Journal of Natural Products
Vol. 51, No. 6, pp. 1080-1083, Nov-Dec 1988

PIMARADIENE DITERPENES FROM *ACANTHOPANAX KOREANUM*

YOUNG H. KIM, BO S. CHUNG,*

College of Pharmacy, Seoul National University, Seoul 151-742, Korea

and USHIO SANKAWA

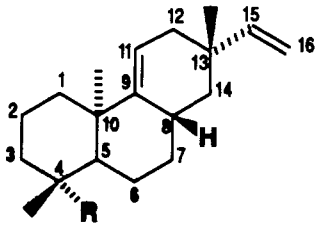
Faculty of Pharmaceutical Sciences, University of Tokyo, Tokyo 113, Japan

ABSTRACT.—The root bark of *Acanthopanax koreanum* has yielded two new (−)-pimaradiene type diterpenes, (−)-primara-9(11),15-dien-19-oic acid [**2**] and (−)-pimara-9(11),15-dien-19-ol 19-acetate [**3**]. Their structures were elucidated by chemical and spectroscopic methods.

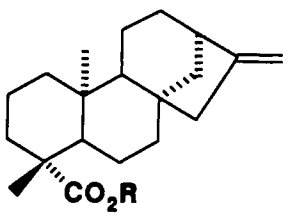
In continuation of our systematic chemical studies of Korean medicinal plants (1), we have undertaken a study of *Acanthopanax koreanum* Nakai (Araliaceae), which has been used as a tonic and sedative as well as in the treatment of rheumatism and diabetes (2). As part of this work, we report here the isolation of six diterpenes, two of which appear to be novel, (−)-pimara-9(11),15-dien-19-oic acid [**2**] and (−)-pimara-9(11),15-dien-19-ol 19-acetate [**3**], as well as four known (−)-pimaradiene and kaurane type diterpenes from the root bark of *A. koreanum*.

RESULTS AND DISCUSSION

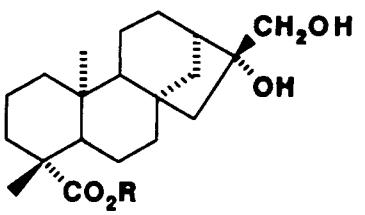
The Et_2O extracts of the root bark of *A. koreanum* were prepared as described in the Experimental section and were chromatographed on Si gel to afford four pimaradiene diterpenes **1**–**4** and a kaurane-type diterpene **5**. *n*-BuOH extracts were also chromatographed over Si gel and gave one kaurane-type diterpene **6**. The first four pimaradiene diterpenes showed a number of spectral features in common. The ir spectra exhibited weak bands at ca. 1645 and 910 cm^{-1} for olefinic absorptions. The ^1H-nmr spectra (Table 1) showed an ABX pattern at the olefinic region (δ 4.82–5.83) which was as-



1 R=CH_2OH
2 R=COOH
3 R=CH_2OAc
4 R=Me
8 R=COOMe



5 R=H
9 R=Me



6 R=H
7 R=Me

TABLE 1. ^{1}H-nmr Data of Compounds **1–4**.

Proton	Compound			
	1	**2**	**3**	**4**
11	5.35 m	5.41 m	5.38 m	5.32 m
15	5.79 dd (17.4, 10.7 Hz)	5.83 dd	5.83 dd	5.79 dd
16*trans*	4.92 dd (17.4, 1.3 Hz)	4.93 dd	4.91 dd	4.87 dd
16*cis*	4.85 dd (10.7, 1.3 Hz)	4.86 dd	4.84 dd	4.82 dd
17	0.98 s	1.00 s	0.97 s	1.00 s
18	0.97 s	1.25 s	0.97 s	0.96 s
19a	3.84 d (10.8)	—	4.30 d	
				0.86 s
19b	3.54 d (10.8)	—	3.98 d	
20	1.05 s	0.96 s	1.08 s	1.05 s
-OAc	—	—	2.05 s	—

signed to a monosubstituted double bond and closely resembled pimaradiene-type diterpenes (3). The multiplet at 5.35 ppm was attributed to the proton of a trisubstituted double bond. The double bond position $\Delta^{9(11)}$ was confirmed by ^{13}C-nmr data (Table 2) and the ms fragmentation pattern which showed a peak attributed to an elimination of 2-methyl-butadiene by a retro-Diels-Alder process of the C ring (4).

