Three New Quassinoids, Ailantinol E, F, and G, from *Ailanthus altissima*

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Three new quassinoids, ailantinol E (1), ailantinol F (2), and ailantinol G (3), and related compounds were isolated from *Ailanthus altissima* grown in Taiwan. Their structures were elucidated from spectral evidence. Each new quassinoid was evaluated for its antitumor promoting effects against Epstein-Barr virus early antigen activation introduced by 12-*O*-tetradecanoylphorbol-13-acetate in Raji cells. The new quassinoids were found to show potent activity without showing any cytotoxicity. The screening for inhibitors against nitric oxide donor action was also conducted using the new quassinoids and some standard samples.

Key words quassinoid; *Ailanthus altissima*; Simaroubaceae; inhibitory effect on Epstein-Barr virus early antigen (EBV-EA) activation

Simaroubaceous plants contain many quassinoids with various biological activities, such as antitumor, antimalarial, antifeedant, insecticidal, antiinflammatory, 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 part of these studies, we investigated the isolation of quassinoids from *Ailanthus altissima* SWINGLE (Simaroubaceae). In previous papers,[15,16)] we reported on the isolation and structural elucidation of four new quassinoids, ailantinols A, B, C, and D, and related compounds from the stem bark of *A. altissima* grown in Japan. Recently, 18 quassinoid glycosides and nine known quassinoids, some of which were isolated from *A. altissima*, were tested for inhibitory activity against human immunodeficiency virus (HIV) replication in H9 lymphocytic cells.[17)] Shinjulactones B and C, and ailantinol A, all of which are quassinoids isolated from *A. altissima*, showed good anti-HIV activities (EC_{50} values of 28.0, 10.6, and 30.0 μM with a therapeutic index of $>$10.0, $>$25.0, and $>$8.2, respectively). In this paper, we report on the isolation and structural elucidation of three new quassinoids, ailantinol E (**1**), ailantinol F (**2**), and ailantinol G (**3**), from *A. altissima* grown in Taiwan. The known quassinoids chapparin,[18)] amarolide-11-acetate,[19)] shinjulactone A,[20)] shinjulactone C,[21,22)] shinjulactone H,[23)] and shinjulactone L,[24)] were also isolated from this plant.

Results and Discussion

The aerial parts of *A. altissima* were extracted with MeOH to afford a crude extract. After evaporation of the solvent, the crude extract was dissolved in aqueous MeOH and re-extracted with *n*-hexane, chloroform, and then *n*-BuOH. The $CHCl_3$ extract was further fractionated using combination Si gel and Sephadex LH-20 column chromatography and preparative HPLC to give three new quassinoids (**1**—**3**, Fig. 1) and several known quassinoids.

Compound **1** was obtained as a colorless amorphous solid. Its IR spectrum showed the presence of hydroxyl (3400 cm^{-1}), δ-lactone (1760 cm^{-1}), and α,β-unsaturated carbonyl (1650 cm^{-1}) groups. Its molecular formula was established to be $C_{21}H_{24}O_7$ from its high-resolution MS (*m/z* 388.1521) and ^{1}H-, ^{13}C-, and distortionless enhancement by polarization transfer (DEPT)-NMR spectra. The ^{1}H-NMR spectrum (Table 1) of **1** showed three quaternary methyl groups [δ_H 1.14 (10-Me), 1.30 (13-Me), and 2.00 (4-Me)], a methoxymethyl [δ_H 3.50], an olefinic proton [δ_H 6.45 (H-3)], three methylene protons, and three methine protons. The ^{13}C- and DEPT-NMR spectral data indicated the presence of two ketone carbonyls [δ_C 211.2 (C-11), 195.9 (C-2)], a lactone

