ent-Kaurane Diterpenoids from *Isodon nervosus*

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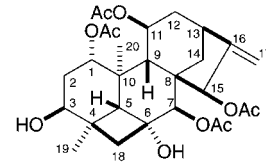
A pentacyclic *ent*-kauranoid, nervonin A (**1**), with an unprecedented cyclobutane moiety in the structure, and nine other new *ent*-kaurane diterpenoids, nervonins B−J (**2**–**10**), along with 10 known ones (**11**–**20**), were isolated from *Isodon nervosus*. Their structures were elucidated by detailed spectroscopic analysis. All diterpenoids were assayed for their cytotoxicity against K562, A549, and HepG2 human cell lines. Compounds **2**, **11**, **16**, **17**, and **20** showed significant cytotoxicity.

ent-Kauranoids are the major secondary metabolites isolated from *Isodon* (Labiatae) plants. These compounds have been demonstrated to exhibit antibacterial, antitumor, anti-inflammatory, and antifeeding activities.[1] The diverse structures and biological activities have prompted numerous studies of these diterpenoids.[2–6]

Isodon nervosus (Hemsl.) Kudo (Labiatae), a perennial herb, is distributed mainly in the south of China. Its stems and leaves have been used to treat hepatitis, fester, and eczema in traditional Chinese medicine.[7] Previous phytochemical investigations on *I. nervosus* collected from Jiangxi, Anhui, and Gansu Provinces led to the discovery of a series of *ent*-kauranoids.[8–20] The secondary metabolites of the genus *Isodon* often differ when grown in different ecological environments.[21–25] Thus, we explored this plant indigenous to Xichang City of Sichuan Province, looking for structurally unique and bioactive *ent*-kauranoids. As a result, a pentacyclic *ent*-kauranoid (**1**) with an unprecedented cyclobutane moiety in the structure and nine other new (**2**–**10**) and 10 known *ent*-kaurane diterpenoids (**11**–**20**) were isolated. Their structures were elucidated by spectroscopic methods and by comparison with reported data. Furthermore, we have carried out the cytotoxic evaluation of diterpenoids **1**–**20** against K562, A549, and HepG2 human cell lines.



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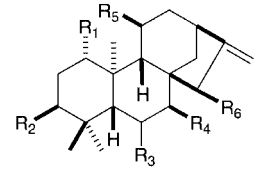

	R_1	R_2	R_3	R_4	R_5	R_6
2	OH	OH	α-OAc	OAc	OAc	=O
3	OH	OH	=O	OAc	OAc	OAc
4	OAc	OH	α-OH	OAc	OAc	OAc
5	OAc	OH	=O	OH	OAc	OH
6	OAc	OH	α-OH	OAc	OH	OH
7	OAc	OH	α-OAc	OAc	OH	OH
8	OH	OH	H	OAc	OAc	OH
9	OH	OH	α-OH	OAc	OAc	OH
10	OH	OH	=O	H	OH	OAc
11	OAc	OH	α-OAc	OAc	OAc	=O
12	OAc	OH	=O	OAc	OAc	OAc
13	OAc	OH	=O	OH	OAc	OAc
14	OH	OAc	α-OH	OAc	OH	OH
15	OH	OAc	H	OAc	OH	OH
16	OAc	OH	α-OH	OAc	OAc	=O
17	OAc	OH	=O	OAc	OAc	=O
18	OAc	OH	β-OH	OAc	OAc	OH
19	OAc	OH	=O	OAc	OAc	OH
20	OH	OH	=O	OAc	OAc	=O

Results and Discussion

Repeated chromatography of the acetone extract of the aerial parts of *I. nervosus* yielded 10 new (**1**–**10**) and 10 known (**11**–**20**) *ent*-kaurane diterpenoids.

Compound **1** was obtained as a white powder. The molecular formula $C_{28}H_{38}O_{10}$, indicating 10 degrees of unsaturation, was determined by the quasi-molecular ion peak at *m/z* 557.2354 [M + Na]$^+$ in the HRESIMS. IR absorption bands at 3443, 1735, 1704, and 1629 cm^{-1} indicated the presence of OH, ester, and double-bond groups. The ^{1}H, ^{13}C NMR, and DEPT spectra displayed signals of two singlet methyl, four methylene, eight methine (five oxygenated), and four quaternary carbons (one oxygenated), one exocyclic double bond, and four acetyl groups. Comparison of the ^{1}H and ^{13}C NMR data of **1** with those of compound **12**[18] showed that they had similar substructures in rings A−D. However, a singlet methyl group and one ketone group in **12** were replaced by a methylene group and an oxygenated quaternary carbon in **1**. Considering the apparent degrees of unsaturation, compound **1** possessed one more ring than **12**. In the HMBC spectrum, H_2-18 correlated to C-3, -4, -6, and -19, and H-5 correlated to C-3, -6, and -18, revealing the presence of a four-membered carbon ring between C-6 and C-18. ROESY correlations of H-5β to H-1β, H-9β, and H-18β, and H-3α to H_3-19 indicated β-orientation of the cyclobutane ring. This conclusion was supported by the selective NOE correlation between H_3-19 and H_3-20. The other substituents

