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DITERPENOIDS AND FLAVONOIDS FROM *OPHRYOSPORUS CHARRUA*

LAURA S. FAVIER, MATÍAS NIETO, OSCAR S. GIORDANO and CARLOS E. TONN*

INTEQUI-CONICET-Facultad de Química, Bioquímica y Farmacia-Universidad Nacional de San Luis-Chacabuco
y Pedernera-5700-San Luis, Argentina

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Key Word Index—*Ophryosporus charrua*; Eupatoriae; diterpenoids; *ent*-labdane; *ent*-halimane; flavonoids.

Abstract—Two new diterpenes and four known flavones were isolated from the aerial parts of *Ophryosporus charrua* (Griseb.) Hieron. The diterpenes were characterized as 2-oxo-3α-hydroxy-*ent*-labd-7-en-15-oic acid and 2-oxo-*ent*-haliman-1(10), 7-dien-15-oic acid. The structures were established by analyses of their ^{1}H and ^{13}C NMR spectra with the aid of 2D experiments and chemical transformations. The flavones were identified as apigenin-7,4′-dimethyl ether, circimaritine, salvigenin, and 7-*O*-methyleriodictyol. © 1997 Elsevier Science Ltd. All rights reserved

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INTRODUCTION

The genus *Ophryosporus* (Asteraceae-Asteroideae-Eupatorieae) consist of some 40 species entirely distributed in South America [1]. Several of them have been studied and have yielded a great number of products. The diterpenoids are usually of the *ent*-labdane type as in *O. chilca* [2], *O. heptantus* [3] and *O. floribundus* [4]. In all the species chemically studied euparine derivatives, chromenes, acetophenones, flavones and flavanones are the common secondary metabolites [5–7]; on the other hand, aerial parts of *O. floribundus* [4] and *O. piquerioides* [5] yielded eudesmanolides.

As part of our search for diterpenoids with latent bioactivity toward insects larvae [8–10], we wish to report here on the isolation and structure determination from the aerial parts of *O. charrua* (Griseb.) Hieron. of two new diterpenes **1** and **2**, the first with an *ent*-labdane framework and the second a rearranged *ent*-labdane diterpenoid with an *ent*-halimane skeleton [11]. The four known flavones, **3**–**6**, were also isolated.

RESULTS AND DISCUSSION

The molecular formula of compound **1** was established by a combination of EI mass spectrometry, ^{1}H NMR and ^{13}C NMR as $C_{20}H_{32}O_4$. The IR spectrum (KBr) showed absorption bands at 3455, 1750, and 1710 cm^{-1} for —OH, —COOH, and C═O groups, respectively. The ^{1}H NMR spectrum was suggestive of a compound with a labdane skeleton; it contained a broad olefinic singlet at δ 5.42 ($W_{1/2} = 9.6$ Hz), a one proton signal at δ 3.92 as a singlet; five methyl groups, one of them secondary as a doublet at δ 1.0 ($J = 6.0$ Hz), three tertiary at δ 0.70, 0.80 and 1.12, and an olefinic methyl group at δ 1.70 allylically coupled with the olefinic proton at δ 5.42 as shown by the ^{1}H–^{1}H COSY spectrum. This experiment also revealed that a doublet at δ 2.70 ($J = 12.0$ Hz) was clearly coupled with a double-doublet centred at δ 2.25 ($J = 12.0$; 1.0 Hz) which showed a long range W coupling with a methyl group at δ 0.70 [12]. The relative downfield chemical shift of the signals at δ 2.25 and 2.70 were considered to be in accordance with a methylene group adjacent to a ketone function. Therefore, this system can be assigned to the protons H-1α (δ 2.70) and H-1β (δ 2.25 ppm) together with the C-20 methyl protons.

The ^{13}C NMR assignments for compound **1** (Table 2) were resolved by a combination of 1D- and 2D-NMR techniques comprising DEPT and XH-CORR as well as by comparison with previously reported data [13–15]. This spectrum showed two carbonyl groups, one of them at δ 210.9 typical of a ketone function; four methyl groups attached to sp^3 carbons; one olefinic methyl group at δ 21.8; five methylene groups; four sp^3 methine carbons, one of them bearing a hydroxyl group with resonance at 82.4 ppm; two olefinic carbons, one of them proton-bearing; and two quaternary sp^3 carbons. The NMR spectroscopic data

