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Absolute Configuration and Complete Assignment of ^{13}C NMR Data for New Sesquiterpenes from *Maytenus chiapensis*

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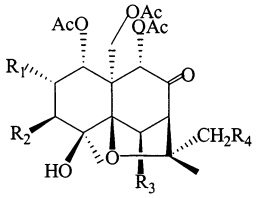
Five new dihydro-β-agarofuran sesquiterpenes (**1**−**5**) were isolated from the leaves of *Maytenus chiapensis*. The structures of **1**−**5** were determined by means of 1D and 2D NMR techniques. A semiselective HMBC technique was applied in order to obtain complete ^{13}C NMR assignments. Absolute configurations were determined by CD studies and chemical correlations and on biogenetic grounds. Compound **4** showed weak activity against a multidrug-resistant *Leishmania tropica* line.

Species of the genus *Maytenus* (Celastraceae) have a long history of use in traditional medicine and agriculture in North Africa, South and Central America, and Central and East Asia.[1] As part of our studies of medicinal plants of this genus, we have previously reported on phenolic[2] and quinone-methide triterpenes,[3] dimeric[4] and trimeric[5] triterpenes, and dihydro-β-agarofuran sesquiterpenes.[6] The last type of compounds are chemotaxonomic indicators in the plant family,[7] and they have attracted a great deal of interest on account of their wide range of biological activities, such as immunosuppression and against the reversal of the multidrug resistance (MDR) phenotype.[8]

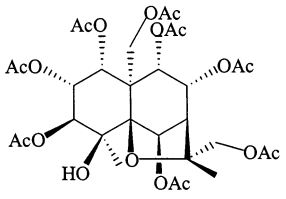
The present paper reports the isolation and structure characterization of five new sesquiterpenes (**1**−**5**) with a dihydro-β-agarofuran skeleton from the leaves of *Maytenus chiapensis* Lundell and the activity of these compounds against a MDR *Leishmania tropica* line, the second most common cause of death among parasitic diseases worldwide.[9]

Compound **1** showed the molecular formula $C_{25}H_{34}O_{13}$ by HREIMS. Its IR spectrum showed absorption bands for hydroxyl (3540 cm^{-1}) and carboxyl groups (1749 cm^{-1}). When **1** was treated with acetic anhydride in pyridine, it was unaltered, a fact which together with a singlet at δ 2.72 in the ^1H NMR spectrum (Table 1), interchangeable with D_2O, confirmed the presence of a tertiary hydroxyl group. All these data indicated that compound **1** is a heptasubstituted dihydro-β-agarofuran sesquiterpene. In its ^1H NMR spectrum was also observed an ABX_2 system (δ 5.47 d, δ 5.34 m, and δ 2.04 m), assignable to protons H-1, H-2, and H-3, respectively; three methine protons appeared as singlets at δ 6.60 (H-6), 2.97 (H-7), and 5.58 (H-9), along with one methylene group (δ 4.47, 4.87, each d), assigned to H-15. These data are compatible with a H-1ax, H-2eq stereochemistry in this type of sesquiterpene, and the multiplicities and ^1H NMR chemical shifts of the H-7 and H-9 signals suggested that they are α to a carbonyl group.

In the elucidation of the chemical structures by NMR spectroscopy a potentially limiting problem with HMBC is that in regions of the ^{13}C spectrum where many resonances fall closely together the resolution may prove insufficient and result in cross-peak overlap. To solve this problem,



1 $R_1 = R_3 = OAc$; $R_2 = R_4 = H$
2 $R_1 = OAc$; $R_2 = R_4 = H$; $R_3 = OH$
3 $R_1 = R_3 = OH$; $R_2 = R_4 = H$
4 $R_1 = R_2 = R_3 = R_4 = OAc$



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a semiselective HMBC technique[10] was applied and the complete ^{13}C NMR chemical shift assignments, including the carbonyl carbons of the ester groups, were made (Table 2).

