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KAURANE DERIVATIVES FROM *ACANTHOPANAX KOREANUM*

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Abstract—Two new *ent*-kaurane derivatives were isolated from the stem bark of *Acanthopanax koreanum*. On the basis of extensive spectral evidence, their structures were elucidated as *ent*-16βH,17-isovalerate-kauran-19-oic acid **1** and *ent*-16βH,17-methyl butanoate-kauran-19-oic acid **2**.

INTRODUCTION

In continuation of our systematic chemical studies of Korean medicinal plants, we have undertaken a study of *Acanthopanax koreanum* Nakai which is used in medicines for elderly people and for the treatment of paralysis, arthritis, rheumatism, lameness and high blood pressure, and as a tonic [1]. We have already reported six diterpenoids, polyacetylenes and lignan compounds from this plant [2, 3]. Further studies on the stem bark of this plant gave two new kaurane diterpenes from the ether fraction.

RESULTS AND DISCUSSION

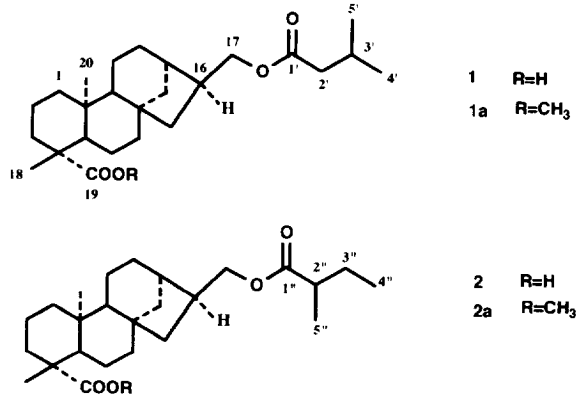
The new diterpenoids were isolated as an inseparable mixture. The EI-MS and HR-mass spectrometry revealed a molecular ion at m/z 404 in accord with a molecular formula of $C_{25}H_{40}O_4$. The IR spectrum showed strong absorptions at 1738 and 1689 cm^{-1} owing to ester carbonyl and carboxyl groups respectively. The ^1H NMR spectrum (Table 1) showed two angular methyl signals (δ0.93 and 1.23) and one doublet (J = 6.6 Hz) of dimethyl protons at δ0.95 due to the isovalerate group. Further methyl signals (d at δ1.14 and t at δ0.90) with a low intensity were present in the upfield region. The ^{13}C NMR spectrum (Table 2) showed more than 25 carbon peaks. From these results, we deduced that the mixture was made up of one major compound (**1**) and one minor compound (**2**) (ratio *ca* 3:1) with similar carbon skeletons. Compounds **1** and **2** were inseparable even by repeated HPLC. However, extensive NMR studies (^1H, ^{13}C NMR, HOMOCOSY, HETEROCOSY and HMBC) revealed that they both had the 17-hydroxy kauran 19-oic acid skeleton. Their exact structures and configurations were determined by careful examination of the ^1H NMR spectra. Two tertiary methyl resonances at δ1.23 and 0.93 and the ^{13}C NMR resonances at δ184.4 (C-19), 29.0 (C-18) and 15.5 (C-20) were typical of the axial C-20 and equatorial C-18 in diterpenoids with a C-19 axial carboxyl acid [4, 5]. In the HOMOCOSY spectrum, the methylene signals at δ3.86 (2H) were correlated to the methine signal at δ2.08 (1H, *m*, overlapping) and suggested the presence of a hydroxymethyl group. The other chemical shifts in the ^1H and ^{13}C NMR

Table 1. ^1H NMR spectral data of compounds **1** and **2** (500 MHz, $CDCl_3$, TMS as int. standard)

	1	2
1	0.82, 1.85	0.82, 1.85
2	1.44, 1.84	1.44, 1.84
3	1.00, 2.14	1.00, 2.14
5	1.04	1.04
6	1.75–1.90	1.75–1.90
7	1.44	1.44
9	0.98	0.98
11	1.58	1.58
12	1.44, 1.56	1.44, 1.56
13	2.03	2.03
14	1.03, 1.85	1.03, 1.85
15	0.95, 1.55	0.95, 1.55
16	2.08	2.08
17	3.86	3.86
18	1.23	1.23
20	0.93	0.93
2′	2.17	
3′	2.09	
4′	0.95	
5′	0.95	
2″		2.36
3″		1.50, 1.56
4″		0.90
5″		1.14

Table 2. ^{13}C NMR spectral data of compounds **1**, **1a**, **2** and **2a** (125 MHz, $CDCl_3$)