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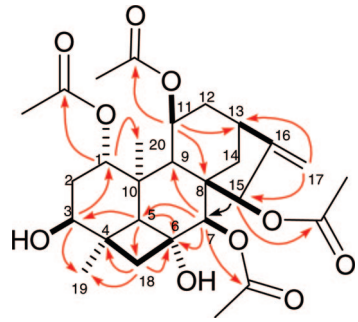

Figure 1. Key HMBC correlations of **1**.

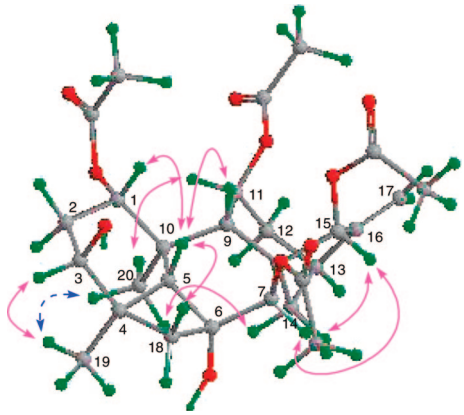

Figure 2. Key NOE (dashed) and ROESY correlations of **1**.

had the same locations and orientations as those in **12**, as elucidated by HMBC and ROESY experiments. (Figures 1 and 2). Thus, compound **1** was determined to be 3β,6α-dihydroxy-1α,7β,11β,15β-tetraacetoxy-6,18-cyclo-*ent*-kaur-16-ene, which represents an unprecedented 6,18-cyclo-*ent*-kaurane pentacyclic skeleton. From a biogenetic point of view, compound **12** could possibly be transformed into compound **1** via a free radical coupling reaction (see Supporting Information).

Compounds **2** and **3** possessed the same molecular formula, $C_{26}H_{36}O_9$ (HRESIMS). Detailed studies of their IR, UV, and 1D and 2D NMR spectra indicated that they were the 1-*O*-deacetyl derivatives of **11**[26] and **12**, respectively. Similarly, compound **5** is the 15-*O*-deacetyl derivative of **13**.[27] Thus, compounds **2**, **3**, and **5** were identified as 1α,3β-dihydroxy-6α,7β,11β-triacetoxy-*ent*-kaur-16-en-15-one, 1α,3β-dihydroxy-7β,11β,15β-triacetoxy-*ent*-kaur-16-en-6-one, and 3β,7β,15β-trihydroxy-1α,11β-diacetoxy-*ent*-kaur-16-en-6-one, respectively.

The ^{13}C NMR data of compound **4** were similar to that of **12**, with the main difference between them in ring B. The carbonyl group (δ_C 207.7) in **12** was replaced by an oxygenated methine (δ_C 68.5) in **4**. In the HMBC spectrum, the proton at δ_H 4.04 correlating to C-7 (δ_C 82.0) and C-10 (δ_C 42.1) confirmed the oxygenated methine assignable to C-6 in **4**. The α-orientation of OH-6 was established by ROESY cross-peaks from H-6β to H-5β and H_3-18. Consequently, compound **4** was elucidated as 3β,6α-dihydroxy-1α,7β,11β,15β-tetracetoxy-*ent*-kaur-16-ene.

Compound **6** ($C_{24}H_{36}O_8$) was presumed to be an isomer of **14**[28] differing in the position of one of the acetyl groups. The signal due to C-1 at δ_H 4.42 (δ_C 78.0) in **14** was shifted downfield to δ_H 5.26 (δ_C 81.2) in **6**, and the signal due to C-3 at δ_H 5.00 (δ_C 80.1) in **14** was shifted upfield to δ_H 3.49 (δ_C 76.4) in **6**. This information implied the presence of an acetoxy group at the C-1α position and a β-orientated OH group at C-3 in **6**. Thus, compound **6** was identified as 3β,6α,11β,15β-tetrahydroxy-1α,7β-diacetoxy-*ent*-kaur-16-ene.

