Two New Quassinoids, Ailantinols C and D, from *Ailanthus Altissima*

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Two new quassinoids, ailantinol C and ailantinol D, were isolated from *Ailanthus altissima*, and their structures were elucidated from spectral evidence.

Simaroubaceous plants contain many quassinoids with various biological activities, such as antitumor, antimalarial, antifeedant, insecticidal, anti-inflammatory, amoebicidal, and herbicidal effects.[1] We are interested in such biologically active compounds and have isolated many quassinoids from plants, such as *Brucea antidysenterica*,[2—8] *Picrasma ailanthoides*,[9—12] and *Brucea javanica*.[13,14] As a part of these studies, we have investigated the isolation of quassinoids from *Ailanthus altissima* Swingle (=*Ailanthus glandulosa* Desf) (Simaroubaceae). In a previous paper,[15] we reported on the isolation and structural elucidation of two new quassinoids, ailantinols A and B (**5**), and related compounds from the stem bark of *A. altissima*. Recently,[16] eighteen quassinoid glycosides and nine known quassinoids, some of which were isolated from *A. altissima*, were tested for inhibitory activity against HIV replication in H9 lymphocytic cells. Shinjulactones C, B, and ailantiol A, all of which are quassinoids of *A. altissima*, showed good anti-HIV activity (EC_{50} values of 10.6, 28, and 30 μM with therapeutic index of > 25, > 10, and > 8.2, respectively). We are interested in the components of the same plant, and carried out a detailed investigation. Herein, we wish to report on the isolation and structural elucidation of two new quassinoids, ailantinol C (**1**) and ailantinol D (**2**), from this same plant. Shinjulactone A (**3**),[17] shinjudilactone (**4**),[18,19] and ailantinol B (**5**)[15] also isolated from this plant, were very useful in the structural elucidation of these new compounds (Chart 1).

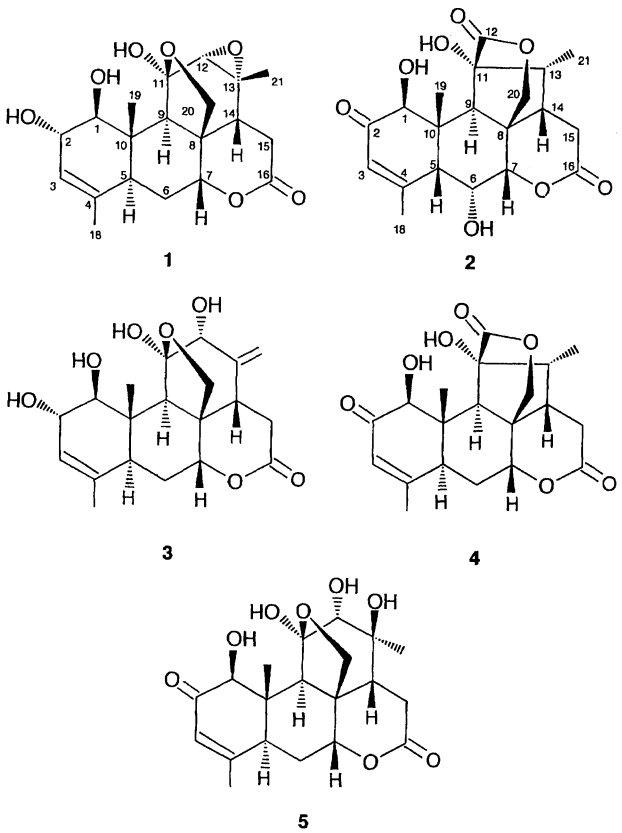

Chart 1. Structures of compounds **1**, **2**, **3**, **4**, and **5**.

Results and Discussion

Compound **1** was obtained as colorless needles. Its IR spectrum showed the presence of hydroxy (3370 cm^{-1}) and δ-lactone (1725 cm^{-1}) groups. Its molecular formula was established as $C_{20}H_{20}O_7$ from its HREIMS (*m/z* 378.1679). Because its ^1H and ^{13}C NMR spectra did not coincide with those any known quassinoid, this compound was assumed to be a quassinoid. We have assigned the name ailantinol C to compound **1**.