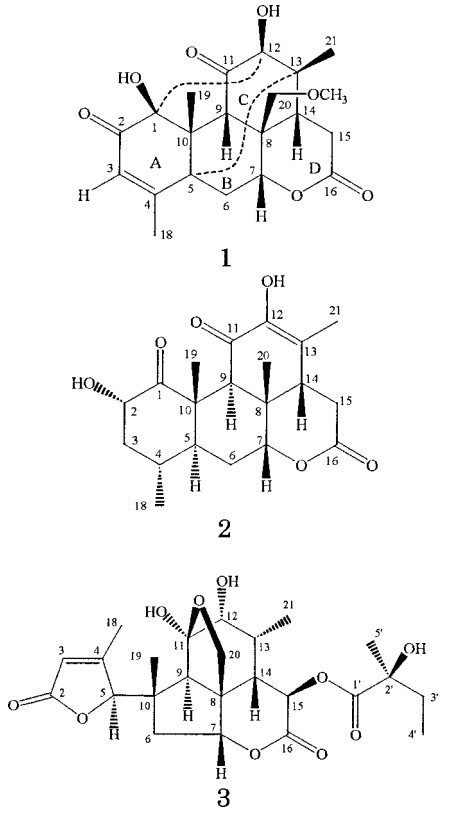

Fig. 1. New Quassinoid Ailantinol E (**1**), Ailantinol F (**2**), Ailantinol G (**3**)

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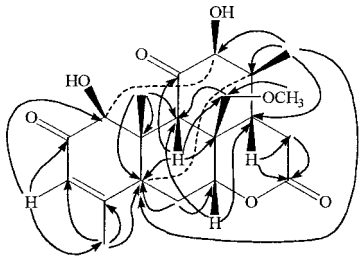

Fig. 2. HMBC Correlations of **1**

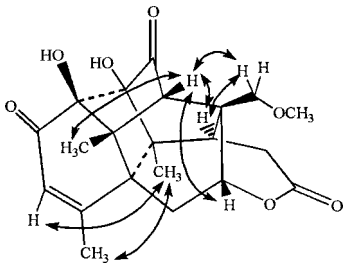

Fig. 3. NOE Correlations of **1**

carbonyl [δ_C 174.8 (C-16)], two olefinic carbons [δ_C 127.2 (C-3), 167.5 (C-4)], and three methylene carbons [δ_C 31.4 (C-6), 33.1 (C-15), 61.1 (C-20)].

Comparison of the ^{1}H- and ^{13}C-NMR data of **1** with those of shinjulactone C revealed that both of ^{1}H- and ^{13}C-NMR spectra were strikingly similar, except for a signal arising from methoxymethyl group. The observation of a long-range correlation between the methoxymethyl group (δ_H 3.50) and C-20 methylene carbon (δ_C 61.1) revealed that the methoxymethyl group was attached to C-20. In the heteronuclear multiple bond connectivity (HMBC) spectrum, the cross peak observed between 13-Me at δ_H 1.30 and C-5 carbon at δ_C 52.2 confirmed C-12 carbon connected to C-5 carbon (Fig. 2). The relative stereochemistry of **1** was confirmed by nuclear Overhauser effect (NOE) correlations, as shown in Fig. 3. The correlation of between 13-Me and 4-Me suggested that the ring A was connected to the ring C. The molecular formula and unsaturation number of **1** obtained from high resolution electron ionization mass spectrum (HR-EI-MS) supported this result. From these data, the structure of **1** was determined as 20-methoxyshinjulactone C.

Compound **2** was obtained as a colorless amorphous solid. Its IR spectrum showed the presence of hydroxyl (3400 cm^{-1}), δ-lactone (1720 cm^{-1}), and α,β-unsaturated carbonyl (1680 cm^{-1}) groups. Its molecular formula was established to be $C_{20}H_{26}O_6$ from its high-resolution MS (*m/z* 362.1730) and ^{1}H-, ^{13}C-, and DEPT-NMR spectra. The ^{1}H-NMR spectrum (Table 1) of **2** showed three quaternary methyl groups [δ_H 1.23 (8-Me), 1.44 (10-Me), and 1.91 (13-Me)], a doublet methyl [δ_H 0.94 (4-Me)], three methylene protons, and six methine protons. The ^{13}C- and DEPT-NMR spectral data indicated the presence of two ketone carbonyls [δ_C 213.2 (C-1), 191.0 (C-11)], a lactone carbonyl [δ_C 169.1 (C-16)], two olefinic carbons [δ_C 125.2 (C-13), 142.9 (C-12)], and three methylene carbons [δ_C 47.5 (C-3), 26.1 (C-6), 31.5 (C-15)].