Compound **7** was determined to be the 6-*O*-acetyl derivative of **6** according to the following information. One more acetyl group was present in the NMR and MS spectra of **7**. The signal of C-6 at δ_H 4.04 (δ_C 69.1) in **6** was shifted downfield to δ_H 5.15 (δ_C 70.4) in **7**, and H-6 showed correlation to one of the acetyl groups in the HMBC spectrum. Compound **7** was assigned the structure 3β,11β,15β-trihydroxy-1α,6α,7β-triacetoxy-*ent*-kaur-16-ene.

In the same way as **6** and **14**, compound **8** was presumed to be an isomer of **15**.[27] The 3β-acetoxy and 11β-OH groups in **15** were replaced by 3β-OH and 11β-acetoxy groups in **8**, which was confirmed by the HMBC correlation between H-11 (δ_H 6.98) and one of the acetyl groups. Thus, compound **8** was determined to be 1α,3β,15β-trihydroxy-7β,11β-diacetoxy-*ent*-kaur-16-ene.

Compound **9** exhibited the molecular formula $C_{24}H_{36}O_8$ as determined by HRESIMS. The ^{1}H and ^{13}C NMR data showed that **9** was related to **8**, with the most notable differences being the disappearance of one methylene and the presence of an additional oxygenated methine. Thus, one more OH group was present in compound **9**. In the HMBC spectrum, the proton at δ_H 3.98 correlating to C-5, C-7, and C-8 indicated that C-6 was substituted by the OH group. The OH was established as α-oriented from ROESY correlations from H-6β to H-5β and H_3-18. Therefore, compound **9** was 1α,3β,6α,15β-tetrahydroxy-7β,11β-diacetoxy-*ent*-kaur-16-ene.

The molecular formula $C_{22}H_{32}O_6$ was assigned to compound **10** by positive HRESIMS, indicating seven degrees of unsaturation. The NMR data of **10** suggested it to be a C-20 nonoxygenated *ent*-kaurane diterpenoid, substituted by one carbonyl group, one acetoxy group, and three OH groups. Analysis of the 2D NMR spectra of **10** enabled placement of the carbonyl group and the acetoxy group at C-6 and C-15β, respectively, and attachment of the three OH groups to C-1α, C-3β, and C-11β, respectively. Consequently, compound **10** was characterized as 1α,3β,11β-trihydroxy-15β-acetoxy-*ent*-kaur-16-en-6-one.

The known *ent*-kaurane diterpenoids, calcicolin A (**11**),[26] nervosanin (**12**),[18] adenanthin K (**13**),[27] forrestin B (**14**),[28] adenanthin E (**15**),[27] weisiensin A (**16**),[29] adenanthin (**17**),[30] forrestin C (**18**),[28] adenanthin J (**19**),[27] and calcicolin B (**20**),[31] were identified by comparing their spectroscopic data with those reported in the literature.

Cytotoxic activity of diterpenoids **1**−**20** was evaluated against K562, A549, and HepG2 human cell lines using the method described in the literature.[32] Compounds **2** and **16** were cytotoxic for all the test cell lines. Compounds **11**, **17**, and **20** showed selective cytotoxicity against K562 and HepG2 cell lines (Table 2). The other diterpenoids were noncytotoxic in these test systems (IC_{50} > 100 μM). The results were consistent with the previous conclusion that the cyclopentanone conjugated with an exomethylene group was indispensable for the cytotoxicity of *ent*-kaurane diterpenoids.[33]

Experimental Section

General Experimental Procedures. Melting points were obtained on an XRC-1 micro melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. UV spectra were carried out on a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used for scanning IR spectroscopy. NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometers with TMS as internal standard. Electrospray-ionization (ESIMS) and high-resolution electrospray-ionization (HRESIMS) mass spectra were acquired on a VG Autospec-3000 mass spectrometer. Semipreparative HPLC was performed on an Agilent 1100 apparatus equipped with a diode-array detector and a Zorbax SB-C_{18} (Agilent, 9.4 mm × 25 cm) column. Column chromatography was performed on silica gel (200–300 mesh, Qingdao Marine Chemical Factory, Qingdao, People's Republic of China), Sephadex LH-20 (General Electric Company, Fairfield, CT), Lichroprep RP-18 gel (40–63 μm, Merck, Darmstadt, Germany), and MCI gel (75–150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan).

