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New Sesquiterpenes With Antifeedant Activity from *Maytenus canariensis* (Celastraceae)

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Abstract· Five new sesquiterpenes with a dihydro-β-agarofuran skeleton were isolated from *Maytenus canariensis* (Celastraceae) and identified. Their structures and absolute configurations were determined by means of chemical correlations, ^{1}H and ^{13}C NMR studies, bidimensional ^{1}H-^{1}H and ^{1}H-^{13}C correlations, NOE experiments, selective INEPT, and CD studies. The new sesquiterpenes were assayed against *Spodoptera littoralis* in an election test and exhibited insect antifeedant activity

Our earlier work on *Maytenus canariensis* (Loes) Kunk et Sund[1] in the context of an intensive study of bioactive metabolites from the Celastraceae, yielded dihydro-β-agarofuran sesquiterpenes with polyhydroxy isoalatol and 4β-hydroxyalatol skeletons[2,3], triterpenes[4,5], and nor-triterpene methylene quinones[6] showing antitumoral activity[7] This species exhibits the characteristic Celastraceae[8] lack of selectivity in the biosynthesis of triterpenes metabolites with ursan, olean, lupan and friedelan skeletons were obtained[2-6] On the other hand, it is specific in biosynthesizing dihydro-β-agarofuran skeleton sesquiterpenes[8].

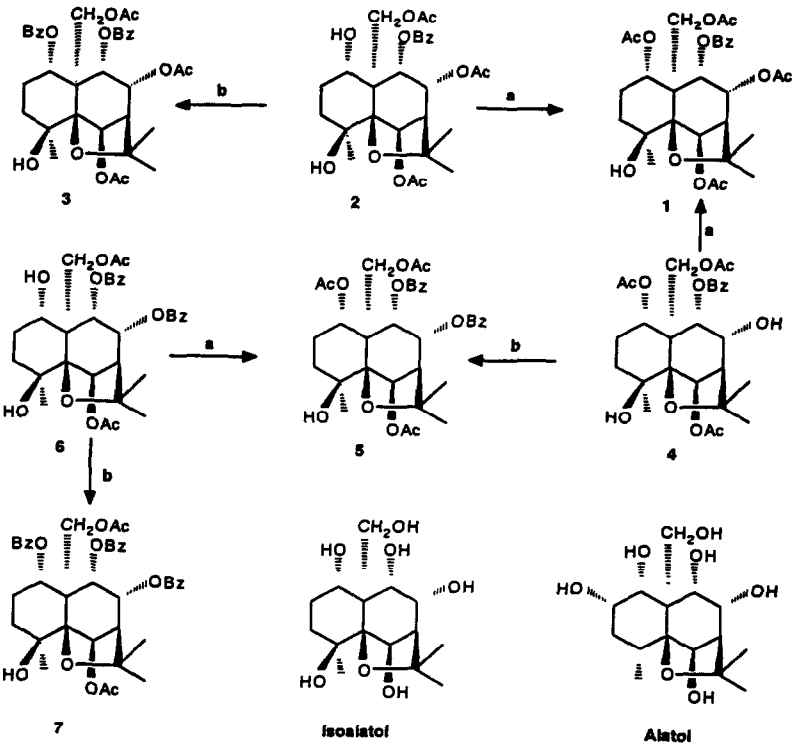
It has been known for quite a while that some species of Celastraceae, such as *Tripterigium wilfordii*[9] and *Celastrus angulatus*[10], safeguard some vegetable species against attack by a wide range of insects Their aerial parts are finely ground, suspended in water and sprayed over the crop to be protected The sesquiterpene celangulin, which has the same sort of skeleton as the products reported here, has shown antifeedant activity against *Spodoptera frugiperda*[10]

This paper reports the isolation and structural elucidation of five new polyester sesquiterpenes and a new semi-synthetic substance Absolute configurations were determined by CD studies, the results of which were on the same lines as those for celorbicol[11] and resolved the doubts raised by the study[12] and later correction of the absolute configuration[13] of malkanguniol It is to be regretted that many studies of compounds of this type have been made without the absolute configuration being determined[8] The new compounds were assayed for antifeedant activity against *Spodoptera littoralis* in an election test[14,15].

