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KAURANE SUCCINATES AND PRENYLATED AROMATICS FROM *ODIXIA ANGUSTA* AND *OZOTHAMNUS OBCORDATUS*

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Key Word Index—*Odixia angusta*; *Ozothamnus obcordatus*; Compositae; diterpenes; kauranes; *nor*-kaurane; prenylated coumaric acids.

Abstract—The aerial parts of *Odixia angusta* afforded five *ent*-kaurane succinates and a *nor*-derivative as well as diprenyl *p*-coumarate and a hemiacetal derived from the latter. *Ozothamnus obcordatus* gave five prenylated *p*-hydroxybenzoic acids probably formed by oxidative cleavage of the coumarates. The structures were elucidated by high field NMR techniques.

INTRODUCTION

Odixia is an Australian genus placed in the tribe Gnaphalieae (Compositae) closely related to *Cassinia* and *Ozothamnus* [1]. As nothing was known on the chemistry we have studied *Odixia angusta* and *Ozothamnus obcordatus*.

RESULTS AND DISCUSSION

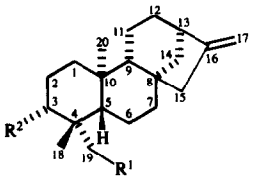
The extract of the aerial parts of *Odixia angusta* (Wakef.) Orch. gave the *ent*-kaurane derivatives **1**–**5**, the *nor*-kaurane **6**, 3,5-diprenyl-*p*-coumaric acid **7** [2] and the derived acid **8**, all isolated as their methyl esters (**1a**–**8a**). The ^{1}H NMR spectral data of **1a** (Table 1) indicated that we were dealing with an *ent*-kaur-16-ene derivative where one of the methyl groups is substituted with a succinyloxy group. A double doublet at δ3.25 showed that a secondary hydroxy group was also present. This was supported by the ^{13}C NMR spectrum (Table 2). The positions of the oxygen functions and the stereochemistry was established by NOED [H-20 with H-19 (7%) and H-19′ (4%); H-18 with H-3 (4%)]. The resulting structure is identical with that given for a diterpene isolated from a Goodeniaceae [3] where, however, no NMR data are presented.

The ^{1}H NMR spectrum of the methyl ester of the main constituent (**2a**) (Table 1) was close to that of **1a**. However, the low field signal at δ3.25 was missing and some chemical shifts were slightly different. In agreement with the molecular formula ($C_{25}H_{38}O_4$) and the ^{13}C NMR spectrum (Table 2), therefore, the 3-desoxy derivative of **1a** was present.

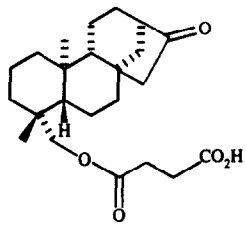
The ^{1}H NMR spectrum of **3a** (Table 1) which had the same molecular formula as **2a**, showed that now the oxygen function is at C-3. The couplings of H-3 indicated the α-orientation of the ester group. The ^{1}H NMR spectrum of **4a** (Table 1) showed that most likely the acetate of **1a** was present. This was established by acetylation of **1a**.

The ^{1}H NMR spectrum of **5a** (Table 1) indicated that an isomer of **1a** must be present with a free hydroxy group at C-19 and the ester group at C-3. Accordingly, the chemical shifts differed in the expected way from those of **1a**.

The molecular formula of **6a** ($C_{24}H_{36}O_5$) already indicated the presence of a *nor*-diterpene as the ^{1}H NMR



	1	2	3	4	5
R^1	OSucc	OSucc	H	OSucc	OH
R^2	OH	H	OSucc	OAc	Succ



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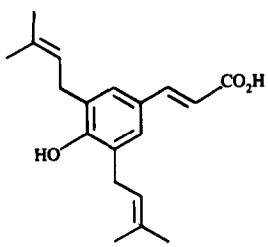

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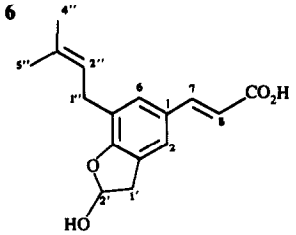


8

1a–**8a** are the methylesters

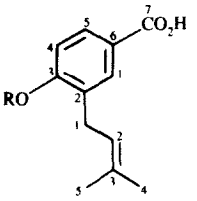
Table 1. ^{1}H NMR spectral data of compounds **1a–6a** (400 MHz, $CDCl_3$, δ-values)

