Structural Elucidation and Absolute Configuration of Novel β-Agarofuran (Epoxyeudesmene) Sesquiterpenes from *Maytenus magellanica* (Celastraceae)

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Six dihydro-β-agarofuran [5,11-epoxy-5β,10α-eudesm-4(14)-ene] sesquiterpenes with a novel substitution pattern were isolated from *Maytenus magellanica* and their structures were elucidated by means of ^{1}H and ^{13}C NMR spectroscopic studies, including ^{1}H–^{13}C heteronuclear correlation (HETCOR), long range correlation spectra with inverse detection (HMBC) and NOE experiments. Their absolute configurations were determined by application of the CD exciton chirality method while hydrolysis and preparation of derivatives provided additional information. One of the sesquiterpenes exhibited significant antifeedant activity against *Spodoptera littoralis*.

As part of an intensive course of research into biologically active metabolites from Celastraceae species used in folk medicine,[1–3] *Maytenus magellanica* Hook[4] was studied. This species grows in the phytogeographical region of the Antarctic forest (Argentina and Chile) and had shown moderate activity (LC_{50} 650 ppm) in the brine shrimp lethality bioassay.[5] Compound **1** showed antifeedant activity[6] against *Spodoptera littoralis* in an election test, although it was not particularly effective against microorganisms[6,†] or viruses.‡

The six metabolites isolated displayed a hitherto unreported substitution pattern on a dihydro-β-agarofuran [5,11-epoxy-5β,10α-eudesm-4(14)ene] skeleton with 1α, 2β, 3β, 4β and 9β substituents. Four of the compounds also had substituents at 6β. The compounds closest in structure to them are those isolated by Kupchan[7] from other Celastraceae: maytolin and maytolidin, with the basic polyhydroxy skeleton of maytol (1α,2α,3β,4β,6β,9β,15-heptahydroxydihydro-β-agarofuran); they are also related to the sesquiterpene core of the macrocyclic alkaloids evoninol,[8] euonyminol[9] and isoeuonyminol.[10]

Regrettably, the absolute configurations of dihydro-β-agarofuran sesquiterpenes have not been reported. Clardy,[11] applying X-ray diffraction techniques to a celorbicol derivative with a heavy atom, gave the absolute configuration of a series of celorbicol derivatives; contradictory results were published regarding the determination of the absolute configuration of malkanguniol[12] although the later corrections[13] agreed in the main with the findings of Clardy. This paper gives an account of the structural elucidation of compounds **1**, **4**, **7**, **9**, **10** and **11** and their absolute configuration based on CD techniques and chemical correlations (Schemes 1 and 2).

Discussion

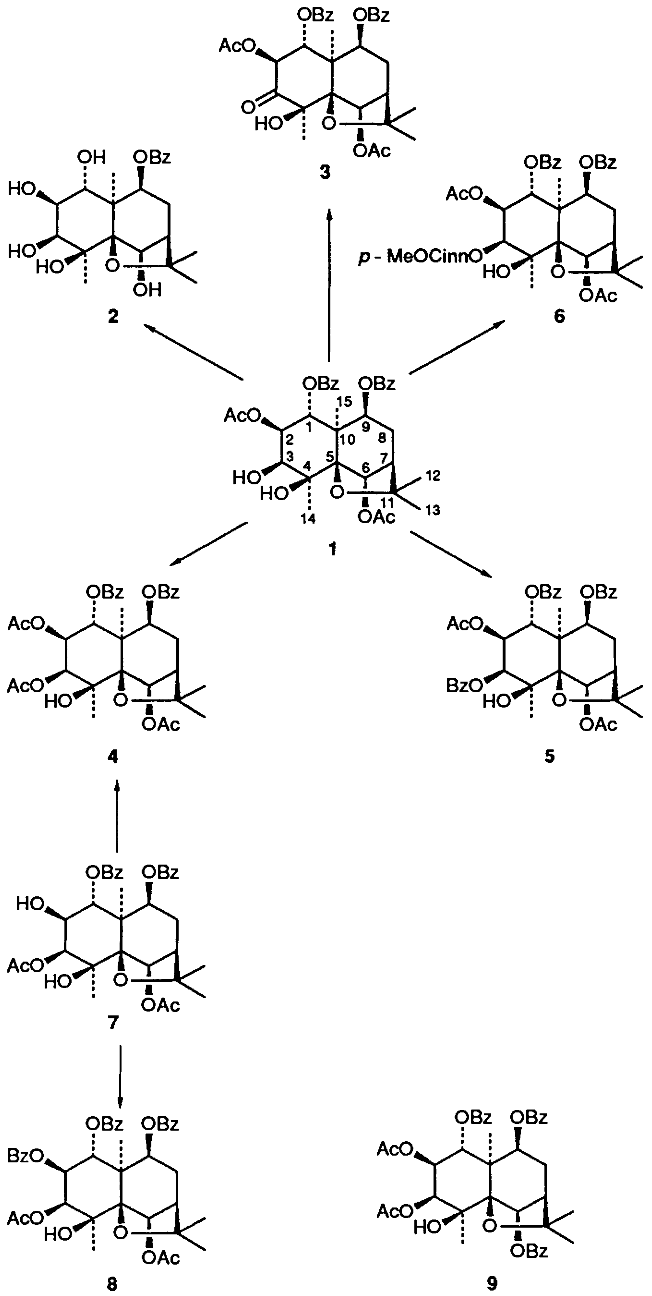
The molecular formula of compound **1** $[\alpha]_D^{20}$ +18.0 10^{-1} deg cm^2 g^{-1} (*c* 0.11, MeOH) was established as $C_{33}H_{30}O_{11}$ by high resolution mass spectrometry), and it showed typical IR absorptions for ester and alcohol groups. The electronic impact mass spectrum showed sharp peaks at *m/z* 105, ($M^+ - 60$) and ($M^+ - 42$) suggesting that benzoate and acetate groups are to be found in the molecule, and this was confirmed by the ^{1}H and ^{13}C NMR spectroscopic data which included signals for 10 aromatic protons between δ_H 7.23 and 7.96, two acetate methyls as singlets at δ_H 1.83 and 2.16, two carboxylic benzoate carbons at δ_C 164.74 and 165.47 and two acetate carboxylic carbons at δ_C 170.37 and 170.55. All these data indicate that this is a polyester sesquiterpene of the type usually found in the Celastraceae.[14]