The relative stereochemistry of **1** was established on the basis of the coupling constants and confirmed by a ROESY experiment (Figure 1), showing NOE effects of H-14 to H-6 and H-15, H-13 to H-9, and H-1 to H-2 and H-9. The absolute configuration of **1** was determined by CD studies with the curve showing a positive Cotton effect at 290 nm ($\Delta\epsilon = +1.8$) corresponding to the n−π* transition of the carbonyl group at C-8. Application of the octant rule indicated that the structure and absolute configuration of **1** is (1*R*,2*S*,4*S*,5*S*,6*R*,7*R*,9*S*,10*S*)-1,2,6,9,15-pentaacetoxy-4-hydroxy-8-oxodihydro-β-agarofuran.

The structures of compounds **2** and **3** were elucidated by spectral methods, ^1H and ^{13}C NMR studies (Tables 1 and 2), $^1H-^1H$ and $^1H-^{13}C$ correlations, ROESY experiments (Figure 1), and chemical correlations. Thus, acetylation of **2** and **3** gave the same product, the spectral data of which were identical to those of **1**.

Compound **4** was isolated as a colorless lacquer with the molecular formula $C_{29}H_{36}O_{16}$ (HREIMS). Its ^1H and ^{13}C NMR data (Tables 1 and 2), assigned by HMBC and

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Table 1. ^{1}H NMR (δ, $CDCl_3$, J are given in Hz in parentheses) Data for Compounds **1**−**5**

position	1	2	3	4	5
H-1	5.47 d (3.2)	5.48 d (3.2)	5.46 d (2.8)	5.69 d (3.1)	5.58d (3.6)
H-2	5.34 m	5.36 m	4.10 br s	5.28 dd (3.1, 2.8)	5.26 dd (3.6, 2.8)
H-3	2.04 m	2.09 m	2.10 m	4.86 d (2.8)	4.82 d (2.8)
OH-4	2.72 s	3.14 s	3.03 s	4.54 s	
H-6	6.60 s	5.28 d (5.4)	5.26 d (5.3)	6.62 s	6.76 s
OH-6		5.22 d (5.4)	5.29 d (5.3)		
H-7	2.97 s	3.09 s	3.06 s (5.3)	3.00 s	2.32 d (3.6)
H-8					5.48 dd (3.6, 5.9)
H-9	5.58 s	5.58 s	5.59 s	5.57 s	5.34 d (5.9)
H-12	1.62 s	1.64 s	1.63 s	5.10 d, 3.89 d (11.7)	4.89 d, 3.94 d (11.6)
H-13	1.49 s	1.49 s	1.49 s	1.47 s	1.56 s
H-14	1.52 s	1.80 s	1.85	1.54 s	1.47 s
H-15	4.87 d, 4.47 d (13.0)	4.95 d, 4.34 d (13.0)	5.00 d, 4.42 d (13.2)	4.84 d, 4.44 d (13.1)	5.25 d, 4.42 d (13.3)
Ac-1	1.94 s	1.94 s	2.04 s	1.94 s	1.88 s
Ac-2	2.11 s	2.12 s		2.15 s	2.12 s
Ac-3				2.19 s	2.15 s
Ac-6	2.14 s			2.14 s	2.13 s
Ac-8					2.16 s
Ac-9	2.10 s	2.05 s	2.07 s	2.13 s	1.98 s
Ac-12				2.11 s	2.11 s
Ac-15	2.02 s	1.95 s	1.93 s	2.02 s	2.25 s

