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SESQUITERPENOID ESTERS FROM THE FRUITS OF *FERULA COMMUNIS*

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Key word Index—*Ferula communis*; Umbelliferae; fruit; daucane esters; 1-oxojaeskeanadiol esters.

Abstract—The fruits of the toxic variety of *Ferula communis* gave a series of 1-oxojaeskeanadiol esters, whose structure was established by spectroscopic data and chemical reactions, including correlation with jaeskeanadiol. Treatment of these esters with potassium hydroxide gave two polienonic products, resulting from the stepwise elimination of the ester group and one water molecule. Both compounds were unstable; the dienone underwent rapid deconjugation via a 1,5-sigmatropic hydrogen shift, whereas the trienone was oxidized in air at the terminal bond of the conjugated system. The toxic prenylated coumarins were not found in the fruits.

INTRODUCTION

Ferulosis is an often lethal poisoning caused by *Ferula communis* L. and described for the first time in Sardinia [1 and refs therein]. In previous studies, we reported the presence of distinct chemical races of *F. communis* subsp. *communis* [2–4]. This infraspecific variability of the secondary metabolism of the plant is probably responsible for the contrasting data on the toxicity of the species reported in the literature, because only plants containing prenylated coumarins can elicit the toxic symptoms of ferulosis, whereas those containing daucane esters are not toxic [5].

The race containing prenylated coumarins is not homogeneous. Based on the ratio of ferulenol to its ω-oxygenated derivatives, two patterns of constituents were detected; one in which ferulenol is the major constituent and one consisting almost exclusively of its more polar ω-oxygenated derivatives [4]. Roots were used in these studies, but a very similar pattern of constituents was also found in leaves. We have now investigated the fruits from different collections of *F. communis* subsp. *communis* from Sardinia.

RESULTS AND DISCUSSION

The prenylated coumarins could not be isolated from any fruit sample examined, whereas the phenylpropane laserin (**1**) [6] and the daucane ester siol anisate (**2**) [2] were always present. However, other compounds had a more specific distribution, and differences between the various collections could thus be detected, although not so striking as those found in leaves and roots. The fruits from the plants containing prenylated coumarins in their roots and leaves gave in fact a series of 1-oxojaeskeanadiol esters which were not contained in the other samples.

The major constituent of these fruits was a *ca* 4:1 crystalline mixture of the 5(*O*)-angeloyl and isovaleroyl esters of 1-oxojaeskeanadiol (**3a** and **3b** respectively). These compounds could not be satisfactorily separated by HPLC or crystallization. Also the 5(*O*)-anisoyl ester of 1-oxojaeskeanadiol, **3c**, was obtained from the more polar fractions. Compound **3a** was previously isolated, as an oil, from a plant of the Compositae family [7]; **3b** and **3c** are new compounds.

The relative and absolute stereochemistry of **3a/3b** was established by correlation with jaeskeanadiol (**4a**). The mixture **3a/3b** was reduced with sodium borohydride, and the allylic alcohols obtained in this way (**5a/b**) were acetylated and then reduced with lithium in liquid ammonia to give the crystalline diol **4a**, identical with a sample of jaeskeanadiol obtained from the reaction of ferutidin (**4b**) with lithium aluminium hydride. The secondary hydroxyl of **5a/b** was β, as shown by analysis of the ^{1}H NMR spectrum ($J_{1,2} \cong 0$ Hz; $\sphericalangle$ H_1–C_1–C_2–H_2 *ca* 90°), and from inspection of models of the starting enone, in which approach of the hydride from the β-face is hindered by the angular methyl. The allylic oxidation of ferutidin (**4b**) gave **6** and not **3c**.