H	1a	2a	3a	4a	5a	6a	
3α	3.25 *dd*	1.84	4.49 *dd*	4.55 *dd*	4.65 *dd*	1.84	*br d*
3β		0.78				0.83	*dt*
13	2.63	2.62	2.64	2.64	2.64	2.40 *ddd*	*br s*
14	1.91	1.93	1.96	1.92	1.92	2.26	*dd*
14′	1.07	1.08	1.10	1.12	1.11	1.43 *m*	*br dd*
15	2.04 *br s*	2.07	2.08	2.06 *br s*	2.06 *br s*	1.98 *dd*	*dt*
15′		2.02	2.03			1.93 *d*	*dt*
17	4.79	4.78	4.79	4.80	4.80	—	*br s*
17′	4.73	4.72	4.73	4.74	4.74	—	*br s*
18	1 12	1.02	1.04	1.05	1.05	1.09	*s*
19	4.39	4.27	0.86	4.35	4.12	4.27	*d*
19′	4.10	3.88		4.19	3.38 *br d*	3.91	*dd*
20	1.00	0.93	0.85	0.99	0.99	0.96	*s*
OMe	3.69	3.66	3.69	3.70	3.70	3.69	*s*
OCOR	2.63	2.62	2.64	2.64	2.64	2.24	*br s*
OAc	—	—	—	2.04	—	—	*s*

J [Hz]: 5,19′ ~ 1; 13,14 ~ 1; 13,14′ = 5; 14,14′ = 11; compounds **1a** and **4a**: 2α,3 = 11; 2β,3 = 5; compounds **2a** and **6a**: 2α,3β = 3α,3β = 14; 2β,3β = 4; compound **3a**: 2α,3 = 10; 2β,3 = 6; compound **4a**: 2α,3 = 9.5; 2β,3 = 7; compound **5a**: 2α,3 = 8.5; 2β,3 = 9.5; compound **6a**: 12,13 = 13,14 ~ 3; 13,14′ = 5; 14,15 = 2.5.

Table 2. ^{13}C NMR spectral data of compounds **1a**, **2a** and *ent*-kaur-16-ene (100.6 MHz, $CDCl_3$, δ-values)

C	1a	2a	*ent*-Kaurene
1	41.2	41.4	41.3
2	27.1	20.3	18.7
3	79.0	36.2	42.0
4	38.7	37.0	33.3
5	55.8	56.7	56.1
6	20.4	20.3	20.3
7	39.4	40.2	40.4
8	43.8	44.0	44.2
9	55.7	56.0	56.1
10	38.7	39.0	39.3
11	18.2	18.1	18.1
12	33.0	33.0	33.3
13	43.7	43.8	44.2
14	39.3	39.5	39.9
15	48.7	48.9	49.2
16	155.4	155.7	156.0
17	102.1	102.9	102.8
18	22.4	27.4	33.7
19	65.4	67.3	21.7
20	17.6	17.9	17.6
OCOR	172.3	172.3	—
	29.2	29.1	
	28.9	28.9	
	172.7	172.7	
OMe	51.8	51.7	



	9	10	11	12	13	14
R	Sen	Ang	*i*Bu	MeBu	*i*Val	H

9a–14a are the methylesters

spectrum (Table 1) shows that again a 19-succinyloxy derivative had to be proposed. The absence of the characteristic exomethylene proton signals led to the assumption that **6a** is a 16-oxo derivative formed by oxidative cleavage of **2a**. This was supported by spin decoupling which shows that the signal at δ2.40 was due to H-13, those at δ1.98 and 1.93 to H-15 and those at δ2.26 and 1.43 to H-14. The latter assignment followed from the presence of a *W*-coupling between H-14 and H-15. Finally, the structure was confirmed by oxidative degradation of **2a** by epoxidation, hydrolysis to the diol and periodate splitting. The resulting ketone was identical with the methyl ester of the natural product. A positive Cotton-effect of the ketone agrees with the presence of *ent*-kauranes.

The structure of **8a** followed from its molecular formula ($C_{17}H_{20}O_4$) and its ^{1}H NMR spectrum (Experimental) which in part was close to that of **7a**. However, only one prenyl group was present. The second substituent of the coumaric acid caused a pair of double doublets at δ3.40 and 3.06 which both showed a vicinal coupling with a three-fold doublet at δ6.13. The latter was further coupled with a doublet at δ3.18 which disappeared after addition of D_2O while the signal at δ6.13 became a clear double doublet. We have named compound **8** odixia acid.

