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ANTIBIOTIC PHENOL NOR-TRITERPENES FROM *MAYTENUS CANARIENSIS*

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Key Word Index—*Maytenus canariensis*; Celastraceae; phenolnortriterpenes; antibiotic activity.

Abstract—The new phenols 6-oxo-tingenol, 3-*O*-methyl-6-oxo-tingenol and 6-oxo-iguesterol were isolated from the root bark of *Maytenus canariensis*. Their structures were determined by ^{1}H and ^{13}C NMR spectroscopic studies, including HMQC, HMBC, DEPT and ROESY and chemical transformations. The synthesis of 6-oxo-tingenol was achieved from tingenone. These compounds exhibit antibiotic activity against Gram-positive bacteria.

INTRODUCTION

Species of Celastraceae have a long history in traditional medicine [1]. Our earlier work on *Maytenus canariensis* (Loes) Kunk et Sund [2], an endemic species from the Canary Islands, in the context of an intensive study of bioactive metabolites, yielded dihydro-β-agarofuran sesquiterpenes with antifeedant activity [3–4], triterpenes [5–6], and nor-triterpene methylene quinones showing antitumoral and antibiotic activities [7].

Gamlath and Gunatilaka [8] isolated from *Kokoona zeylanica* (Celastraceae), phenolic nortriterpenes with the pristimerin skeleton. Phenolic compounds with the tingenone-type skeleton have been reported [9] only as dimer constituents, but related compounds with the iguesterin skeleton has never been isolated.

This paper reports the isolation and structural elucidation of new phenolic nortriterpenes with the tingenone or iguesterin skeleton, i.e. 6-oxo-tingenol (**1**), 3-*O*-methyl-6-oxo-tingenol (**2**) and 6-oxo-iguesterol (**3**). The synthesis of **1** was achieved from tingenone. The known methylene quinone triterpenes, iguesterin, pristimerin, tingenone and 22β-hydroxy-tingenone, and the phenol 6-oxo-pristimerol, already known as a synthetic product [10], were also isolated. The new compounds were assayed for antimicrobial activity.

RESULTS AND DISCUSSION

Compound **1** was assigned the molecular formula $C_{28}H_{36}O_4$, based on its EI-mass spectral data and the HREI-mass spectral data of its 2,3-*O*-dimethyl derivative **5**. The IR spectra of **1** showed absorption bands for hydroxyl groups (3460 cm^{-1}), a ketone (1708 cm^{-1}) and a conjugated ketone (1638 cm^{-1}). The ^{1}H NMR spectrum (Table 1) displayed signals for six methyls, which included four angular methyls, one doublet at δ 0.91 and one on an aromatic ring at δ 2.56, assigned to a periplanar position to a carbonyl because of its chemical shift [10]. Signals for two singlets protons at δ 6.79 and 6.18 were assigned to a proton on an aromatic ring and an α proton to a conjugated ketone, respectively. These data confirmed the structure of 6-oxo-tingenol for this product. Compound **1** was methylated with diazomethane affording 2-*O*-methyl-6-oxo tingenol (**4**) and 2,3-*O*-dimethyl-6-oxo-tingenol (**5**) (Tables 2 and 3). The structure of **4** was confirmed by a NOE effect observed between the methoxyl and H-1 in a ROESY experiment. Compound **1** was then synthesized from tingenone (**6**) by the route shown in Scheme 1.

Compound **2** had similar spectroscopic data to those of 2-*O*-methyl-6-oxo-tingenol (**4**) (Table 1). The only difference was the chemical shift of the methoxy group from δ 3.97 to 3.81 in the ^{1}H NMR spectrum. A ROESY experiment on **2** showed a NOE effect between the methyl on C-4 and the methoxy group on the aromatic ring, establishing the structure of **2** as 3-*O*-methyl-6-oxo-tingenol, which was confirmed by the treatment of **2** with CH_2N_2 to afford 2,3-*O*-dimethyl-6-oxo-tingenol (**5**).

