), 44.32 (s), 44.41 (d), 44.51 (s), 51.62 (q, -OMe), 51.62 (q, -OMe), 178.91 (s), 179.33 (s); for other signals, see Table 2; FAB-MS (%): 928 (M^+)(100), 663 (45), 464 (90).

7,8-Dihydro-scutionin-αA (2). Yellow amorphous solid, m.p 186-187°; $[\alpha]_D^{20}$ + 254.7 (c 1.7, $CHCl_3$); CD λ_{max}(MeOH) nm ($\Delta\varepsilon$): 280.4 (+9.07), 340 (+2.02); IR ν_{max} ($CHCl_3$) cm^{-1}: 3500, 2998, 2871, 1724, 1674, 1462, 1383, 1194, 1030, 768, 733; UV λ_{max}(MeOH) nm(log ε): 286 (3.70), 229.5 (3.89), 216.5 (3.93); ^1H NMR ($CDCl_3$): 0.75 (3H, s), 0.86 (3H, s), 0.99 (3H, s), 1.00 (3H, s), 1.07 (3H, s), 1.12 (3H, s), 1.19 (3H, s), 1.21 (3H, s), 1.26 (3H, s), 1.43 (1H, m, overlapping signal), 4.85 (1H, s, int. with D_2O), for other signals, see Table 1; ^{13}C NMR ($CDCl_3$): 11.08 (q), 15.99 (q), 16.80 (q), 16.98 (q), 17.47 (q), 22.11 (q), 22.15 (q), 22.80 (q), 24.11 (t), 28.17 (t), 28.30 (t), 29.87 (t), 29.96 (t), 29.99 (t), 30.16 (s), 30.40 (s), 30.51 (t), 30.59 (t), 30.65 (t), 31.18 (t), 31.65 (q), 31.78 (q), 32.17 (q), 32.78 (q), 32.31 (q), 35.98 (t), 36.02 (t), 36.47 (t), 38.87 (s), 38.98 (s), 39.02 (s), 40.09 (s), 40.50 (s), 40.58 (s), 44.42 (d), 44.65 (d), 51.57 (q, -OMe), 55.64 (q, -OMe), 179.03 (s), 179.27 (s), for other signals, see Table 2; FAB-MS (%): 930 (M^+) (51), 665 (60), 462 (100).

7,8-Dihydro-scutionin β A (3). Yellow lacquer; $[\alpha]_D^{20}$ -144.0 (c 0.15, $CHCl_3$); CD λ_{max}(MeOH) nm ($\Delta\varepsilon$): 268.2 (-3.24), 229.6 (+6.10); IR ν_{max} ($CHCl_3$) cm^{-1}: 3447, 3022, 2925, 1720, 1674, 1625, 1603, 1213, 1095, 784, 655; UV λ_{max}(MeOH) nm(log ε): 285 (3.91), 225 (4.10), 203 (4.18); ^1H NMR ($CDCl_3$): 0.79 (3H, s), 0.81 (3H, s), 0.95 (3H, s), 1.05 (3H, s), 1.07 (3H, s), 1.09 (3H, s), 1.18 (6H, s), 1.57 (3H, s), 4.92 (1H, s, int. with D_2O), for other signals, see Table 1; FAB-MS (%): 930 (M+) (72), 665 (87), 467 (100).

7,8-Dihydro-scutionin α B (4). Yellow lacquer; $[\alpha]_D^{20}$ +208.93 (c 0.8, $CHCl_3$);); CD λ_{max}(MeOH) nm ($\Delta\varepsilon$): 277.4 (+5.86); IR ν_{max} ($CHCl_3$) cm^{-1}: 3400, 2996, 2949, 2872, 1724, 1674, 1645, 1460, 1376, 1200, 1140, 966; UV λ_{max}(MeOH) nm (log ε): 283.5 (3.75); ^1H NMR ($CDCl_3$): 0.79 (3H, s), 0.81 (3H, s), 0.99 (3H, s), 1.06 (3H, s), 1.10 (3H, s), 1.18 (6H, s), 1.59 (3H, s), 4.91 (1H, s, int. with D_2O), for other signals, see Table 1; ^{13}C NMR ($CDCl_3$): 10.79 (q), 15.96 (q), 16.85 (q), 17.03 (q), 17.41 (q), 22.01 (q), 22.06 (q), 22.89 (q), 24.13 (t), 28.23 (t), 28.42 (t), 29.43 (t), 29.68 (t), 29.88 (t), 29.93 (t), 30.04 (t), 30.14 (s), 30.38 (s), 30.58 (t), 31.01 (t), 31.68 (q), 31.80 (q), 32.12 (q), 32.25 (t), 35.98 (t), 36.03 (t), 36.39 (t), 37.39 (s), 38.85 (s), 38.89 (s), 38.97 (s), 40.04 (s), 40.50 (s), 44.47 (d), 44.61 (d), 51.58 (q, -OMe), 51.60 (q, -OMe), 91.28 (s), 179.01 (s), 179.04 (s), for other signals, see Table 2; FAB-MS (%): 930 (85) (M+), 665 (100), 467 (90).