Treatment with potassium hydroxide of the crystalline mixture **3a/b** gave two products, neither of which corresponded to the expected ketol resulting from the hydrolysis of the ester group. The least polar product was the trienone **7**, and the more polar product the dienone **8**. These compounds were formed from **3a/b** by the sequential loss of angelic or isovalerianic acid of one water molecule. Both **7** and **8** are unstable; the trienone was oxidized in air at the terminal bond of the conjugated

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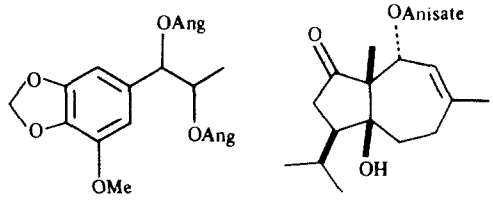
system, giving the epoxide **9**, whereas the dienone underwent deconjugation via a 1,5-hydrogen shift, affording the diene **10**. Compound **7** had a very high optical rotation (+484).

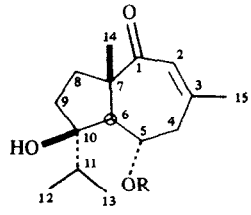
As minor constituents the angelate esters of webbiol (**11**) and jaeskeanadiol (**4c**) as well as the bis-angelate ester of 8α-hydroxyjaeskeanadiol (**12a**) were isolated. Compounds **11** and **4c** are known [8, 9], whereas **12a** is new.

No difference in the constituents of the fruits was found between plants containing ferulenol and its ω-oxygenated derivatives in their roots and leaves, and those containing only the ω-oxygenated derivatives. However, the fruits of the non-toxic variety of *F. communis* subsp. *communis* did not contain 1-oxojaeskeanadiol esters, but only jaeskeanadiol and siol esters (G. Appendino and M. G. Valle, unpublished data). Thus, in spite of the absence of prenylated coumarins and the presence of compounds belonging to the same class, differences between the toxic and the atoxic races of *F. communis* subsp. *communis* exist also at the level of the fruits, suggesting that variations in