Compound **3** proved to be similar 6-oxo-tingenol (**1**), except for the E ring, where differences were clearly seen with **3** having a vinylic proton as a broad singlet at δ 5.24 and a methyl on a double bond at δ 1.61 as a singlet in its ^{1}H NMR spectrum (Table 1), i.e. signals that were identical to the ^{1}H NMR spectrum of iguesterin. The spectroscopic data of its dimethyl ether

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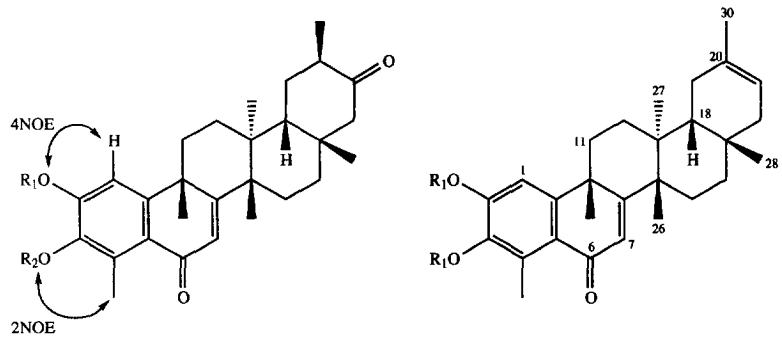


	1	2	4	5
R_1	H	H	CH_3	CH_3
R_2	H	CH_3	H	CH_3

	3	9
R_1	H	CH_3

derivative 2,3-*O*-dimethyl-6-oxo-iguesterol (**9**), confirmed the structure of **3** as 6-oxo-iguesterol (Tables 1–3).

Compounds **1–3** and the derivatives **5**, **8** and **9** were tested on Gram-positive and Gram-negative bacteria, and the yeast *Candida albicans*. All of the compounds assayed were inactive (MIC $> 100\ \mu g\ ml^{-1}$) against the Gram-negative bacteria and the yeast. Compounds **1–3** showed antibiotic activity against *B. subtilis*, with a MIC (minimal inhibitory concentration) of 12–14, 35–39 and 25 $\mu g\ ml^{-1}$, respectively; **1** was also active against *S. aureus* with a MIC of 40–50 $\mu g\ ml^{-1}$. These results suggest that the antibiotic activity may be associated with the presence of free hydroxyl groups in ring A; compound **1**, with two hydroxyl groups was about three times more active than **2**, with one methoxy group; while the dimethyl derivative **5** and the diacetate **8**, were inactive.

EXPERIMENTAL

General procedures. ^1H and ^{13}C NMR: 200 and 50 MHz, respectively; HMBC was: 400 MHz; MS: VG Micromass LTD-ZAB-2F and/or on an HP 5930 A at 70 eV.

Isolation of metabolites. The plant was gathered in Icod, Tenerife, in November 1992 and a voucher specimen is on file with the Departamento de Biología Vegetal, Universidad de La Laguna. Overstem rootbark (1 kg) was extracted with petrol–Et_2O (1:1) in a Soxhlet and the extract (18 g) was repeatedly chromatographed to afford compounds **1** (10.2 mg), **2** (2.5 mg), **3** (7.3 mg), iguesterin (50 mg), pristimerin (40 mg), tingenone (60 mg), 22β-hydroxy-tingenone (5 mg) and 6-oxo-pristimerol (15 mg).

Antimicrobial activity. Bacillus subtilis CECT 39, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* CECT 99, *Salmonella typhimurium* UBC 1 and the yeast *C. albicans* UBC2 were used. The strains were maintained on Nutrient Agar (Oxoid) or Sabouraud (Oxoid). The bacterial cultures were grown in Nutrient Broth (Oxoid) and the yeast culture in YEPD medium. The MICs were determined in liquid medium as previously described [11]. The different compounds were added in a soln of DMSO, and tubes with the same proportion of DMSO were used as controls. The

Table 1. ^1H NMR (200 MHz) data (*d*, $CDCl_3$) of compounds **1–5** and **7–9** (*J* are given in Hz in brackets)

	1	2	3	4	5	7	8	9
H-1	6.79 *s*	6.97 *s*	6.92 *s*	6.81 *s*	6.85 *s*	7.02 *s*	7.26 *s*	6.84 *s*
H-7	6.18 *s*	6.28 *s*	6.23 *s*	6.29 *s*	6.29 *s*	5.74 *dd* (1.6, 6.0)	6.33 *s*	6.24 *s*
Me-23	2.56 *s*	2.68 *s*	2.67 *s*	2.65 *s*	2.67 *s*	2.08 *s*	2.58 *s*	2.60 *s*
Me-25	1.52 *s*	1.60 *s*	1.54 *s*	1.60 *s*	1.63 *s*	1.38 *s*	1.65 *s*	1.58 *s*
Me-26	1.32 *s*	1.37 *s*	1.35 *s*	1.38 *s*	1.40 *s*	1.30 *s*	1.41 *s*	1.38 *s*
Me-27	0.94 *s*	1.02 *s*	0.53 *s*	1.02 *s*	1.03 *s*	1.02 *s*	1.04 *s*	0.57 *s*
Me-28	0.94 *s*	1.02 *s*	0.99 *s*	1.02 *s*	1.02 *s*	1.00 *s*	1.03 *s*	1.00 *s*
Me-30	0.91 *d* (5.9)	1.01 *d* (5.9)	1.61 *s*	0.98 *d* (5.9)	0.99 *d* (5.9)	0.98 *d* (5.9)	1.00 *d* (5.9)	1.60 *s*
-OMe								
2				3.97 *s*	3.94 *s*			3.92 *s*
3		3.81 *s*			3.77 *s*			3.77 *s*
-OCC-Me								
2						2.33 *s*	2.37 *s*	
3						2.28 *s*	2.33 *s*	