The ^{1}H NMR spectra (Table 1) were assigned by a thorough study of the chemical shifts and confirmed by a COSY experiment. The ^{13}C spectra (see Table 2) were resolved by DEPT experiments and ^{1}H–^{13}C correlations which, together with NOE experiments (Fig. 1), made it possible to determine the substitution positions as 1α, 2β, 3β, 4β, 6β and 9β. The regiosubstitution characteristics were elicited by a two dimensional ^{1}H–^{13}C NMR experiment [correlation *via* heteronuclear zero and double quantum coherence, optimized on long range couplings with low-pass *J*-filter to suppress one bond correlations with no decoupling during acquisition[15] (HMBC)[16] (Fig. 2)] which located the benzoates at C-1 and C-9, the acetates at C-2 and C-6, and the hydroxy groups at C-3 and C-4, since the 1-H (δ 6.25) and 9-H (δ 5.01) were clearly three-bond-coupled with the carboxylic carbons of two benzoates; 2-H (δ 5.32) and 6-H (δ 5.59) exhibited coupling with the carboxylic carbons of the two acetates. Three bond and some two bond coupling with other carbons was also observed (Table 3). These data agreed with the proposed structure and were supported by chemical means as hydrolysis of **1** with a 0.1 mol dm^{-3} solution of $NaHCO_3$ gave the monobenzoate **2** and oxidation with Jones' reagent gave the 3-oxo-derivative **3**. To obtain derivatives with different ester substitutions at C-3, the acetyl-derivative **4** was prepared, proving identical to one of the natural products isolated from *M. magellanica* (see ^{1}H NMR spectroscopic data in Table 1).

The absolute configuration of compound **1** was resolved by the dibenzoate chirality method, an extension of the circular dichroism exciton chirality method.[17,18] The dihedral angle between the two benzoate chromophores is approximately 150°

† Compound **1** was assayed on *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* CECT 39, *Escherichia coli* CECT 99, *Salmonella sp.* CECT 456, *Saccharomyces cerevisiae* X 2180 A, *Candida albicans* and *Pseudomonas aeruginosa* (from the Dept. of Microbiology of the University of Vancouver, BC).

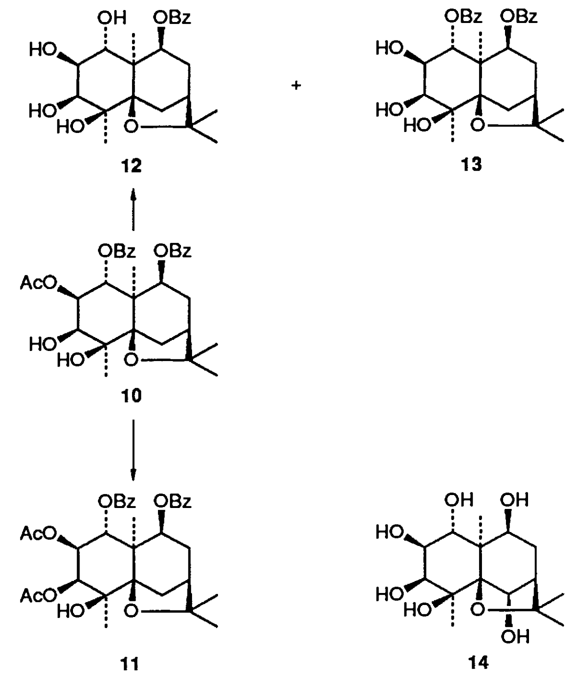
‡ *Herpes simplex* virus Type 1, KOS strain (HSV-1), Vesicular Stomatitis Virus, Indiana strain (VSV) and HeLa (uterus neck cancer) cells were used.



