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A COSTIC ACID GUAIANYL ESTER AND OTHER CONSTITUENTS OF *PODACHAENIUM EMINENS**

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Key Word Index—*Podachaenium eminens*; Compositae; Heliantheae; eudesmanolides; guaianolides; germacrolide; costic acid guaianyl ester; sesquiterpene lactones.

Abstract—In addition to known compounds, the aerial parts of *Podachaenium eminens* contained the guaianolide named eminensin A, the eudesmanolide eminensin B and podachaenin, a compound formed through esterification of an hydroxyguaianolide by costic acid. Structures of these compounds were established by analysis of their spectroscopic data and, in the case of eminensin A, it was confirmed by X-ray crystallographic analysis.

INTRODUCTION

In former articles on *Podachaenium eminens* (Lag) Sch. Bip., the presence of guaianolides, which are 3, 7- or 13-hydroxy derivatives of dehydrocostus lactone has been described [1, 2]. The analyzed populations were collected in Guatemala and Morelos, México. We have examined another Mexican population from the State of Sinaloa, México. In the present paper, we describe the isolation and structure determination of three new and three known sesquiterpene lactones. One of the new compounds, podachaenin (**6**) is an unusual ester of an eudesmane, costic acid, and a 3-hydroxyguaianolide. Two similar esters, but constituted by two pseudoguaiane units, have been identified as components of *Ambrosia* species [3, 4].

RESULTS AND DISCUSSION

The aerial parts of *P. eminens* afforded sitosterol and stigmasterol together with known lactones, desacetoxymatricarin (**1**) [5–7], 2-acetoxy-3α, 4α-epoxy-3, 4-dihydrokauniolide (**2**) [8] and tamaulipin B-acetate (**4**) [9, 10]. These compounds were identified by comparison of their spectral and physical properties with those published. The ^{13}C NMR data of **2** and **4** are given in Table 2. None of the previously reported sesquiterpene lactones [1, 2] were isolated from this population.

Eminensin A (**3**), $C_{15}H_{16}O_4$ (EI–MS), showed IR bands for α-methylene-γ-lactone (1773 cm^{-1}) and α, β-unsaturated cyclopentanone (1717, 1629 cm^{-1}). Its ^1H NMR spectrum was similar to that of **1**, except for the appearance of a singlet at δ 3.36, instead of that for the vinylic H-3 of **1** and the upfield shift of the signal for the C-4 methyl group which appeared at δ 1.78 as a sharp singlet. The above suggested that **3** contains a C-3–C-4-epoxide instead of the Δ^3-double bond of **1**. This was confirmed by the ^{13}C NMR spectrum which showed the signals for C-3 and C-4 at δ 63.1 (*d*) and 62.3 (*s*), respectively. COSY, HETCOR and long-range HETCOR NMR spectra confirmed the structure of eminensin A as **3** and allowed us to assign the ^1H and ^{13}C signals listed in Tables 1 and 2.

The stereochemistry at C-5, C-6 and C-7 was based on the large coupling constants of H-6 ($J_{5,6}=10.5$ Hz and $J_{6,7}=9.8$ Hz) as well as on the NOEs between (i) H-5 and H-7 and (ii) H-6 and H-8β, H-15 observed in the NOESY spectrum, which also showed a NOE between H-3 and H-15, thus establishing the orientation of the C-3–C-4-epoxy group as α. This was confirmed by means of the X-ray crystallographic analysis of **3**.

Eminensin A crystallized with two chemically identical but crystallographic different molecules in the asymmetric unit. Figure 1 shows the α-orientation of the oxirane ring. According to the Cremer and Pople [11] parameters, the seven-membered rings adopt chair conformations: molecule A; ($q_2=0.372$, $\phi_2=185.07$, $q_3=0.673$, $\phi_3=358.97$), molecule B; ($q_2=0.410$, $\phi_2=190.69$, $q_3=0.691$, $\phi_3=359.77$), while the cyclopentanone rings are essentially planar and the γ-lactone rings can be best described as twisted

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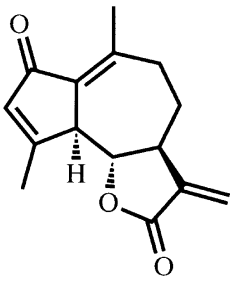