Table 2. ^{13}C NMR (50 MHz) data (δ, $CDCl_3$) of compounds **5**, **8** and **9**

C	5	8	9
1	105.8 *d*	117.4 *d*	105.9 *d*
2	156.2 *s*	145.2 *s*	156.0 *s*
3	146.4 *s*	139.6 *s*	146.2 *s*
4	134.3 *s*	134.0 *s*	134.1 *s*
5	124.0 *s*	127.6 *s*	123.7 *s*
6	187.5 *s*	186.7 *s*	187.7 *s*
7	126.7 *d*	125.9 *d*	126.1 *d*
8	170.3 *s*	170.9 *s*	171.4 *s*
9	40.6 *s*	40.1* *s*	40.6 *s*
10	154.4 *s*	154.9 *s*	154.9 *s*
11	34.8 *t*	34.0 *t*	35.6 *t*
12	30.6 *t*	30.0 *t*	30.2 *t*
13	40.6 *s*	40.2* *s*	40.6 *s*
14	44.7 *s*	43.4 *s*	44.8 *s*
15	28.8 *t*	28.4 *t*	28.8 *t*
16	35.9 *t*	35.4 *t*	37.5 *t*
17	30.1 *s*	38.1 *s*	30.8 *s*
18	43.9 *d*	44.5 *d*	42.7 *d*
19	32.4 *t*	31.9 *t*	27.9 *t*
20	42.3 *d*	41.8 *d*	132.4 *s*
21	214.0 *s*	213.5 *s*	119.9 *d*
22	53.0 *t*	52.5 *t*	35.2 *t*
23	14.4 *q*	14.5 *q*	14.4 *q*
25	39.0 *q*	38.4 *q*	39.2 *q*
26	21.2 *q*	20.7 *q*	21.4 *q*
27	20.2 *q*	19.8 *q*	15.7 *q*
28	33.0 *q*	32.5 *q*	33.1 *q*
30	15.5 *q*	15.1 *q*	24.0 *q*

Data are based on HMQC and HMBC.
*Interchangeable values.

cultures were incubated at 37° in a rotatory shaker and growth was measured by viable counting on Nutrient Agar or Sabouraud plates.

6-Oxo-tingenol (**1**). Amorphous pale yellow lacquer; UV λ_{max} (EtOH) nm; 298, 264; IR ν_{max}($CHCl_3$) cm^{-1}: 3460, 1772, 1708, 1638; ^1H NMR ($CDCl_3$) δ: 2.32 (1H, *m*), 2.72 (1H, *d*, $J = 14.2$ Hz), for other signals, see Table 1; EI-MS *m/z* (rel. int.): 436 [M^+] (47), 421 (55), 187 (75), 120 (40), 109 (58), 69 (100).

3-O-*methyl*-6-*oxo-tingenol* (**2**). Amorphous pale yellow lacquer; $[\alpha]_D^{20}$ −26.9° ($CHCl_3$, *c* 0.026); UV λ_{max} (EtOH) nm: 305, 263; IR ν_{max} ($CHCl_3$) cm^{-1}: 3378, 1731, 1713, 1643; ^1H NMR ($CDCl_3$) δ: 2,50 (1H, *m*), 2.91 (1H, *d*, $J = 14.2$ Hz), for other signals, see Table 1; EIMS *m/z* (rel. int.): 436 [M − 14]$^+$ (2), 435 (5), 321 (2), 231 (21), 165 (16), 115 (15), 105 (80), 55 (100).