Scheme 1

in compound **1** (calculated from *J* value data and by molecular mechanics calculations using the PC model)[19] and the compound was therefore considered suitable for CD study; the CD spectrum showed a split CD curve with extrema at the right-hand wavelength, *i.e.* the first Cotton effect was located at 237.3 nm ($\Delta\varepsilon$ +21.2), and the second at 220.0 nm ($\Delta\varepsilon$ −8.0) (see Table 4).

The CD spectrum of the benzoyl and *p*-methoxycinnamoyl derivatives of **1**, compounds **5** and **6**, respectively, confirmed the absolute configuration established for **1**. The tribenzoate **5** did not show any split CD curve, as the opposite 1,3 and 1,9 pairwise interactions cancelled each other out and the 3,9 pairwise interaction was almost coplanar. The CD spectrum of bichromophore **6** (1,9-dibenzoyl-3-*p*-methoxycinnamoyl derivative), on the other hand, did exhibit the expected Cotton effects, negative at 306.0 nm produced by the 1,3 hetero pairwise interaction and positive and negative at 239.8 and 224.8 nm, respectively, due to the homobenzoate interaction, and the resulting curve is as shown (see Table 4 and Fig. 3).



Scheme 2

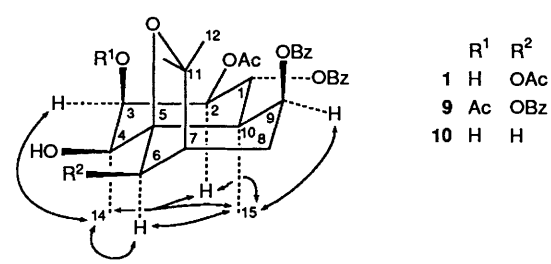


Fig. 1 NOE enhancements observed for compounds **1**, **9** and **10**

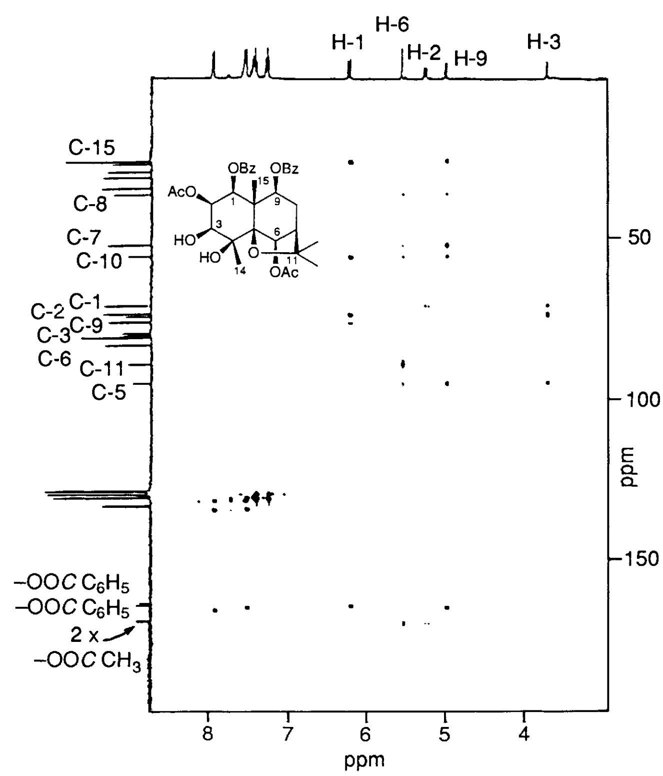


Fig. 2 Selected portion of HMBC spectra of compound **1**

Table 1 ^1H NMR spectroscopic data for compounds **1–13** (200 MHz, $CDCl_3$)[a]