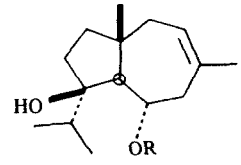


1

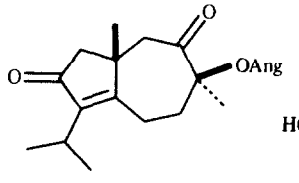
2



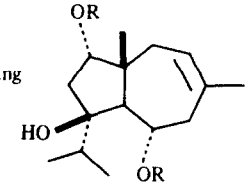
R	
3a	Ang
3b	*i*Val
3c	Anisate



R	
4a	H
4b	Anisate
4c	Ang



11



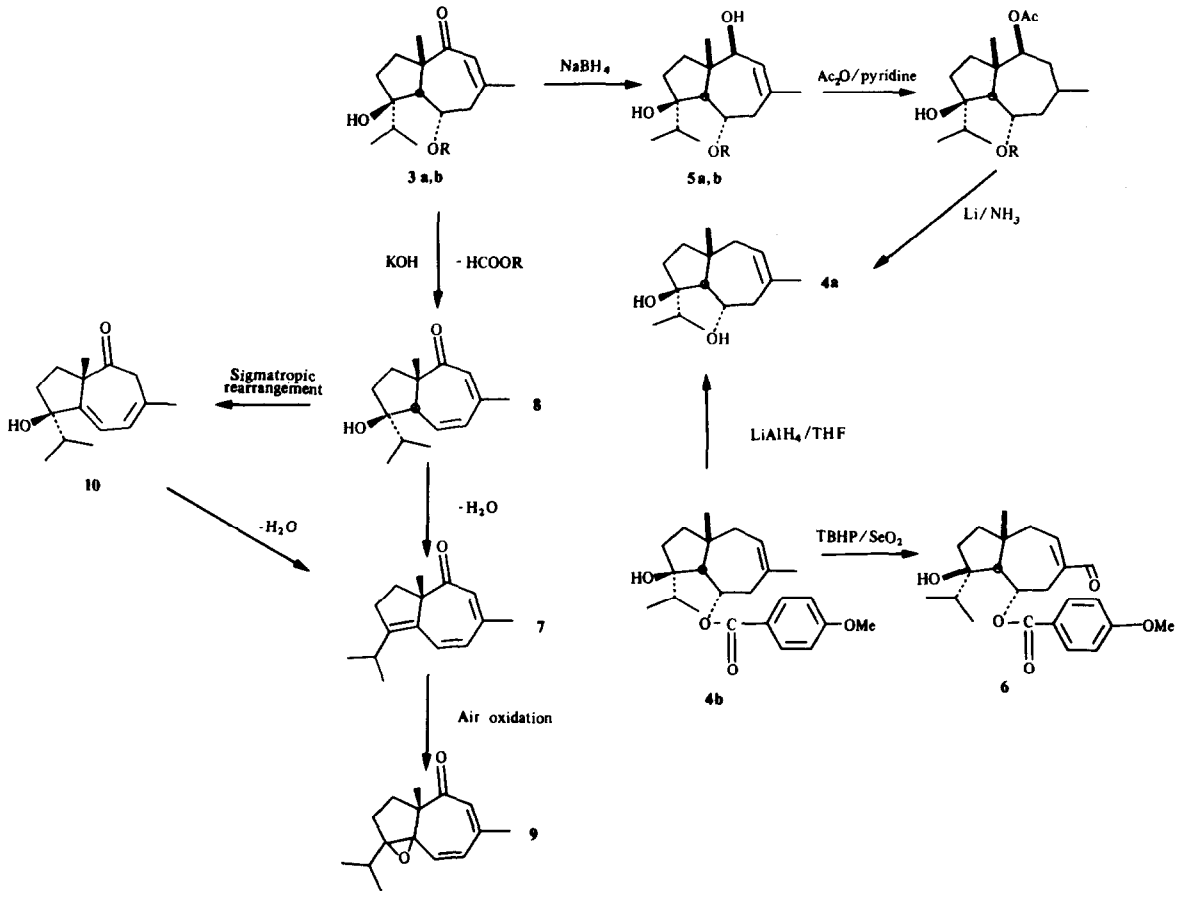
R	
12a	An
12b	H

Table 1. ^{13}C NMR data of compounds **3a–6**, **7**, **9** and **12b** (62.89 MHz, $CDCl_3$, TMS as int. standard)

C	**3a**	**3b**	**3c**	**6**	**7**	**9**	**12b** (Acetone-d_6)
1	208.45 *s*	208.02 *s*	208.78 *s*	30.72 *t*	198.91 *s*	201.50 *s*	44.78 t^a
2	128.51 *d*	128.51 *d*	128.70 *d*	155.15 *d*	128.08 d^a	128.89 d^a	127.08 *d*
3	149.43 *s*	49.43 *s*	150.03 *s*	140.23 *s*	158.24 s^b	145.10 *s*	137.18 *s*
4	37.99 t^a	38.13 t^a	38.39 t^a	42.36 t^a	127.08 d^a	128.85 d^a	37.65 t^b
5	69.65 *d*	69.86 *d*	70.47 *d*	70.04 *d*	124.68 d^a	135.40 d^a	73.34 d^c
6	50.00 *d*	49.75 *d*	50.36 *d*	59.43 *d*	145.18 s^b	74.98 s^b	51.98 *d*
7	55.03 *s*	54.83 *s*	55.33 *s*	43.64 *s*	60.68 *s*	55.77 *s*	47.09 *s*
8	36.76 t^a	36.39 t^a	36.92 t^a	41.27 t^a	31.13 t^c	21.68 t^c	69.19 d^c
9	31.13 t^a	31.13 t^a	31.25 t^a	31.22 t^a	28.13 t^c	27.51 t^c	31.82 t^b
10	84.20 *s*	84.04 *s*	84.55 *s*	86.24 *s*	132.75 s^b	67.11 s^b	87.91 *s*
11	36.16 *d*	36.16 *d*	36.45 *d*	36.93 *d*	27.76 *d*	28.75 *d*	39.49 *d*
12	18.01 q^b	18.01 q^b	18.35 q^b	18.31 q^b	20.89 q^d	18.36 q^d	17.79 q^d
13	17.06 q^b	17.06 q^b	17.31 q^b	17.26 q^b	21.12 q^d	18.70 q^d	18.77 q^d
14	17.93 q^b	18.06 q^b	18.17 q^b	20.35 q^b	24.81 q^d	21.34 q^d	20.90 q^d
15	27.76 *q*	28.02 *q*	28.53 *q*	192.88 *d*	27.00 *q*	27.40 *q*	28.63 *q*