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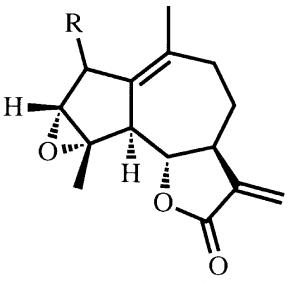

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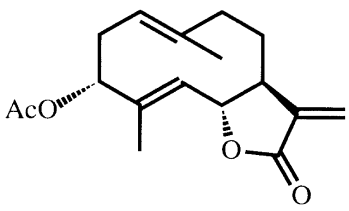
3 R = =O



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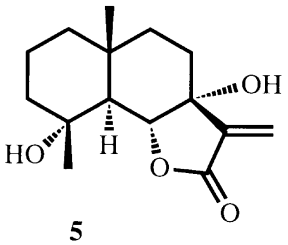

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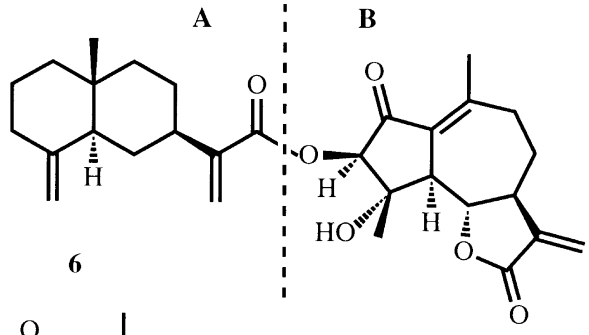

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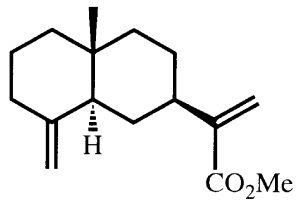

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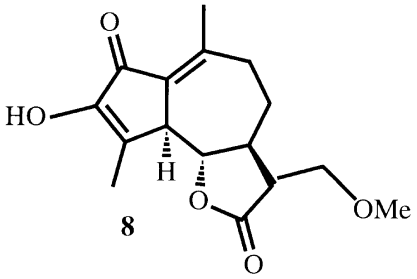

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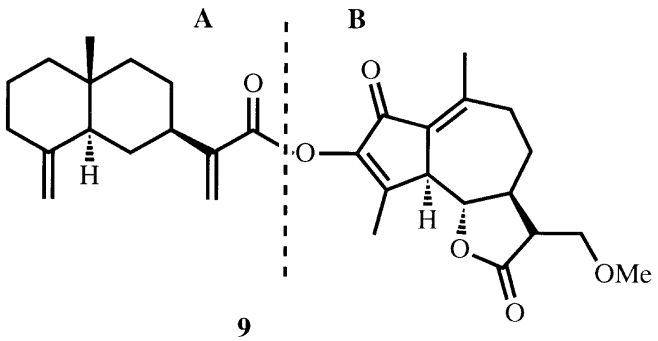

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conformations, molecule A: ($q_2 = 0.344$, $\theta = 21.61$); molecule B; ($q_2 = 0.331$, $\theta = 16.04$).

The second new lactone, eminensin B (**5**), $C_{15}H_{22}O_4$ (EI–MS) exhibited IR bands for a hydroxyl group, α-methylene-γ-lactone and double bond. Its structure was deduced as a 4,7-dihydroxy-6,12-eudesmanolide based on the ^1H, ^{13}C, COSY and HETCOR NMR spectra. The ^1H NMR spectrum showed the presence of a secondary (δ 1.32 *s*, H-15) and a tertiary (δ 0.99 *s*, H-14) methyl groups; the former on a carbon (C-4) bearing an hydroxy group. The singlets at δ 6.17 and 5.69 were assigned to the exomethylene protons (H-13, H-13′) conjugated with the γ-lactone and the doublet at δ 4.15 to the proton under the lactone ring closure (H-6). This last signal correlated (COSY spectrum) with the doublet at δ 2.38 assigned to H-5. The multiplicity of H-6, H-13 and H-13′ led us to propose the presence of an hydroxy group at C-7. The above was confirmed by the ^{13}C NMR spectrum which showed signals for three carbons bearing oxygenated functions at δ 83.1 *d*, 75.1 *s* and 71.6 *s*. They were assigned to C-6, C-7 and C-4, respectively and confirmed by long-range HETCOR NMR. The coupling constant ($J_{5,6} = 12.3$ Hz) and the observed NOEs