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. 4. The relative stereochemistry of **2** was confirmed by NOE correlations, as shown in Fig. 5. Correlations between H-5/H-9 and H-5/4-Me indicated that H-5, H-9, and

Table 1. ^{1}H-NMR Spectra of Ailantinol E (**1**), Ailantinol F (**2**), and Ailantinol G (**3**)

Proton	Compound		
	1[a]	**2**[b]	**3**[a]
H-2	—	4.86 dd (12, 8)	—
H-3	6.45 br s	(α) 1.13 m	5.92 d (2)
	—	(β) 2.50 ddd (12, 8, 4)	—
H-4	—	2.00—2.07 m	—
H-5	—	1.48 m	4.96 s
H-6	(α) 2.38 dd (18, 14)	(β) 1.85 m	(α) 2.30 dd (16, 4)
	(β) 2.53 dd (14, 10)	(α) 2.00—2.07 m	(β) 2.96 d (16)
H-7	4.75 t (9)	4.29 t (3)	4.85 d (4)
H-9	3.03 s	3.35 s	3.39 s
H-12	—	—	4.02 t (4)
H-13	—	—	2.53 m
H-14	2.78 m	2.42 dd (12, 6)	2.75 dd (10, 6)
H-15	(α) 3.04 dd (18, 8)	(α) 2.61 dd (18, 12)	6.27 d (10)
	(β) 3.81 dd (18, 3)	(β) 3.01 dd (18, 6)	—
H-20	4.17 d (10)	—	3.89 d (9)
	4.53 d (10)	—	3.97 d (9)
4-Me	2.00 br s	0.94 d (7)	2.45 s
8-Me	—	1.23 s	—
10-Me	1.14 s	1.44 s	1.51 s
13-Me	1.30 s	1.91 s	1.38 d (7)
20-OMe	3.50 s	—	—
H-3′	—	—	2.03 m
	—	—	2.15 m
3′-Me	—	—	1.23 t (7)
2′-Me	—	—	1.71 s

a) Measured in C_5D_5N. *b*) Measured in $CDCl_3$.

Table 2. ^{13}C-NMR Spectra of Ailantinol E (**1**), Ailantinol F (**2**), and Ailantinol G (**3**)

Carbon	Compound		
	1[a]	**2**[b]	**3**[a]
C-1	88.4 (C)	213.2 (C=O)	—
C-2	195.9 (C=O)	69.8 (CH)	172.6 (C=O)
C-3	127.2 (CH)	47.5 (CH_2)	119.0 (CH)
C-4	167.5 (C)	28.2 (CH)	169.9 (C)
C-5	52.2 (C)	47.0 (CH)	92.1 (CH)
C-6	31.4 (CH_2)	26.1 (CH_2)	45.7 (CH_2)
C-7	65.6 (CH)	81.9 (CH)	83.7 (CH)
C-8	49.5 (C)	37.1 (C)	58.2 (C)
C-9	54.1 (CH)	45.6 (CH)	45.3 (CH)
C-10	55.6 (C)	48.0 (C)	46.2 (C)
C-11	211.2 (C=O)	191.0 (C=O)	111.2 (C)
C-12	94.5 (C)	142.9 (C)	80.7 (CH)
C-13	55.1 (C)	125.2 (C)	34.1 (CH)
C-14	40.9 (CH)	46.9 (CH)	43.4 (CH)
C-15	33.1 (CH_2)	31.5 (CH_2)	70.8 (CH)
C-16	174.8 (C=O)	169.1 (C=O)	167.9 (C=O)
C-18	22.6 (Me)	18.3 (Me)	16.0 (Me)
C-19	13.8 (Me)	14.8 (Me)	18.3 (Me)
C-20	61.1 (CH_2)	23.2 (Me)	72.1 (CH_2)
C-21	14.1 (Me)	15.2 (Me)	14.9 (Me)
20-OCH_3	51.5 (Me)	—	—
C-1′	—	—	176.4 (C=O)
C-2′	—	—	75.2 (C)
C-3′	—	—	33.8 (CH_2)
C-4′	—	—	8.4 (Me)
C-5′	—	—	25.8 (Me)

a) Measured in C_5D_5N. *b*) Measured in $CDCl_3$.

4-Me had α-orientations. Correlations between H-2/10-Me, 10-Me/8-Me, 8-Me/H-7, and H-7/H-14 indicated that H-2, H-7, H-14, 8-Me, and 10-Me had β-orientations. On the basis of these data, the structure of **2** was determined as demethylpicrasin B.