Table 2. ^{13}C NMR (δ, $CDCl_3$) Data[a] for Compounds **1**−**5**

position	1	2	3	4	5
C-1	74.35 d	74.40 d	72.40 d	71.58 d	73.13d
C-2	68.05 d	67.75 d	67.61 d	68.77 d	69.01 d
C-3	42.02 t	41.11 t	42.66 t	75.11 d	75.83 d
C-4	69.75 s	72.24 s	72.40 s	69.37 s	69.29 s
C-5	93.21 s	92.50 s	92.89 s	94.44 s	92.93 s
C-6	74.59 d	76.27 d	76.25 d	73.72 d	73.93 d
C-7	64.83 d	66.41 d	66.36 d	62.24 d	50.71 d
C-8	197.50 s	199.12 s	199.06 s	196.32 s	68.96 d
C-9	78.79 d	78.80 d	79.26 d	78.44 d	70.95 d
C-10	52.65 s	52.12 s	52.13 s	52.16 s	51.90 s
C-11	84.81 s	84.90 s	84.69 s	86.13 s	84.04 s
C-12	29.22 q	29.75 q	29.71 q	69.62 t	69.43 t
C-13	24.56 q	25.24 q	25.11 q	18.67 q	18.30 q
C-14	25.17 q	24.70 q	24.77 q	24.01 q	23.25 q
C-15	60.54 t	60.22 t	60.53 t	60.28 t	60.28 t
Ac-1	20.66 q	20.50 q	20.52 q	20.50 q	20.50 q
	169.24 s	169.36 s	169.27 s	168.68 s	169.30 s
Ac-2	21.13 q	21.10 q		20.94 q	20.80 q
	169.35 s	169.25 s		167.97 s	168.68 s
Ac-3				20.75 q	20.83 q
				168.91 s	169.50 s
Ac-6	21.35 q			21.24 q	20.96 q
	169.14 s			168.80 s	169.80 s
Ac-8					20.96 q
					170.03 s
Ac-9	20.22 q	20.12 q	20.78 q	20.78 q	20.46 q
	169.22 s	169.20 s	169.20 s	168.72 s	168.90 s
Ac-12				20.15 q	21.44 q
				170.34 s	170.70 s
Ac-15	20.1 q	20.62 q	20.05 q	20.42 q	21.22 q
	169.72 s	169.72 s	169.90 s	169.11 s	170.10 s

[a] Data are based on DEPT, HSQC, and HMBC experiments.

semiselective HMBC experiments, indicate that it is a polyester sesquiterpene with seven acetates, two more than **1**. Its ^{1}H NMR spectrum was similar to that of **1**, except for the signals for H-3 (δ 4.86, d, J = 2.8 Hz) and H-12 (δ 5.10 and 3.89, each d, J = 11.7 Hz), indicating both C-3 and C-12 to be acetylated. This assignment and the relative stereochemistry of **4** were determined on the basis of the coupling constants and confirmed by a ROESY experiment,

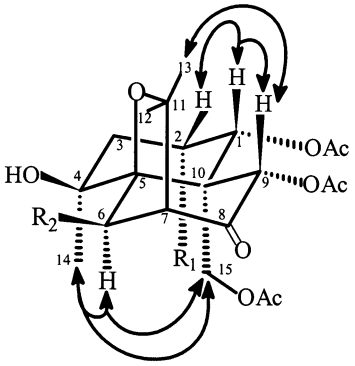

Figure 1. ROESY correlations for compounds **1**−**3**.

showing in addition to the correlations observed for compounds **1**−**3** (Figure 1), a NOE effect of H-1 to H-3.

The structure of compound **5** was elucidated by spectral methods, including HREIMS, IR, UV, ^{1}H and ^{13}C NMR, and 2D ^{1}H−^{1}H and ^{1}H−^{13}C experiments (Tables 1 and 2). All of these data indicated that **5** is euonyminol octaacetate,[11] this being the first time that it has been reported as a natural compound. An example of the use of the semiselective HMBC technique is given in Figure S1, where the carbonyl region from the conventional HMBC is compared with that from the semiselective HMBC experiment, for compound **5**. The aim was to obtain specific assignments for each carbonyl group throughout connectivity with their geminal protons. This allowed all carbons to be assigned, even though the eight ester carbonyl resonances fall within a 2 ppm window.

The absolute stereochemistry of compounds **4** and **5** could be related, on the basis of biogenetic grounds, to compound **1**, except for the chiral centers C-3 and C-8, which were determined by analysis of coupling constants and ROESY experiments.