Table 1. ^{1}H NMR spectral data of compounds **3**, **5**–**9** (300 MHz, $CDCl_3$, TMS as int. standard)

H	**3**	**5**	**6A**	**6B**	**7**	**8**	**9A**	**9B**
1	—	1.3–1.5 *m*	1.28 *m*	—	1.3 *m*	—	a	—
2	—	1.4–1.7 *m*	1.58 *m*	—	1.6 *m*	—	a	—
2′	—	1.4–1.7 *m*	1.58 *m*	—	1.6 *m*	—	a	—
3	3.36 *s*	1.8 *m*	2.30 *br d* 13	5.58 *s*	2.30 *br d* 13.5	—	2.31 *br d* 15	—
3′		1.5 *m*	2.01 *m*	—	2.01 *m*	—	2.05 *m*	—
5	3.45 *br d* 10.5	2.38 *d* 12.3	1.89 *br d* 11.4	3.30 *br d* 10.3	1.90 *br d* 12.3	3.32 *d* 10	1.78 *br d* 12	3.49 *d* 10
6	3.65 *dd* 10.5, 10	4.15 *d* 12.3	1.72 *m* 1.28 *m*	3.88 *t* 10.3	1.60 *m* 1.28 *m*	3.55 *t* 10	a	3.70 *t* 10
7	2.86 *dddd* 12, 10, 6, 3.2	—	2.57 *m*	2.86 *br t* 10.3	2.55 *br t t* 11.6, 3.5	2.40 *m*	2.5 *m*	a
8	2.10 *br d* 12	2.00 *m*	1.72 *m*	2.23 *br d* 14.4	1.6 *m*	2.12 *m*	a	a
8′	1.43 *br dt* 12, 12	1.8 *m*	1.72 *m*	1.48 *m*	1.6 *m*	1.31 *br dt* 12, 12	a	a
9	2.42 *m*	1.9 *m*	1.48 *m*	2.5 *m*	1.5 *m*	2.47 *m*	a	a
9′	2.42 *m*	1.3 *m*	1.28 *m*	2.5 *m*	1.3 *m*	2.33 *ddd* 14.5, 6, 1.8	a	a
13	6.17 *d* 3.3	6.17 *s*	6.32 *br s*	6.29 *d* 3.3	6.14 *d* 1	3.75 *dd* 10, 4	6.40 *s*	3.77 *dd* 10, 3.6
13′	5.43 *d* 3	5.69 *s*	5.78 *t* 1	5.57 *d* 3	5.55 *t* 1.2	3.69 *dd* 10, 2.7	5.77 *s*	3.67 *dd* 10, 3
14	2.34 *br s*	0.99 *s*	0.75 *s*	2.35 *d* 3	0.74 *s*	2.47 *s*	0.75 *s*	2.45 *s*
15	1.78 *s*	1.32 *s*	4.70 *br q* 1.5 4.43 *br q* 1.5	1.36 *s*	4.70 *br q* 1.2 4.70 *br q* 1.2	2.17 *s*	4.71 *br q* 1.5 4.44 *br q* 1.5	2.16 *s*
OH		2.96 *s*		2.80 *s*	3.75 *s*	3.39 *s*		3.40 *s*
OMe		3.46 *s*				5.80 *br*		

[a]Overlapped signals between δ 1.1 and 2.5.

Table 2. ^{13}C NMR spectral data of compounds **2**–**7** (75 MHz, $CDCl_3$)[a]