Ester groups: **3a** (angelate): 167.62 *s*, 139.08 *d*, 127.37 *s*, 20.18 *q*, 15.50 *q*. **3b** (isovalerate): 172.40 *s*, 43.52 *t*, 25.28 *d*, 22.17 *q*. **3c** (anisate): 166.24 *s*, 165.76 *d*, 131.69 *d*, 122.28 *d*, 113.88 *d*, 55.47 *q*. **6** (anisate): 166.15 *s*, 163.45 *s*, 131.60 *d*, 122.80 *d*, 113.58 *d*, 55.25 *q*.

[a–d] Interchangeable signals within a column.



Scheme 1. Reaction of **3a, b** (R = Ang and *i*Val) with potassium hydroxide and chemical correlation with jaeskeanadiol (**4a**).

secondary metabolism as a whole is characteristic of this species.

EXPERIMENTAL

CC: silica gel 60 (70–230 mesh, Merck). HPLC: microporasyl column (80 × 3 cm) (Waters) coupled to a Waters differential refractometer R 401; ^{1}H and ^{13}C NMR: 250 and 62.89 MHz respectively.

Plant material. Fruits of the toxic variety of *F. communis* subsp. *comunis* were collected in July 1988 at Olliena (NU) (sample A) and at Caprera island (sample B), and were identified by V.P.; leaves and roots from the plants were also analysed. Although a different pattern of prenylated coumarins was found in these organs, the constituents of the fruits were identical. The extraction of the fruits from sample B (Caprera) is reported as representative. Voucher specimens of the fruits are kept at the laboratory of Torino University.

Isolation of the constituents. Dried fruits (320 g) were powdered and extracted with CH_2Cl_2 (*ca* 1 l, × 3). After removal of the solvent, 19 g of extract (6%) was obtained as a black paste. The latter was dissolved in CH_2Cl_2 and 10 g of silica gel (35–70 mesh, Merck) added. After removal of the solvent (rotary evaporator), the residue was loaded onto a column packed with silica gel (70–230 mesh, 100 g). Elution was started with hexane–EtOAc (19:1). The following compounds were obtained: (A) laserin (**1**) (2 g, 0.65%, hexane–EtOAc 19:1); (B) a crude mixture of **4c**, **11**, **12a** and stigmasterol (hexane–EtOAc 9:1); (C) **3a, b** (0.803 g, 0.25%, hexane–EtOAc 9:1); (D) **2** (0.458 g, 0.14%, hexane–EtOAc 4:1); (E) **3c** (0.190 g, 0.059%, hexane–EtOAc 9:1). Yields refer to chromatographically and spectroscopically pure compounds. Analytical samples of **3a, b** and **3c** were obtained by crystallization from hexane at −5°. The ratio **3a**:**3b** (*ca* 4:1, from ^{1}H NMR analysis) was not changed by repeated crystallization. Part of fraction B was further purified by HPLC (hexane–EtOAc 4:1). Compound **11** was thus obtained as crystals, and **4c** as an oil. Compound **12a** could only be thoroughly purified from long-chain aliphatic compounds after conversion to the triol **12b** by treatment wih $LiAlH_4$.

