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LABDANE DITERPENES FROM AN *ACACIA* SPECIES

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Key Word Index—*Acacia* sp.; Leguminosae; diterpenes; (13*E*)-labd-13-ene-3α,8β,15-triol; (13*E*)-3α,8β-dihydroxy-labd-13-en-15-oic acid.

Abstract—Two new diterpenes, (13*E*)-labd-13-ene-3β,8α,15-triol and (13*E*)-3β,8α-dihydroxylabd-13-en-15-oic acid have been isolated from an unclassified *Acacia* sp. Chemical and spectroscopic evidence for their structure is presented. The known labdanes, sclareol, 13-*epi*-sclareol and (13*E*)-labd-13-ene-8α,15-diol were also isolated.

INTRODUCTION

In continuation of our investigation of the chemistry of Western Australian plants, we have examined the constituents of the resin of an unclassified species of the genus *Acacia*, a voucher specimen (No 00117633) of which has been deposited in the Western Australian Herbarium. Five labdane diterpenes were isolated: sclareol (**1**), 13-*epi*-sclareol (**2**) and (13*E*)-lab-13-ene-8α,15-diol (**3**) are known compounds; (13*E*)-labd-13-ene-3β,8α,15-triol (**4**) and (13*E*)-3β,8α-dihydroxylabd-13-en-15-oic acid (**8**) are new labdanes. Chemical and spectroscopic evidence for the structure and absolute stereochemistry of these new compounds is presented in this report.

RESULTS AND DISCUSSION

The ether extract of the leaves and terminal branches of *Acacia* sp. was partitioned into a neutral and acid fractions. Alumina chromatography of the neutral portion afforded fractions of sclareol (**1**) and 13-*epi*-sclareol (**2**) in a 1:1 ratio as evidenced by ^{1}H and ^{13}C NMR spectroscopy. The ^{13}C NMR spectrum of this mixture was compared with that reported for sclareol [1] and this allowed the ^{13}C NMR signals for 13-*epi*-sclareol to be assigned (Table 1). A second neutral compound isolated was shown to be labd-13-ene-8α,15-diol (**3**) by comparison of mp and $[\alpha]_D$ values with those published [2] and by comparison of the ^{1}H and ^{13}C NMR spectra with those of an authentic sample [3] of the enantiomeric diol [the $[\alpha]_D$ values published for both enantiomers, +0.7° [3] and −0.5° [2] are extremely low. More meaningful values are obtained if optical rotations are taken at 365 nm (see Experimental)].

The most polar compound isolated was shown to be a triol (**4**), $C_{20}H_{36}O_3$, since it formed a diacetate (**5**) whose IR spectrum showed absorption for a hydroxyl group. The ^{1}H NMR spectrum of **4** was essentially similar to that of **3** except for the presence of extra signals for a hydroxymethine proton (δ3.25, $W_{1/2}$ = 18 Hz) and a downfield shift (δ0.87–0.99) for one of the three tertiary methyl groups. Comparison of the ^{13}C NMR spectra of **3** and **4** suggested that the extra hydroxyl group was located at C-3 since significant chemical shift differences were observed for the carbons assigned to ring-A and its pendant groups (Table 1). Interrelation of **4** with the known [4] diol **6** confirmed this and also established the absolute configuration of **4**. To this end the diacetate **5** was treated with at 0° to give a mixture of **7** and its Δ^8-isomer (2:1) which on treatment with lithium aluminium hydride gave the corresponding diols. Chromatography of the mixture on alumina–silver nitrate (9:1) afforded a small amount of the diol **6**, mp 161–163° $[\alpha]_D$ + 24.8° (lit. [4], mp 164–165°, for the enantiomer [5], mp 160.5–162°, $[\alpha]_D$ − 27°).

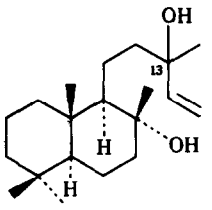
The configuration at C-8 in **4** can be assigned from consideration of the ^{1}H and ^{13}C NMR spectra. The chemical shift of the C-10 methyl in the ^{1}H NMR spectrum of **4** occurs at δ0.82 indicating the absence of a 1,3-diaxial interaction with the C-8 hydroxyl group which otherwise would result in a downfield shift to δ0.95 [6]. More significantly, in the ^{13}C NMR spectra of 8-hydroxylated labdanes an equatorial hydroxyl group has a deshielding effect on C-17 of 6.5 ppm compared to the axial, C-17 resonating at δ24.0 [1, 7] instead of δ30.5 [8]. This is consistent with effects observed [9] on model systems. In addition C-9 is shielded by 2.6 ppm when the hydroxyl at C-8 is equatorial (δ61.4 vs δ58.8). Since the ^{13}C NMR spectrum of **4** shows C-17 at 23.8 and C-9 at δ62.4 the hydroxyl at C-8 is equatorial (8*R*-configuration).