C	**2**	**3**	**4**	**5**	**6A**	**6B**	**7**	**8**
1	132.2 *s*	131.0 *s*	125.5 *d*	40.5 *t*	41.9 *t*	126.7 *s*	41.9 t	128.7 *s*
2	74.4 *d*	197.7 *s*	31.5 *t*	19.4 *t*	23.5 *t*	195.6 *s*	23.5 *t*	188.8 *s*
3	64.1 *d*	63.1 *d*	80.9 *d*	40.0 *t*	36.9 *t*	82.9 *d*	36.8 *t*	135.4 *s*
4	64.7 *s*	62.3 *s*	139.8 *s*	71.6 *s*	150.6 *s*	75.3 *s*	150.6 *s*	152.1 *s*
5	52.0 *d*	50.4 *d*	122.5 *d*	48.6 *d*	50.0 *d*	54.6 *d*	49.9 *d*	47.9 *d*
6	80.9 *d*	80.2 *d*	77.0 *d*	83.1 *d*	29.9 *t*	82.2 *d*	30.0 *t*	85.7 *d*
7	54.1 *d*	54.2 *d*	50.6 *d*	75.1 *s*	39.9 *d*	50.0 *d*	39.7 *d*	50.5 *d*
8	24.9 *t*	24.8 *t*	28.0 *t*	27.3 *t*	27.4 *t*	24.9 *t*	27.3 *t*	25.9 *t*
9	34.9 *t*	37.3 *t*	41.0 *t*	38.1 *t*	41.2 *t*	38.5 *t*	41.1 *t*	37.3 *t*
10	142.6 *s*	159.1 *s*	139.1 *s*	37.2 *s*	36.0 *s*	158.2 *s*	35.9 *s*	154.1 *s*
11	138.9 *s*	138.4 *s*	139.1 *s*	141.6 *s*	145.3 *s*	137.8 *s*	146.0 *s*	46.9 *d*
12	169.0 *s*	168.8 *s*	169.6 *s*	169.8 *s*	168.8 *s*	166.3 *s*	167.9 *s*	174.9 *s*
13	118.2 *t*	118.6 *t*	119.6 *t*	119.9 *t*	123.7 *t*	120.2 *t*	122.3 *t*	68.3 *t*
14	22.3 *q*	22.8 *q*	16.3 *q*	19.0 *q*	16.4 *q*	24.7 *q*	16.3 *q*	22.1 *q*
15	19.2 *q*	18.8 *q*	16.3 *q*	24.1 *q*	105.6 *t*	17.6 *q*	105.4 *t*	14.0 *q*
OMe or	170.9 *s*		170.0 *s*				51.7 *q*	59.3 *q*
OAc	20.7 *q*		21.1 *q*					

[a] Multiplicities were established by DEPT pulse sequence.

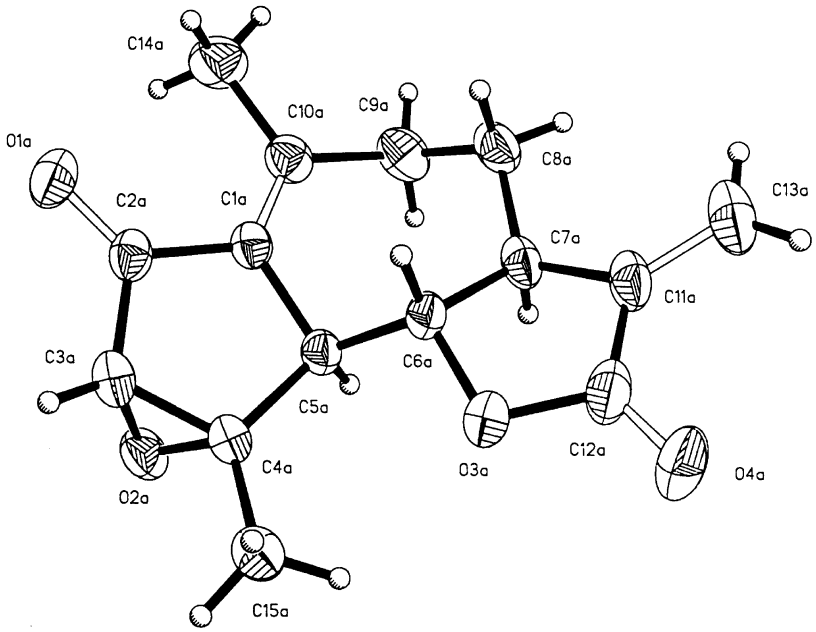

Fig. 1. Ortep-like drawing of Eminensin A (Molecule A). Thermal ellipsoids at 30% probability level.

between (i) H-14 and H-6, H-8 and H-15 and (ii) H-15 and H-6 established the stereochemistry of eminensin B as depicted in (**5**).