	δ_H							
	1-H	2-H	3-H	6-H	9-H	2-OAc	3-OAc	6-OAc
1	6.25 d (11.3)	5.32 dd (2.5, 11.3)	3.77 d (2.5)	5.59 s	5.01 d (6.4)	1.83	—	2.16
2	4.20 d (3.4)	3.63[b]	3.63[b]	4.52 s	5.11 d (6.5)	—	—	—
3	6.06 d (11.7)	5.73[b] (11.7)	—	5.70 s	5.14 d (6.6)	2.15	—	2.15
4	6.28 d (11.2)	5.28 dd (2.8, 11.2)	5.18 d (2.8)	5.57 s	5.03 d (6.1)	1.74	2.31	2.14
5	6.48 d (10.3)	5.38 dd[b]	5.41 d[b]	5.66 s	5.05 d (6.1)	1.73	—	2.14
6	6.42 d (10.1)	5.37 dd[b]	5.30 d (2.5)	5.63 s	5.06 d (6.1)	1.76	—	2.15
7	6.00 d (11.2)	4.03 dd (3.2, 11.2)	5.23 d (3.2)	5.54 s	5.04 d (6.6)	—	2.33	2.13
8	6.46 d (11.2)	5.45 dd (2.8, 11.2)	5.38 d (2.8)	5.60 s	5.09 d (5.9)	—	2.27	2.16
9	6.34 d (11.2)	5.32 dd (2.9, 11.2)	5.24 d (2.9)	5.73 s	5.09 d (6.0)	1.75	2.34	—
10	6.24 d (11.2)	5.31 dd (2.4, 11.2)	3.85 d (2.4)	—	5.07 d (6.0)	1.84	—	—
11	6.27 d (10.9)	5.30 dd (2.8, 10.9)	5.27 d[b] (2.8)	—	5.03 d (5.8)	1.73	2.29	—
12	4.21 d (10.4)	3.64 m[b]	3.64 m[b]	—	5.17 d (4.8)	—	—	—
13	5.94 d (10.8)	3.86 m[b]	3.86 m[b]	—	5.08 d (5.2)			

[a] The data were confirmed by COSY experiments. [b] Overlapping signals.

Table 2 ^{13}C NMR spectroscopic data for compounds **1**, **9**, **10** and **11** (50 MHz, $CDCl_3$)[a]

	δ_C			
	1	**9**	**10**	**11**
C-1	67.91	67.97	68.20	69.01
C-2	70.51	68.57	71.08	70.02
C-3	77.84	76.82	77.79	75.95
C-4	71.33	70.71	70.98	68.12
C-5	92.63	91.34	92.14	89.89
C-6	80.21	80.85	31.08	30.30
C-7	48.28	48.51	42.46	42.97
C-8	31.66	31.21	32.40	32.75
C-9	73.04	72.72	73.67	73.36
C-10	51.85	51.36	48.40	47.96
C-11	86.32	85.41	85.64	84.11
C-12	29.57	30.05	29.84	29.99
C-13	26.00	26.25	24.53	24.47
C-14	24.18	23.98	23.95	24.01
C-15	20.87	20.73	20.92	19.92

[a] Values based on ^1H–^{13}C, long-range, correlation and DEPT experiments

Table 3 Three-bond ^1H–^{13}C couplings in compound **1**

1-H	C-2,[a] C-9, C-10, C-15, O-*CO*-C_6H_5
2-H	C-1,[a] O-*CO*-Me
3-H	C-1, C-2,[a] C-5
6-H	C-5,[a] C-7,[a] C-8, C-10, C-11, O-*CO*-Me
9-H	C-5, C-7, C-8,[a] C-10[a], C-15, O-*CO*-C_6H_5

[a] Two bond coupling enhancement observed.

The natural product **7**, when treated with acetic anhydride in pyridine, afforded a product identical to **4** thus establishing its absolute configuration; when **7** was benzoylated, the tribenzoate **8** was obtained and its spectral and analytical data (see Table 1) agree with the structure proposed.

Table 4 Circular dichroism data for compounds **1**, **5**, **6**, **9** and **13** (MeCN)

Compound	$\lambda_{ext}/\Delta\varepsilon$	$\lambda_{ext}/\Delta\varepsilon$	$\lambda_{ext}/\Delta\varepsilon$	$\Delta\varepsilon = 0$
1	237.3/21.1	220.0/−8.0		227.3
5	227.8/−14.1			
6	239.8/13.2	224.8/−9.0	306.0/−12.7	251.0, 232.2
9	236.6/16.0	218.6/−4.9		226.2
13	236.5/21.1	221.3/−9.7		227.5

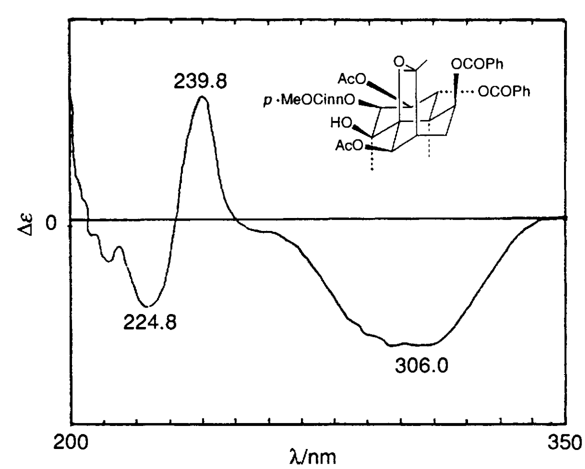


Fig. 3 CD spectra of compound **6**

A CD study aided the structural elucidation of the metabolite **9**. The analytical and spectral data of **9** indicated that its structure was isomeric to those of the tribenzoates **5** and **8**. The CD curve of **9** had a first positive Cotton effect at 236.6 nm ($\Delta\varepsilon$ +16.0) and a second negative one at 218.6 ($\Delta\varepsilon$ −4.9). From a comparison of the CD curves of compounds **1** and **9** it was inferred that the lesser intensities of the Cotton effects for the latter derived from the weak, negative 1,6 interaction since the 6,9 pairwise interaction was null due to the coplanarity of the benzoyl groups (Table 4).