The extract of aerial parts of *Ozothamnus obcordatus* DC. afforded aromadendrene and a mixture of the acids **9–13**. After addition of diazomethane the methyl esters **9a–11a** could be separated by HPLC while the esters **12a** and **13a** were obtained as a mixture. The ^{1}H NMR spectrum of **9a** (Table 3) indicated that a senecioate of a prenylated methylhydroxybenzoate was present. The chemical shifts of the aromatic protons were in agreement with a prenylated *o*- or *p*-hydroxybenzoate. Partial saponification gave methyl-3-prenyl-4-hydroxybenzoate

Table 3. ^{1}H NMR spectral data of compounds **9a**–**14a** (400 MHz, $CDCl_3$, δ-values)

H	9a	10a	11a	12a	13a	14a	
1	7.92	7.94	7.92	7.91	7.82		*d*
4	7.10	7.13	7.07	7.07	7.08	6.83	*d*
5	7.89	7.91	7.90	6.89	7.81		*dd*
1′	3.28	3.28	3.26	3.26	3.38		*br d*
2′	5.22	5.22	5.24	5.22	5.31		*qqt*
4′	1.72	1.73	1.75	1.74	} 1.78		*br s*
5′	1.68	1.68	1.69	1.69	}		*br s*
OCOR	5.94 *qq*	6.31 *qq*	2.34 *qq*	1.86 *dtq*	2.47 *d*	—	
	2.23 *d*	2.08 *dq*	1.34 *d*	1.63 *dtq*	2.25 *dqq*	—	
	2.01 *d*	2.06 *dq*		1.03 *t*	1.07 *d*	—	
				1.32 *d*			
OMe	3.90	3.91	3.90	3.91	3.91	3.88	*s*

J [Hz]: 1,5 = 2; 4,5 = 8.5; 1′,2′ = 7; 1′,4′ = 1′,5′ = 2′,4′ = 2′,5′ = 1.5.

as clearly followed from the absence of a hydrogen bonded hydroxy proton signal in the ^{1}H NMR spectrum. The ^{13}C NMR data (Experimental) also agree with the proposed structure **9a**.

The ^{1}H NMR spectra of **10a**–**13a** (Table 3) show that the corresponding angelate, isobutyrate, 2-methylbutyrate and isovalerate were present. It is interesting that the nature of the ester residues shows a small but clear influence on the chemical shifts of all protons. The acids **9**–**13** most likely are formed by degradation of the corresponding prenylated *p*-coumarates.

The chemistry of the *Odixia* and the *Ozothamnus* species does not show a very close relationship. The diterpene succinates may be of chemotaxonomic relevance as these esters are relatively rare in Compositae. In the Gnaphalieae they have been observed from Australian *Helipterum* [4], *Helichrysum* [5], *Craspedia* [5] and *Myriocephalus* species [6]. However, in these cases esters of beyeranes or pimaranes were present. From *Relhania* species labdane succinates were isolated [7].

The *p*-hydroxybenzoic acid derivatives **9**–**13** may be an indication of a relationship of *Ozothamnus* to *Odixia* as prenylated *p*-coumarates, the possible precursors of **9**–**13**, are present. The latter have been reported from *H. diosmifolium* (= *Ozothamnus diosmifolius*) together with several very different compounds. The proposed relationship to *Cassinia* is not supported by our chemical results.

EXPERIMENTAL

The air-dried aerial parts of *Odixia angusta* (70 g, voucher Nordenstam and Anderberg 1342, deposited in the Herbarium of Stockholm, collected in autumn 1989 in Australia) were extracted with MeOH–Et_2O–petrol (1:1:1). The extract obtained was defatted with MeOH and sepd as reported previously [9]. CC gave 3 frs. The first one gave, after addition of CH_2N_2, by TLC (Et_2O–petrol, 1:3), 450 mg **2a** (R_f 0.65) and a mixt. which afforded by TLC (EtOAc–petrol, 1:19) 5 mg **7a**, identified by comparing the 400 MHz ^{1}H NMR spectrum with that of authentic material and a mixt. of **2a** and **3a** which gave by HPLC (MeOH–H_2O, 9:1, always RP 8, flow rate 3 ml min^{-1}), 30 mg **3a** (R_t 9.1 min) and 50 mg **2a** (R_t 9.8 min). The second CC fr. gave, after addition of CH_2N_2, by TLC (Et_2O–petrol, 1:1) 30 mg **4a** (R_f 0.60) and 2 mixts (2/2 and 2/3). HPLC of 2/2 (MeOH–H_2O, 9:1) gave 2 mg **8a** (R_t 1.3 min) and 3 mg **6a** (R_t 2.3 min). HPLC of 2/3 (MeOH–H_2O, 9:1) afforded 8 mg **5a** (R_t 3.9 min) and 10 mg **1a** (R_t 4.1 min). TLC (Et_2O–petrol, 1:1) of the last CC fr. gave, after addition of CH_2N_2, 190 mg **1a** (R_f 0.42).