1-Oxojaeskeanadiol angelate/isovalerate (**3a, b**). Crystals from hexane (*ca* 4:1 mixture); mp 79°; $[\alpha]_D^{25}$ +97.5 ($CHCl_2$; *c* 1.2); IR ν_{max}^{KBr} cm^{-1}: 3400, 1710, 1635, 1380, 1260, 1230, 1165, 1040; UV λ_{EtOH}^{max} nm: 252; ^{1}H NMR (250 MHz, $CDCl_3$, TMS): **3a** δ6.01 (*br s*, H-2), 5.37 (*ddd*, $J_{5,6}$ = 9.5, $J_{4a,5}$ = 5.4, $J_{4b,5}$ = 2.7, H-5), 3.00 (*dd*, $J_{4a,b}$ = 16.1, $J_{4a,5}$ = 5.4, H-4a), 2.44 (*d*, $J_{5,6}$ = 9.5, H-6), 2.37 (*dd*, $J_{4a,b}$ = 16.1, $J_{4b,5}$ = 2.7, H-4b), 1.91 (*br s*, H-15), 1.39 (*s*, H-14), 0.94 and 0.86 (each *d*, $J_{11,12(13)}$ = 6.5 Hz, H-12 and H-13); angelate: 6.16 *qq*, 2.04 *dq*, 1.91 *dq*. For **3b** δH-5 = 5.63, *i*Val: 2.20 *m*, 2.10 *m*, 0.98 *d*, and 0.94 *d*; EIMS 70 eV, *m/z* (rel. int.): 334 $[C_{20}H_{30}O_4]^+$ $[M]^+$ (**3a**) (1), 291 (25), 234 (50), 191 (82), 149 (90), 83 (100).

1-Oxojaeskeanadiol anisate (**3c**). Crystals (from hexane), mp 161°, $[\alpha]_D^{25}$: +91 (CH_2Cl_2; *c* 0.88); λ_{max}^{EtOH} nm: 252; IR ν_{max}^{KBr} cm^{-1}: 3500, 1690, 1660, 1610, 1280, 1180, 850, 780; ^{1}H NMR (250 MHz, $CDCl_3$, TMS): δ6.02 (*br s*, H-2), 5.85 (*ddd*, *J* = 9.6; 5.3 and 2.7, H-5), 3.03 (*dd*, *J* = 16.2 and 2.5, H-4a), 2.55 (*d*, *J* = 9.6, H-6), 2.46 (*dd*,

J = 16.2 and 2.7, H-4b), 2.00 (*br s*, H-15), 1.37 (*s*, H-14), 0.87 and 0.85 (each *d*, J = 6.7, H-12 and H-13); anisate: 7.98 and 6.95 (AA'BB' system), 3.90 *s*; EIMS 70 eV, *m/z* (rel. int.): no molecular ion, 343 $[M-31]^+$ (10), 234 (42), 149 (78), 135 (100), 96 (84).

Chemical correlation of 1*-oxojaeskeanadiol esters and jaeskeanadiol.* (i) A 431 mg sample of crude **3a, b** was dissolved in 3 ml MeOH and an excess of $NaBH_4$ (*ca* 100 mg) was added portionwise. The reaction was followed by TLC (hexane–EtOAc 3:2), and after 5 hr satd NH_4Cl was added, and the reaction mixture extracted with CH_2Cl_2. After washing with brine and drying ($MgSO_4$), the semisolid residue was separated by CC (10 g silica gel, hexane–EtOAc 4:1) to give 216 mg (41%) of crude **5a, b**. Crystallization from hexane afforded pure **5a** as a white powder, mp 78°, $[\alpha]_D^{25}$: −48 (CH_2Cl_2; *c* 0.83); IR ν_{max}^{KBr} cm^{-1}:3500, 3300, 1690, 1640, 1240, 1175, 1160, 1050, 1020, ^{1}H NMR (250 MHz, $CDCl_3$, TMS: δ 5.40 (*br s*, H-2), 5.13 (*td*, $J_{5,6} = J_{5,4a} = 10.6$, $J_{5,4b} = 2.7$, H-5), 4.05 (*br s*, H-1), 1.83 (*br s*, H-15), 1.10 (*s*, H-14), 0.92 and 0.91 (each *d*, $J_{11,12(13)} = 6.7$); angelate: 6.12 *q*, 2.02 *br d*, 1.89 *br s*; EIMS 70 eV, *m/z* (rel. int.): 336 ($C_{20}H_{32}O_4$) $[M]^+$ (10), 319 (10), 293 (22), 193 (65), 132 (60), 83 (100).