Compounds **10** and **11** were related to each other as the acetylation of **10** led to **11**. These compounds were extraordinarily similar to products **1** and **4**; analysis of their analytical and spectroscopic data show them to be 6-deoxyacetyl-**1** and -**4**, respectively (see Table 1).

The CD curve of the hydrolysis product **13**, a 1,9-dibenzoate obtained together with **12** when compound **10** was subjected to treatment with 0.1 mol dm^{-3} $NaHCO_3$, showed a split curve with a first positive Cotton effect at 236.5 nm ($\Delta\varepsilon$ +21.1) and a second negative one at 221.3 nm ($\Delta\varepsilon$ −9.7) so that the absolute configuration of all these compounds could be deduced (Table 4).

The basic polyhydroxy skeleton of the natural compounds **1**, **4**, **7** and **9** exhibited a new type of substitution and we suggest that this polyol be designated magellanol **14** while products **10** and **11** must therefore have the basic polyhydroxy skeleton of 6-deoxymagellanol.

Although the compounds described above show some degree of complexity, none of the metabolites obtained from the Celastraceae have anything like the fantastic complexity of those isolated and elucidated by the team at the University of Nottingham[20] in their wide-ranging and interesting work on the subject, which suggests that perhaps the single-species genus *Catha edulis*,[21] by virtue of its very uniqueness, generates highly individual metabolites.

Experimental

M.p.s are uncorrected. IR spectra were taken on a PE 681 spectrophotometer and ^1H and ^{13}C NMR spectra on a Bruker WP-200 SY in $CDCl_3$ (at 200 and 50 MHz, respectively) while the HMBC was taken on a Bruker at 400 MHz. *J* Values are given in Hz. Optical rotations were measured on a Perkin-Elmer 241 automatic polarimeter $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}; mass spectra were recorded on a VG Micromass LTD-ZAB-2F and/or on an HP 5930 A at 70 eV. UV spectra were run on a Perkin-Elmer 550-SE and CD spectra on a Jasco J-600 spectropolarimeter.

Plant Collection.—The plant was gathered in January 1987 in the Novena region, in the province of Temuca, on the slopes of the volcano Osorno in Chile and a voucher specimen is on file with the Facultad de Ciencias, Universidad de Chile, Santiago.

Extraction and Isolation.—The aerial part of the plant (4 kg) was extracted with EtOH (10 dm^3) at room temp. for a week. The extract (250 g) was repeatedly chromatographed to afford the following products: **1** (80 mg); **4** (8 mg); **7** (10 mg); **9** (28 mg); **10** (60 mg) and **11** (24 mg).

(1R,2S,3S,4S,5S,6R,7R,9S,10R)-2β,6β-*Diacetoxy*-1α,9β-*dibenzoyloxy*-3β,4β-*dihydroxydihydro*-(15α)-β-*agarofuran* **1**. This compound was isolated as a crystalline solid: m.p. 114–116 °C; $[\alpha]_D^{20}$ +18.0 (*c* 0.11, MeOH); $\nu_{max}(CHCl_3)/cm^{-1}$ 3500, 3490, 3000, 2950, 1730, 1720, 1450, 1360, 1280, 1240, 1170, 1130, 1100, 1020 and 710; λ_{max}(EtOH)/nm 272, 230 and 200; δ_H 1.49 (6 H, s), 1.57 (3 H, s), 1.61 (3 H, s), 2.20 (1 H, m), 2.45 (1 H, m) and 7.23–7.96 (10 H, m), for other signals see Table 1; δ_C (see Table 2); *m/z* (%) 550 (M^+ − $MeCO_2H$, 1), 490 (1), 446 (1), 428 (1), 410 (2), 386 (1), 368 (1), 325 (1), 306 (2) and 105 (100) (Found: M^+, 610.2407. $C_{33}H_{38}O_{11}$ requires *M*, 610.2401).