After repeated chromatography on Sephadex LH-20 and silica gel, five new metabolites (**1**, **2**, **4**, **6** and **7**) were isolated (see Scheme 1) Compound **1** was assigned the structure and configuration 1S,4S,5S,6R,7R,8R,9S,10S-9-benzoyloxy-1,6,8,15-tetra-acetoxy-4-hydroxydihydro-β-agarofuran for the folowing reasons based on its ^{1}H, ^{13}C NMR and high resolution mass spectral data it was assigned the formula $C_{30}H_{38}O_{12}$, its ^{1}H NMR spectrum (see Table 1) displayed four acetate methyls as singlets at δ 2 31, 2 13, 2 06 and 1 46 and in ^{13}C NMR, signals appeared at 170 65 and 169 96 x 3 for four carboxy carbons, there were signals for five protons at δ 7 49 (3H, m), and 8 04 (2H, m) in the aromatic region of the ^{1}H NMR spectrum,

and a signal for a conjugated carboxy carbon was observed at 164.85 in the ^{13}C NMR spectrum (see Table 2) Treatment of 1 with acetic anhydride and pyridine at room temperature yielded unaltered starting material still exhibiting an IR absorption band for a hydroxy group These data pointed to the presence of four acetate groups, a benzoate group and an alcohol on a tetra-substituted carbon in the molecule Compound 1 thus clearly possessed a dihydro-β-agarofuran skeleton. The substituent positions were determined as 1α, 4β, 6β, 8α, 9α and 15 from a careful study of the coupling constants[8] and double resonance experiments (see Table 1) and were confirmed by a NOE difference experiment involving the irradiation of H-6 and Me-12 (see Figure 1)

Scheme 1



In order to determine the regiosubstitution characteristics, a selective INEPT experiment (see Table 3) was carried out[16] which established the position of the benzoate group in the molecule. All the protonated carbon atoms could be assigned by DEPT and HETCOR spectra and the J-modulated selective INEPT technique, whereby a particular proton is irradiated with a soft proton pulse, resulting in magnetization transfer and selective enhancement of carbon atoms three bonds away from the irradiated proton Whenever an acetate is found facing a benzoate in positions C-1, C-9 or vice versa, the acetate methyl must undergo a shift, in this case to δ 1 45, due to the magnetic anisotropy generated by the aromatic moiety of the benzoate group Accordingly, H-1 and H-9 were irradiated (see Table 3) These data unambiguously established the structure of this compound as that proposed for 1, while the absolute configuration was determined by study of the CD curve of product **6** which was chemically correlated with 1 (see Scheme 1)

Table 1: ^{1}H NMR (200 MHz) Data (δ, $CDCl_3$) of 1-7 (J are given in Hz in the brackets)

	1	2	3	4	5	6	7
H-1	5.32 dd (4 4,11 8)	4 21 m (4 0,12.5)	5.56 dd (4 0,12.1)	5.31 dd (4.3,11 1)	5.38 dd	4 20 m	5 69 dd (4 4,11 5
OH-4	2.65 s	2.70 s	2.70 s	2.70 s	2.70 s	2.75 s	2 74 s
H-6	6 83 s	6.31 s	6 86 s	6 76 s	6 97 s	6 41 s	6 99 s
H-7	2.38 d (4 0)	2.44 d (4 2)	2.37 d (4 1)	2.36 d (4 4)	2.59 d (3 3)	2.59 d (4 0)	2.60 d (4 0)
H-8	5.57 dd (4 0,5 8)	5.57 dd (4 2,5 6)	5.54 dd (4 1,5 8)	4 40 m	5 80*	5 83 dd (4 0,5.5)	5 79 dd (4 0,5 8)
H-9	5 67 d (5 8)	5 84 d (5 6)	5 76 d (5 8)	5.58 d (5 7)	5 80*	5 97 d (5 5)	5 90 d (5 8)
H-15	4.52-5 02 d_{AB}(13 4)	4 81-4 97 d_{AB}(13 0)	4 72-5 22 d_{AB}(13 3)	4 63-4 95 d_{AB}(13 0)	4 74-4 84 d_{AB}(13 3)	4 86-5 04 d_{AB}(12.8)	4 91-5 0 d_{AB}(13 0)
Me-12	1 66 s	1 68 s	1 68 s	1.57 s	1 73 s	1 72 s	1 74 s
Me-13	1.55 s	1.53 s	1.57 s	1.54 s	1 60 s	1.58 s	1 62 s
Me-14	1 37 s	1 41 s	1 41 s	1.36 s	1 36 s	1 39 s	1 40 s
-OOC-Me							
1	1 45 s			1 47 s	1 49 s		
6	2.13 s	2 14 s	2 14 s	2 12 s	2 04 s	1 97 s	2.08 s
8	2 05 s	2 13 s	2.04 s				
15	2.31 s	2 17 s	2.35 s	2 33 s	2 15 s	2 13 s	2 16 s