The ^1H NMR signals (Table 1) of **1** were similar to those of shinjulactone A (**3**), except for the positions of the H-9, H-12, and H-14 signals, the presence of an additional methyl at C-13, and the absence of an exo-methylene at this position. The H-9, H-12, and H-14 signals in **1** appeared at higher field by 0.78, 1.01, and 0.53 ppm, respectively, compared to those in **3**. These observations suggested that **1** has an epoxy group between C-12 and C-13.

Table 1. ^{1}H NMR Spectra[a] of Compounds **1**—**5**

Proton	Compound				
	1[b]	**2**[b]	**3**[b]	**4**[b]	**5**[b]
H-1	3.36 d (8)	5.46 s	4.02 (8)	4.23 s	4.42 s
H-2	4.55 br	—	4.62 m	—	—
H-3	5.77 br s	6.24 br s	5.72 br s	6.10 br s	6.12 br s
H-5	2.43 brd (12.4)	2.76 br s	2.67 br d (13)	3.22 br d (13)	3.03 br d (13)
H-6α	2.00 br dd (14.4, 2)	—	2.07 ddd (15, 2.5, 2.5)	2.31 ddd (15, 3, 2.5)	2.19 ddd (15, 3.5, 3)
H-6β	1.89 br dd (14.4, 12.4)	4.76 dd (5.6, .5.2)	1.92 ddd (15, 13, 2.5)	2.08 ddd (15, 13, 2.5)	2.07 ddd (15, 3, 3)
H-7	4.43 br	4.45 d (5.2)	4.57 t (2.5)	4.81 t (2.5)	4.62 dd (3.5, 3)
H-9	2.52 s	4.12 s	3.30 s	2.72 s	3.37 s
H-12	3.58 br s	—	4.59 s	—	4.16 br d (5)
H-13	—	2.14 dq (7.2, 4.4)	—	2.18 quin (7)	—
H-14	2.27 br dd (9.2, 9.2)	2.06 ddd (13.2, 5.2, 4.4)	2.80 dd (13.5, 5,5)	2.28 ddd (10.5, 7, 1)	2.55 dd (14.5, 5.5)
H-15α	2.93 br d (9.2)	3.03 dd (16.4, 13.2)	3.57 dd (18, 13.5)	3.13 dd (16, 10.5)	3.29 dd (18.5, 14.5)
H-15β	2.93 br d (9.2)	2.94 dd (16.4, 5.2)	2.90 dd (18, 15.5)	2.70 dd (16, 1)	3.09 dd (18.5, 5.5)
H-20α	3.97 d (9.6)	4.62 d (12)	4.17 d (8)	4.79 d (12)	4.78 d (8)
H-20β	3.72 d (9.6)	4.46 d (12)	3.67 d (8)	4.31 d (12)	4.14 d (8)
H-21	—	—	5.20 d (2)	—	—
	—	—	5.28 d (2)	—	—
4-Me	1.59 br s	1.91 s	1.60 br s	1.80 br s	1.76 br s
10-Me	1.53 s	1.49 s	1.68 s	1.24 s	1.61 s
13-Me	1.35 s	1.30 d (7.2)	—	1.24 d (7)	1.67 s
6-OH	—	8.68 d (5.6)	—	—	—
12-OH	d)	—	d)	—	7.60 d (5)

a) Values are in ppm (C_5D_5N). The coupling constant (J values) in parentheses are Hz. b) 400 MHz. c) 500 MHz. d) Not assignable.