9β-*Benzoyloxy*-1α,2β,3β,4β,6β-*pentahydroxydihydro*-(15α)-β-*agarofuran* **2**. Compound **1** (25 mg) was dissolved in MeOH (5 cm^3) and $NaHCO_3$ (0.1 mol dm^{-3}; 2 cm^3) was added. The mixture was heated at 60 °C for 3 h, stirred and left to cool, evaporated almost to dryness and extracted with EtOAc, to give compound **2** (12 mg) as an oil after chromatography; δ_H 1.28 (3 H, s), 1.55 (3 H, s), 1.58 (3 H, s), 1.62 (3 H, s), 2.20 (2 H, overlapping signals), 7.51 (3 H, m) and 8.03 (2 H, m), for other signals see Table 1; *m/z* (%) 407 (M^+ − Me, 42), 389 (19), 371 (1), 317 (1), 300 (1), 285 (9), 267 (6), 249 (9) and 105 (100).

2β,6β-(*Diacetoxy*-1α,9β-*benzoyloxy*-4β-*hydroxy*-3-*oxodihydro*-(15α)-β-*agarofuran* **3**. Compound **1** (40 mg) was dissolved in acetone (7 cm^3) and freshly prepared Jones' reagent (4 drops) was added at room temp. while stirring; excess reagent was destroyed by adding a few drops of isopropyl alcohol and the reaction product was extracted to afford compound **3** (39.6 mg); δ_H 1.50 (3 H, s), 1.63 (3 H, s), 1.85 (3 H, s), 1.98 (3 H, s), 2.21 (1 H, m), 2.50 (1 H, m), 3.55 (1 H, s) and 7.26–7.90 (10 H, m), for other signals see Table 1; *m/z* (%) 608 (M^+, 1), 593 (1), 548 (3), 530 (1), 486 (3), 471 (8), 444 (6), 443 (2), 426 (4), 384 (4), 279 (9) and 105 (100) (Found: M^+, 608.2213. $C_{33}H_{36}O_{11}$ requires *M*, 608.2169).

(1R,2S,3S,4S,5S,6R,7R,9S,10R)-2β,3β,6β-*Triacetoxy*-1α,9β-*dibenzoyloxy*-4β-*hydroxydihydro*-(15α)-β-*agarofuran* **4**. This compound was isolated as an oil: $[\alpha]_D^{20}$ +143.4 (*c* 0.05, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3448, 3018, 2359, 2339, 2099, 1733, 1637, 1452, 1368, 1283, 1245, 1219, 1178, 1096 and 1025; λ_{max}(EtOH)/nm 286, 274 and 240; δ_H 1.49 (3 H, s), 1.54 (3 H, s), 1.55 (3 H, s), 1.60 (3 H, s), 2.25 (1 H, m), 2.45 (1 H, m), 3.61 (1 H, s) and 7.23–7.97 (10 H, m), for other signals see Table 1; *m/z* (%) 637 (M^+ − Me, 1), 592 (2), 550 (6), 515 (10), 470 (2), 455 (2), 428 (7), 410 (4), 368 (3) and 105 (100) (Found: M^+, 637.2311. $C_{34}H_{37}O_{12}$ requires *M*, 637.2337).

2β,6β-*Diacetoxy*-1α,3β,9β-*tribenzoyloxy*-4β-*hydroxydihydro*-(15α)-β-*agarofuran* **5**. When compound **1** (12 mg) was treated with benzoyl chloride in pyridine overnight, worked up and purified by chromatography, compound **5**, (6 mg) was obtained as an amorphous solid: $[\alpha]_D^{20}$ −27.9 (*c* 0.12, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3520, 2945, 2920, 1720, 1595, 1445, 1365, 1280, 1230, 1110 and 710; λ_{max} (EtOH)/nm 280, 273 and 227; δ_H 1.54 (3 H, s), 1.59 (3 H, s), 1.61 (3 H, s), 1.67 (3 H, s), 2.32 (1 H, m), 2.48 (2 H, m), 3.62 (1 H, s) and 7.26–8.47 (15 H, m), for other signals see Table 1; *m/z* (%) 714 (M^+, 1), 699 (1), 654 (1), 577 (4), 532 (2), 490 (1), 475 (1) and 105 (100) (Found: M^+, 714.2688. $C_{40}H_{42}O_{12}$ requires *M*, 714.2700).

2β,6β-*Diacetoxy*-1α,9β-*dibenzoyloxy*-4β-*hydroxy*-3β-p-*methoxycinnamoyloxydihydro*-(15α)-β-*agarofuran* **6**. Compound **1** (8 mg) was refluxed with an excess of *p*-methoxycinnamoyl chloride acid in pyridine and the mixture worked up and purified, to yield compound **6** (4 mg); δ_H 1.49 (3 H, s), 1.57 (3 H, s), 1.60 (3 H, s), 1.65 (3 H, s), 3.60 (1 H, s), 3.90 (3 H, s), 6.44–8.10 (2 H, d_{AB}, *J* 15.8), 6.99–7.62 (4 H, d_{AB}, *J* 8.8) and 7.19–7.54 (10 H, m), for other signals see Table 1.