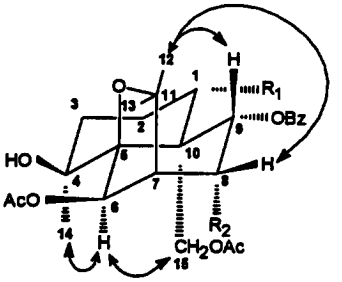
* Superimposable

Table 2. ^{13}C NMR (50 MHz) Data (δ, $CDCl_3$) of 1, 3 and 7

	1	3	7
1	78 30 d	78.59 d	78.69 d
2	25 00 t	25 17 t	25 31 t
3	37 87 t	37 94 t	38 05 t
4	70 59 s	70 59 s	70 79 s
5	92.09 s	92.20 s	92.25 s
6	75 28 d	75 45 d	75 42 d
7	53 24 d	53 24 d	53 58 d
8	70 22 d	70 17 d	71 18 d
9	72.72 d	72.67 d	72.99 d
10	52.18 s	52.67 s	52.52 s
11	82,71 s	82.68 s	82,90 s
12	24 41 q	24 27 q	24 53 q
13	29 35 q	29 45 q	29.62 q
14	22.77 q	22.75 q	22.68 q
15	60 52 t	60 58 t	61 37 t

Data are based on ^{1}H-^{13}C bidimensional experiments

Figure 1: n O e experiment of 1, 3 and 7



	R_1	R_2
1	OAc	OAc
3	OBz	OAc
7	OBz	OBz

Table 3: Selective INEPT Data for **1** and **7**

Compound	Irradiated protons δ_H	Observed carbons δ_C
1	H-1 (5 32)	-OOC-CH_3 (170 65), C-10* (52 18), C-15 (60 52)
	H-9 (5 67)	-OOC-C_6H_5 (164 85), C-1 (78.30), C-10* (52 18), C-15 (60.52)
7	H-6 (6 99)	-OOC-CH_3 (169 99), C-11 (82 90)
	H-15 (4 96)	-OOC-CH_3 (171 16), C-5 (92 25), C-9 (72 99), C-10* (52.52)

* Two-bond coupling enhancement observed

The CD spectrum[17] of **6** showed a split-curve with a first negative Cotton effect at 236 9 nm ($\Delta\varepsilon$= -13 1) and a second positive Cotton effect at 219 0 nm ($\Delta\varepsilon$= +8 3) due to the coupling of the two benzoate chromophores on C-8α and C-9α Thus, the proposed structure was confirmed and the absolute configuration shown to be that given in the figure The chemically-correlated natural products **1**, **2** and **4** and the semi-synthetic **5** all proved to have the same configuration (see Table 1 and Scheme 1)

The natural products **2**, **4**, **6** and **7** were correlated both with each other and with product **1** as shown in the scheme 2 Product **2** could be acetylated to give compound **1** and benzoylated to form product **3** which we had already obtained earlier[2] Acetylation of the natural product **4** gave a product identical to **1** while benzoylation gave product **5** identical to that obtained when compound **6** was acetylated When **6** was benzoylated, it afforded **7**

A selective INEPT study of compound **7** (see Table 3) showed that the three benzoate chromophore groups were in the positions 1, 8, 9 and the acetates at 6 and 15, a NOE difference experiment on H-6 and Me-12 confirmed the relative configuration of the molecule while its absolute configuration was established by studying its CD curve A split-curve was observed with a first negative Cotton effect at 235 1 nm ($\Delta\varepsilon$= -26 4) and a second positive Cotton effect at 219 8 nm ($\Delta\varepsilon$= +15 1) Comparison of these effects with those of the CD curve of compound **6** revealed that they were almost twice as broad, which is only possible if the chromophore groups are in the positions 1α, 8α, 9α The absolute configuration of **7** was determined accordingly The basic polyhydroxy skeleton of compounds **1**, **2**, **4**, **6** and **7** is that of isoalatol[2], an isomer of alatol[18]

The five new products were assayed in order to determine their antifeedant activity on larvae of the Egyptian cottonleaf worm *Spodoptera littoralis* (Boisduval), using the leaf disk method[14,15] Activity at concentrations of 10 μg/cm^2 was observed

EXPERIMENTAL

The plant was collected in Icod, Tenerife in October 1986 and a voucher specimen is on file with the Departamento de Biología Vegetal, Facultad de Ciencias Biológicas, Universidad de La Laguna.