Compound **3** was obtained as colorless needles. The molecular formula, $C_{24}H_{32}O_{10}$, was determined by its high-resolution MS (*m/z* 480.1986) and ^{1}H-, ^{13}C-, and DEPT-NMR spectra. Its IR spectrum showed the presence of a hydroxyl (3420 cm^{-1}) and γ-lactone (1720 cm^{-1}). The IR and UV spectra indicated the presence of an α,β-unsaturated γ-lactone group. In the γ-lactone group, an α-proton at δ_H 5.92 was long-range coupled with a lactonic proton H-5 (δ_H 4.96) and 4-Me (δ_H 2.45) in ^{1}H–^{1}H COSY spectrum. The ^{13}C-NMR spectrum of **3** confirmed the presence of 24 carbons including γ-lactone (δ_C 172.6), δ-lactone (δ_C 167.9), and ester carbonyl (δ_C 176.4). The complete assignments of NMR signals were made by a combination of 2D NMR spectra (HMBC, Fig. 6). In the HMBC spectrum, cross peak observed between C-11 at δ_C 211.2 and H-20 at δ_H 4.17, 4.53 confirmed the partial structure of the ether linkage at C-11 to C-20. The observation of long range correlation between 10-Me/C-5, H-6/C-5 confirmed partial structure of a γ-lactone moiety. The position of the ester side chain was confirmed by long-range correlations between H-15 (δ_H 6.27) and C-1′ (δ_C 176.4).

These spectral data suggested that 3 possesses 1,2-seco-1-nor-6(5→10)*abeo*-picrasan-2,5-olide skeleton.[25] The relative configuration was established by NOE correlations, as shown in Fig. 7. In addition, the relative stereochemistry of **3** was confirmed by a single crystal X-ray diffraction analysis. An ORTEP drawing of the final X-ray model of **3** is given in Fig. 8.

The antitumor activity of each new quassinoid was tested in a short-term *in vitro* assay of 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced Epstein-Barr virus early antigen (EBV-EA) activation in Raji cells. Their inhibitory effects on the activation of the virus genome, and the viability of the Raji cells, are shown in Table 3. All of the compounds tested