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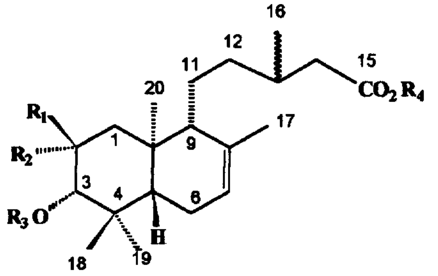
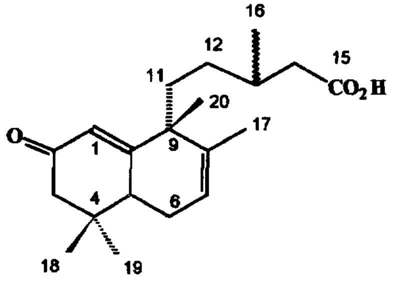
* Author to whom correspondence should be addressed.



1 R_1, R_2 = =O $R_3 = R_4 = H$
1a R_1, R_2 = =O $R_3 = H$ $R_4 = Me$
1b R_1, R_2 = =O $R_3 = Ac$ $R_4 = Me$
1c $R_1 = R_3 = R_4 = H$ $R_2 = OH$
1d $R_1 = H$ R_2, R_3 = acetonide $R_4 = Me$



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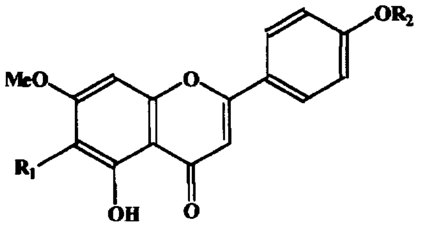
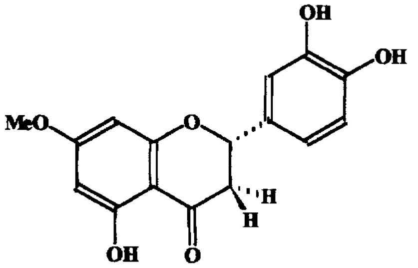


3 $R_1 = H$ $R_2 = Me$
4 $R_1 = OMe$ $R_2 = H$
5 $R_1 = OMe$ $R_2 = Me$



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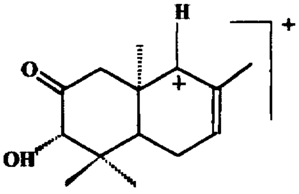
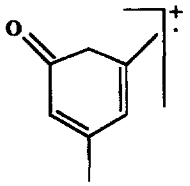
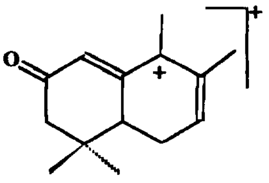


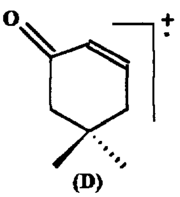
(A)
m/z 221



(B)
m/z 122



(C)
m/z 203



(D)
m/z 124

of the methyl ester **1a** (Tables 1 and 2) were also in agreement with a labdane type diterpene. Both **1** and **1a** gave the same EI mass spectrum fragment at *m/z* 221 (A) indicative of the loss of the side-chain by an allylic cleavage at the C-9/C-11 bond. The ion at *m/z* 122 (B) may be due to the retro-Diels–Alder fragmentation in the decalin ring simultaneously with the loss of the hydroxyl group and one of the geminal methyl groups at C-4.

The presence of one hydroxyl group was, in principle, established using the information presented above as well as by the characteristic downfield shift of the signal at δ 3.92 in the ^1H NMR spectra of **1** which appeared at δ 4.85 upon acetylation of its

Table 1. ^{1}H NMR spectral data of diterpenoids (200 MHz, $CDCl_3$, δ values in ppm, J values in Hz)