The aerial part of the plant (7 kg) was extracted with cold EtOH and 100 g of this extract was chromatographed on Sephadex LH-20 using n-hexane-$CHCl_3$-MeOH (2 1 1) as eluant, followed by repeated chromatographed on silica gel with mixtures of n-hexane-Et_2O, n-hexane-EtOAc and n-hexane-dioxan to give **1** (50 mg), **2** (7 mg), **3** (52 mg), **4** (8 mg), **5** (5 mg), **6** (7 mg) and **7** (23 mg) IR data were taken in $CHCl_3$ The products used for CD curves were purified by HPLC using a semi-preparative μ Porosil column and mixtures of n-hexane-EtOAc and n-hexane-dioxan as eluants

1S,4S,5S,6R,7R,8R,9S,10S-9-Benzoyloxy-1,6,8,15-tetra-acetoxy-4-hydroxy-dihydro-β-agarofuran (1) - Was obtained as a white amorphous solid, mp 75-76° (from $CHCl_3$), $[\alpha]_D^{25}$= -21.7° ($CHCl_3$, c= 0.35), UV λ_{max} (EtOH) nm 280, 274, IR ν_{max} cm^{-1} 3561, 3013, 1737, 1700, 1370, 1277, 1245, 1233, 1093, 790, 732,

^{1}H NMR ($CDCl_3$) δ: 7 49 (3H, m, Ar), 8 04 (2H, m, Ar), for other signals, see Table 1; EIMS m/z (%). 530 [M^+ - 60] (29), 488 (5), 470 (1), 428 (1), 366 (1), 288 (1), 246 (7), 105 (100); HREIMS [M^+ - HOAc] at m/z 530 2137 (calc. for $C_{28}H_{34}O_{10}$, 530.2123).

1S,4S,5S,6R,7R,8R,9S,10S-9-Benzoyloxy-6,8,15-triacetoxy-1,4-dihydroxy-dihydro-β-agarofuran (2) - Was obtained as an oil, $[\alpha]_D^{25}$= +25.0º ($CHCl_3$, c= 0.22), UV λ_{max} (EtOH) nm· 279, 270, IR ν_{max} cm^{-1} 3480, 2990, 1760, 1740, 1710, 1360, 1270, 1240, 1110, 1090, 1040, 750, 710, ^{1}H NMR ($CDCl_3$) δ. 7.46 (3H, m, Ar), 7 97 (2H, m, Ar), for other signals, see Table 1, EIMS m/z (%) 530 [M^+ - 18] (6), 488 (2), 470 (1), 446 (1), 430 (1), 428 (2), 368 (2), 366 (2), 328 (9), 268 (5), 246 (7), 224 (4), 164 (47), 105 (100).

Acetylation of 2 - Acetic anhydride (4 drops) was added to compound **2** (3 mg) dissolved in pyridine (2 drops) and the mixture was left at room temperature for 16 hours, EtOH (3 X 2 0 ml) was added and carried almost to dryness in a rotavapor and this process was repeated with C_6H_6 (3 X 2.0 ml), to give product **1** (3 mg)

Benzoylation of 2 - Compound **2** (4 mg) was dissolved in dry pyridine (0.5 ml) and benzoyl chloride (6 drops) and some crystals of 4-dimethylamino-pyridine were added under argon atmosphere The mixture was heated at 60ºC for 15 hours, poured over water, extracted with EtOAc and purified on a preparative column on silica gel with a mixture of C_6H_6-EtOAc, to give compound **3** (3 5 mg)

1S,4S,5S,6R,7R,8R,9S,10S-9-Benzoyloxy-1,6,15-triacetoxy-4,8-dihydroxy-dihydro-β-agarofuran (4) - Was obtained as an oil, $[\alpha]_D^{25}$= -23.3º ($CHCl_3$, c= 0.25), UV λ_{max} (EtOH) nm 280, 270, IR ν_{max} cm^{-1} 3450, 2920, 1735, 1725, 1365, 1270, 1225, 750, 710, ^{1}H NMR ($CDCl_3$) δ: 7 50 (3H, m, Ar), 8.05 (2H, m, Ar), for other signals, see Table 1, EIMS m/z (%) 530 [M^+ - 18] (9), 488 (12), 470 (1), 428 (5), 410 (1), 368 (2), 328 (4), 306 (4), 246 (16), 105 (100), HREIMS [M^+ - H_2O] at m/z 530.2102 (Calc for $C_{28}H_{34}O_{10}$, 530.2098)