Table 1. ^{13}C NMR Data of Compounds **1**–**10** (δ in ppm)

no.	1	2	3	4	5	6	7	8	9	10
1	80.6 d	75.9 d	75.1 d	80.0 d	78.5 d	81.2 d	81.0 d	76.1 d	76.3 d	74.8 d
2	34.5 t	34.8 t	34.9 t	32.1 t	32.2 t	32.4 t	32.2 t	38.1 t	35.8 t	35.7 t
3	74.8 d	76.7 d	76.2 d	76.6 d	76.0 d	76.4 d	76.0 d	75.8 d	77.3 d	76.7 d
4	43.0 s	37.7 s	36.2 s	37.8 s	36.3 s	37.9 s	37.7 s	37.7 s	37.9 s	36.8 s
5	39.4 d	40.0 d	49.6 d	41.1 d	48.7 d	41.2 d	40.3 d	39.3 d	41.1 d	57.4 d
6	78.2 s	70.4 d	207.2 s	68.5 d	211.0 s	69.1 d	70.4 d	25.1 t	69.8 d	210.2 s
7	84.0 d	70.3 d	86.7 d	82.0 d	86.0 d	81.6 d	76.3 d	79.3 d	81.3 d	52.8 t
8	48.8 s	48.2 s	51.1 s	44.6 s	51.3 s	45.3 s	45.7 s	48.1 s	45.6 s	47.9 s
9	50.6 d	56.2 d	50.2 d	49.7 d	48.5 d	52.0 d	51.4 d	50.9 d	50.2 d	57.1 d
10	38.5 s	43.8 s	50.2 s	42.1 s	48.3 s	42.0 s	42.4 s	43.9 s	43.2 s	47.8 s
11	69.5 d	71.1 d	70.2 d	69.2 d	69.7 d	66.5 d	66.3 d	72.1 d	71.7 d	65.8 d
12	38.6 t	37.7 t	38.7 t	39.5 t	39.4 t	42.5 t	42.1 t	39.7 t	39.3 t	42.8 t
13	39.0 d	36.4 d	37.8 d	38.5 d	37.9 d	38.9 d	38.7 d	38.8 d	38.5 d	37.7 d
14	37.1 t	35.7 t	33.9 t	36.6 t	32.9 t	36.2 t	35.5 t	35.5 t	35.5 t	36.5 t
15	80.2 d	205.0 s	79.2 d	79.8 d	82.6 d	81.3 d	80.7 d	81.5 d	81.7 d	82.1 d
16	151.4 s	149.3 s	150.1 s	151.3 s	154.9 s	156.0 s	155.7 s	158.8 s	156.0 s	152.4 s
17	107.0 t	113.4 t	107.9 t	106.1 t	106.8 t	106.9 t	106.9 t	105.4 t	106.3 t	109.6 t
18	47.3 t	28.3 q	26.4 q	28.5 q	26.1 q	28.6 q	28.5 q	29.0 q	28.6 q	26.8 q
19	21.5 q	23.6 q	22.6 q	24.2 q	22.4 q	24.4 q	23.6 q	22.4 q	24.4 q	22.0 q
20	12.2 q	14.3 q	13.4 q	14.9 q	14.7 q	14.7 q	14.6 q	13.9 q	14.0 q	13.7 q
Ac	171.0 s	172.1 s	172.3 s	171.0 s	170.4 s	171.2 s	171.1 s	170.8 s	170.8 s	170.8 s
	170.6 s	169.6 s	170.0 s	170.8 s	168.3 s	171.1 s	170.1 s	169.1 s	170.5 s	21.3 q
	170.4 s	169.1 s	169.4 s	170.3 s	21.6 q	21.7 q	169.2 s	21.7 q	21.7 q	
	169.8 s	21.5 q	21.6 q	170.0 s	21.4 q	21.4 q	21.7 q	21.6 q	21.4 q	
	21.7 q	21.4 q	21.4 q	21.8 q			21.4 q			
	21.6 q	21.3 q	20.4 q	21.6 q			21.3 q			
	21.4 q			21.4 q						
	20.9 q			21.1 q						