7,8-Dihydro-scutionin β B (5). Yellow lacquer; $[\alpha]_D^{20}$ -69.81 (c 0.5, $CHCl_3$);); CD λ_{max}(MeOH) nm ($\Delta\varepsilon$): 255.6 (-2.66); IR ν_{max} ($CHCl_3$) cm^{-1} :3400, 3024, 2949, 1724, 1673, 1218, 1142, 1095, 792; UV λ_{max}(MeOH) nm (log ε): 283.0 (3.75), 260.5 (3.67); ^1H NMR ($CDCl_3$): 0.80 (3H, s), 0.95 (3H, s), 1.03 (3H, s), 1.06 (3H, s), 1.08 (3H, s), 1.11 (3H, s), 1.19 (6H, s), 1.59 (3H, s), 4.78 (1H, s, int. with D_2O), for other signals, see Table 1; ^{13}C NMR ($CDCl_3$): 10.79 (q), 16.31 (q), 16.83 (q), 17.34 (q), 22.24 (q), 23.77 (t), 24.32 (q), 24.70 (q), 28.21 (t), 28.81 (t), 29.92 (t), 29.93 (t), 30.20 (s), 30.47 (t), 30.61 (t), 31.07 (t), 31.14 (t), 31.58 (q), 31.72 (q), 31.79 (q), 32.11 (q), 32.17 (q), 35.96 (t), 36.06 (t), 36.20 (t), 36.33 (t), 36.38 (t), 38.81 (s), 38.86 (s), 38.98 (s), 39.37 (s), 40.52 (s), 40.52 (s), 44.48 (d), 44.53 (d), 51.52 (q, -OMe), 51.58 (q, -OMe), 147.14 (s), 179.04 (s), 179.04 (s), for other signals, see Table 2; FAB-MS (%): 930 (M+) (77), 665 (100), 467 (60).

Scutidin a A (6). Yellow amorphous solid, m.p 193-194°; $[\alpha]_D^{20}$ + 278.6 (c 4.1, $CHCl_3$); CD λ_{max}(MeOH) nm ($\Delta\varepsilon$): 337.6 (+1.57), 299.0 (+1.53), 251.4 (-2.42); IR ν_{max} ($CHCl_3$) cm^{-1}: 3400, 3014, 2950, 1726, 1642, 1464, 1308, 1225, 1142, 750; UV λ_{max}(MeOH) nm (log ε): 380.6 (3.80), 299.0 (4.01), 253.0 (4.12); ^1H NMR ($CDCl_3$): 0.56 (3H, s), 0.62 (3H, s) 1.06 (3H, s), 1.10 (3H, s), 1.16 (3H, s), 1.18 (3H, s), 1.19 (3H, s), 1.29 (3H, s), 1.42 (3H, s), 1.54 (3H, s), 5.04 (1H, int. with DO_2), for other signals, see Table 1; ^{13}C NMR ($CDCl_3$): 13.25 (q), 18.36 (q), 18.50 (q), 20.75 (q), 22.10 (q), 22.44 (q), 28.27 (q), 28.45 (t), 29.44 (t), 29.66 (t), 29.71 (t), 29.87 (t), 30.45 (s), 30.45 (s), 30.92 (t), 30.97 (t), 31.52 (q), 31.57 (q), 32.72 (q), 32.83 (t), 32.87 (q), 34.17 (t), 34.73 (t), 34.89 (t), 34.93 (q), 36.28 (t), 36.32 (t), 37.54 (q), 38.14 (s), 38.92 (s), 40.36 (s), 40.52 (s), 44.09 (d), 44.18 (d) 44.55 (s), 44.62 (s), 51.59 (q, -OMe), 51.69 (q, -OMe), 178.81 (s), 179.26 (s), for other signals, see Table 2; FAB-MS (%):942 (M+) (10), 481 (55), 464 (90), 450 (100).