H	**1**	**1a**	**1b**	**1c**	**1d**	**2**
1α	2.70 (*d*, *J* = 12.0)	2.68 (*d*, *J* = 12.0)	2.51 (*d*, *J* = 12.0)			6.20 (*s*)
1β	2.25 (*dd*, *J* = 12.0, 1.0)	2.28 (*dd*, *J* = 12.0, 1.0)	2.25 (*d*, *J* = 12.0)			
2				4.15 (*dd*, *J* = 6.0, 4.5)	4.18 (*ddd*, *J* = 13.8, 9.2, 6.9)	
3	3.92 (*s*)	3.95 (*s*)	4.85 (*s*)	3.22 (*d*, *J* = 4.5)	3.85 (*d*, *J* = 6.9)	
6	2.00 (2H, *m*)*	2.00 (2H, *m*)*	1.90 (2H, *m*)			2.0 (*m*)
7	5.42 (*m*)†	5.45 (*m*)†	5.40 (*m*)†	5.40 (*m*)†	5.42 (*m*)†	5.50 (*m*)†
14	2.20 (*m*)*	2.20 (*m*)*	2.10 (*m*)*	2.20 (*dd*, *J* = 15.0, 7.5)	2.11 (*dd*, *J* = 16.0, 6.9)	2.21 (*dd*, *J* = 16.0, 8.0)
14′	2.35 (*dd*, *J* = 14.0, 5.0)	2.30 (*m*)*	2.20 (*m*)*	2.37 (*dd*, *J* = 15.0, 7.5)	2.32 (*dd*, *J* = 16.0, 5.8)	2.38 (*dd*, *J* = 16.0, 8.0)
16	1.00 (*d*, *J* = 6.0)	0.98 (*d*, *J* = 6.6 Hz)	0.95 (*d*, *J* = 6.5)	1.00 (*d*, *J* = 6.0)*	0.94 (*d*, *J* = 6.0)*	1.05 (*d*, *J* = 6.0)*
17	1.70 (*br s*)	1.71 (*br s*)	1.70 (*br s*)	1.68 (*br s*)	1.68 (*br s*)	1.70 (*br s*)
18	0.80 (*s*)	0.72 (*s*)	0.85 (*s*)	1.00 (*s*)*	0.99 (*s*)*	1.12 (*s*)
19	1.12 (*s*)	1.15 (*s*)	1.10 (*s*)	1.10 (*s*)	1.03 (*s*)	1.20 (*s*)
20	0.70 (*s*)	0.70 (*s*)	0.80 (*s*)	1.00 (*s*)*	0.81 (*s*)	1.00 (*s*)*
Ac			2.15 (*s*)			
COOMe		3.70 (*s*)	3.60 (*s*)		3.68 (*s*)	
2Me-acetonide					1.32 (*s*), 1.50 (*s*)	

* Overlapped.
† $W_{1/2}$ = 9.6–10.4 Hz.

Table 2. ^{13}C NMR data for compounds **1**, **1a**, **1c**, **1d** and **2** (50.3 MHz), $CDCl_3$

C	**1**	**1a**	**1c**	**1d**	**2**
1	51.2 *t*	51.3 *t*	45.1 *t*	31.3 *t*	124.5 *d*
2	210.9 *s*	210.8 *s*	72.8 *d*	73.7 *d*	210.7 *s*
3	82.4 *d*	82.4 *d*	80.0 *d*	83.1 *d*	38.7 *t*
4	43.4 *s**	43.5 *s**	37.5 *s**	36.7 *s**	43.4 *s*
5	48.6 *d*	48.6 *d*	49.8 *d*	47.5 *d*	47.9 *d*
6	24.5 *t*	24.4 *t*	22.5 *t*	22.7 *t*	23.0 *t*
7	122.1 *d*	122.1 *d*	122.5 *d*	123.7 *d*	122.2 *d*
8	134.7 *s*	134.7 *s*	135.5 *s*	137.0 *s*	135.0 *s*
9	55.0 *d*	55.1 *d*	56.1 *d*	51.9 *d*	37.8 *s*
10	44.9 *s**	44.8 *s**	36.0 *s**	36.5 *s**	143.8 *s*
11	23.6 *t*	23.6 *t*	26.7 *t*	24.8 *t*	24.7 *t*
12	38.7 *t*	38.7 *t*	38.5 *t*	35.5 *t*	29.6 *t*
13	30.8 *d*	30.8 *d*	30.8 *d*	31.0 *d*	30.8 *d*
14	41.3 *t*	41.5 *t*	42.3 *t*	41.5 *t*	41.2 *t*
15	178.5 *s*	173.4 *s*	177.2 *s*	175.5 *s*	178.6 *s*
16	19.5 *q*	19.5 *q*	21.2 *q*	19.6 *q**	22.2 *q*
17	21.8 *q*	21.7 *q*	22.5 *q*	22.3 *q*	22.1 *q*
18	15.7 *q*	15.7 *q*	17.5 *q*	19.8 *q**	19.5 *q*
19	28.1 *q*	28.1 *q*	31.0 *q*	25.0 *q*	24.9 *q*
20	14.5 *q*	14.5 *q*	15.6 *q*	17.3 *q*	14.9 *q*
$CO_2\underline{Me}$		51.4 *q*		51.4 *q*	
Ketal carbon				108.0 *s*	
2Me-acetonide				27.4 *q*	
				28.6 *q*	