Plant Material. The aerial parts of *Isodon nervosus* were collected in Xichang City of Sichuan Province, People's Republic of China, in August 2005. The sample was identified by Prof. Xi-Wen Li, and a voucher specimen (KIB 050810304) has been deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The air-dried, milled plant material (1.5 kg) was soaked with acetone (3 × 4 L, each 3 days) at room temperature and filtered. The filtrate was evaporated in vacuo to afford a residue, which was dissolved in H_2O (2 L), and then extracted with petroleum ether (2 × 2 L) and ethyl acetate (4 × 2 L), sequentially. The EtOAc extract (38.0 g) was decolorized using MCI gel CHP-20 and eluted with 90% MeOH−H_2O to yield a yellow gum (27.5 g). The gum was separated on a silica gel column, eluted with $CHCl_3$−Me_2CO (1:0 → 0:1, gradient system), to yield six fractions, A−F. Fraction B (10.7 g) was separated into three subfractions, B1−B3, by a silica gel column eluted with petroleum ether−isopropyl alcohol (30:1, 20:1, 10:1). Compounds **11** (1.3 g) and **17** (2.0 g) were crystallized from subfractions B3 and B1, respectively. Subfraction B2 (3.7 g) was purified by a silica gel column, eluted with petroleum ether−EtOAc (2:1, 1:1), to give compound **12** (1.2 g). Compound **16** (1.6 g) was crystallized from fraction C (2.7 g). Then, the mother liquid of **16** and fractions D (0.2 g), E (1.0 g), and F (1.4 g) were subjected to repeated column chromatography (including silica gel, Sephadex LH-20, RP-18, and semipreparative HPLC) to afford compounds **1** (1.8 mg), **2** (6.0 mg), **3** (25.2 mg), **4** (17.5 mg), **5** (3.2 mg), **6** (24.7 mg), **7** (31.8 mg), **8** (5.1 mg), **9** (7.2 mg), **10** (3.9 mg), **13** (130.8 mg), **14** (11.7 mg), **15** (5.0 mg), **18** (24.1 mg), **19** (13.6 mg), and **20** (13.2 mg).

Nervonin A (1): white powder; $[\alpha]^{27}_D$ −21.7 (*c* 0.64, MeOH); UV (MeOH) λ_{max}(log ϵ) 205 (3.82) nm; IR (KBr) ν_{max} 3443, 2980, 2935, 1735, 1704, 1629 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.54 (1H, br s, H-15α), 5.01 (1H, overlap, H-11α), 5.00 (1H, overlap, H-1β), 4.95 (1H, br s, H-17a), 4.82 (1H, br s, H-17b), 4.31 (1H, s, H-7α), 3.70 (1H, br s, H-3α), 2.67 (1H, br s, H-13α), 2.44 (1H, d, J = 13.0 Hz, H-14α), 2.41 (1H, br s, H-9β), 2.30 (1H, br s, H-5β), 2.10 (1H, d, J = 10.6 Hz, H-18β), 2.01 (1H, overlap, H-12β), 1.94 (1H, m, H-2β), 1.86 (1H, overlap, H-2α), 1.84 (1H, m, H-12α), 1.56 (3H, s, Me-19), 1.49 (1H, br d, J = 10.6 Hz, H-18α), 1.46 (1H, m, H-14β), 1.31 (3H, s, Me-20), 2.17, 2.04, 2.01, and 1.91 (each 3H, s, 4 × OAc); ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; positive ESIMS *m/z* 557 [M + Na]$^+$, 1091 [2M + Na]$^+$; positive HRESIMS [M + Na]$^+$ *m/z* 557.2354 (calcd for $C_{28}H_{38}O_{10}Na$ [M + Na]$^+$, 557.2362).

Nervonin B (2): white powder; $[\alpha]^{16}_D$ –42.9 (*c* 0.07, MeOH); UV (MeOH) λ_{max}(log ϵ) 238 (3.87) nm; IR (KBr) ν_{max} 3577, 3441, 2979, 2952, 2936, 2889, 1747, 1728, 1705, 1647 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.88 (1H, d, J = 4.1 Hz, H-11α), 5.82 (1H, br s, H-17a), 5.24 (1H, br s, H-17b), 5.05 (2H, overlap, H-6β, 7α), 3.99 (1H, br d, J = 11.8 Hz, H-1β), 3.49 (1H, br s, H-3α), 3.08 (1H, d, J = 3.3 Hz, H-13α), 2.71 (1H, d, J = 12.3 Hz, H-14α), 2.30 (1H, m, H-12α), 2.12 (1H, overlap, H-2α), 2.08 (2H, overlap, H-5β, 9β), 1.94 (1H, m, H-12β), 1.81 (1H, m, H-2β), 1.77 (3H, s, Me-20), 1.64 (1H, m, H-14β), 1.01 (3H, s, Me-19), 0.97 (3H, s, Me-18), 2.19, 2.10, and 1.92 (each 3H, s, 3 × OAc); ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; positive ESIMS: *m/z* 515 [M + Na]$^+$, 1007 [2M + Na]$^+$; positive HRESIMS [M + Na]$^+$ *m/z* 515.2244 (calcd for $C_{26}H_{36}O_9Na$ [M + Na]$^+$, 515.2257).