The configuration of C-13 was confirmed by the chemical shift of C-17 (ca. δ 22.27), which was due to an axial methyl group having a γ-gauche effect (5), and by comparison with the chemical shift of similar pimaradiene diterpenes (6). The ir, eims, ^{1}H-, and ^{13}C-nmr data of **1** were in good accord with those of the previous report (5). NOe difference spectroscopy established all the chemical configurations of **1**. Irradiation of H-17 showed clear nOe's with H-15, H-16 *trans*, and H-8. Irradiation of H-18

TABLE 2. ^{13}C-nmr Data of Compounds **1** and **2** (90 MHz, $CDCl_3$, δ).

Carbon	Compound	
	1	**2**
1	41.05 t	41.87 t
2	17.98 t	18.86 t
3	35.41 t	37.99 t
4	38.33 s	44.15 s
5	46.25 d	47.95 d
6	19.08 t	20.29 t
7	26.83 t	27.71 t
8	28.94 d	28.62 d
9	151.17 d	149.75 d
10	37.90 s	38.37 s
11	115.70 d	116.50 d
12	37.53 t	37.41 t
13	34.79 s	34.78 s
14	41.57 t	41.74 t
15	150.19 d	150.05 d
16	109.00 t	109.10 t
17	22.43 q	22.15 q
18	26.49 q	28.51 q
19	64.77 t	185.04 s
20	25.97 q	22.33 q

gave nOe's with H-5, and irradiation of H-20 did not give nOe's with H-5 and H-8. These data meant that H-5, H-8, and C-18 had to be located on the same side of the molecule as the methyl group at C-13.

Acetylation of **1** with Ac_2O/pyridine gave a monoacetate which had the same chemical and spectral data as **3**. The molecular formula of **2** was determined as $C_{20}H_{30}O_2$ by elemental analysis and eims. Analysis of the spectral data of **2** suggested it was a diterpenoid closely related to **1**. Thus, when the ^{1}H- and ^{13}C-nmr spectra were compared to those of **1**, there was a disappearance of the C-19 methylene group signal of **1** (^{1}H nmr, δ 3.54 and 3.84; ^{13}C nmr, δ 64.77), and the appearance of a carboxyl group (^{13}C nmr, δ 185.04). On methylation, compound **2** afforded the methyl ester **8**. The structural determinations of **2**, **3**, and **4** were based on the similarities of their ir, ms, ^{1}H-, and ^{13}C-nmr data with the analogous data of **1**. Compounds **2** and **3** are reported for the first time in nature, but compound **4** has been isolated previously (5).

The fifth diterpene **5** showed a number of spectral features of a kaurane diterpene derivative. The ir spectrum exhibited weak bands at ca. 3060 and 1655 cm^{-1} for an exocyclic double bond and at ca. 1690 cm^{-1}, typical of a carboxylic acid. The ^{1}H-nmr spectra showed a broad singlet (2H) at δ 4.75, confirming the presence of an exocyclic double bond. By comparison of ms and ^{13}C-nmr data with those published (7), compound **5** was identified as (−)-kaur-16-en-19-oic acid.