The major acidic component isolated from *Acacia* sp. appeared, from the spectral data of the more soluble methyl ester, to be the dihydroxy acid **8** and this was confirmed by reduction of the methyl ester **9** with aluminium hydride which gave a triol identical with the natural product **4**. The 3-acetoxy derivative (**10**) of **9** and the 3-oxo derivative (**11**) of **8** were prepared for ^{13}C NMR spectral analysis (Table 1).

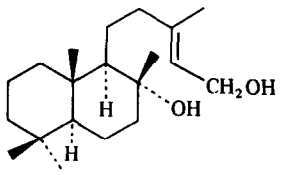
EXPERIMENTAL

General experimental details have been described [10].

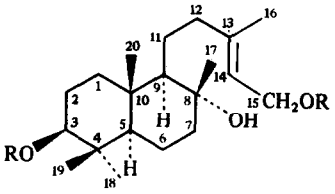
Isolation of metabolites from Acacia *sp.* Leaves and terminal branches of a sample (220 g) of *Acacia* sp., collected north of Hyden, Western Australia, were extracted with Et_2O. The extract was partitioned into 8% aq. $NaHCO_3$ soluble (4.48 g), 10% aq. NaOH soluble (8.55 g) and neutral fractions (25.65 g). A portion (20 g) of the neutral fraction was partitioned between 5% aq.



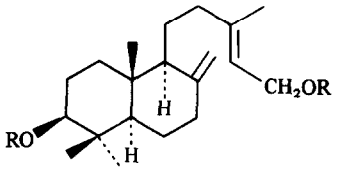
1 13*S*
2 13*R*



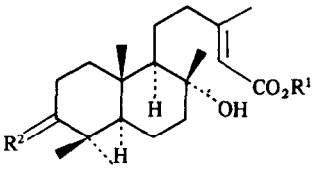
3



4 R = H
5 R = Ac



6 R = H
7 R = Ac



	R^1	R^2
8	H	αH, βOH
9	Me	αH, βOH
10	Me	αH, βOAc
11	H	O

MeOH and petrol and a portion of the MeOH soluble (8 g from 15.9 g) fraction was chromatographed on alumina (neutral, act. III). Elution with EtOAc–CH_2Cl_2 (1:9) gave a yellow oil (633 mg) which was purified by prep. TLC to give the mixture of sclareol (**1**) and 13-*epi*-sclareol (**2**, 351 mg), mp 93–94° (lit. [11] 95°).

Elution with EtOAc–CH_2Cl_2 (1:1) yielded a fraction of the diol **3** (180 mg) which crystallized from Et_2O as cubes, mp 128°, $[\alpha]_{589}$ −0.5° (*c* 0.8, $CHCl_3$), $[\alpha]_{578}$ 0.0°, $[\alpha]_{546}$ 0.0°, $[\alpha]_{436}$ +0.9°, $[\alpha]_{365}$ +2.3° (lit. [8], mp 129°, $[\alpha]_D$ −0.5°). Optical rotation measurements on a sample of the enantiomer showed $[\alpha]_D$ +0.7 [3], $[\alpha]_{578}$ 0.0° (*c* 0.9, $CHCl_3$), $[\alpha]_{546}$ −0.7°, $[\alpha]_{436}$ −1.4°, $[\alpha]_{365}$ −3.5°. ^{1}H NMR (90 MHz, $CDCl_3$): δ0.80 (6H, *s*, Me-19 and Me-20), 0.87 (3H, *s*, Me-18), 1.13 (3H, *s*, Me-17), 1.70 (3H, *d*, *J* = 1.0 Hz, Me-16), 4.13 (2H, *d*, *J* = 7.0 Hz, H_2-15), 5.45 (*t*, *J* = 7.0 Hz, H-14); MS(EI) *m/z* (rel. int.): 290 $[M-18]^+$ (1), 275 (13), 157 (17), 204 (16), 191 (43), 137 (40), 123 (44), 121 (42), 109 (75), 107 (41), 95 (79), 93 (52), 81 (100).