The extract of the aerial parts of *O. obcordatus* (68 g, voucher Nordenstam and Anderberg 1107 Herb. Stockholm, collected in Autumn 1989 in Australia) gave 2 frs by CC. The first one yielded by TLC 20 mg aromadendrene and the second fr. a complex mixt. of acids. TLC gave the acid **9** still containing **10**. After addition of CH_2N_2 HPLC (MeOH–H_2O, 4:1) afforded 10 mg **11a** and a mixt. One-tenth of the latter gave by repeated HPLC 33 mg **9a** R_t (9.2 min), 30 mg **10a** (R_t 9.6 min) and 6 mg **12a** and **13a** *ca* 1:2, (R_t 10.0 min).

3α-*Hydroxy*-19-*succinyloxy*-ent-*kaur*-16-*ene* (**1**). Isolated as its methyl ester **1a**; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600 (OH), 1740 (CO_2R), 1660, 890 (C=CH_2); MS *m/z* (rel. int.): 418.272 [M]$^+$ (8) (calc. for $C_{25}H_{38}O_5$: 418.272), 400 [M−H_2O]$^+$ (4), 286 [M−RCO_2H]$^+$ (75), 268 [286−H_2O]$^+$ (25), 115 [RCO]$^+$ (100). Acetylation (DMAP, $CHCl_3$, Ac_2O) gave **4a**, identical with the natural acetate.

19-*Succinyloxy*-ent-*kaur*-16-*ene* (**2**). Isolated as its methyl ester **2a**; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1740 (CO_2R), 1650, 880 (C=CH_2); MS *m/z* (rel. int.): 402.277 [M]$^+$ (18) (calc. for $C_{25}H_{38}O_4$: 402.277), 270 [M−RCO_2H]$^+$ (66), 255 [270−Me]$^+$ (38), 115 [RCO]$^+$ (100); $[\alpha]_D^{24°}$ −53 ($CHCl_3$; *c* 2.72). 10 mg **2a** in 2 ml Et_2O was stirred for 1 hr with 50 mg *m*-chloroperbenzoic acid. The epoxide obtained was hydrolysed by stirring in H_2O–dioxane (1:1) with a drop of 1 M H_2SO_4 for 15 min at 20°. The crude diol was stirred in 2 ml MeOH–H_2O (4:1) with 30 mg $NaIO_4$ for 15 min. HPLC (MeOH–H_2O, 9:1) gave 2 mg of the methyl ester **6a**; ^{1}H NMR identical with that of the natural product.

3α-*Succinyloxy*-ent-*kaur*-16-*ene* (**3**). Isolated as its methyl ester **3a**; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1740 (CO_2R), 1660, 880 (C=CH_2); MS *m/z* (rel. int.): 402.277 [M]$^+$ (17) (calc. for $C_{25}H_{38}O_4$: 402.277), 270 [M−RCO_2H]$^+$ (43), 255 [270−Me]$^+$ (40), 115 [RCO]$^+$ (100); $[\alpha]_D^{24°}$ −57 ($CHCl_3$; *c* 0.74).

3α-*Acetoxy*-19-*succinyloxy*-ent-*kaur*-16-*ene* (**4**). Isolated as its methyl ester **4a**; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1745 (CO_2R), 1660, 880 (C=CH_2); MS *m/z* (rel. int.): 460.282 [M]$^+$ (17) (calc. for $C_{27}H_{40}O_6$: 460.282), 400 [M−HOAc]$^+$ (12), 328 [M−RCO_2H]$^+$ (28), 268 [328−HOAc]$^+$ (100), 253 [268−Me]$^+$ (33), 115 [RCO]$^+$ (85).

19-*Hydroxy*-3α-*succinyloxy*-ent-*kaur*-16-*ene* (**5**). Isolated as its methyl ester **5a**; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600 (OH), 1740 (CO_2R); MS *m/z* (rel. int.): 418.272 [M]$^+$ (3) (calc. for $C_{25}H_{38}O_5$: 418.272), 400 [M−H_2O]$^+$ (2), 286 [M−RCO_2H]$^+$ (25), 256 [286−CH_2O]$^+$ (65), 115 [RCO]$^+$ (100).

19-*Succinyloxy*-17-nor-ent-*kauran*-16-*one* (**6**). Isolated as its

methyl ester **6a**; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1745 (C=O, CO_2R); MS *m/z* (rel. int.): 404.257 $[M]^+$ (5) (calc. for $C_{24}H_{36}O_5$: 404.257), 389 $[M-Me]^+$ (0.3), 272 $[M-RCO_2H]^+$ (62), 259 $[M-CH_2OCOR]^+$ (86), 115 $[RCO]^+$ (100), 107 (90); CD (MeOH): $\Delta\varepsilon_{295}+1.3$.