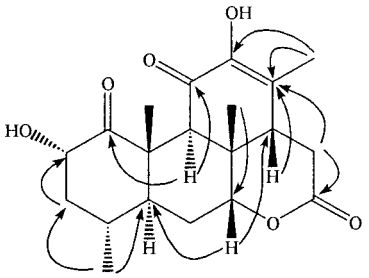

Fig. 4. HMBC Correlations of **2**

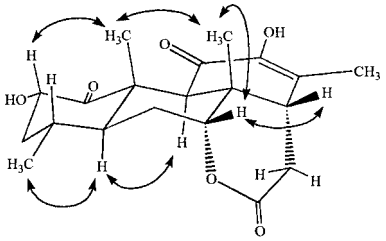

Fig. 5. NOE Correlations of **2**

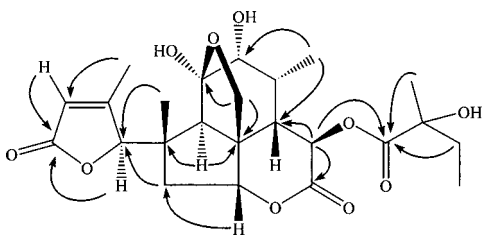

Fig. 6. HMBC Correlations of **3**

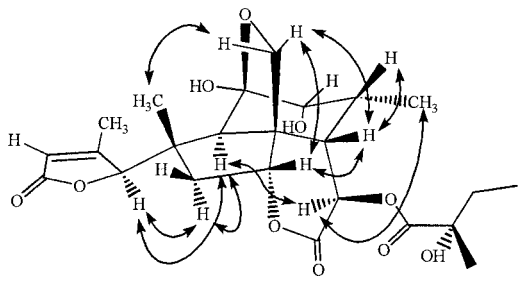

Fig. 7. NOE Correlations of **3**

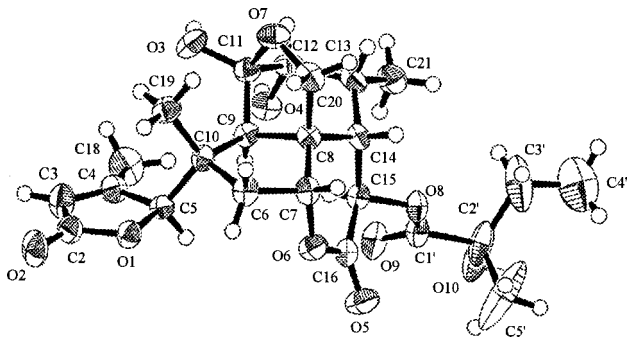

Fig. 8. ORTEP Drawing of Compound **3**

Table 3. Inhibitory Effects of Quassinoids against TPA Induced EBV-EA Activation

Compound	% EBV-EA positive cells				IC_{50}[c]
	Compound concentration (mol ratio/32 pmol TPA)				
	1000	500	100	10	
Ailantinol E (**1**)	9.1±0.9[a] (80)[b]	35.8±2.0	77.3±1.1	98.5±0.3	221
Ailantinol F (**2**)	7.4±0.8 (80)	33.4±1.5	74.0±1.8	95.7±0.4	180
Ailantinol G (**3**)	15.7±1.1 (60)	39.7±1.7	81.4±1.9	100±0.3	285

a) Values represent percentages relative to the positive control value (100%) (n=3, and ±S.D.). *b*) Values in parentheses are the viability of Raji cells. *c*) The IC_{50} values were calculated by probit-transformation technique.

Table 4. Screenig for Inhibitor against NOR1 Action

Treated compound	Concentration (nmol)	Ratio of inhibitory
Positive control NOR1	350	1.0
Ailantinol E (**1**)	350	2.9
Ailantinol F (**2**)	350	3.0
Ailantinol G (**3**)	350	1.7
Carboxy-PIT0	350	8.0
	35	2.7
α-Tocopherol	350	3.4
Trolox	350	4.2
Ascorbic acid	350	5.0
Curcumin	350	2.1
Quercetin	350	2.9
EGCG	350	3.1
Glycyrrhizic acid	350	2.2
Betanin	350	1.2

Cells viability of all samples show the more than 70%.

showed inhibitory effects on EBV activation even at a 1×10^2 mol ratio, and only weak cytotoxicity against Raji cells was observed for all compounds, even at a 1×10^3 mol ratio.

The screening for inhibitors against NOR1; {(±)-(*E*)-4-methyl-2-[(*E*)-hydroxyimino]-5-nitro-6-methoxy-3-hexenamid} action was also conducted using the new quassinoids and some standard samples. The ratios of inhibitory potency are shown in Table 4. Ailantinol E (**1**) and F (**2**) showed the same level of activity as quercetin and epigallocatechingallate (EGCG), which are known to have antioxidant properties.

Experimental

General Experimental Procedures Melting points were determined on a MRK air-bath-type melting point apparatus and are uncorrected. IR spectra were recorded on a JASCO IR-810 spectrophotometer and UV spectra were obtained on a Hitachi 320-S or Shimadzu UV 3101 PC spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were determined on a JEOL JNM-A400 in C_5D_5N or $CDCl_3$ using tetramethylsilane (TMS) as an internal standard. Mass spectra (MS) were recorded on a Hitachi M-80 instrument. Precoated silica gel plates (Merck, $60F_{254}$) 0.25 mm thick were used for analytical TLC and plates 1 mm thick were used for preparative TLC. Components on TLC were detected using a UV lamp (254 and 365 nm). Analytical HPLC was performed on a Tosoh liquid chromatograph equipped with a UV detector set at 254 nm and reverse-phase column (TSK-gel ODS-80Ts) using a mixed solvent of MeOH/H_2O. Preparative HPLC was carried out on a Tosoh liquid chromatograph equipped with a reverse-phase column (Lichrosorb RP-18) at 254 nm using the same solvent as used for analytical HPLC.

Plant Material In 1985, the aerial parts of *A. altissima* (330 kg) were collected in Taiwan and identified by Professor K. Kondo, Laboratory of Plant Chromosome and Gene Stock, Faculty of Science, Hiroshima University. A voucher specimen has been deposited in the Faculty of Integrated Arts and Science, Hiroshima University.