Elution with MeOH–EtOAc (1:9) gave a yellow oil (490 mg) which was purified by rapid silica gel filtration and prep. TLC to give the triol **4** (156 mg), mp 160–161°, $[\alpha]_D$ −3.3° (*c* 0.5; $CHCl_3$); (Found: $[M-18]^+$, 306.257. $C_{20}H_{36}O_3$ requires $[M-18]^+$, 306.2559). ^{1}H NMR (90 MHz, $CDCl_3$): δ0.76 (3H, *s*, Me-19), 0.82 (3H, *s*, Me-20), 0.99 (3H, *s*, Me-18), 1.14 (3H, *s*, Me-17), 1.70 (3H, *s*, Me-16), 3.25 (*m*, $W_{h/2}$ = 18 Hz, H-3), 4.15 (*d*, *J* = 7.0 Hz, H_2-15), 5.43 (*tq*, *J* = 7.0, 1.0 Hz, H-14); MS(EI) *m/z* (rel. int.): 306 $[M-18]^+$ (7), 291 (6), 288 (8), 273 (4), 255 (5), 243 (6), 220 (7), 208 (38), 207 (42), 190 (98), 175 (97), 147 (53), 135 (76), 81 (100).

A portion of the $NaHCO_3$ soluble fraction (4.48 g) was triturated with hot $CHCl_3$ and filtered to give a white residue (1.88 g) which crystallized from EtOAc to give the hydroxy acid **8** (1.30 g) as cubes, mp 123–124.5°, $[\alpha]_D$ +10.4° (*c* 1.9; MeOH); (Found: C, 70.80; H, 10.32. $C_{20}H_{34}O_4$ requires C, 70.95; H, 10.13%).

Conversion of the triol **4** *to* (13E)-*labda*-8(17),13-*diene*-3,15-*diol* (**6**). The triol **4** (109 mg) was treated with Ac_2O in C_5H_5N for 20 hr to give the diacetate **5** as an oil (Found: $[M-HOAc]^+$, 348.265. $C_{24}H_{40}O_5$ requires $[M-HOAc]^+$, 348.2664). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3605, 1740 and 1730; ^{1}H NMR (90 MHz, $CDCl_3$): δ0.83 (6H, *s*, Me-19 and Me-20), 0.86 (3H, *s*, Me-18), 1.12 (3H, *s*, Me-17), 1.69 (3H, *s*, Me-16), 2.01 (6H, *s*, acetoxymethyl protons), 4.41 (*m* (*br*), H-3), 4.51 (2H, *s*, H_2-15), 5.26 (*t* (*br*) H-14); MS(EI) *m/z* (rel. int.): 348 $[M-60]^+$ (3), 193 (20), 190 (72), 147 (60), 140 (97), 135 (80), 120 (41), 119 (67), 43 (100). The diacetate **5** (169 mg) in dry C_5H_5N (5 ml) and $POCl_3$ (1 ml) was stirred at 0° for 18 hr. The products (146 mg) recovered appeared from the

Table 1. ^{13}C NMR spectra of labdane derivatives [20.1 MHz, $CDCl_3$ or CD_3OD (4 and 11)]

C	1	2	3	4	9	10	11
1	39.7	39.7	39.9	39.5	38.0	37.7	39.8
2	19.0*	19.0*	20.6*	27.8	27.1	23.8	34.8
3	42.1	42.1	42.1	79.6	78.6	80.7	219.3
4	33.3	33.3	33.3	40.1	38.9	38.9	48.5
5	56.1	56.1	56.3	56.6	55.1	55.2	56.3
6	18.4*	18.4*	18.5*	21.3*	20.2*	20.2*	22.2*
7	44.0	44.2	42.9†	44.2	44.2	44.2	44.3
8	74.9	74.9	74.2	74.8	74.0	73.9	74.5
9	61.8	62.1	61.3	62.4	61.1	61.2	61.3
10	39.3	39.3	39.3	39.9	38.9	37.8	39.5
11	20.5*	20.5*	23.7	25.3*	23.6*	23.7*	25.1*
12	45.1	45.1	44.6†	45.1	44.6	44.6	45.1
13	73.6	74.1	140.7	140.9	161.0	161.0	162.2
14	146.7	145.1	123.6	124.4	114.9	115.0	116.4
15	111.0	111.9	59.2	59.5	167.4	167.4	170.3
16	26.4	29.5	16.5	16.4	19.1	19.1	19.2
17	24.1	24.4	24.0	23.8	24.0	24.0	23.6
18	33.5	33.5	33.5	28.7	28.2	28.2	26.8
19	21.5	21.5	21.6	16.2	15.9	16.5	21.7
20	15.4	15.4	15.5	16.1	15.4	15.6	15.4
Others					50.7	50.7	
						171.0	
						21.2	

*†Values with identical superscripts in any one column may be interchanged.