Table 2. ^{13}C NMR Spectra[a] of Compounds **1**—**5**

Carbon	Compound									
	1[b]		**2**[b]		**3**[c]		**4**[c]		**5**[c]	
C-1	82.8	(CH)	80.5	(CH)	83.6	(CH)	83.8	(CH)	84.6	(CH)
C-2	72.0	(CH)	197.1	(C=O)	72.7	(CH)	196.9	(C=O)	197.6	(C=O)
C-3	127.1	(CH)	126.4	(CH)	127.1	(CH)	126.3	(CH)	126.3	(CH)
C-4	134.4	(C)	157.5	(C)	134.8	(C)	162.0	(C)	162.3	(C)
C-5	41.5	(CH)	52.8	(CH)	49.6	(CH)	42.2	(CH)	42.6	(CH)
C-6	25.5	(CH_2)	70.5	(CH)	26.2	(CH_2)	27.0	(CH_2)	26.1	(CH_2)
C-7	78.7	(CH)	80.4	(CH)	79.2	(CH)	73.9	(CH)	78.2	(CH)
C-8	44.9	(C)	42.2	(C)	45.8	(C)	48.4	(C)	46.5	(C)
C-9	45.2	(CH)	53.5	(CH)	48.0	(CH)	55.0	(CH)	44.8	(CH)
C-10	41.5	(C)	44.0	(C)	44.9	(C)	43.0	(C)	45.3	(C)
C-11	107.4	(C)	79.2	(C)	110.5	(C)	78.7	(C)	110.7	(C)
C-12	68.0	(CH)	172.8	(C=O)	80.7	(CH)	173.5	(C=O)	83.0	(CH)
C-13	59.0	(C)	53.1	(CH)	148.0	(C)	45.6	(CH)	74.2	(C)
C-14	43.5	(CH)	45.5	(CH)	41.9	(CH)	53.6	(CH)	49.0	(CH)
C-15	28.6	(CH_2)	36.1	(CH_2)	35.4	(CH_2)	32.9	(CH_2)	31.8	(CH_2)
C-16	169.6	(C=O)	170.8	(C=O)	169.6	(C=O)	170.6	(C=O)	170.2	(C=O)
C-18	21.3	(Me)	22.8	(Me)	21.2	(Me)	22.1	(Me)	22.4	(Me)
C-19	10.8	(Me)	16.8	(Me)	10.6	(Me)	10.6	(Me)	10.7	(Me)
C-20	72.8	(CH)	77.3	(CH_2)	72.7	(CH_2)	76.2	(CH_2)	71.0	(CH_2)
C-21	21.9	(Me)	14.3	(Me)	118.0	(CH_2)	13.8	(Me)	26.2	(Me)

a) Values are in ppm (C_5D_5N). b) 100 MHz. c) 22.5 MHz.

The ^{13}C NMR signals (Table 2) observed for C-1 to C-11, C-14, and C-16 to C-20 in **1** were nearly identical to those of **3**. The DEPT NMR spectrum of **1** showed that the C-13 is a quaternary carbon and that the C-21 is a methyl carbon, whereas these carbons in **3** are olefinic and correspond to the *exo*-methylene.

The chemical-shift values (δ = 68.0 and 59.0) of C-12 and C-13 in **1** suggested that these carbons were connected to an oxygen atom. However, the C-12 and C-13 signals appeared at a higher field by 15.0 and 15.2 ppm, respectively, than those in ailantinol B (**5**), which has OH groups at C-12 and C-13. Moreover, a coupling constant of $^1J_{C-H}$ = 181.9 Hz was observed for the doublet signal at δ = 68.0 (C-12). These observations also suggested that **1** has an epoxy group

between C-12 and C-13. From these data, the structure of **1** was assumed to be as shown.

This structure was confirmed from the HMBC data of **1**; the ^{13}C–^{1}H long-range correlations are depicted by arrows in Fig. 1. The relative stereochemistry of **1** was confirmed by NOE correlations, as shown in Fig. 2.

Compound **2** was isolated as colorless needles. Its IR spectrum showed the presence of hydroxy (3380 cm^{-1}), δ-lactone (1750 and 1735 cm^{-1}), and α,β-unsaturated carbonyl (1680 cm^{-1}) groups. Its UV spectrum showed an absorption maximum at 236 nm due to a conjugated enone system. Its molecular formula was established by HREIMS to be $C_{20}H_{24}O_8$, which has one more oxygen than that ($C_{20}H_{24}O_7$) of shinjudilactone (**4**).

The ^{1}H NMR signals of **2** (Table 1) were similar to those of **4**, except for the positions of the H-1, H-5, H-6, H-9, and 10-Me signals. The H-1, H-6β, H-9, and 10-Me signals of **2** appeared at lower field by 1.23, 2.68, 1.40, and 0.25 ppm, respectively, than those of **4**; however, the H-5 signal in **2** shifted to a higher field by 0.46 ppm compared to that in **4**.