The third new compound isolated from *P. eminens* was podachaenin (**6**). Its IR spectrum revealed the presence of hydroxy groups (3583 cm^{-1}), α,β-unsaturated-γ-lactone (1775 cm^{-1}), α,β-unsaturated ester (1721 cm^{-1}) and α,β-unsaturated cyclopentanone (1730 cm^{-1}). The mass spectrum exhibited a molecular ion at m/z 494 in accordance with a molecular formula $C_{30}H_{38}O_6$. The ^1H and COSY NMR spectra showed two independent spin systems, indicating that (**6**) was constituted by two sesquiterpenic units, designated as (**A**) and (**B**). This was supported by the fragments at m/z 233, 217 and 278, 260 for fragments (**A**), (**A**)–16, and (**B**), (**B**)–H_2O, respectively. The fragment (**A**) corresponded to the esterifying acid which was easily identified by the combination of 1D and 2D-homo and heteronuclear NMR spectra as costic acid, an eudesmane-type sesquiterpene isolated from *Costus* root oil (*Saussurea lappa*) [12]. The spin system of fragment (**B**) was quite similar to that of eminensin A (**3**) but, in (**6**), the H-3 singlet signal appeared at δ 5.58 and the C-4 methyl group was shifted upfield (δ 1.36 *s*). In the ^{13}C NMR spectrum, the signals for C-3 and C-4 appeared at δ 82.9 *d* and 75.3 *s*, which was in agreement with a structure in which C-3 was the carbon bearing the ester group and an hydroxy group was bonded to C-4, thus establishing the gross structure (**6**) for podachaenin. The stereochemistry of (**6**) was based on the observed NOEs between (i) H-5 and H-7 and (ii) H-13 and H-14 for fragment **A** and between (i) H-3 and H-5, (ii) H-5 and H-7 and (iii) H-6 and H-15 for fragment **B**. The long-range HETCOR NMR spectrum gave further support for this structure but the definitive confirmation came from the hydrolysis of **6** (K_2CO_3/MeOH), which afforded three major compounds. The less polar product was identified as the methyl ester of costic acid (**7**) by comparison with the published data [12]. The most polar compound was the guaiane derivative (**8**), which was formed from fragment **B** by the conjugated addition of MeOH to the α,β-unsaturated-γ-lactone and dehydration of the C-4-hydroxy group, as indicated by the 1D and 2D-homo and heteronuclear NMR spectra. The ^1H NMR spectra of the third product isolated from this reaction was the sum of the signals of the costic moiety (**7**) and those of **8**. Its mass spectrum showed a molecular ion of m/z 508 ($C_{31}H_{42}O_7$). These facts established the structure of this derivative as **9**.

EXPERIMENTAL

Plant material

Podachaenium eminens (Lag) Sch. Bip. was collected 3 km west of Santa Rita, along the Highway Mazatlán–Durango, México. A voucher specimen is deposited in the Herbarium of the Instituto de Biología, UNAM (MEXU-499853).

Extraction and isolation

Dried and ground aerial parts of the plant (80.5 g) were extracted with Me_2CO to give, after solvent evaporation, 4.19 g of extract. This extract was chromatographed on a silica gel column eluted with hexane–EtOAc gradient. Frs eluted with hexane–EtOAc 9:1 afforded after CC (silica gel G, hexane–EtOAc 19:1) sitosterol and stigmasterol (24.4 mg) and **1**

(6.2 mg) [5–7]. Repeated CC (silica gel G, hexane–EtOAc, 9:1 or $CHCl_3$) of frs eluted with hexane–EtOAc (17:3 and 4:1) gave 17.3 mg of **2** [8], 33.9 mg of **3** and 32.3 mg of **4** [9, 10]. 118.5 mg of **6** were isolated from frs eluted with hexane–EtOAc (3:1 and 7:3), after CC (silica gel G, hexane–EtOAc, 7:3). Compound **5** (10.3 mg) was isolated from frs eluted with hexane–EtOAc 3:2 and 2:3, after CC (silica gel G, hexane–EtOAc, 7:3).

Eminensin A (**3**)

Colorless crystals from hexane–EtOAc; mp 186–188° $[\alpha]_D$ −33.7°(*c*, 0.166, $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1773, 1717, 1629, 1138, 992, 949. EI–MS *m*/*z* (rel. int.): 260 ($C_{15}H_{16}O_4$, 100), 245 (15), 231 (11), 215 (22), 203 (30), 201 (26), 189 (20), 175 (13), 162 (32), 151 (36) 149 (32), 135 (25), 124 (29), 111 (22), 91 (26), 77 (19), 69 (22), 53 (25), 43 (30), 41 (27).