Extraction and Isolation The aerial parts of *A. altissima* were extracted with MeOH. The MeOH extract (24 kg) was obtained by evaporation of the solvent. Part of the MeOH extract (2.6 kg) was dissolved in MeOH–H_2O (2 : 1) and then extracted with *n*-hexane to give an *n*-hexane extract (716 g). Then the MeOH–H_2O layer was extracted with $CHCl_3$ and *n*-BuOH successively to give a $CHCl_3$ extract (514 g), an *n*-BuOH extract (216 g), and finally an H_2O-soluble residue (624 g). Si gel column chromatography of the $CHCl_3$ extract eluted with AcOEt–Et_2O (1 : 1) (54 l), gave 53 fractions (frs. A1—A53, total 123 g), that with $CHCl_3$–MeOH–H_2O (50 : 14 : 3, lower phase) (90 l) gave 90 fractions (frs. C1—C90, total 219 g), and that with MeOH (13 l) gave eight fractions (frs. M1—M8, total 14 g). Each fraction was checked by analytical TLC and HPLC. Frs. A30—39 were combined (3.7 g) and then subjected to column chromatography on Sephadex LH-20 to give 11 fractions. Fraction 5 (650 mg) was further purified with preparative HPLC (MeOH–H_2O, 3 : 7) to provide two new quassinoids, ailantinol F (**2**, 9.0 mg, 0.000025%), and ailantinol G (**3**, 15 mg, 0.000042%). Frs. A40—53 were combined (2.8 g) and purified in the same way as frs. A30—39 to give a new quassinoid, ailantinol E (**1**, 8.6 mg, 0.000024%). Six known quassinoids, chapparin, amarolide-11-acetate, shinjulactone A, shinjulactone C, shinjulactone H, and shinjulactone L were also obtained from the $CHCl_3$ extract, each weight and yield were 6.0 mg (0.000017%), 18.0 mg (0.00005%), 4.5 mg (0.000013%), 31.0 mg (0.000087%), 30.0 mg (0.000084%), and 6.8 mg (0.000019%) respectively.

Ailantinol E (**1**): Colorless amorphous powder; mp 144—146 °C; $[\alpha]_D^{25}$ −166° (*c*=0.14, MeOH); UV λ_{max} (MeOH) nm (ε): 250 (6200); IR (KBr) cm^{-1}: 3400 (OH), 1760 (C=O), and 1650 (C=O); EI-MS *m/z*: 388 (M^+); HR-EI-MS *m/z*: 388.1521 (Calcd for $C_{21}H_{24}O_7$: 388.1520); ^{1}H-NMR (C_5D_5N) δ: 1.14 (3H, s, 10-Me), 1.30 (3H, s, 13-Me), 2.00 (3H, br s, 4-Me), 2.38 (1H, dd, *J*=18, 14 Hz, H-6α), 2.53 (1H, dd, *J*=14, 10 Hz, H-6β), 2.78 (1H, m, H-14), 3.03 (1H, s, H-9), 3.04 (1H, dd, *J*=18, 8 Hz, H-15α), 3.50 (3H, s, -OMe), 3.81 (1H, dd, *J*=18, 3 Hz, H-15β), 4.17 (1H, d, *J*=10 Hz, H-20), 4.53 (1H, d, *J*=10 Hz, H-20′), 4.75 (1H, t, *J*=9 Hz, H-7), 6.45 (1H, br s, H-3); ^{13}C-NMR (C_5D_5N) δ: 13.8 (q, C-19), 14.1 (q, C-21), 22.6 (q, C-18), 31.4 (t, C-6), 33.1 (t, C-15), 40.9 (d, C-14), 49.5 (s, C-8), 51.5 (q, -OMe), 52.2 (s, C-5), 54.1 (d, C-9), 55.1 (s, C-13), 55.6 (s, C-10), 61.1 (t, C-20), 65.6 (d, C-7), 88.4 (s, C-1), 94.5 (s, C-12), 127.2 (d, C-3), 167.5 (s, C-4), 174.8 (s, C-16), 195.9 (s, C-2), 211.2 (s, C-11).