The ^{13}C NMR signals of **2** (Table 2) were similar to those of **4**, except for the positions of the C-5, C-6, C-7, and C-8 signals. The three former signals were shifted to lower field by 10.6, 43.5, and 6.5 ppm, respectively, and the latter signal was shifted to a higher field by 6.2 ppm compared with the corresponding carbon signals in **4**. The DEPT NMR spectra of **2** and **4** indicated that the C-6 in **2** is a methine carbon, while the C-6 in **4** is a methylene carbon. The addition of an OH group at C-6 was consistent with the NMR and MS data. From these facts, the structure of **2** was assumed to be that shown.

The structure of **2** was confirmed by ^{13}C–^{1}H long-range correlations in its HMBC NMR spectrum, as depicted by arrows in Fig. 3.

The relative stereochemistry of **2** was confirmed by NOE correlations, as shown in Fig. 4. The following differential NOE measurements of **2** also supported the stereochemical assignment: irradiation at H-5 (δ = 2.76) resulted in an enhancement of the 10-Me, 4-Me, H-7, and H-20α signals (δ = 1.49, 1.91, 4.45, and 4.62) and irradiation at H-9 (δ = 4.12) resulted in an enhancement of the H-1 signal (δ = 5.46).

Compound **2** was acetylated with Ac_2O in pyridine to

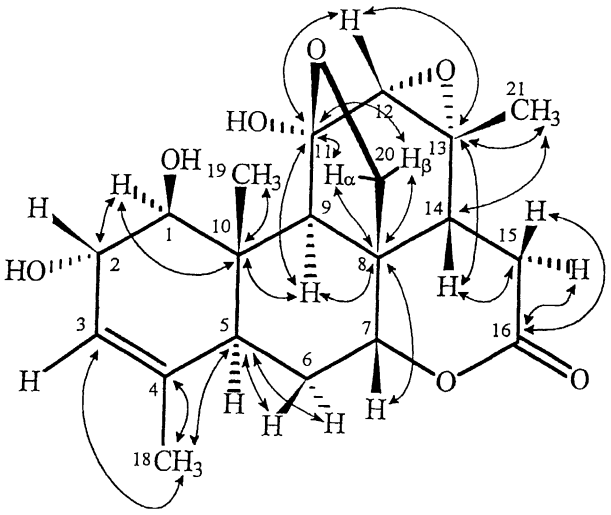

Fig. 1. ^{13}C–^{1}H long-range correlations in the HMBC spectrum of **1**.

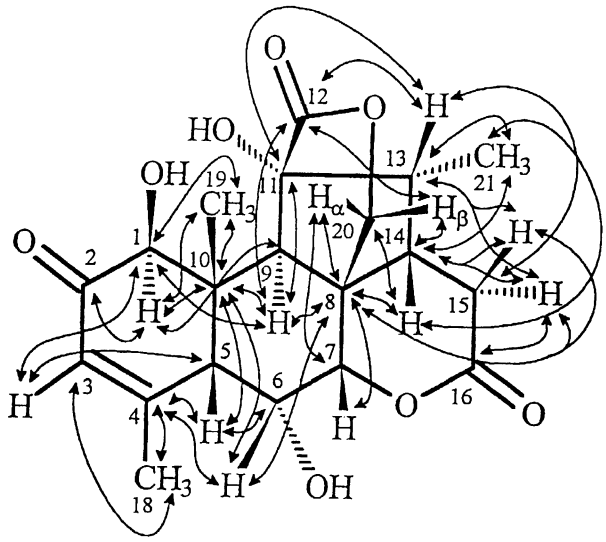

Fig. 3. ^{13}C–^{1}H long-range correlations in the HMBC spectrum of **2**.

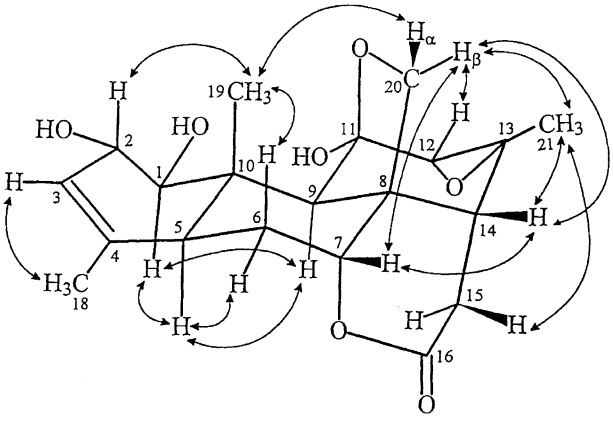

Fig. 2. NOE correlations of **1**.