* Values may be interchangeable in each column.

methyl ester (Compound **1b**, Table 1). This result was in agreement with the presence of one secondary hydroxyl group in the decalin moiety. The ^{1}H–^{1}H COSY experiment performed on compound **1b**, also showed the same coupling pattern between H-1α, H-1β and the long range coupling with H-20 aforementioned for compound **1**. Moreover, the allylic coupling between the H-7 and C-17 methyl protons, as well as the vicinal coupling of H-7 with the protons at C-6 which appeared as a multiplet centred at δ 1.90, were also observed.

The stereochemistry of the hydroxyl group at C-3 was proposed from the ^{1}H NMR data of compound **1c** obtained by reduction of **1** with $NaBH_4$ in methanol for ten minutes. This spectrum showed a doublet (J = 4.5 Hz) centred at δ 3.22 clearly coupled (COSY spectrum) with a double doublet at δ 4.15 (J = 6.0 and 4.5 Hz), these signals were assigned to H-3 and H-2, respectively. This coupling pattern can be explained by the hydroxyl groups at C-2 and C-3 possessing a *cis*-relationship as a consequence of a stereoselective reduction of the carbonyl group at C-2 in compound **1**. This is the only way to explain the small coupling constant of H-2β with H-1α, H-1β and H-3β. NMR simulation by computer [16] also afforded information about the average of dihedral angles and the corresponding J data and was used to check the values obtained from the ^{1}H NMR measurements for the aforementioned protons. The observed stereoselectivity in this reaction can be rationalized if the C-20 methyl group has the α-axial configuration in the proposed *ent*-labdane skeleton. In this circumstance, nucleophilic attack from the *Re* face of the carbonyl group at C-2 is considerably hindered by the presence of the axial C-19 and C-20 methyl groups [17].

Additional proof was obtained when the methyl ester (**1c**) of **1** was treated in acetone with catalytic amounts of sulphuric acid to yield, after purification, only the acetonide **1d**. The ^{1}H NMR of this compound (Table 1) showed a one proton *ddd* (J = 13.8, 9.2 and 6.9 Hz) at δ 4.18 and a doublet at δ 3.85 (J = 6.9 Hz) which were in agreement with H-2 and H-3, respectively; on the other hand, the methyl protons of the acetonide part showed singlet resonances at δ 1.50 and 1.32. The ^{13}C NMR data (Table 2) showed the ketal carbon signal of the acetonide function at δ 108.0 as well as the respective methyl group carbons at δ 27.4 and 28.6. The other signals were in agreement with the proposed structure for the derivative under discussion.

To our knowledge, all the labdanes isolated from the genus *Ophryosporus* have been placed in the *ent*-series [2–4]. From *O. floribundus* have been obtained some 9,10-*seco*-*ent*-labdanes, which have *R* configuration at C-5 [4]. These products could be assigned as biogenetical precursors of the *ent*-labdane skeleton present in other compounds isolated from the same plant. If we accept that the ring formation from the precursor to give the decalin system proceeds without change of configuration at C-5, it is justified to assume the presence of *ent*-labdanes in this species. On this biogenetic basis as well as by comparison of the NMR data with previously reported compounds [2–4] an *ent*-labdane skeleton is proposed for **1**. Hence, on taking into account all the above data, compound **1**, in which the configuration at C-13 could not be determined, has the structure of 2-oxo-3α-hydroxy-*ent*-labd-7-en-15-oic acid, namely ophryoic acid.

Compound **2** was isolated as an oil and assigned the molecular formula $C_{20}H_{30}O_3$ by means of the EI mass spectrometry, ^{13}C NMR and ^{1}H NMR spectral data. The UV spectrum exhibited absorption maxima at 207.0 nm (ε: 2616) and 249 nm (sh). The IR spectrum showed absorption bands at 1740 and 1715 cm^{-1} indicating the presence of a —COOH group and ketone group, respectively. In the ^{1}H NMR spectrum of **2** (Table 1), two signals due to olefinic protons, one of them as a sharp singlet at δ 6.20, and the other one as a broad multiplet ($W_{1/2}$ = 9.6 Hz) at δ 5.50 were observed. A three-proton broad singlet at δ 1.70, three methyl groups as singlets at δ 1.20, 1.12, and 1.00 as well as a three-proton doublet at δ 1.05 (J = 6.0 Hz) superimposed on the singlet at δ 1.00, were also present.