	1	1a	2	2a
1	40.7 *t*	40.8	40.7 *t*	40.8
2	19.1 *t*	19.1	19.1 *t*	19.1
3	37.8 *t*	38.1	37.8 *t*	38.1
4	43.7 *s*	43.7	43.7 *s*	43.7
5	57.0 *d*	57.0	57.0 *d*	57.0
6	22.4 *t*	22.6	22.4 *t*	22.6
7	41.6 *t*	41.6	41.2 *t*	41.6
8	44.9 *s*	44.9	44.9 *s*	44.9
9	55.3 *d*	55.3	55.3 *d*	55.3
10	39.6 *s*	39.5	39.6 *s*	39.5
11	18.8 *t*	18.7	18.8 *t*	18.7
12	31.2 *t*	31.2	31.2 *t*	31.2
13	38.6 *d*	39.5	38.6 *d*	39.5
14	37.1 *t*	37.1	37.1 *t*	37.1
15	45.0 *t*	45.0	45.0 *t*	45.0
16	39.5 *d*	39.4	39.6 *d*	39.6
17	68.4 *t*	68.3	68.3 *t*	68.2
18	29.0 *q*	28.7	29.0 *q*	28.7
19	184.4 *s*	178.0	184.4 *s*	178.0
20	15.5 *q*	15.3	15.5 *q*	15.3
1′	173.5 *s*	173.2		
2′	43.6 *t*	45.5		
3′	25.8 *d*	25.7		
4′	22.4 *q*	22.4		
5′	22.4 *q*	22.4		
1″			177.0 *s*	176.8
2″			41.2 *t*	41.1
3″			26.8 *d*	26.7
4″			11.7 *q*	16.6
5″			16.7 *q*	11.6
OMe		51.0		51.0



spectra were compared with those of *ent*-16α,17-hydroxy kauran-19-oic acid [6] which gave almost the same values as the unknowns, except for those for C-16 and C-17. These results mean that the isovaleric group and methylbutanoic group must be attached to the C-17 hydroxymethyl moiety. Alkaline hydrolysis of these compounds provided *ent*-16β,17-hydroxy kauran-19-oic acid. The configuration of C-16 was determined by comparison of the chemical shift of an AB system of hydroxy methylene signals (H-17a at δ3.37 and H-17b at δ3.40) with those of several known compounds [6–9]. The presence in the EI-mass spectrum of ions at *m/z* 404 $[M]^+$ and 302 [M − isovaleric acid or methylbutanoic acid]$^+$ strongly supported the structures assigned to **1** and **2**. From these spectral data, these compounds were identified as ent-16β*H*,17-isovalerate-kauran 19-oic acid (**1**) and ent-16β*H*,17-methylbutanoate-kauran 19-oic acid (**2**).

EXPERIMENTAL

General. Mps: uncorr.; ^1H NMR: 300 and 500 MHz; MS: HP 5989A spectrometer.

Plant material. The stem bark (2 kg) of *A. koreanum* was collected from the Hanla Mt., Jeju Do, Korea. Voucher specimens are deposited in our laboratory.

Extraction and isolation. Air-dried stem barks were extracted with MeOH. The MeOH extract was evapd *in vacuo* and fractionated with Et_2O and *n*-BuOH. The Et_2O extract was chromatographed on silica gel with hexane–EtOAc (10:1). A total of 20 fractions (A–T) was collected. Fraction T (450 mg) was repeatedly chromatographed (CC) on silica gel, eluting with hexane–EtOAc (10:1). Needle crystals (134 mg) were obtained by recrystallization in hexane.

ent-16β*H*,17-*isovalerate-kauran* 19-*oic acid* (**1**) *and* ent-16β*H*,17-*methylbutanoate-kauran* 19-*oic acid* (**2**). Mp 169–171°, $C_{25}H_{40}O_4$ (Found: C, 74.49; H, 10.12. Requires C, 74.26; H, 9.90). IR ν_{max}^{KBr} cm^{-1}: 2934, 1738, 1689, 1466, and 1186; EI-MS 70 eV *m/z* (rel. int.): 404 $[M]^+$ (3), 386 (22), 358 (61), 302 [M − isovaleric acid, M − methylbutanoic acid]$^+$ (60); ^1H and ^{13}C NMR: Tables 1 and 2.

Methylation of compounds **1** *and* **2**. The mixture of **1** and **2** (30 mg) was dissolved in Et_2O and methylated with CH_2N_2 at room temp. The usual work-up yielded 20 mg of **1a** and **2a**. These methyl esters were not clearly separated by GC-MS (data not shown). EI-MS 70 eV *m/z* (rel. int.): 418 $[M]^+$ (3), 386 [M − MeOH]$^+$ (11), 358 (57), 316 (20); ^1H NMR (500 MHz, $CDCl_3$): δ0.74 (*s*, H-20), 0.83 (*t*, *J* = 7.34 Hz, H-4″), 0.88 (*d*, *J* = 6.61 Hz, H-4′,5′), 1.06 (*d*, *J* = 6.97 Hz, H-5″), 1.09 (*s*, H-18), 3.57 (*s*, -OMe); ^{13}C NMR: Table 2.

Alkaline hydrolysis. The mixture of **1** and **2** (15 mg) was treated with 2 M NaOH in MeOH at room temp. for 4 hr, then the soln was poured into H_2O and extracted with EtOAc. The EtOAc layer was concd and chromatographed on silica gel (CH_2Cl_2–MeOH, 50:1) to afford *ent*-16β*H*,17-hydroxy kauran 19-oic acid, mp 187–189°. ^1H NMR (300 MHz, $CDCl_3$): δ1.21 (3H, *s*, Me-20), 1.23 (3H, *s*, Me-18), 3.37 (1H, *d*, *J* = 7.7 Hz, H-17a), 3.40 (1H, *d*, *J* = 7.7 Hz, H-17b); EI-MS *m/z* (rel. int.): 320 $[M]^+$ (3), 302 (14), 274 (14), 261 (5).

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