7,8-Dihydro-scutidin a B (7). Yellow crystalline solid, m.p 81-82°; $[\alpha]_D^{20}$ + 3.33 (c 0.2, $CHCl_3$); CD λ_{max} (MeOH) nm ($\Delta\varepsilon$): 290.8 (+4.22), 248.2 (-2.08); IR ν_{max} ($CHCl_3$) cm^{-1}: 3000, 1690, 1600, 1580, 1450, 1410, 1310, 1280, 1170, 930, 710, 660; UV λ_{max}(MeOH) nm (log ε): 297.2 (3.51), 250.6 (3.45); ^1H NMR ($CDCl_3$): 0.62 (3H, s), 0.76 (3H, s), 0.99 (3H, s), 1.06 (3H, s), 1.08 (3H, s), 1.11 (3H, s), 1.18 (3H, s), 1.19 (3H, s), 1.30 (3H, s), 1.54 (3H, s), 4.93 (1H, s, int. with D_2O), for other signals, see Table 1; ^{13}C NMR ($CDCl_3$): 13.01 (q), 15.69 (q), 16.53 (q), 18.23 (q), 20.53 (q), 21.77 (q), 22.40 (q), 23.84 (t), 28.01 (t), 28.21 (t), 29.41 (s), 29.52 (t), 29.59 (t), 29.87 (s), 30.19 (t), 30.19 (t), 30.31 (t), 30.68 (t), 31.29 (q), 31.35 (q), 31.97 (q), 32.58 (q), 33.88 (t), 34.63 (t), 35.70 (t), 35.70 (t), 36.07 (t), 37.08 (s), 37.33 (q), 38.56 (s), 38.68 (q), 40.18 (s), 40.25 (s), 43.94 (d), 44.31 (d), 44.36 (s), 51.23 (q, -OMe), 51.32 (q, -OMe), 178.66 (s), 178.88 (s), for other signals, see Table 2; FAB-MS (%):944 (M+) (10).

Netzascutionin α A (8). Yellow amorphous solid, m.p 119-120°; $[\alpha]_D^{20}$ + 437.79 (c 0.7, $CHCl_3$); CD λ_{max} (MeOH) nm ($\Delta\varepsilon$): 390.6 (+7.01), 276.0 (+7.96), 251.0 (-1.92); IR ν_{max} ($CHCl_3$) cm^{-1}: 3500, 3024, 2950, 1720,

1662, 1462, 1225, 1218, 1144, 782, 769, 755; UV λ_{max}(MeOH) nm (log ε): 398.0 (3.57), 273.5 (3.48); ^{1}H NMR ($CDCl_3$): 0.80 (3H, s), 0.87 (3H, s), 1.01 (3H, s), 1.07 (3H, s), 1.12 (3H, s), 1.15 (3H, s) 1.18 (3H, s), 1.20 (6H, s), 1.70 (3H, s), 4.93 (1H, s, int. with D_2O), for other signals, see Table 1; ^{13}C NMR ($CDCl_3$): 10.92 (q), 16.97 (q), 17.49 (q), 19.79 (q), 22.17 (q), 22.27 (q), 23.00 (q), 27.23 (q), 28.44 (q), 28.67 (t), 29.99 (t), 29.99 (t), 30.39 (s), 30.65 (t), 31.20 (t), 31.57 (q), 31.79 (q), 32.20 (q), 33.67 (s), 33.97 (t), 35.61 (t), 35.96 (t), 36.11 (t), 36.25 (t), 36.44 (t), 36.50 (t), 37.96 (t), 38.98 (s), 39.03 (s), 40.59 (s), 42.11 (s), 42.66 (s), 44.04 (d), 44.45 (d), 51.59 (q, -OMe), 51.84 (q, -OMe), 128.56 (s), 134.66 (s), 179.34 (s), 179.34 (s), for other signals, see Table 2; FAB-MS (%): 926 (M^+) (8), 661 (10), 462 (100).