The ^{13}C NMR spectra (Table 2), resolved with the aid of DEPT, XH-CORR and COLOC experiments, showed the presence of two carbonyl groups at δ 210.7 and 178.6, in agreement with ketone and carboxyl groups, respectively. Four olefinic carbon atoms, two of them proton-bearing, were in accord with two trisubstituted olefinic bonds; the first between C-7-C-8, as in **1** and, the second, conjugated with the ketone carbonyl group. The ^{13}C NMR spectra also showed five methylene carbons, five methyl groups, two sp^3 methines, and two sp^3 quaternary carbon atoms.

The ^{1}H–^{1}H COSY spectrum revealed cross-peaks between the olefinic proton at δ 5.50 with the broad methyl singlet at δ 1.70 as well as with the multiplet at δ 2.0 assigned to the C-6 methylene group. From this 2D experiment, the homoallylic coupling between the methylene protons and the olefinic methyl group at δ 1.70 was observed. All the above data suggested a B ring similar to that proposed for compound **1**. Similarly, the sequence H-16, H-13 and H-14 (eight line multiplet) was established; accordingly, no functionalities were at C-6 and C-14. All these data were consistent with the α,β-unsaturated ketone being located on ring A with the more deshielding olefinic proton at C-1. Additional support was obtained by COLOC which showed long range coupling between both C-2 and C-10 with H-1 as well as of this proton with the C-3 methylene group. Consequently, the structure of **2** belongs to the *ent*-halimane series [11].

The ^{13}C NMR spectra showed from the chemical shifts that C-10 (β carbon in the enone moiety) was shielded, whereas C-2 (carbonyl group) was deshielded with respect to other compounds with these functionalities. This fact can be explained by taking account of the fact that carbonyl and alkenyl carbon shifts in α,β-unsaturated ketones may be used to differentiate between planar and twisted conjugated systems [18]. In the compound under discussion, the carbonyl group at C-2 may be placed out of the plane determined by the C-1, C-10, C-5 and C-9 carbons, to avoid the ring strain in the decalin fragment. In order to confirm this, we performed an AM1 calculation for **2** [19]; this theoretical calculation predicted that the most stable conformation for ring A is the one in which the carbonyl group is down 13.45° from the plane. Furthermore, this is consistent with the observed ketone carbonyl group IR absorption at 1715 cm^{-1} instead of the typical band at low frequency for a non-twisted conjugated carbonyl system, as well as the hypsochromic shift with low molar absorptivity observed in the UV spectrum (see Experimental).

Finally, the EI-mass spectrum of the methyl ester of compound **2** displayed important ions at m/z 203 (ion C) and 124 (ion D). These fragments could be accounted for as a consequence of the loss of the side-chain from the molecular ion in the first case and from cleavage of the C-9/C-10 and C-5/C-6 bonds to afford the ion at m/z 124.

Hence, on taking into account all of the above data as well as biogenetic considerations, compound **2**, in which the configuration at C-13 could not be determined, has the structure of 2-oxo-*ent*-haliman-1(10),7-dien-15-oic acid, namely charruoic acid.

Finally, the flavones apigenin-7,4′-dimethyl ether (**3**), circimaritine (**4**), salvigenin (**5**), and 7-*O*-methyleriodictyol (**6**), were isolated and their structures determined by spectroscopic means [20–23] and by comparison with authentic samples.

EXPERIMENTAL

General. ^{1}H NMR: $CDCl_3$ at 200.13 MHz; ^{13}C NMR: 50.23 MHz; COSY, XH-CORR, and COLOC experiments were resolved using standard software. EIMS: 70 eV; CC: Silica gel G 70–230 mesh and Kieselgel 60 H; TLC: Silica gel F_{254} 0.2 thick plates using C_6H_6–dioxane–AcOH (30:5:1); HPLC: silica gel 25–40 μm (10 mm i.d. × 25 cm) with *n*-hexane–EtOAc (7:3), flow 0.5 ml min^{-1}, frs of 0.1 ml each, detection by differential refractometer.

Plant material. Specimens of *O. charrua* were collected at Quebrada de los Cóndores (San Luis, Argentina) on April 1995, a herbarium sample is deposited in the Herbario of the Universidad Nacional de San Luis, L.A. Del Vitto *et al.* 6205 (UNSL).