Ailantinol F (**2**): Colorless amorphous powder; mp 93—95 °C; $[\alpha]_D^{25}$ +23.3° (*c*=0.06, MeOH); UV λ_{max} (MeOH) nm (ε): 276 (11100); IR (KBr) cm^{-1}: 3400 (OH), 1720 (C=O), and 1680 (C−O); EI-MS *m/z*: 362 (M^+); HR-EI-MS *m/z*: 362.1730 (Calcd for $C_{20}H_{26}O_6$: 362.1727); ^{1}H-NMR ($CDCl_3$) δ: 0.94 (3H, d, *J*=7 Hz, 4-Me), 1.13 (1H, m, H-3α), 1.23 (3H, s, 8-Me), 1.44 (3H, s, 10-Me), 1.48 (1H, m, H-5), 1.85 (1H, m, H-6β), 1.91 (3H, s, 13-Me), 2.00—2.07 (2H, m, H-4, H-6α), 2.42 (1H, dd, *J*=12, 6 Hz, H-14), 2.50 (1H, ddd, *J*=12, 8, 4 Hz, H-3β), 2.61 (1H, dd, *J*=18, 12 Hz, H-15α), 3.01 (1H, dd, *J*=18, 6 Hz, H-15β), 3.35 (1H, s, H-9), 4.29 (1H, t, *J*=3 Hz, H-7), 4.86 (1H, dd, *J*=12, 8 Hz, H-2); ^{13}C-NMR ($CDCl_3$) δ: 14.8 (q, C-19), 15.2 (q, C-21), 18.3 (q, C-18), 23.2 (q, C-20), 26.1 (t, C-6), 28.2 (d, C-4), 31.5 (t, C-15), 37.1 (s, C-8), 45.6 (d, C-9), 46.9 (d, C-14), 47.0 (d, C-5), 47.5 (t, C-3), 48.0 (s, C-10), 69.8 (d, C-2), 81.9 (d, C-7), 125.2 (s, C-13), 142.9 (s, C-12), 169.1 (s, C-16), 191.0 (s, C-11), 213.2 (s, C-1).

Ailantinol G (**3**): Colorless needles; mp 230—232 °C (decomp.); $[\alpha]_D^{25}$ +80.0° (*c*=0.12, MeOH); UV λ_{max} (MeOH) nm (ε): 213 (14400); IR (KBr) cm^{-1}: 3420 (OH), 1720 (C=O); EI-MS *m/z*: 480 (M^+); HR-EI-MS *m/z*: 480.1986 (Calcd for $C_{24}H_{32}O_{10}$: 480.1993); ^{1}H-NMR (C_5D_5N) δ: 1.23 (3H, t, *J*=7 Hz, 3′-Me), 1.38 (3H, d, *J*=7 Hz, 13-Me), 1.51 (3H, s, 10-Me), 1.71 (3H, s, 2′-Me), 2.03 (1H, m, H-3′), 2.15 (1H, m, H-3′), 2.30 (1H, dd, *J*=16, 4 Hz, H-6α), 2.45 (3H, s, 4-Me), 2.53 (1H, m, H-13), 2.75 (1H, dd, *J*=10, 6 Hz, H-14), 2.96 (1H, d, *J*=16 Hz, H-6β), 3.39 (1H, s, H-9), 3.89 (1H, d, *J*=9 Hz, H-20), 3.97 (1H, d, *J*=9 Hz, H-20′), 4.02 (1H, t, *J*=4 Hz, H-12), 4.85 (1H, d, *J*=4 Hz, H-7), 4.96 (1H, s, H-5), 5.92 (1H, d, *J*=2 Hz, H-3), 6.27 (1H, d, *J*=10 Hz, H-15); ^{13}C-NMR (C_5D_5N) δ: 8.4 (q, C-4′), 14.9 (q, C-21), 16.0 (q, C-18), 18.3 (q, C-19), 25.8 (q, C-5′), 33.8 (t, C-3′), 34.1 (d, C-13), 43.4 (d, C-14), 45.3 (d, C-9), 45.7 (t, C-6), 46.2 (s, C-10), 58.2 (s, C-8), 70.8 (d, C-15), 72.1 (t, C-20), 75.2 (s, C-2′), 80.7 (d, C-12), 83.7 (d, C-7), 92.1 (d, C-5), 111.2 (s, C-11), 119.0 (d, C-3), 167.9 (s, C-16), 169.9 (s, C-4), 172.6 (s, C-2), 176.4 (s, C-1′).