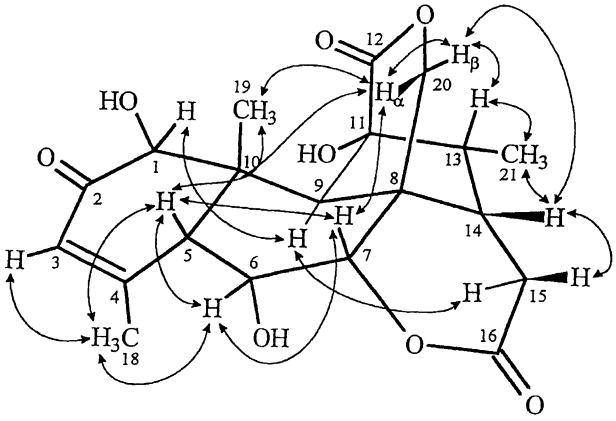

Fig. 4. NOE correlations of **2**.

afford an acetate. The ^{1}H NMR spectrum of the acetate showed an acetoxy signal ($\delta = 2.35$), together with three methyl signals ($\delta = 1.92$, 1.60, and 1.14; 4-Me, 10-Me, and 13-Me). The H-1 methine signal ($\delta = 7.08$) appeared at lower field by 1.62 ppm than that of **2**. Thus, the OH group at C-1 was acetylated. An NOE experiment of the acetate also supported the structure of **2**.

Experimental

The melting points were determined on an MRK air-bath type melting-point apparatus, and are uncorrected. Specific rotations were obtained on a JASCO DIP-370 digital polarimeter ($L = 0.5$ dm). IR and UV spectra were recorded on JASCO IR-810 and Hitachi 320-S spectrophotometers, respectively. ^{1}H and ^{13}C NMR spectra were determined on Varian VXR-500, JASCO GSX-500, or JEOL ALPHA-400 instruments in C_5D_5N using TMS as an internal standard. The mass spectra were recorded on a Hitachi M-80 instrument. Si gel (Merck, type 60, 70-320 mesh) and Sephadex LH-20 (Pharmacia) were used for CC. Precoated silica-gel plates (Merck 60F$_{254}$) of 0.25 mm thickness were used for analytical TLC, and plates of 1 and 2 mm thickness were used for prep. TLC. The components were detected on TLC plates using a UV lamp (245 and 365 nm). Analytical HPLC was performed on a Tosoh liquid chromatograph equipped with a UV detector at 254 nm and a reverse-phase column (TSK-gel ODS-80Ts) using a mixed solvent of MeOH/H_2O. Preparative HPLC was carried out on Tosoh or Gilson liquid chromatographs equipped with a reverse-phase column (Dynamax-60A and/or Lichrosorb RP-18) at 254 nm using the same solvents as those employed for analytical HPLC.

Plant Material. In 1982, *Ailanthus altissima* Swingle = *Ailanthus glandulosa* Desf (Simaroubaceae) was planted in the Higashisenda campus of Hiroshima University. In 1990, the plant was cut down and the stem bark was collected for extraction. A voucher specimen was deposited at the above campus.

Materials. Shinjulactone A (**3**), shinjudilactone (**4**), and ailantinol B (**5**) used for this study were isolated from *A. altissima*, and their structures was elucidated from spectral evidence.[19]