Nervonin C (3): colorless needles; 240–241 °C; $[\alpha]^{16}_D$ +6.7 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ϵ) 204 (3.78) nm; IR (KBr) ν_{max} 3534, 3453, 2950, 2934, 2880, 1732, 1728, 1629 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.69 (1H, d, J = 4.8 Hz, H-11α), 5.57 (1H, s, H-15α), 5.26 (1H, d, J = 6.0 Hz, OH-1α), 5.04 (1H, J = 2.0 Hz, H-17a), 4.87 (1H, J = 2.0 Hz, H-17b), 4.26 (1H, s, H-7α), 4.25 (1H, m, H-1β), 3.37 (1H, br s, H-3α), 3.17 (1H, s, H-5β), 2.73 (1H, d, J = 2.6 Hz, H-13α), 2.54 (1H, br s, H-9β), 2.05 (1H, overlap, H-2α), 2.03 (1H, overlap, H-12β), 1.90 (1H, m, H-12α), 1.82 (1H, m, H-2β), 1.79 (1H, m, H-14α), 1.46 (1H, m, H-14β), 1.27 (3H, s, Me-19), 1.05 (3H, s, Me-20), 0.76 (3H, s, Me-18), 2.17, 2.05 and 1.99 (each 3H, s, 3 × OAc); ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; positive ESIMS *m/z* 515 [M + Na]$^+$, 1007 [2M + Na]$^+$; positive HRESIMS [M + Na]$^+$ *m/z* 515.2263 (calcd for $C_{26}H_{36}O_9Na$ [M + Na]$^+$, 515.2257).

Nervonin D (4): white powder; $[\alpha]^{16}_D$ +2.7 (*c* 0.20, MeOH); UV (MeOH) λ_{max}(log ϵ) 204 (3.73) nm; IR (KBr) ν_{max} 3483, 2963, 2936, 2875, 1735, 1720, 1637 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.58 (1H, d, J = 4.9 Hz, H-11α), 5.49 (1H, br s, H-15α), 5.13 (1H, dd, J = 11.5, 4.3 Hz, H-1β), 4.88 (1H, br s, H-17a), 4.74 (1H, br s, H-17b), 4.64 (1H, d, J = 4.6 Hz, H-7α), 4.04 (1H, br s, H-6β), 3.45 (1H, t, J = 2.7 Hz, H-3α), 2.63 (1H, br s, H-13α), 2.42 (1H, overlap, H-14α),

Table 2. Cytotoxic Activity of Compounds **2**, **11**, **16**, **17**, and **20**[a]

compound	K562	A549	HepG2
2	1.18	1.53	1.16
11	2.39	12.2	0.51
16	4.11	1.88	1.43
17	1.05	8.07	0.32
20	1.55	8.50	0.52
cisplatin	1.14	3.84	1.27

[a] Results are expressed as IC_{50} values in μM.

2.41 (1H, br s, H-9β), 2.02 (1H, overlap, H-2α), 2.01 (1H, overlap, H-12β), 1.88 (1H, overlap, H-12α), 1.87 (1H, overlap, H-2β), 1.73 (1H, br s, H-5β), 1.52 (3H, s, Me-20), 1.43 (1H, m, H-14β), 1.22 (3H, s, Me-19), 0.89 (3H, s, Me-18), 2.16, 2.02, 1.94 and 1.84 (each 3H, s, 4 × OAc); ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; positive ESIMS *m/z* 559 $[M + Na]^+$, 1095 $[2M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 559.2507 (calcd for $C_{28}H_{40}O_{10}Na$ $[M + Na]^+$, 559.2519).