The new compounds have the basic polyhydroxylated skeletons of 3,12-dideoxyevoninol (**1**−**3**),[12] evoninol (**4**),[12] and euonyminol (**5**), respectively.[12]

Compounds **1**−**5** were tested against a MDR *Leishmania tropica* line overexpressing a P-glyprotein-like transporter, to determine their ability to revert the resistance phenotype and to modulate intracellular drug accumulation. From the series, only compound **4** showed weak activity (growth inhibition 28% at 60 μM). Probably the compounds are too bulky to bind at the active site, and these results seem to suggest, in agreement with previous observations,[8] that the steric properties for the same class of sesquiterpenes can modulate their activity.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 automatic polarimeter, and the $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. UV spectra were collected on a JASCO V-560 spectrometer. CD spectra were run on a JASCO J-600 spectropolarimeter. IR (film) spectra were recorded on a Bruker IFS 55 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-500, a Bruker Avance 400, or a Bruker Avance 300 spectrometer. A EBURP 90° pulse (4 ms) was chosen for carrying out the semiselective HMBC experiments. EIMS and HREIMS were recorded on a Micromass Autospec spectrometer. Purification was performed using silica gel (particle size 40−63 μM, Merck, and HPTLC-Platten-Sil 20 UV_{254}, Panreac) and Sephadex LH-20 (Pharmacia) and was monitored by TLC (1500/LS 25 Schleicher and Schüell foil). The compound subjected to CD was purified by HPLC using a semipreparative μ-Porasil column (Waters, 10 μM, 19 mm × 25 cm) and eluted with toluene−acetone (8:2).

Plant Material. *Maytenus chiapensis* was collected at Parque Nacional El Imposible, El Salvador, in August 1999, and was identified by Prof. Edi Montalvo, and a voucher specimen (ISB-88) is on file at the Jardin Botánico, El Salvador.

Extraction and Isolation. The leaves of the plant (2.1 kg) were extracted with EtOH in a Soxhlet apparatus. Evaporation of the solvent under reduced pressure provided 400.2 g of crude extract, which was partitioned into a CH_2Cl_2−H_2O (1:1, v/v) solution. Removal of the CH_2Cl_2 from the organic-soluble extract under reduced pressure yielded 77.0 g of residue, which was chromatographed on a silica gel column, using mixtures of *n*-hexane−EtOAc of increasing polarity as eluant to afford 54 fractions. Fractions 49−51 (4.0 g) were subjected to column chromatography over Sephadex LH-20 (*n*-hexane−$CHCl_3$−MeOH, 2:1:1) and silica gel (CH_2Cl_2−acetone of increasing polarity). Preparative HPTLC developed with toluene−acetone (7:3) was used to purify the new compounds **1** (41.0 mg, R_f 0.50), **2** (81.7 mg, R_f 0.45), **3** (2.9 mg, R_f 0.36), **4** (5.0 mg, R_f 0.36), and **5** (10.0 mg, R_f 0.30).

(1*R*,2*S*,4*S*,5*S*,6*R*,7*R*,9*S*,10*S*)-1,2,6,9,15-Pentaacetoxy-4-hydroxy-8-oxodihydro-*β*-agarofuran (1): colorless lacquer; $[\alpha]^{25}_D$ −1.3° (*c* 0.77, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 264 (2.81) nm; CD λ_{ext} (MeCN) nm 290 ($\Delta\epsilon$ = +1.8); IR ν_{max} (film) 3540, 2929, 1749, 1433, 1371, 1235, 1228, 1083, 1040, 757 cm^{-1}; ^{1}H NMR ($CDCl_3$), see Table 1; ^{13}C NMR ($CDCl_3$), see Table 2; EIMS *m*/*z* 542 (M^+, 1), 527 (1), 500 (11), 482 (20), 440 (67), 380 (8), 263 (7), 156 (100), 114 (47), 83 (34); HREIMS *m*/*z* 542.19931 (calcd for $C_{25}H_{34}O_{13}$, 542.19994).