Extraction and isolation. Dried aerial parts (2.2 kg) were extracted with MeOH. The extract was concd *in vacuo* and the resultant syrup was dissolved in a mixt. of $MeOH–H_2O$ (9:1) and partitioned against *n*-hexane. The alcoholic phase was concd and the residue dissolved in $CHCl_3$. From this extract, the acidic fr. was recovered by extraction with aq. $NaHCO_3$ followed by acidification and partitioning against Et_2O. After several CC and HPLC purifications of the ethereal extract, compounds **1** (316 mg) and **2** (83 mg) were isolated as oils.

2-Oxo-3α-hydroxy-ent-*labd-7-en-15-oic acid* (**1**). Oil, $[\alpha]_D^{25}$ −2.4° (Cl_3CH; *c* 0.43). IR ν_{max}^{KBr} cm^{-1} 3455, 2950, 1750, 1710, 1205, 1180, 820; ^{1}H and ^{13}C NMR: Tables 1 and 2; EIMS: *m/z* (rel. int.): 336 (34) $[M]^+$, 321 (27) $[M–Me]^+$, 318 (16) $[M–H_2O]^+$, 263 (32), 222 (64), 221 (44) [ion A]$^+$, 203 (54) [ion A–H_2O]$^+$, 122 (86) [ion B]$^+$, 107 (100), 96 (22), 81 (75), 69 (73), 55 (49), 43 (72).

2-Oxo-3α-hydroxy-ent-*labd-7-en-15-oic methyl ester* (**1a**). A satd soln of CH_2N_2 in Et_2O (2.5 ml) was added to a soln of compound **1** (50 mg) in Et_2O (5 ml) and the mixt. was kept at room temp. for 1 hr. Removal of the solvent *in vacuo* yielded a residue which was subjected to CC to give the corresponding methyl ester (**1a**) (39 mg) as an oil. IR ν_{max}^{KBr} cm^{-1} 3450, 2930, 1730, 1710, 1200, 1180, 820; ^{1}H and ^{13}C NMR: Tables 1 and 2; EIMS: *m/z* (rel. int.): 350 (16) $[M]^+$, 335 (10) $[M–CH_3]^+$, 317 (5), 221 (11) [ion A]$^+$, 203 (41) [ion A–H_2O]$^+$, 129 (30), 122 (44) [ion B]$^+$, 107 (39), 69 (41), 43 (100).

2-Oxo-3α-acetoxy-ent-*labd-7-en-15-oic methyl ester* (**1b**). Compound **1a** (25 mg) was acetylated with Ac_2O–pyridine overnight and, after usual work-up and preparative TLC, 18 mg of the compound **1b** were recovered as an oil. ^{1}H NMR: Table 1.

2α,3α-Dihydroxy-ent-*labd-7-en-15-oic acid* (**1c**). Compound **1** (55 mg) was dissolved in MeOH (4.0 ml) and an excess of $NaBH_4$ was added. After 10 min with stirring at room temp. the solvent was removed under vacuum and the residue was acidified with diluted HCl and extracted with Et_2O. The ethereal extract was purified by CC and 44 mg of **1c** were recovered as an oil. ^{1}H and ^{13}C NMR: Tables 1 and 2; EIMS: *m/z* (rel. int.): 320 (3) $[M–H_2O]^+$, 251 (2), 205 (5), 182 (14), 122 (28), 107 (19), 95 (28), 91 (16), 81 (23), 69 (32), 55 (65), 43 (100).

Acetonide derivative **1d**. The methyl ester of **1c** (25 mg) was treated overnight with Me_2CO with catalytic amounts of H_2SO_4. The acid was neutralized with $NaHCO_3$ and the resulting oil was partitioned against brine and Et_2O, and **1d** purified by prep. TLC. ^{1}H and ^{13}C NMR: Tables 1 and 2.

2-Oxo-ent-*haliman-1(10),7-dien-15-oic acid* (**2**). Oil, $[\alpha]_D^{25}$ −4.7° (Cl_3CH; *c* 0.50). UV λ_{max}^{MeOH} nm (log ε): 207 (3.41), 249 sh; IR ν_{max}^{KBr} cm^{-1}: 2920, 1740, 1715, 1660, 1220, 870; ^{1}H and ^{13}C NMR: Tables 1 and 2; EIMS (as methyl ester): *m/z* (rel. int.): 332 (2) $[M]^+$, 203 (12) [ion C]$^+$, 196 (9), 152 (55), 124 (85) [ion D]$^+$, 109 (36) [ion D–CH_3]$^+$, 91 (27), 69 (37), 43 (100).

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