Nervonin E (5): colorless needles; 199–200 °C; $[\alpha]^{16}_D$ 0 (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log ϵ) 206 (3.85) nm; IR (KBr) ν_{max} 3506, 3454, 2987, 2957, 2935, 1737, 1720, 1634 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.59 (1H, d, J = 4.4 Hz, H-11α), 5.45 (1H, dd, J = 11.0, 5.0 Hz, H-1β), 5.17 (1H, br s, H-17a), 5.02 (1H, br s, H-17b), 4.34 (1H, br d, J = 11.9 Hz, H-15α), 4.26 (1H, br s, OH-7β), 4.00 (1H, s, H-5β), 3.66 (1H, br s, H-7α), 3.40 (2H, overlap, H-3α, OH-15β), 2.65 (2H, overlap, H-9β, 13α), 1.93–1.86 (4H, overlap, H_2-2, H_2-12), 1.65 (1H, d, J = 12.5 Hz, H-14α), 1.32 (3H, s, Me-19), 1.29 (1H, overlap, H-14β), 1.17 (3H, s, Me-20), 0.98 (3H, s, Me-18), 2.06 and 1.93 (each 3H, s, 2 × OAc); ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; positive ESIMS *m/z* 473 $[M + Na]^+$, 923 $[2M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 473.2150 (calcd for $C_{24}H_{34}O_8Na$ $[M + Na]^+$, 473.2151).

Nervonin F (6): white powder; $[\alpha]^{16}_D$ +3.7 (*c* 0.33, MeOH); UV (MeOH) λ_{max} (log ϵ) 207 (3.72) nm; IR (KBr) ν_{max} 3434, 2931, 2878, 1715, 1638 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.26 (1H, dd, J = 11.6, 4.0 Hz, H-1β), 5.11 (1H, br s, H-17a), 5.06 (1H, br s, H-17b), 4.68 (1H, d, J = 2.9 Hz, H-7α), 4.54 (1H, br s, H-11α), 4.11 (1H, br s, H-15α), 4.04 (1H, br s, H-6β), 3.49 (1H, br s, H-3α), 2.62 (1H, br s, H-13α), 2.33 (1H, d, J = 13.0 Hz, H-14α), 2.30 (1H, br s, H-9β), 2.09 (1H, overlap, H-2α), 2.08 (1H, overlap, H-12α), 1.85 (3H, overlap, H-2β, 5β, 12β), 1.47 (3H, s, Me-20), 1.33 (1H, br d, J = 13.0 Hz, H-14β), 1.24 (3H, s, Me-19), 0.94 (3H, s, Me-18), 2.15 and 2.06 (each 3H, s, 2 × OAc); ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; positive ESIMS: *m/z* 475 $[M + Na]^+$, 927 $[2M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 475.2311 (calcd for $C_{24}H_{36}O_8Na$ $[M + Na]^+$, 475.2307).

Nervonin G (7): white powder; $[\alpha]^{16}_D$ –12.3 (*c* 0.26, MeOH); UV (MeOH) λ_{max} (log ϵ) 207 (3.78) nm; IR (KBr) ν_{max} 3434, 2977, 2935, 2879, 1742, 1639 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.28 (1H, dd, J = 11.5, 4.2 Hz, H-1β), 5.15 (1H, br s, H-6β), 5.07 (1H, br s, H-17a), 5.03 (1H, br s, H-17b), 4.82 (1H, d, J = 3.3 Hz, H-7α), 4.56 (1H, d, J = 5.1 Hz, H-11α), 4.14 (1H, br s, H-15α), 3.47 (1H, br s, H-3α), 2.61 (1H, d, J = 2.9 Hz, H-13α), 2.34 (1H, br s, H-9β), 2.15 (2H, overlap, H-5β, 14α), 2.06 (1H, overlap, H-12β), 2.04 (1H, overlap, H-2α), 1.86 (2H. overlap, H-2β, 12α), 1.44 (3H, s, Me-20), 1.25 (1H, m, H-14β), 0.99 (3H, s, Me-19), 0.96 (3H, s, Me-18), 2.15, 2.08 and 2.06 (each 3H, s, 3 × OAc); ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; positive ESIMS *m/z* 517 $[M + Na]^+$, 1011 $[2M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 517.2403 (calcd for $C_{26}H_{38}O_9Na$ $[M + Na]^+$, 517.2413).