^1H NMR spectrum to contain a mixture of the $\Delta^{8(17)}$-diacetate **7** and the Δ^8-diacetate in a 2:1 ratio. A portion of this mixture (94 mg) was dissolved in Et_2O (5 ml) and treated with $LiAlH_4$ (45 mg) at 0° for 5 min. The product (62 mg) was adsorbed on a column of 10% $AgNO_3$–alumina and elution with pentane–Et_2O gave the diol **6**, mp 161–163°, $[\alpha]_D + 24.8°$ (*c* 0.2; $CHCl_3$) (lit. [4] mp 164–165°), for the enantiomer [5], mp 160.5–162° and $[\alpha]_D$ −27° have been reported). ^1H NMR (80 MHz, $CDCl_3$): δ0.69 (3H, *s*, Me-20), 0.77 (3H, *s*, Me-19), 0.99 (3H, *s*, Me-18), 1.67 (3H, *s* (*br*), Me-16), 3.35 (X part of ABX, $J_{AX} + J_{BX}$ 17 Hz, H-3), 4.14 (2H, *d*, J = 7 Hz, H_2-15), 4.53 and 4.85 (H_2-17), 5.39 (*t* (*br*), J = 7 Hz, H-14); MS(EI) *m/z* (rel. int.): 306 $[M]^+$ (1), 291 (12), 288 (7), 273 (25), 255 (13), 187 (11), 175 (14), 135 (79), 107 (100).

Derivatives of 3β,8α-dihydroxylabd-13-en-15-oic acid (**8**). (i) Treatment of **8** with ethereal CH_2N_2 yielded the methyl ester **9** as a colourless oil, bp (block temp.) 230–240°/0.2 mm, $[\alpha]_D + 6.8°$ (*c* 1.5; $CHCl_3$); (Found: C, 71.60; H, 10.31. $C_{21}H_{36}O_4$ requires C, 71.55; H, 10.45%). ^1H NMR (90 MHz, $CDCl_3$): δ0.76 (3H, *s*, Me-19), 0.80 (3H, *s*, Me-20), 0.99 (3H, *s*, Me-18), 1.16 (3H, *s*, Me-17), 2.17 (3H, *d*, J = 1.0 Hz, Me-16), 3.22 (*m*, $W_{h/2}$ = 18 Hz, H-3), 3.67 (*s*, methoxy protons), 5.69 (*s* (*br*), H-14); MS(EI) *m/z* (rel. int.): 352 $[M]^+$ (1), 334 (1), 316 (2), 220 (11), 207 (24), 203 (11), 190 (32), 175 (22), 135 (31), 123 (90), 82 (100). (ii) The methyl ester **9** was treated with Ac_2O–C_5H_5N to give the diacetate **10** as an oil; (Found: $[M - HOAc]^+$, 334.249. $C_{22}H_{38}O_5$ requires $[M - HOAc]^+$, 334.2508). ^1H NMR (80 MHz, $CDCl_3$): δ0.84 (6H, *s* (*br*), Me-19 and Me-20), 0.87 (3H, *s*, Me-18), 1.16 (3H, *s*, Me-17), 2.05 (*s*, acetoxymethyl protons), 2.15 (3H, *d*, J = 1.0 Hz, Me-16), 3.68 (*s*, MeO protons), 4.43 (*m*, H-3), 5.68 (*q*, J = 1.0 Hz, H-14); MS(EI) *m/z* (rel. int.): 376 $[M - 18]^+$ (1), 334 (3), 316 (5), 303 (5), 262 (11), 190 (60), 189 (28), 175 (37), 147 (29), 82 (100). (iii) The dihydroxy acid **8** (100 mg) in dry DMF (5 ml) and pyridinium dichromate (500 mg) was stirred at 0° for 20 hr. The product (95 mg) recovered crystallized from EtOAc as cubes of the keto acid **11**, mp 180–181°, $[\alpha]_D + 18.8°$ (*c*, 0.4, $CHCl_3$); (Found: C, 71.09; H, 9.20. $C_{20}H_{32}O_4$ requires C, 71.38; H, 9.59%). ^1H NMR (90 MHz, $CDCl_3$): δ0.96 (3H, *s*, Me-20), 1.03 (3H, *s*, Me-19), 1.11 (3H, *s*, Me-18), 1.23 (3H, *s*, Me-17), 2.18 (3H, *d*, J = 1.0 Hz, Me-16), 5.72 (*s* (*br*), H-14); MS(EI) *m/z* (rel. int.): 336 $[M]^+$ (1), 318 (6), 290 (5), 209 (8), 191 (19), 100 (10), 98 (34), 95 (35), 82 (100).

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