EBV-EA Activation The inhibition of EBV-EA activation was assayed using a methods previously reported.[26] The cells were incubated at 37 °C for 48 h in a medium containing *n*-butyric acid (4 mM), TPA (32 pM), and various amounts of test compounds. Smears were made from the cell suspensions and the EBV-EA inducing cells were stained by means of an indirect immunofluorescence technique.[27]

In each assay, at least 500 cells were counted, and the number of stained cells (positive cells) was recorded. Triplicate assays were performed for each data point. The EBV-EA inhibitory activity of the test compound was compared with that of the control experiment with butyric acid plus TPA. In control experiments, the EBV-EA activities were generally around 40%, and these values were taken as a positive control. The viability of the cells was assayed by the trypan-blue staining method. For the determination of cytotoxicity, the cell viability was required to be more than 60%.[28]

Screening for Inhibitors of NOR1 Activity Cultured Chang Liver cells 5×10^5/ml derived from human liver were cultured in Eagle's minimum essential medium. NOR1 was added to the culture and incubated for 1 h in a CO_2 incubator as a control. For the screening assay, the scavengers or naturally occurring compounds were added to the culture dish 1 min before the addition of NOR1. The ratio of inhibitory potency was calculated as: % transformed cells (NOR1 alone)/% transformed cells (NOR1 alone+screening sample).

Crystal Data of 3. Data Collection A colorless prismatic crystal of

$C_{24}H_{32}O_{10}$ having approximate dimensions of 0.10×0.10×0.12 mm was mounted on a glass fiber. All measurements were made on a RIGAKU AFC-7R diffractometer with graphite monochromated Mo$K\alpha$ radiation. Cell constants and an orientation matrix for data collection, obtained from a least-squares refinement using the setting angles of 25 carefully centered reflections in the range $30.07<2\theta<4.98°$, corresponded to a primitive monoclinic cell with dimensions: $a=14.630(2)$ Å, $b=6.994(2)$ Å, $\beta=105.63(2)°$, $c=11.759(3)$ Å, $V=1158.6(5)$ Å^3. For $Z=2$ and F.W.=480.51, the calculated density is 1.38 g/cm^3. Based on the systematic absences of: $0k0$: $k\pm2n$ packing considerations, a statistical analysis of intensity distribution, and the successful solution and refinement of the structure, the space group was determined to be: $P2_1$ (#4). The data were collected at a temperature of 25±1 °C using the ω–2θ scan technique to a maximum 2θ value of 55.0°. Omega scans of several intense reflections, made prior to data collection, had an average width at half-height of 0.25° with a take-off angle of 6.0°. Scans of $(1.47+0.30\tan\theta)°$ were made at speeds ranging from 8.0 to 16.0°/min (in ω). The weak reflections ($I<10.0\sigma(I)$) were rescanned (maximum of seven scans) and the counts were accumulated to ensure good counting statistics.

Data Reduction Of the 2976 reflections that were collected, 2865 were unique ($R_{int}=0.000$); equivalent reflections were merged. The intensities of three representative reflections were measured after every 150 reflections. No decay correction was applied. The linear absorption coefficient, μ, for Mo$K\alpha$ radiation is 1.1 cm^{-1}. The data were corrected for Lorentz and polarization effects. A correction for secondary extinction[29] was applied (coefficient=67.139000).

Structure Solution and Refinement The structure was solved by direct methods[30] and expanded using Fourier techniques.[31] The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were refined using the riding model. The final cycle of full-matrix least-squares refinement[32] on F was based on 1877 observed reflections ($I>3.00\sigma(I)$) and 340 variable parameters and converged (largest parameter shift was 0.00 times its esd) with unweighted and weighted agreement factors of: $R=\Sigma||F_o|-|F_c||/\Sigma|F_o|=0.046$, $R_w=[\Sigma w(|F_o|-|F_c|)^2/\Sigma wF_o^2]^{1/2}=0.033$.

The standard deviation of an observation of unit weight[33] was 2.04. The weighting scheme was based on counting statistics. Plots of $\Sigma w\,(|F_o|-|F_c|)^2$ versus $|F_o|$, reflection order in data collection, $\sin\theta/\lambda$, and various classes of indices showed no unusual trends. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.47 and -0.26 e$^-$Å^3, respectively. Neutral atom scattering factors were taken from Cromer and Waber.[34] Anomalous dispersion effects were included in Fcalc[35]; the values for $\Delta f'$ and $\Delta f''$ were those of Creagh and McAuley.[36] The values for the mass attenuation coefficients are those of Creagh and Hubbell.[37] All calculations were performed using the CrystalStructure[38,39] crystallographic software package.

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