(1*R*,2*S*,4*S*,5*S*,6*R*,7*R*,9*S*,10*S*)-1,2,9,15-Tetraacetoxy-4,6-dihydroxy-8-oxodihydro-*β*-agarofuran (2): colorless lacquer; $[\alpha]^{25}_D$ +3.6° (*c* 2.21, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 270 (2.60) nm; IR ν_{max} (film) 3403, 2978, 1752, 1371, 1232, 1140, 1052, 758 cm^{-1}; ^{1}H NMR ($CDCl_3$), see Table 1; ^{13}C NMR ($CDCl_3$), see Table 2; EIMS *m*/*z* 500 (M^+, 8), 440 (28), 380 (11), 352 (19), 292 (8), 156 (100), 114 (73), 83 (60); HREIMS *m*/*z* 500.18608 (calcd for $C_{23}H_{32}O_{12}$, 500.18937).

Acetylation of 2. Compound **2** (2.0 mg), dissolved in pyridine (2 drops), was treated with Ac_2O (4 drops), and the mixture was left at room temperature for 16 h, evaporated to dryness under reduced pressure, and purified by preparative TLC with a mixture of *n*-hexane−AcOEt (1:1) to give a product (1.5 mg) whose spectroscopic data were identical to those of compound **1**.

(1*R*,2*S*,4*S*,5*S*,6*R*,7*R*,9*S*,10*S*)-1,9,15-Triacetoxy-2,4,6-trihydroxy-8-oxodihydro-*β*-agarofuran (3): colorless lacquer; $[\alpha]^{25}_D$ −3.8° (*c* 0.34, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 265 (2.12) nm; IR ν_{max} (film) 3536, 2925, 2853, 1736, 1375, 1235, 1136, 1045, 751 cm^{-1}; ^{1}H NMR ($CDCl_3$), see Table 1; ^{13}C NMR ($CDCl_3$), see Table 2; EIMS *m*/*z* 440 (M^+ − 18, 8), 380 (9), 338 (8), 278 (6), 165 (30), 114 (75), 83 (100); HREIMS *m*/*z* 440.17069 (calcd for $C_{21}H_{28}O_{10}$, 440.16825).

Acetylation of 3. Compound **3** (1.0 mg) was treated under the same conditions as described above to give a product (0.9 mg) whose spectroscopic data were identical to those of compound **1**.

(1*R*,2*S*,3*S*,4*S*,5*S*,6*R*,7*R*,9*S*,10*R*)-1,2,3,6,9,12,15-Heptaacetoxy-4-hydroxy-8-oxodihydro-*β*-agarofuran (4): colorless lacquer; $[\alpha]^{25}_D$ +1.0° (*c* 0.52, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 264 (2.90) nm; IR ν_{max} (film) 3462, 2926, 1751, 1458, 1371, 1229, 1040, 757 cm^{-1}; ^{1}H NMR ($CDCl_3$), see Table 1; ^{13}C NMR ($CDCl_3$), see Table 2; EIMS *m*/*z* 640, (M^+ − 18, 1), 585 (25), 567 (41), 465 (48), 363 (73), 321 (65), 231 (59), 105 (75), 60 (100); HREIMS *m*/*z* 640.19572 (calcd for $C_{29}H_{36}O_{16}$, 658.20034).

1α,2α,3*β*,6*β*,8α,9α,12,15-Octaacetoxy-4*β*-hydroxydihydro-*β*-agarofuran (5): colorless lacquer; $[\alpha]^{25}_D$ −5.9° (*c* 1.11, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 220 (3.93) nm; IR ν_{max} (film) 3444, 2969, 1738, 1645, 1370, 1233, 1045 cm^{-1}; ^{1}H NMR ($CDCl_3$), see Table 1; ^{13}C NMR ($CDCl_3$), see Table 2; EIMS *m*/*z* 684 (M^+ − 18, 1), 642 (6), 629 (45), 611 (48), 540 (28), 509 (53), 449 (34), 407 (100), 347 (65), 257 (80), 245 (88), 215 (73); HREIMS *m*/*z* 684.22867 (calcd for $C_{31}H_{40}O_{17}$, 684.22655).

In Vitro Multidrug-Resistant Assays. All compounds have been tested for efficacy as potential modulators in a multidrug-resistant *Leishmania tropica* line according to an established protocol.[8]

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Supporting Information Available: HMBC spectra for compound **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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