6-Oxo-iguesterol (**3**). Amorphous yellow lacquer; $[\alpha]_D^{20}$ +75.0° ($CHCl_3$, *c* 0.24); UV λ_{max} (EtOH) nm: 302, 201; IR ν_{max} ($CHCl_3$) cm^{-1}: 3413, 1637, 1578; ^1H NMR ($CDCl_3$) δ: 5.24 (1H, *s*), for other signals, see Table 1; EIMS *m/z* (rel. int.): 420 [M]$^+$ (4), 405 (5), 241 (13), 201 (29), 105 (39), 68 (100); HR-EIMS *m/z* at 420.2668 (calc. for $C_{28}H_{36}O_3$, 420.2664).

Methylation of 6-oxo-tingenol (**1**). Compound **1** (10.2 mg) was treated with CH_2N_2 and purified by prep. TLC to give compounds **4** (2.3 mg) and **5** (4.9 mg).

2-O-Methyl-6-oxo-tingenol (**4**). Amorphous pale yellow lacquer; $[\alpha]_D^{20}$ −78.3° ($CHCl_3$, *c* 0.046); UV λ_{max} (EtOH) nm: 294, 262; IR ν_{max} ($CHCl_3$) cm^{-1}: 3319, 1708, 1643; ^1H NMR ($CDCl_3$) δ: 2.45 (1H, *m*), 2.91 (1H, *d*, $J = 14.5$ Hz), 5.72 (1H, *s*), for other signals, see Table 1; EIMS *m/z* (rel. int.): 435 [M − 15]$^+$ (1), 256 (7), 231 (17), 165 (20), 104 (29), 95 (79), 55 (100).

2,3-O-*Dimethyl-6-oxo-tingenol* (5). Amorphous pale yellow lacquer; $[\alpha]_D^{20}$ −58.2° ($CHCl_3$, *c* 0.098); UV λ_{max} (EtOH) nm: 295, 251; IR ν_{max} ($CHCl_3$) cm^{-1}: 1708, 1649; ^1H NMR ($CDCl_3$) δ: 2.92 (1H, *d*, $J =$ 14.3 Hz), for other signals, see Table 1; ^{13}C NMR ($CDCl_3$) δ: 56.0 *q* (OMe), 60.8 *q* (OMe), for other signals, see Table 2; EIMS *m/z* (rel. int.); 464 [M]$^+$ (65), 449 (100), 405 (9), 245 (19), 229 (17), 165 (17), 55 (37); HR-EIMS *m/z* at 464.2948 (calc. for $C_{30}H_{40}O_4$, 464.2927).

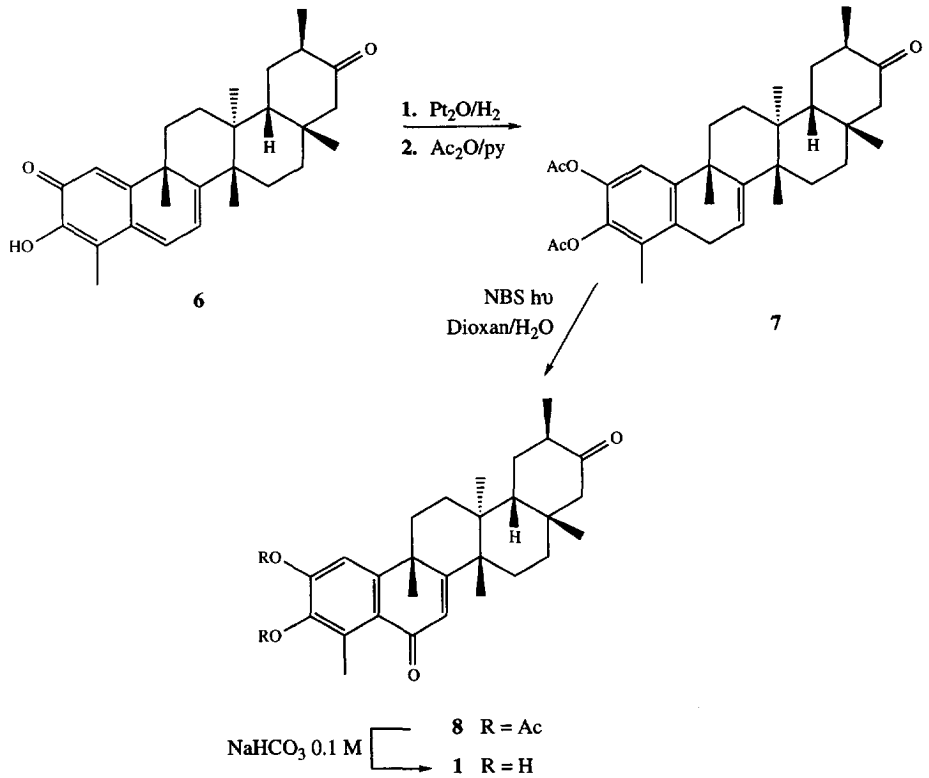
2,3-*Diacetoxy-tingenol* (**7**). Tingenone (200 mg) was hydrogenated using Pt_2O as catalyst and acetylated with Ac_2O/py in an inert atmosphere; purification of the crude reaction by CC and prep. TLC yielded **7** (105 mg); ^1H NMR ($CDCl_3$) δ: 2.52 (1H, *m*), 2.94 (1H, *d*, $J = 14.2$ Hz), 3.12 (1H, *dd*, $J = 1.6$, 19.0 Hz), 3.48 (1H, *dd*, $J = 6.0$, 19.0 Hz), for other signals, see Table 1; EIMS *m/z* (rel. int.): 506 [M]$^+$ (3), 491 (42), 448 (34), 436 (24), 341 (32), 406 (41), 229 (79), 201 (60), 187 (100); HR-EIMS *m/z* at 506.3037 (calc. for $C_{32}H_{42}O_5$, 506.3032).