The last diterpene **6**, mp 272–274°, *m/z* $[M]^+$ 336, showed hydroxy bands at 3380 cm^{-1} and a carboxyl carbonyl band at 1696 cm^{-1}. The ^{1}H- and ^{13}C-nmr data suggested that this compound had one carboxy group (^{13}C nmr δ 179.49) and a diol system [^{1}H nmr δ 3.98 and 4.08 (d, J = 10.8 Hz); ^{13}C nmr δ 81.34 (C-16), 66.16 (C-17)]. Compound **6** easily formed a methyl ester **7** with CH_2N_2. All the chemical and spectral data were identical with those reported (8,9) for *ent*-16β,17-dihydroxykauran-19-oic acid and its methyl ester.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The melting points are uncorrected. Ir spectra were recorded on KBr discs (Beckmann IR-20A spectrometer). The nmr spectra were obtained in $CDCl_3$ or C_6D_5N (varian FT-80A, JEOL JMN FX-100, Nicolet NT 360, and Bruker AM 500 spectrometers) using TMS as an internal standard. Mass spectra (70 eV, Hewlett Packard HP 5985 B GC/MS system) were taken with a direct inlet.

PLANT MATERIAL.—The root bark (8 kg) of *A. koreanum* was collected from the Hanla Mt. of Jeju-Do in Korea in September 1982. The plant was verified by Dr. Bo S. Chung, Seoul National University. Voucher specimens are deposited in our laboratory.

EXTRACTION AND ISOLATION.—Air-dried root bark was extracted with MeOH (9 liters × 3). The MeOH extract was evaporated in vacuo and fractionated with Et_2O and *n*-BuOH. The Et_2O extract was chromatographed on SiO_2 with *n*-hexane-EtOAc (20:1→5:1). A total of 14 fractions (A–N) were collected. From fraction E (6 g), eluting with C_6H_6-EtOAc (95:5), compound **1** (75 mg) was obtained. Fraction B (160 g) eluted with *n*-hexane-EtOAc (10:1) afforded compound **2** (3 g), compund **3** (27 mg), and compound **5** (1.2 g). Fraction A, also eluted with *n*-hexane-EtOAc (20:1), contained compound **4** (8 mg). Compound **6** (42 mg) was isolated from the *n*-BuOH extract by Si gel cc using the solvent mixture of $CHCl_3$-MeOH (6:1) and C_6H_6-EtOH (9:1) successively.

(−)-PIMARA-9(11),15-DIEN-19-OL [**1**].—Compound **1** was recrystallized from *n*-hexane as white needles; mp 73–74°, $[\alpha]^{25}D$ −14.94° ($CHCl_3$, c = 0.19); ir ν max cm^{-1} 3360 (-OH), 1645, 910 (olefinic); ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* (rel. int. %) $[M]^+$ 288 (1.4), $[M-Me]^+$ 273 (5.3), $[M-CH_2OH]^+$ 257 (10.3), 241 (17.0), $[M-C_5H_8]^+$ 220 (26.0), $[M-CH_2OH-C_5H_8]^+$ 189 (41.6), 173 (17.9), 119 (58.3). Acetylation of **1** (30 mg) with Ac_2O/pyridine (overnight, room temperature) gave the corresponding monoacetate (18 mg), colorless oil; ^{1}H nmr ($CDCl_3$) δ 0.97 (6H, s, H-17, 18), 1.08 (3H, s, H-20), 2.05 (3H, s, -OAc), 3.98 (1H, d, J = 10.8 Hz, Ha-19), 4.30 (1H, d, J = 10.8 Hz, Hb-19), 4.84 (1H, dd, J = 10.7, 1.3 Hz, H-16*cis*), 4.91 (1H, dd, J = 17.4, 1.3 Hz, H-16*trans*), 5.38 (1H, m, H-11), 5.83 (1H, dd, J = 17.4, 10.7 Hz, H-15); eims *m/z* (rel. int. %) $[M]^+$ 330 (49.5), $[M-Me]^+$ 315 (74.7), 288 (2.1), 257 (21.4), 202 (29.7), 187 (69.0), 173 (43.0), 107 (100.0).