Extraction and Isolation of Ailantinols C(1) and D(2). The stem bark of *A. altissima* (fresh material, 33 kg) was cut into small pieces and soaked in MeOH (76 l) for one year at room temperature. A MeOH extract (2.5 kg) was obtained by evaporation of the solvent. The extract was then partitioned between MeOH/H_2O (2 : 1) and hexane to give a hexane extract (201 g). The MeOH/H_2O layer was then extracted with $CHCl_3$ to give a $CHCl_3$ extract (221 g). Silica gel CC of the $CHCl_3$ extract (221 g) eluting with EtOAc/Et_2O (1 : 1, v/v) (13 l) gave 95 fractions, then with MeOH (13 l) gave 27 fractions. Each fraction was checked by analytical TLC and HPLC; combination gave 19 subfractions: Fr-1 (5.94 g), -2 (6.67 g), -3 (4.89 g), -4 (5.53 g), -5 (6.42 g), -6 (6.47 g), -7 (4.35 g), -8 (4.99 g), -9 (4.55 g), -10 (4.02 g), -11 (4.85 g), -12 (3.29 g), -13 (5.83 g), -14 (6.01 g), -15 (15.2 g, MeOH), -16 (16.1 g, MeOH), -17 (15.0 g, MeOH), -18 (19.6 g, MeOH), and -19 (18.7 g, MeOH). Prep. TLC ($CHCl_3$/MeOH/H_2O, 50 : 14 : 3) of subfraction 9 (4.55 g) gave seven fractions: Fr. 9-1 (0.56 g), 9-2 (1.55 g), 9-3 (1.46 g), 9-4 (0.59 g), 9-5 (0.56 g), 9-6 (0.39 g), and 9-7 (0.12 g). Fr. 9-3 was subjected to prep. HPLC (MeOH/H_2O, 4 : 6) to give eight fractions: Fr. 9-3-1 (531 mg), 9-3-2 (142 mg), 9-3-3 (117 mg), 9-3-4 (136 mg), 9-3-5 (93 mg), 9-3-6 (194 mg), 9-3-7 (34 mg), and 9-3-8 (89 mg). Fr. 9-3-2 (142 mg) was further subjected to repeat prep. HPLC (MeOH/H_2O, 3 : 7) to afford the new quassinoid ailantinol C (**1**, 5.7 mg, 0.000017%) as colorless needles. Subfraction 12 (3.29 g) was subjected to prep. TLC (EtOAc/Et_2O, 1 : 1) to give six fractions: Fr. 12-1 (0.28 g), 12-2 (1.77 g), 12-3 (1.09 g), 12-4 (0.35 g), 12-5 (0.31 g), and 12-6 (0.36 g). Prep. HPLC (MeOH/H_2O, 2 : 8) of Fr. 12-2 (1.77 g) gave nine fractions: Fr. 12-2-1 (301 mg), 12-2-2 (172 mg), 12-2-3, (95 mg) 12-2-4 (143 mg), 12-2-5 (36 mg), 12-2-6 (93 mg), 12-2-7 (198 mg), 12-2-8 (95 mg), and 12-2-9 (55 mg). Fr. 12-2-3 (95 mg) was subjected to prep. HPLC (MeOH/H_2O, 15 : 85) to afford the new quassinoid ailantinol D (**2**, 15.7 mg, 0.000047%) as colorless needles.

Ailantinol C [1]. Colorless needles: mp 188 °C (decomp); $[\alpha]_D^{20}$ −140° (*c* 0.041, MeOH), IR (KBr) ν_{max} 3370 (OH), 3220 (OH), and 1720 (δ-lactone C=O) cm^{-1}; HREIMS *m/z* [M−H_2O]$^+$ 378.1679 ($C_{20}H_{26}O_7$, error 0.2); ^{1}H and ^{13}C NMR data see Tables 1 and 2.

Ailantinol D. [2]. Colorless needles: mp 200—203 °C; $[\alpha]_D^{26}$ −55.0° (*c* 0.047, EtOH); UV (EtOH) λ_{max} (ε) 236 (7040) nm; IR (KBr) ν_{max} 3380 (OH), 1750 and 1735 (δ-lactone C=O), and 1680 (α,β-unsaturated C=O) cm^{-1}; EIMS *m/z* [M]$^+$ 392 (11); HREIMS *m/z* [M]$^+$ 392.1469 ($C_{20}H_{24}O_8$, error 0.0); ^{1}H and ^{13}C NMR data see Tables 1 and 2.

Acetylation of 2. Compounds **2** (3.2 mg) was acetylated with Ac_2O (0.5 ml) in pyridine (0.5 ml) at 25 °C for 10 h. Crude product (4.9 mg) was obtained after the addition of MeOH (1 ml) and evaporation of the solvent. Purification of the crude product by prep. HPLC afforded an acetate of **2** (2.2 mg, 62% yield).