Reaction of 4-α-hydroxy-pristimerin (9) with pristimerin. 15 mg (0.03 eq) of pristimerin in 20 ml of dry CH_2Cl_2 were treated with 12 mg (0.025 eq.) of **9**[11] and one drop of $SnCl_4$ as catalyst. The mixture of reaction was stirred under reflux for 72 h. The solvent was removed under vacuum. The crude of reaction was purified by TLC preparative (n-hex: AcOEt; 2:1) to give the corresponding dimer derivative (**10**) as pale yellow lacquer (3 mg) (10%); CD λ_{max} (MeOH) nm (Δε): 341.2 (+7.90), 287.0 (+3.68), 247.4 (-16.8); IR ν_{max} ($CHCl_3$) cm^{-1}: 3447, 3028, 2920, 1720, 1640, 1603, 1541, 1372, 1230, 1201, 752; UV λ_{max} (MeOH) nm: 366; ^{1}H NMR ($CDCl_3$): 0.59 (3H, s), 0.66 (3H, s), 1.09 (6H, s), 1.21 (6H, s), 1.39 (3H, s), 1.48 (3H, s), 1.52 (3H, s), 3.60 (3H, s), 3.63 (3H, s), 5.86 (1H, s), 5.99 (1H, s), 6.02 (1H, d, J=6.0 Hz), 6.17 (1H, d, J=6.0 Hz), 6.61 (1H, d, J=6.0 Hz), 6.67 (1H, d, J=6.0 Hz). FAB-MS (%): 944 (M^+) (20), 463 (90), 391 (100).

REFERENCES

1. Moujir, L.M; Navarro-Gutiérrez, A.M.; González, A.G.; Ravelo, A.G.; Luis, J.G. *Antimicrobial Agents and Chemotherapy*, **1991**, *35*, 211-213.
2. González, A.G.; Jiménez, J.S.; Moujir, L.M.; Ravelo, A.G.; Luis, J.G.; Bazzocchi, I.L.; Navarro-Gutiérrez, A.M. *Tetrahedron*, **1992**, *48*, 769-774.
3. González, G.J.; Monache, D.G.; Monache, D.F.; Marini-Bettolo, B.G. *J. Ethnopharmacology*, **1982**, *5*, 73-77.
4. Simões, C.M.O.; Mentz, A.L.; Schenkel, P.E.; Irgang, E.B.; Stehmann, R.J. *Plantas da Medicina Popular no Rio Grande do Sul*, Ed. da Universidade/ Universidade Federal do Rio Grande do Sul, **1986.**
5. Lourteig, A.; O' Donnell, C.A. *Nature*, **1955**, *1*, 181.
6. González, M.D. *Catalogo de Plantas Medicinales Usadas en Paraguay*; Asunción, **1981**.
7. Alvarenga, N.S. Estudio fitoquímico de especies del género *Maytenus* (Celastraceae): *M. canariensis* y *M. scutioides*, University of La Laguna 1995.

8. González, A.G.; Alvarenga, N.S; Ravelo, A.G.; Bazzocchi, I.L.; Ferro, E.A.; Navarro, A.G.; Moujir, L. *Bioorg. Med. Chem.* (in press).
9. Laschat, S. *Angew. Chem. Int. Ed. Engl.*, **1996**, *35*, 289-291.
10. Shirota, O.; Morita, H.; Takeya, K.; Itokawa, H. *Tetrahedron*, **1995**, *51*, 1107-1120.
11. González, A.G., Alvarenga, N.L.; Estévez-Braun, A.; Ravelo, A.G.; Estévez -Reyes, R. *Tetrahedron*, **1996** (send to publish).
12. Harada, N; Nakanishi, K. *Circular Dichroic Spectroscopy-Exciton Coupling in Organic Stereochemistry*; University Science Books: Mill Valley, CA, **1983**.
13. González A.G.; Alvarenga N.L.; Rodriguez F.; Ravelo A.G.; Jiménez , I.A.; Bazzocchi, I.L.; Gupta M. P. *Nat. Prod. Lett.*, **1995**, *7*, 209-218.
14. González, A.G.; Ravelo, A.G.; Bazzocchi, I.L.; Jiménez, J.; González, C.M.; Luis, J.G.; Ferro, E.A.; Navarro, A.G.; Moujir, L.M.; de Las Heras, F.G. *Il Farmaco*, **1988**, *43*, 501-505.
15. Mosmann, T. *J. Immunol. Methods*, **1983**, *65*, 55.

ACKNOWLEDGMENTS

We are indebted to the Gobierno Autónomo Canario, CYCIT (Project SAF 94-0239) and Colegio Libre Emerito for financial help. We thank Prof. Trujillo Vázquez for carrying out the CD spectrum and Prof. T. Sierra- Travieso and the staff of the mass spectrometry of the University of Zaragoza for carrying out the FAB-MS spectra.

(*Received in UK* 23 *April* 1996; *accepted* 23 *May* 1996)