(ii) A 703 mg sample of crude **5a, b** was dissolved in 3.5 ml dry pyridine, and 3 ml Ac_2O added. After stirring overnight, ice and MeOH were added, followed, after *ca* 10 min, by water, and the reaction mixture extracted with CH_2Cl_2. After washing with satd $NaHCO_3$, satd. $CuSO_4$ and brine, the organic phase was dried ($MgSO_4$) and evapd. The residue was purified by CC (10 g silica gel, hexane–EtOAc 1:1) to give 456 mg of a mixture of the acetates of **5a,b** (yield: 60%).

(iii) To a soln of Li (47 mg) in *ca* 15 ml liquid NH_3 (distilled from Na amide), a soln of 100 mg (0.27 mmol) of the acetates of **5a,b** in 1 ml dry THF was added. The blue soln was stirred at −40° for 2 hr, and then quenched by the addition of solid NH_4Cl. NH_3 was removed by evapn, and then water and CH_2Cl_2 were added. After washing with brine, the organic phase was dried ($MgSO_4$) and evapd. The residue was purified by CC (5g silica gel, hexane–EtOAc 9:1) to give 6.5 mg **4a** (yield 10%), identical (IR, ^{1}H NMR, $[\alpha]_D^{25}$) with a sample of jaeskeanadiol prepared by treatment of **4b** with $LiAlH_4$ (THF, 5 mol equivalents, 0°, quenching with NH_4Cl, yield 74%).

Allylic oxidation of ferutidin (**4b**). To a suspension of 150 mg SeO_2 (1.34 mmol, 0.5 mol, equivs) in 5 ml dry CH_2Cl_2, 734 μl of 70% aq. TBHP (5.36 mg, 2 mol. equivs) were added. The soln. was stirred for 10 min at room temp. and when all the SeO_2 had dissolved, a soln of 1.05 g **4b** (2.68 mmol) was added. The soln was stirred for 48 hr at room temp. under N_2, and then worked-up by the addition of H_2O and CH_2Cl_2. The organic phase was washed with satd Na_2SO_3, brine and then dried. After purification by CC (15 g silica gel, hexane–EtOAc 4:1), 345 mg (yield 33%) **6** were obtained. Crystals from ether, mp 124°, $[\alpha]_D^{25}$: +38 (CH_2Cl_2; *c* 1.1); IR ν_{max}^{KBr} cm^{-1}: 3590, 2720, 1705, 1690, 1680, 1645, 1610, 1520, 1270, 1175, 1120, 860, 780; ^{1}H NMR (250 MHz,$CDCl_3$, TMS): δ 9.40 (*s*, H-15), 6.85 (*m*, H-2), 5.26 (*td*, J = 10.1; 10.1 and 4.0, H-5), 3.26 (*dd*, J = 14.0 and 4.0, H-4a), 2.09 (*d*, J = 10.1, H-6), 1.12 (*s*, H-14), 0.92 and 0.83 (each *d*, J = 7.1, H-12 and H-13); anisate: 7.98 and 6.94 (AA'BB' system), 3.85 (*s*).

Treatment of **3a, b** *with base.* A 1.50 g sample of **3a, b** was solved in 20 ml 5% methanolic KOH, and the soln was stirred under N_2 for 62 hr. The black reaction mixture was worked-up by diln with H_2O, and extracted with CH_2Cl_2. After washing with brine (twice) and drying, the organic phase was evapd, and the residue was separated by CC (20 g silica gel, hexane–EtOAc 9:1 as eluant) to give 296 mg **7** (yield: 31%) and 106 mg of crude dienone **8** (yield 11%). The latter was further purified by HPLC (hexane–EtOAc 4:1).