Odixia acid (**8**). Isolated as its methyl ester **8a**; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600 (OH), 1740 (CO_2R); MS *m/z* (rel. int.): 288.137 $[M]^+$ (100) (calc. for $C_{17}H_{20}O_4$: 288.137), 259 (72), 257 (38), 233 (76), 203 (61), 173 (36), 115 (43), 91 (21); ^{1}H NMR ($CDCl_3$): δ 7.17 (*br s*, H-2, H-6), 7.63 (*d*, H-7), 6.23 (*d*, H-8), 3.40 and 3.06 (*dd*, H-1′), 6.13 (*ddd*, H-2′), 3.18 (*d*, OH), 3.30 (*br d*, H-1″), 5.30 (*tqq*, H-2″), 1.76 (*br s*, H-4″), 1.71 (*br s*, H-5″); *J* [Hz]: 7,8 = 16; 1′$_1$,2′ = 17; 1′$_2$,2′ = 6; 1′$_2$,2′ = 2; 2′,OH = 5; 1″,2″ = 7; 2″,4″ = 2″,5″ = 1.5; $[\alpha]_D^{24^\circ}-9$ ($CHCl_3$; *c* 0.1).

3-*Prenyl-4-hydroxybenzoic acid senecioate* (**9**). MS *m/z* (rel. int.): 288.136 $[M]^+$ (0.6) (calc. for $C_{17}H_{20}O_4$: 288.136), 232 $[M-C_4H_8]^+$ (5), 188 $[M-RCO_2H]^+$ (21), 83 $[RCO]^+$ (100), 55 $[83-CO]^+$ (36). Purified as its methyl ester **9a**; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1745 (PhOCOC=C), 1730 ($PhCO_2R$); MS *m/z* (rel. int.): 302.152 $[M]^+$ (1) (calc. for $C_{18}H_{22}O_4$: 302.152), 271 $[M-OMe]^+$ (0.5), 219 $[M-COC_4H_7]^+$ (3), 83 $[RCO]^+$ (100), 55 $[83-CO]^+$ (57); ^{13}C NMR ($CDCl_3$, C-1–C-7): δ 133.5, 131.5, 127.5, 160.7, 121.0, 128.4, 164.1; C-1′–C-5′: 28.7, 122.5, 134.0, 25.6, 17.8; OSen: 166.6, 114.6, 152.6, 27.6, 20.5; OMe: 52.0.

3-*Prenyl-4-hydroxybenzoic acid angelate* (**10**). Isolated as its methyl ester **10a**; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1745 (PhOCOC=C), 1730 ($PhCO_2R$); MS *m/z* (rel. int.): 302.152 $[M]^+$ (1) (calc. for $C_{18}H_{22}O_4$: 302.152), 271 (0.5), 219 (6), 83 (100), 55 (53). To 50 mg **2a** in 2 ml MeOH–dioxane (3:1), 100 mg KOH in MeOH–H_2O (3:1) was added. After addition of dil H_2SO_4 usual work-up gave 30 mg **14a**; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3610 (OH), 1730 ($PhCO_2R$); MS *m/z* (rel. int.): 220.105 $[M]^+$ (56) (calc. for $C_{13}H_{16}O_3$: 220.105), 189 $[M-OMe]^+$ (17), 165 $[M-C_4H_7]^+$ (100), 161 $[189-CO]^+$ (30), 133 $[165-MeOH]^+$ (28), 105 $[133-CO]^+$ (31).

3-*Prenyl-4-hydroxybenzoic acid isobutyrate* (**11**). Isolated as its methyl ester **11a**; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1770 (PhOCOR), 1730 ($PhCO_2R$); MS *m/z* (rel. int.): 290.152 $[M]^+$ (3) (calc. for $C_{17}H_{22}O_4$: 290.152), 219 $[M-COR]^+$ (32), 71 $[RCO]^+$ (100).

3-*Prenyl-4-hydroxybenzoic acid-*[*2-methyl-butyrate and isovalerate*] (**12** *and* **13**). Isolated as their methyl esters **12a** and **13a** which could not be separated; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1775 (PhOCOR), 1730 (CO_2R); MS *m/z* (rel. int.): 304.167 $[M]^+$ (1) (calc. for $C_{18}H_{24}O_4$: 304.167), 219 $[M-COR]^+$ (20), 85 $[RCO]^+$ (45), 57 $[85-CO]^+$ (100).

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