2,3-*Diacetoxy-6-oxo-tingenol* (**8**). To a stirred suspension of 2,3-diacetoxy-tingenol (**7**) (105 mg) and $CaCO_3$ (105 mg) in dioxane (18 ml) containing water (5 ml), *N*-bromosuccinimide (NBS) (105 mg) was added all at once and the mixture was irradiated at room temp. with a 100-W tungsten filament lamp and stirred for a further 3 hr (control by TLC). After that time, the reaction mixt. was filtered into water (60 ml),

Table 3. Three-bond ^1H–^{13}C couplings (HMBC) of compounds **5**, **8** and **9**

H	5	8	9
1	C-2*, C-3, C-5, C-9, C-10*	C-2*, C-3, C-5, C-9	C-2*, C-3, C-5, C-9
7	C-5, C-6*, C-8*, C-9	C-5, C-9, C-14	C-5, C-9, C-14
22	C-20, C-21*		
23	C-3, C-4*, C-5	C-3, C-4*, C-5	C-3, C-4*, C-5
30	C-19, C-20*, C-21	C-19, C-20*, C-21	C-19, C-21

*Two-bond ^1H–^{13}C coupling enhancement observed.



Scheme 1. Chemical synthesis of compounds **1** and **8** from **6**.

extracted with EtOAc, and taken to dryness. Purification by prep. TLC yielded **8** (96 mg): $[\alpha]_D^{20}$ −57.5° ($CHCl_3$, *c* 0.098); UV λ_{max} (EtOH) nm: 275; IR ν_{max} ($CHCl_3$) cm^{-1}: 1772, 1702, 1649; ^1H NMR ($CDCl_3$) δ: 2.51 (1H, *m*), 2.93 (1H, *d*, $J = 14.4$ Hz), for other signals, see Table 1; ^{13}C NMR ($CDCl_3$) δ: 20.2 *q*, 20.7 *q*, 167.9 *s*, 168.1 *s*, for other signals, see Table 2; EIMS *m/z* (rel. int.): 520 $[M]^+$ (21), 436 (100), 421 (71), 260 (13), 215 (45), 121 (20), 55 (63); HR-EIMS at *m/z* 520.2823 (calc. for $C_{32}H_{40}O_6$, 520.2825).

2,3-O-*Dimethyl-6-oxo-iguesterol* (**9**). Compound **3** (7.3 mg) was treated with CH_2N_2 yielding after prep TLC **9** (4.9 mg) as an amorphous yellow lacquer; $[\alpha]_D^{20}$ −72.5° ($CHCl_3$, *c* 0.098); UV λ_{max} (EtOH) nm: 304, 276; IR ν_{max} ($CHCl_3$) cm^{-1}: 1647, 1585; ^1H NMR ($CDCl_3$) δ: 5.24 (1H, *s*), for other signals, see Table 1; ^{13}C NMR ($CDCl_3$) δ: 56.0 *q* (OMe), 60.7 *q* (OMe), for other signals, see Table 2; EIMS *m/z* (rel. int.): 448 $[M]^+$ (100), 433 (86), 245 (14), 216 (10), 165 (6), 91 (21), 67 (13); HR-EIMS at *m/z* 448.2977 (calc. $C_{30}H_{40}O_3$, 448.2978).

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