Acetylation of 4 - Compound **4** (3 mg) was treated under the same conditions as described above, to give **1** (3 mg)

Benzoylation of 4 - Compound **4** (5 mg) was treated as described above and afforded compound **5** (4 mg)

1S,4S,5S,6R,7R,8R,9S,10S-8,9-Dibenzoyloxy-1,6,15-triacetoxy-4-hydroxy-dihydro-β-agarofuran (5) - Was obtained as an oil when compound **4** was benzoylated or compound **6** was acetylated $[\alpha]_D^{25}$= -54 4º ($CHCl_3$, c= 0 25), UV λ_{max} (EtOH) nm 280, 270, IR ν_{max} cm^{-1}· 3564, 2930, 2854, 1736, 1602, 1369, 1317, 1227, 1094, 759, 712, ^{1}H NMR ($CDCl_3$) δ 7 42 (6H, m, Ar), 7.96 (4H, m, Ar), for other signals, see Table 1; EIMS m/z (%) 592 [M^+ - 60] (3), 588 (2), 546 (1), 531 (3), 488 (4), 471 (1), 246 (4), 218 (2), 202 (6), 164 (8), 105 (100), HREIMS [M]$^+$ at m/z 652.2671 (Calc for $C_{35}H_{40}O_{12}$, 652 2656).

1S,4S,5S,6R,7R,8R,9S,10S-6,15-Diacetoxy-8,9-dibenzoyloxy-1,4-dihydroxy-dihydro-β-agarofuran (6) - Was obtained as an oil, $[\alpha]_D^{25}$= -39 7º ($CHCl_3$, c= 0 37), CD λ_{max} (MeCN) nm 236 9 (Δε= -13 1), 226.2 (Δε= 0), 219 0 (Δε= +8 3), UV λ_{max} (EtOH) nm 282, 274, IR ν_{max} cm^{-1} 3608, 3016, 1729, 1369, 1234, 1106, 756, ^{1}H NMR ($CDCl_3$) δ 7 31 (3H, m, Ar), 7 50 (3H, m, Ar), 7.84 (2H, m, Ar), 8 06 (2H, m, Ar), for other signals, see Table 1, EIMS m/z (%) 592 [M^+ - 18] (2), 493 (1), 488 (1), 470 (1), 428 (2), 410 (1), 368 (1), 250 (1), 208 (1), 206 (5), 164 (34), 105 (100), HREIMS [M^+ - H_2O] at m/z 592.2342 (calc for $C_{33}H_{36}O_{10}$, 592 2346)

Acetylation of 6 - Compound **6** (3 mg) was treated under the same conditions as described above and **5** (3 mg) was obtained

Benzoylation of 6 - Compound **6** (4 mg) was treated as described above and gave compound **7** (4 mg)

1S,4S,5S,6R,7R,8R,9S,10S-6,15-Diacetoxy-1,8,9-tribenzoyloxy-4-hydroxy-dihydro-β-agarofuran (7) - Was obtained as a white amorphous solid, mp 174-175ºC (from $CHCl_3$), $[\alpha]_D^{25}$= +52.7º ($CHCl_3$, c= 2 3), CD λ_{max} (MeCN) nm 235.1 (Δε= -26 4), 226 2 (Δε= 0), 219.8 (Δε= +15.1), UV λ_{max} (MeCN) nm· 227, IR ν_{max}

cm^{-1}: 3561, 3019, 1728, 1370, 1268, 1236, 1094, 734, 709; ^1H NMR ($CDCl_3$) δ: 6.86 (3H, m, Ar), 7 11 (4H, m, Ar), 7.34 (2H, m, Ar), 7 54 (3H, m, Ar), 7.98 (3H, m, Ar), for other signals, see Table 1; EIMS m/z (%) 714 $[M]^+$ (1), 592 (1), 550 (1), 544 (1), 502 (1), 488 (1), 380 (1), 321 (1), 105 (100); HREIMS $[M]^+$ at m/z 714 2609 (calc for $C_{40}H_{42}O_{12}$, 714 2602)

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