Acetate of 2. Colorless amorphous solid: mp 106—109 °C; IR (KBr) ν_{max} 3400 (OH), 1750 (γ-lactone C=O), 1735 (δ-lactone C=O), 1680 (α,β-unsaturated C=O) cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz) $\delta = 1.14$ (3H, s, Me-13), 1.61 (3H, s, Me-10), 1.92 (3H, s, Me-4), 2.35 (3H, s, AcO), 2.82 (1H, br s, H-5), 4.03 (1H, s, H-9), 4.47 (1H, d, $J = 5.2$ Hz, H-7), 4.76 (1H, d, $J = 5.2$ Hz, H-6β), 4.49 (1H, d, $J = 12$ Hz, H-20β), 4.73 (1H, d, $J = 12$ Hz, H-20α), 6.02 (1H, s, H-3), 7.08 (1H, s, H-1); EIMS *m/z* [M]$^+$ 434 (100).

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References

1) M. Okano, N. Fukamiya, and K. Lee, in "Studies in Natural Products Chemistry," ed by Atta-ur-Rahman, Elsevier Science Publishers B. V., Amsterdam (1990), Vol. 7, p. 369.

2) M. Okano, K. H. Lee, I. H. Hall, and F. E. Boettner, *J. Nat. Prod.*, **44**, 470 (1981).

3) N. Fukamiya, M. Okano, K. Tagahara, T. Aratani, Y. Muramoto, and K. H. Lee, *J. Nat. Prod.*, **50**, 1075 (1987).

4) M. Okano, N. Fukamiya, T. Aratani, M. Ju-Ichi, and K. H. Lee, *J. Nat. Prod.*, **48**, 972 (1985).

5) N. Fukamiya, M. Okano, K. Tagahara, T. Aratani, and K. H. Lee, *J. Nat. Prod.*, **51**, 349 (1988).

6) K. Imamura, N. Fukamiya, M. Okano, K. Tagahara, and K. H. Lee, *J. Nat. Prod.*, **56**, 2091 (1993).

7) M. Okano, N. Fukamiya, T. Toyota, K. Tagahara, and K. H. Lee, *J. Nat. Prod.*, **52**, 398 (1989).

8) T. Toyota, N. Fukamiya, M. Okano, K. Tagahara, J. J. Chang, and K. H. Lee, *J. Nat. Prod.*, **53**, 1526 (1990).

9) M. Okano, T. Fujita, N. Fukamiya, and T. Aratani, *Bull. Chem. Soc. Jpn.*, **58**, 1793 (1985).

10) T. Matsuzaki, N. Fukamiya, M. Okano, T. Fujita, K.

Tagahara, and K. H. Lee, *J. Nat. Prod.*, **54**, 844 (1991).
11) M. Daido, N. Fukamiya, M. Okano, and K. Tagahara, *J. Nat. Prod.*, **55**, 1643 (1992).
12) M. Daido, N. Fukamiya, M. Okano, and K. Tagahara, *J. Nat. Prod.*, **58**, 605 (1995).
13) K. H. Lee, Y. Imakura, Y. Sumida, R. Y. Wu, I. H. Hall, and H. C. Huang, *J. Org. Chem.*, **44**, 2180 (1979).
14) N. Fukamiya, M. Okano, M. Miyamoto, K. Tagahara, and K. H. Lee, *J. Nat. Prod.*, **55**, 468 (1992).
15) H. Kubota, N. Fukamiya, T. Hamada, M. Okano, K. Tagahara, and K. H. Lee, *J. Nat. Prod.*, **59**, 683 (1996).
16) M. Okano, N. Fukamiya, K. Tagahara, M. Cosentino, T. T. Lee, S. M. Natschke, and K. H. Lee, *Bio-Org. Med. Chem. Lett.*, **6**, 701 (1996).
17) H. Naora, M. Ishibashi, T. Furuno, T. Tsuyuki, T. Murae, H. Hirota, T. Takahashi, A. Itai, and Y. Iitaka, *Bull. Chem. Soc. Jpn.*, **56**, 3694 (1983).
18) M. Ishibashi, T. Tsuyuki, T. Murae, H. Hirota, T. Takahashi, A. Itai, and Y. Iitaka, *Bull. Chem. Soc. Jpn.*, **56** 3683 (1983).
19) M. Ishibashi, T. Murae, H. Hirota, H. Naora, T. Tsuyuki, T. Takahashi, A. Itai, and Y. Iitaka, *Chem. Lett.*, **1981**, 1597.