Nervonin H (8): white powder; $[\alpha]^{16}_D$ –6.6 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ϵ): 206 (3.81) nm; IR (KBr) ν_{max} 3444, 2942, 2875, 1733, 1710, 1636 cm^{-1}; ^{1}H NMR (C_5D_5N, 500 MHz) δ 6.98 (1H, d, J = 4.3 Hz, H-11α), 6.27 (1H, d, J = 5.0 Hz, OH-1α), 6.11 (1H, d, J = 3.7 Hz, OH-3β), 5.27 (1H, br s, H-17a), 5.23 (1H, br s, H-7α), 4.99 (1H, br s, H-17b), 4.64 (1H, dd, J = 10.5, 4.7 Hz, H-1β), 4.48 (1H, d, J = 10.7 Hz, H-15α), 3.77 (1H, br s, H-3α), 3.72 (1H, d, J = 10.7 Hz, OH-15β), 2.70 (1H, br s, H-9β), 2.52 (2H, overlap, H-5β, 13α), 2.37 (1H, m, H-2α), 2.22 (1H, m, H-2β), 2.07–1.98 (2H, m, H_2-12), 1.84 (1H, d, J = 12.5 Hz, H-14α), 1.81–1.68 (2H, m, H_2-6), 1.30 (3H, s, Me-20), 1.21 (1H, dd, J = 12.5, 2.0 Hz, H-14β), 1.14 (3H, s, Me-18), 0.91 (3H, s, Me-19), 2.09 and 1.81 (each 3H, s, 2 × OAc); ^{13}C NMR (C_5D_5N, 125 MHz), see Table 1; positive ESIMS *m/z* 459 $[M + Na]^+$, 475 $[M + K]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 459.2363 (calcd for $C_{24}H_{36}O_7Na$ $[M + Na]^+$, 459.2358).

Nervonin I (9): white powder; $[\alpha]^{17}_D$ –18.9 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ϵ) 206 (3.75) nm; IR (KBr) ν_{max} 3443, 2932, 1716, 1638 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.17 (1H, d, J = 5.1 Hz, H-11α), 5.13 (1H, br s, H-17a), 5.02 (1H, br s, H-17b), 4.74 (1H, d, J = 2.8 Hz, H-7α), 4.13 (d, J = 10.0 Hz, H-15α), 3.98 (1H, overlap, H-6β), 3.96 (1H, overlap, H-1β), 3.48 (1H, br s, H-3α), 2.64 (1H, br s, H-13α), 2.41 (1H, d, J = 12.7 Hz, H-14α), 2.19 (1H, br s, H-9β), 2.10 (1H, overlap, H-2α), 2.07 (1H, m, H-12α), 1.83 (1H, br d, J = 14.5 Hz, H-12β), 1.77 (1H, br d, J = 14.3 Hz, H-2β), 1.72 (1H, br s, H-5β), 1.44 (3H, s, Me-20), 1.35 (1H, br d, J = 12.7 Hz, H-14β), 1.23 (3H, s, Me-19), 0.94 (3H, s, Me-18), 2.12 and 2.02 (each 3H, s, 2 × OAc); ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; positive ESIMS *m/z* 475 $[M + Na]^+$, 927 $[2M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 475.2298 (calcd for $C_{24}H_{36}O_8Na$ $[M + Na]^+$, 475.2307).

Nervonin J (10): white powder; $[\alpha]^{16}_D$ –48.2 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ϵ) 204 (3.61) nm; IR (KBr) ν_{max} 3433, 2939, 1737, 1637 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.26 (1H, s, H-15α), 5.19 (1H, br s, H-17a), 4.98 (1H, br s, H-17b), 4.92 (1H, br s, H-11α), 4.32 (1H, dd, J = 11.5, 4.8 Hz, H-1β), 3.38 (1H, br s, H-3α), 2.70 (2H, br s, H-5β, 13α), 2.68 (1H, d, J = 14.0 Hz, H-7β), 2.39 (1H, br s, H-9β), 2.20 (1H, d, J = 14.0 Hz, H-7α), 2.16 (1H, m, H-12β), 1.98 (1H, m, H-2α), 1.87 (1H, m, H-12α), 1.86–1.79 (2H, overlap, H-2β, 14α), 1.39 (1H, m, H-14β), 1.22 (3H, s, Me-19), 1.03 (6H, s, Me-18, 20), 2.26 (3H, s, OAc); ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; positive ESIMS *m/z* 415 $[M + Na]^+$, 807 $[2M + Na]^+$; positive HRESIMS $[M + Na]^+$ *m/z* 415.2089 (calcd for $C_{22}H_{32}O_6Na$ $[M + Na]^+$, 415.2096).

Cytotoxicity Assay. The cytotoxicity of **1**−**20** was tested against K562 (chronic myelogenous leukemia), A549 (lung cancer), and HepG2 (hepatocellular carcinoma) human cell lines using the method described in the literature,[32] with cisplatin as the positive control. Results are expressed as IC_{50} values (concentration required to inhibit cell growth by 50%) in μM, and data were obtained from triplicate experiments.

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Supporting Information Available: NMR and mass spectra and a proposed biosynthetic pathway for nervonin A (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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