Compound **7**, orange oil, $[\alpha]_D^{25}$ +484 (!) (CH_2Cl_2; *c* 1.0), UV λ_{max}^{EtOH} nm: 378, 246; IR $\nu_{max}^{liquid\ film}$ cm^{-1}: 1650, 1615, 1450, 1380, 1330, 1270, 1030, 860, 795; ^{1}H NMR (250 MHz, $CDCl_3$, TMS: δ 6.63 (*d*, J = 11.6, H-5), 6.02 (*br s*, H-2), 5.74 (*d*, J = 11.6, H-4), 2.92 (*heptet*, J = 6.7, H-11), 2.68 (*dt*, J = 13.2; 8.2 and 8.2, H-9a), 2.36 (*m*, H-9b), 2.03 (*s*, H-15), 1.60 (*m*, H-8a), 1.17 (*s*, H-14), 1.05 and 0.99 (each *d*, J = 6.7, H-12 H-13); EIMS 70 eV, *m/z* (rel. int.): 216 ($C_{15}H_{20}O$) $[M]^+$ (30), 201 $[M-15]^+$ (45), 173 (52), 161 (100), 145 (60).

Compound **8**, oil, IR $\nu_{max}^{liquid\ film}$ cm^{-1}: 3400, 1700, 1640, 1450, 1380, 1030, 860, 770; ^{1}H NMR (250 MHz, $CDCl_3$, TMS: δ 6.53 (*dd*, J = 11.0 and 4.2, H-5), 6.08 (*d*, J = 11.0, H-4), 6.08 (*d*, J = 11.0, H-4), 6.02 (*br s*, H-2), 2.02 (*br s*, H-15), 1.12 (*s*, H-15), 0.94 and 0.89 (each *d*, J = 6.8, H-12 and H-13). Compounds **7** and **8** could not be stored for more than a few days at room temp. Compound **7** was air-oxidized (both in soln and in the solid state) to the epoxide **9**, whereas **8** was deconjugated in soln to the ketodiene **10**. The latter could be stored for a few weeks at refrigerator temp. without elimination of H_2O and formation of the trienone **7**. Compound **9**, crystalline yellowish product, mp 107° (from hexane), $[\alpha]_D^{25}$ +267 (CH_2Cl_2; *c* 1.5). UV λ_{max}^{EtOH} nm: 295. IR ν_{max}^{KBr} cm^{-1}: 1720, 1650, 1590, 1455, 1385, 1280, 1030, 920; ^{1}H NMR (250 MHz, $CDCl_3$, TMS): δ 6.23 (*d*, J = 10.8, H-5), 6.05 (*br s*, H-2), 5.85 (*d*, J = 10.8, H-4), 2.05 (*s*, H-15), 1.15 (*s*, H-14), 1.12 and 1.01 (each *d*, J = 6.9, H-12 and H-13) **10**, oil, IR $\nu_{max}^{liquid\ film}$ cm^{-1}: 3400, 1700, 1640, 1450, 1380, 1180, 1030, 840, 740; ^{1}H NMR (250 MHz, $CDCl_3$, TMS): δ 6.10 (*m*, H-4 + H-5), 3.12 (*br s*, H-2a,b), 1.92 (*br s*, H-15), 1.28 (*s*, H-14), 0.99 and 0.92 (each *d*, J = 6.9, H-12 and H-13).

Treatment of **12a** *with* $LiAlH_4$. To a suspension of an excess $LiAlH_4$ (200 mg) in 5 ml dry THF, a 230 mg sample of impure **12a** in 5 ml dry THF was added dropwise at 0°. After stirring under N_2 for 1 hr at room temp., the reaction was quenched by the careful addition of EtOAc and then diluted with H_2O. After further addition of EtOAc, the organic phase was sepd, washed with brine (twice) and dried ($MgSO_4$). The residue crystallized spontaneously, affording 51 mg **12b** as large cubes, mp 170°, $[\alpha]_D^{25}$ −109 (CH_2Cl_2; *c* 0.88), IR ν_{max}^{KBr} cm^{-1}: 3570, 3300, 1670, 1470, 1015, 990, 980, 970; ^{1}H NMR (250 MHz, $CDCl_3$, TMS): δ 5.71 (*br d*, J = 7.2, H-2), 4.04 (*tt*, J = 10.5; 10.5; 3.0 and 3.0, H-5), 3.76 (*d*, J = 7.7, H-8), 2.49 (*d*, J = 10.5, H-6), 1.79 (*br s*, H-15), 1.02 (*s*, H-14), 0.94 (6H, *d*, J = 7.1, H-12 and H-13).

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