Eminensin B (**5**)

Colorless crystals from hexane–EtOAc; mp 184–186° $[\alpha]_D$ +82.6° (*c*, 0.213, $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3589, 3348, 1773, 1670, 1390, 1136, 1063, 1031, 991, 977, 879. EI–MS *m*/*z* (rel.int): 266 ($C_{15}H_{22}O_4$,3) 251 (54), 233 (72), 205 (22), 202 (19), 190 (17), 163 (100), 135 (18), 123 (12), 109 (16), 85 (13), 71 (14), 55 (20), 43 (44), 41 (18).

Podachaenin (**6**)

Colorless crystals from hexane–EtOAc; mp 242–245° $[\alpha]_D$ +66.1° (*c*, 0.121, $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3583, 1775, 1730, 1721, 1645, 1616, 1381, 1132, 1108, 996, 947, 890. EI–MS *m*/*z* (rel. int): 494 ($C_{30}H_{38}O_6$, 4), 479 (3), 476 (8), 448 (2), 368 (4), 313 (2), 278 (4), 260 (47), 244 (31), 233 (76), 217 (100), 201 (19), 147 (24), 133 (19), 121 (37), 107 (35), 95 (52), 81 (34), 67 (33), 55 (22), 43 (24).

Hydrolysis of podachaenin (**6**)

K_2CO_3 (98.9 mg) was added to a soln of **6** (105.4 mg) in MeOH (10 ml). The reaction mixture was left to stand by 20 min, neutralized with HCl 5%, extracted with EtOAc, washed with H_2O and dried over anh. Na_2SO_4. From the reaction mixture compounds, **7** (19.2 mg), **8** (18.7 mg) and **9** (16.7 mg) were isolated after prep. TLC eluted with hexane–EtOAc (4:1).

Costic acid methyl ester (**7**)

Colorless oil; IR as reported [12]; EI–MS *m*/*z* (rel. int): 248 ($C_{16}H_{24}O_2$, 25), 233 (24), 217 (16), 201 (18), 185 (22), 173 (24), 147 (31), 121 (39), 105 (44), 95 (50), 91 (65), 81 (56), 69 (59), 55 (92), 43 (85), 41 (100).

Compound **8**

Pale yellow gum; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3486, 1783, 1714, 1672, 1626, 1408, 1368, 1175, 1075, 1003. EI–MS *m*/*z* (rel. int): 292 ($C_{16}H_{20}O_5$, 42), 278 (8), 260 (5), 247 (19), 214 (8), 175 (15), 161 (13), 147 (11), 121 (10), 117 (10), 105 (16), 91 (32), 79 (25), 77 (24), 55 (29), 45 (100), 43 (74).

Compound **9**

Pale yellow gum; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3525, 1783, 1739, 1695, 1675, 1630, 1383, 1118, 1083, 1001, 890. EI–MS *m*/*z* (rel. int): 508 ($C_{31}H_{40}O_6$, 39), 408 (9), 368 (7), 292 (45), 260 (5), 247 (9), 233 (12), 217 (100), 189 (31), 147 (28), 133 (33), 121 (45), 81 (63), 67 (56), 55 (63), 45 (88), 41 (67).

X-ray crystallography

Eminensin A (**3**) gave crystals from hexane–EtOAc: $C_{15}H_{16}O_4$, orthorhombic, $V = 3275.1(5)$ Å^3, space group $P2_12_12_1$ (No. 19) with cell constants $a = 9.3575(5)$ Å, $b = 15.833(1)$ Å, $c = 17.895(2)$ Å, $D_c = 1.304$ g cm^{-3} and 8 molecules in the unit cell. The data collection was performed using monochromated CuKα radiation 1.54178 Å. The structure was solved by direct methods (SIR92) [13]. Refinement (SHELXL-97) [14] by least-squares with anisotropic displacement parameter for all non-hydrogen atoms resulted in $R = 0.0477$, $wR = 0.1226$. A weighting scheme was used according to $w = 1/[\sigma^2(F_o^2) + (0.0831 \times P)^2 + 0.39 \times P]$ where $P = (F_o^2 + 2F_c^2)/3$.

Hydrogen atoms were treated isotropically in a riding model only those bonded at O were refined in a restrained way. 344 Parameters were considered in the full least-squares refinement using 3524 reflections (*hkl* plus Friedel pairs, $2\theta \leq 113.5°$). The absolute configuration was determined by Flack's method [15] (Flack parameter = 0.2(3)); orientation at the asymmetric carbons as given in Fig. 1. Listing of positional and displacement parameters, tables of bond distances and angles have been submitted as supplementary material to be deposited in the Cambridge Crystallographic Data Centre.

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