(−)-PIMARA-9(11),15-DIEN-19-OIC ACID [**2**].—Compound **2** was an amorphous powder, mp 135–136°. Found C 79.2%, H 10.1%, $C_{20}H_{30}O_2$ required C 79.5%, H 9.9%; ir ν max cm^{-1} 3290 (-OH), 1690 (C=O), 1638 (C=C), 1460, 1260, 1075, 965; eims *m/z* (rel. int. %) $[M]^+$ 302 (17.4), $[M-Me]^+$ 287 (32.3), 241 (40.4), 234 (34.4), 219 (11.5), 201 (10.0), 189 (49.9), 173 (44.1); ^1H nmr see Table 1; ^{13}C nmr see Table 2.

Reaction of **2** (1.5 g) with CH_2N_2 gave the methylester **8** (470 mg), yellowish oil; $[\alpha]^{25}D$ −35.98 ($CHCl_3$, $c=0.8$); ir ν max cm^{-1} 2940, 1728 (C=O), 1635 (C=C), 1465, 1225, 1150, 910; ^1H nmr δ 0.92 (3H, s, H-20), 0.98 (3H, s, H-17), 1.20 (3H, s, H-18), 3.63 (3H, s, -OMe), 4.86 (1H, dd, H-16*cis*), 4.93 (1H, dd, H-16*trans*), 5.40 (1H, m, H-11), 5.83 (1H, dd, H-15); ^{13}C nmr δ 41.90 (C-1), 19.16 (C-2), 38.38 (C-3), 44.30 (C-4), 48.05 (C-5), 20.45 (C-6), 28.42 (C-7), 28.71 (C-8), 149.72 (C-9), 38.38 (C-10), 116.44 (C-11), 37.50 (C-12), 34.81 (C-13), 41.90 (C-14), 149.90 (C-15), 109.00 (C-16), 22.27 (C-17), 27.89 (C-18), 177.56 (C-19), 22.27 (C-20), 51.40 (-OMe).

(−)-PIMARA-9(11),15-DIEN-19-OL 19-ACETATE [**3**].—Compound **3** was a slightly yellow oil: $[\alpha]^{25}D$ −8.82 ($CHCl_3$, $c=0.34$); eims *m/z* (rel. int. %) $[M]^+$ 330 (8.4), $[M-Me]^+$ 315 (24.2), $[M-HOAc]^+$ 270 (8.1), $[M-CH_2OAc]^+$ 257 (25.3); ^1H nmr see Table 1.

(−)-PIMARA-9(11),15-DIENE [**4**].—Compound **4** was a yellowish oil: ir ν max cm^{-1} 2960, 1640, 1460, 1375, 998, 910; eims *m/z* (rel. int. %) $[M]^+$ 272 (4.2), $[M-Me]^+$ 257 (19.9), 243 (9.6), 189 (36.0), 175 (22.9), 161 (34.7); ^1H nmr see Table 1.

(−)-KAUR-16-EN-19-OIC ACID [**5**].—Compound **5** was a cubic crystal: mp 177–178°, $[\alpha]^{25}D$ −97.45 ($CHCl_3$, $c=0.55$); ir ν max cm^{-1} 3420 (-OH), 1690 (-COOH), 1655, 875 (-C=CH_2); eims *m/z* (rel. int. %): $[M]^+$ 302 (42.2), 287 (40.3), 259 (55.1); ^1H nmr ($CDCl_3$) δ 0.95 (3H, s, H-20), 1.25 (3H, s, H-18), 2.63 (1H, m, H-13), 4.75 (2H, m, H-17); ^{13}C nmr ($CDCl_3$) δ 40.7 (t, C-1), 19.1 (t, C-2), 37.7 (t, C-3), 43.7 (s, C-4), 57.0 (d, C-5), 21.8 (t, C-6), 41.3 (t, C-7), 44.2 (s, C-8), 55.1 (d, C-9), 39.6 (s, C-10), 18.4 (t, C-11), 33.1 (t, C-12), 43.8 (d, C-13), 39.7 (t, C-14), 48.9 (t, C-15), 155.6 (s, C-16), 103.0 (t, C-17), 28.9 (q, C-18), 185.0 (s, C-19), 15.6 (q, C-20).