(1R,2S,3S,4S,5S,6R,7R,9S,10R)-3β,6β-*Diacetoxy*-1α,9β-*dibenzoyloxy*-2β,4β-*dihydroxydihydro*-(15α)-β-*agarofuran* **7**. This compound was isolated as a thick oil: $[\alpha]_D^{20}$ +47.3 (*c* 0.11, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3532, 3025, 2958, 2924, 2857, 1733, 1446, 1370, 1286, 1243, 1108, 1015 and 710; λ_{max}(EtOH)/nm: 288, 278 and 230; $\delta_H(CHCl_3 + D_2O)$ 1.47 (6 H, s), 1.54 (3 H, s), 1.56 (3 H, s), 2.29 (1 H, m), 2.48 (1 H, m) and 7.21–7.77 (10 H, m), for other signals see Table 1; *m/z* (%) 610 (M^+, 1), 595 (2), 577 (3), 550 (5), 532 (2), 473 (2), 428 (5), 410 (2), 386 (2), 249 (2) and 105 (100) (Found: M^+, 610.2390. $C_{33}H_{38}O_{11}$ requires *M*, 610.2367).

3β,6β-*Diacetoxy*-1α,2β,9β-*tribenzoyloxy*-4β-*hydroxydihydro*-(15α)-β-*agarofuran* **8**. This compound (1.8 mg) was obtained from compound **7** (2.1 mg) by benzoylation; δ_H 1.50 (3 H, s), 1.61 (3 H, s), 1.66 (3 H, s), 3.65 (1 H, s) and 7.15–7.98 (15 H, m), for other signals see Table 1; *m/z* (%) 700 (M^+ − Me − H^+, 1), 577 (3), 430 (2), 415 (2), 368 (4), 257 (6) and 105 (32) (Found: M^+, 577.2081. $C_{32}H_{33}O_{10}$ requires *M*, 577.2089).

(1R,2S,3S,4S,5S,6R,7R,9S,10R)-2β,3β-*Diacetoxy*-1α,6β,9β-

tribenzoyloxy-4β-*hydroxydihydro*-(15α)-β-*agarofuran* **9**. This compound was isolated as an oil; $[\alpha]_D^{20}$ +69.6 (*c* 0.33, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3530, 2920, 1710, 1595, 1445, 1275, 1250, 1110 and 710; λ_{max}(EtOH)/nm 280, 271 and 228; δ_H 1.52 (3 H, s), 1.56 (3 H, s), 1.57 (3 H, s), 1.65 (3 H, s), 2.40 (1 H, m), 2.57 (1 H, m), 3.84 (1 H, s) and 7.26–8.24 (15 H, m), for other signals see Table 1; δ_C (see Table 2); *m/z* (%) 714 (M^+, 1), 699 (1), 654 (1), 612 (1), 577 (3), 532 (1), 490 (1), 410 (2), 368 (2) and 105 (100) (Found: M^+, 714.2701. $C_{40}H_{42}O_{12}$ requires *M*, 714.2727).

(1R,2S,3S,4S,5R,7R,9S,10R)-2β-*Acetoxy*-1α,9β-*dibenzoyloxy*-3β,4β-*dihydroxydihydro*-(15α)-β-*agarofuran* **10**. This compound could not be crystallized with any of the usual solvents; $[\alpha]_D^{20}$ +71.81 (*c* 0.19, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3500, 3450, 2980, 2910, 1740, 1710, 1590, 1450, 1370, 1368, 1310, 1270, 1120, 1110, 1020, 1000, 750 and 710; λ_{max}(EtOH)/nm 277, 224 and 197; δ_H 1.36 (3 H, s), 1.44 (3 H, s), 1.45 (3 H, s), 1.51 (3 H, s), 3.42 (1 H, s), 3.85 (1 H, d, *J* 2.4) and 7.26–8.01 (10 H, m), for other signals see Table 1; δ_C (see Table 2); *m/z* (%) 552 (M^+, 11), 537 (3), 492 (1), 477 (1), 430 (4), 388 (2), 370 (4), 355 (1), 266 (4), 248 (7) and 105 (100) (Found: M^+, 552.2361. $C_{31}H_{36}O_9$ requires *M*, 552.2363).

(1R,2S,3S,4S,5R,7R,9S,10R)-2β,3β-*Diacetoxy*-1α,9β-*dibenzoyloxy*-4β-*hydroxydihydro*-(15α)-β-*agarofuran* **11**. This compound was isolated as an oil; $[\alpha]_D^{20}$ +84.2 (*c* 0.6, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3525, 3029, 2920, 2364, 2315, 2295, 1746, 1725, 1706, 1631, 1448, 1368, 1279, 1239, 1115, 1065, 1021, 887, 756 and 709; λ_{max}(EtOH)/nm 281, 271 and 227; δ_H 1.37 (3 H, s), 1.44 (3 H, s), 1.48 (6 H, s), 3.44 (1 H, s) and 7.24–8.02 (10 H, m), for other signals see Table 1: *m/z* (%) 594 (M^+, 2), 579 (14), 534 (1), 492 (1), 457 (14), 412 (3), 370 (14), 248 (15) and 105 (100) (Found: M^+, 594.2456. $C_{33}H_{38}O_{10}$ requires *M* 594.2448).