Reaction of **5** (100 mg) with CH_2N_2 gave the methylester **9** (70 mg), white crystals: mp 76–78°; ir ν max cm^{-1} 1718 (C=O), 1655, 1240, 1205, 1150, 878; eims *m/z* (rel. int. %) $[M]^+$ 316 (80.0), 273 (40.5), 257 (54.0), 241 (40.5), 131 (34.7), 123 (37.6), 121 (51.1), 91 (100.0); ^1H nmr ($CDCl_3$) δ 0.81 (3H, s, H-20), 1.18 (3H, s, H-20), 3.65 (3H, s, COOMe), 4.78 (2H, m, H-17).

ENT-16β,17-DIHYDROXY-(−)-KAURAN-19-OIC ACID [**6**].—Compound **6** existed as white needle-like crystals, mp 272–274°. Found C 71.1%, H 9.4%, $C_{20}H_{32}O_4$ requires C 71.5%, H 9.5%; ir ν max cm^{-1} 3380 (-OH), 1696 (-COOH); eims *m/z* (rel. int. %) $[M]^+$ 336 (10.7), 305 (100.0), 287 (14.3), 259 (24.7), 241 (23.3); ^1H nmr (pyridine-d_5) 1.17, 1.26 (-Me), 3.98 and 4.08 (d, $J=10.8$ Hz, $-CH_2OH$); ^{13}C nmr (pyridine-d_5) 40.96 (C-1), 19.69 (C-2), 38.62 (C-3), 43.77 (C-4), 56.96 (C-5), 22.80 (C-6), 42.66 (C-7), 44.83 (C-8), 56.20 (C-9), 40.00 (C-10), 18.90 (C-11), 26.66 (C-12), 45.76 (C-13), 37.62 (C-14), 53.74 (C-15), 81.34 (C-16), 66.16 (C-17), 29.24 (C-18), 179.49 (C-19), 15.94 (C-20).

Reaction of **6** (20 mg) with CH_2N_2 gave the methylester **7** (15 mg); eims *m/z* (rel. int.%) 332 (8.0), 319 (32.1), 273 (100.0), 295 (45.2); ^1H nmr ($CDCl_3$) δ 0.81 (-Me), 1.16 (-Me), 3.65 (-OMe), 3.63 and 3.78 (d, $J=11$ Hz, Ha and Hb-17).

LITERATURE CITED

1. B.S. Chung, Y.H. Kim, H.K. Lee, J.W. Kim, J.C. Kim, Y.H. Lee, and W.K. Yoo, *Proc. Asian Symp. Med. Plants Spices, 5th*, 385, 1984.
2. L.M. Perry and J. Metzger, "Medicinal Plants of East and Southeast Asia," MIT Press, Cambridge, MA and London, 1980, p. 41.
3. E. Wenkert and P. Bead, *J. Am. Chem. Soc.*, **83**, 998 (1961).
4. P.R. Jefferies and T. Ratajczak, *Aust. J. Chem.*, **26**, 173 (1973).
5. S.K. Fernanda, V. Wagner, O. Fernando, and F.R. Nidia, *Phytochemistry*, **25**, 1240 (1986).
6. E. Wenkert and B.L. Buckwalter, *J. Am. Chem. Soc.*, **94**, 4367 (1972).
7. C.M. Hasen, T.M. Healey, and P.G. Waterman, *Phytochemistry*, **21**, 1365 (1982).
8. K. Yamasaki, H. Kohda, T. Kobayashi, R. Kasai, and O. Tanaka, *Tetrahedron Lett.*, **13**, 1005 (1976).
9. W. Herz, P. Kulanthaivel, and K. Watanabe, *Phytochemistry*, **22**, 2021 (1983).

Received 23 February 1988