9β-*Benzoyloxy*-1α,2β,3β,4β-*tetrahydroxydihydro*-(15α)-β-*agarofuran* **12**. Compound **10** (30 mg) was hydrolysed with $NaHCO_3$ (0.1 mol dm^{-3}; 2 cm^3) in MeOH (5 cm^3) at room temp. stirred for 20 min, taken almost to dryness and extracted with EtOAc and purified, to give compounds **12** (5 mg) and **13** (6 mg) as major products, δ_H 1.21 (3 H, s), 1.31 (3 H, s), 1.35 (3 H, s), 1.49 (3 H, s), 3.24 (1 H, s), 7.52 (3 H, m) and 8.06 (2 H, m), for other signals see Table 1; *m/z* (%) 406 (M^+, 3), 391 (55), 373 (15), 284 (4), 266 (14) and 105 (100).

1α,9β-*Dibenzoyloxy*-2β,3β,4β-*trihydroxydihydro*-(15α)-*agarofuran* **13**. δ_H 1.25 (3 H, s), 1.37 (3 H, s), 1.45 (3 H, s), 1.47 (3 H, s), 3.33 (1 H, s) and 7.23–7.87 (10 H, m); *m/z* (%) 510 (M^+, 3), 492 (5), 477 (12), 459 (1), 388 (7), 370 (3), 266 (4) and 105 (100).

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References

1 A. G. González, A. G. Ravelo, I. L. Bazzocchi, J. J. Mendoza, C. M. González, J. G. Luis, E. A. Ferro, A. Gutiérrez, L. Moujir and F. G. de las Heras, *Il Farmaco*, 1983, **43**, 501.
2 A. G. González, C. M. González, A. G. Ravelo, A. Gutiérrez, E. Navarro and J. Boada, *Rev. Latinoamer. Quim.*, 1988, **19**, 36.
3 L. Moujir, A. M. Gutiérrez-Navarro, A. G. González, A. G. Ravelo and J. G. Luis, *Antimicrob. Agents Chemother.*, 1991, **35**, 211.
4 A. Lourteig and C. A. O'Donnell, *Natura*, 1955, **1**, 181.
5 B. N. Meyer, N. R. Ferrigni, J. E. Putnam, L. B. Jacobsen, D. E. Nichols and J. L. McLaughlin, *Planta Medica*, 1982, **45**, 31.
6 M. P. Nuñez, Ph.D. Thesis, Universidad de La Laguna, 1991.
7 S. M. Kupchan and R. M. Smith, *J. Org. Chem.*, 1977, **42**, 115.
8 A. A. Sánchez, J. Cafdenas, M. S. García, R. Toscano and L. Rodríguez, *Phytochemistry*, 1986, **25**, 2647.
9 I. Q. Taylor, Jr. and W. H. Watson, *Acta Crystallogr., Sect. B*, 1977, **33** 3176.
10 Y. Shizuri, H. Wada, K. Yamada and Y. Hirata, *Tetrahedron*, 1973, 1795.
11 C. R. Smith, Jr., R. W. Miller, D. Weisleder, W. K. Rohwedder, N. Eickman and J. Clardy, *J. Org. Chem.*, 1976, **41** 3264.
12 H. J. den Hertog, Jr., C. Kruk, D. D. Nanavati and S. Dev, *Tetrahedron Lett.*, 1974, **26**, 2219.
13 H. Lotter, R. Brüning and H. Wagner, *Tetrahedron Lett.*, 1978, **35**, 3243.
14 R. Brüning and H. Wagner, *Phytochemistry*, 1978, **17**, 1821.
15 A. Bax and M. F. Summers, *J. Am. Chem. Soc.*, 1986, **108**, 1093.
16 Software AMX 400, Bruker Instruments.
17 N. Harada and K. Nakanishi, *Circular Dichroism Spectroscopy Exciton Coupling in Organic Stereochemistry*, University Science Books, Mill Valley, CA, 1983.
18 N. Harada and K. Nakanishi, *J. Am. Chem. Soc.*, 1969, **91**, 2989.
19 PC Model from Serena Software, PO Box 3076, Bloomington, Indiana 47402–3076.
20 L. Crombie, D. Toplis, D. A. Whiting, Z. Rozsá, J. Hahmann and K. Szundrei, *J. Chem. Soc., Perkin Trans. 1*, 1986, 53 and references therein.
21 T. Loesener *Die Natürlichen Pflanzefamilien III* (5), eds. A. Engler and K. Prantl, Leipzig, 1978, p. 189.

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