Cytotoxic Diterpenoids from Vietnamese Medicinal Plant *Croton tonkinensis* Gagnep.

Phan Minh Giang,[a,b] Phan Tong Son,[a] Yumi Hamada,[b] and Hideaki Otsuka*,[b]

[a] *Faculty of Chemistry, College of Natural Science, Vietnam National University; 19 Le Thanh Tong, Hanoi, Vietnam: and* [b] *Graduate School of Biomedical Sciences, Hiroshima University; 1–2–3 Kasumi, Minami-ku, Hiroshima 734–8551, Japan.*
Received September 29, 2004; accepted November 25, 2004

Six new *ent*-kaurane-type diterpenoids were isolated from the leaves of the endemic Vietnamese medicinal plant *Croton tonkinensis* Gagnep. (Euphorbiaceae) together with three known *ent*-11α-acetoxy-7β,14α-dihydroxykaur-16-en-15-one (1), *ent*-kaur-16-en-15-one 18-oic acid (5) and *ent*-18-hydroxykaur-16-ene (7). Their structures were determined by spectroscopic analyses to be *ent*-7β-acetoxy-11α-hydroxykaur-16-en-15-one (2), *ent*-18-acetoxy-11α-hydroxykaur-16-en-15-one (3), *ent*-11α-acetoxykaur-16-en-18-oic acid (4), *ent*-15α,18-dihydroxykaur-16-ene (6), *ent*-11α,18-diacetoxy-7β-hydroxykaur-16-en-15-one (8), and *ent*-(16*S*)-1α,14α-diacetoxy-7β-hydroxy-17-methoxykauran-15-one (14). *ent*-Kaurane-type diterpenoids from *Croton tonkinensis* 2—4, 6, and 9—13, were tested for toxicity in the brine shrimp lethality assay. Compounds 9, 10, and 12 demonstrated significant activity, compounds 2, 3, 6, and 11 showed weak activity, and compounds 4 and 13 were inactive.

Key words *Croton tonkinensis*; Euphorbiaceae; *ent*-kaurane-type diterpenoid; brine shrimp lethality test; cytotoxicity

In recent years, eight tetracyclic *ent*-kaurane-type diterpenoids were isolated by us from the leaves of *Croton tonkinensis* Gagnep. (Euphorbiaceae).[1,2] In our continuing search for more bioactive compounds from this plant, six new diterpenoids **2**—**4**, **6**, **8**, and **14** were isolated together with three known *ent*-kauranes *ent*-11α-acetoxy-7β,14α-dihydroxykaur-16-en-15-one (**1**), *ent*-kaur-16-en-15-one 18-oic acid (**5**), and *ent*-18-hydroxykaur-16-ene (**7**). The known compounds were identified by comparison of their spectroscopic data (^{1}H-, ^{13}C-NMR) with published values.[3—5] Compounds **2**—**4** and **6**, along with diterpenoids **9**—**13**, which were previously isolated from *C. tonkienesis*,[1,2] were tested for their cytotoxicity in the brine shrimp lethality assay. This paper deals with the structure elucidation of the new compounds and the results of the brine shrimp lethality assay.

Results and Discussion

Compound **2** was isolated as an amorphous powder, $[\alpha]_D^{25}$ $-127.3°$ ($c=0.20$, $CHCl_3$). Its molecular formula was determined to be $C_{22}H_{32}O_4$ by negative-ion high-resolution (HR)-FAB-MS (*m*/*z* 359.2183 $[M-H]^-$, Calcd 359.2222). The IR spectrum indicated the presence of hydroxyl (3446 cm^{-1}), ester (1734 cm^{-1}), and conjugated ketone (1649 cm^{-1}) functional groups. The ^{1}H- (Table 1) and ^{13}C-NMR (Table 2) spectroscopic data of **2** showed the presence of 22 carbons which were assignable to three tertiary methyl groups [δ_H 0.82 (s), 0.93 (s), 1.04 (s); δ_C 21.6, 33.4, 17.8, respectively], a ketone (δ_C 206.5), conjugated with an exocyclic methylene [δ_H 5.29 (s), 5.88 (s); δ_C 113.5, 149.6], an acetyl group [δ_H 1.94 (s); δ_C 169.7, 21.1], six methylene groups, five methine groups including two oxygenated (δ_H 4.07, δ_C 65.6, and δ_H 5.16, δ_C 73.6), and three quaternary carbons. Considering the similarity of the ^{13}C-NMR spectroscopic data of **2** with those of the diterpenoid **9**,[1] **2** was suggested to belong to the *ent*-kaurane series. The acetyl group was assigned at C-7 (δ_C 73.6) on the basis of the heteronuclear multiple bond correlation (HMBC) cross-peaks (Fig. 2) from H-7 (δ 5.16) to C-15 (δ 206.5), from H-7 to C-8 (δ 54.5), from H-7 to the carbonyl carbon of the acetyl group (δ 169.7), and from H-5 (δ 1.10) to C-7 (δ 73.6). Cross-peaks in the HMBC spectrum of **2** between H-11 (δ 4.07, br d, $J=5.1$ Hz) and C-8 (δ 54.5) and C-13 (δ 36.4), between H-9 (δ 1.41) and C-11 (δ 65.6), between 11-OH (δ_H 1.58) and C-12 (δ 41.7) placed the remaining hydroxyl group at C-11. The NOEs observed in the nuclear Overhauser enhancement spectroscopy (NOESY) experiment (Fig. 2) between H-5 (δ 1.10) and H-7β axial (δ 5.16, dd, $J=12.4$, 4.6 Hz), between H-5 and H-9 (δ 1.41), and between H-11 and H-1α (δ 1.87), Me-20 (δ 1.04), and between Me-20 and Me-19 (δ 0.82) established the unambiguous relative stereochemistry of **2** as shown. The CD spectrum showed the first negative ($\Delta\varepsilon$ -0.19 at 340 nm), the second positive ($\Delta\varepsilon$ $+0.62$ at 235 nm), and the third negative ($\Delta\varepsilon$ -2.11 at 206 nm) Cotton effects, characteristic of an *ent*-11α-hydroxykaur-16-en-15-one.[5] Thus the absolute structure of **2** was determined to be *ent*-7β-acetoxy-11α-hydroxykaur-16-en-15-one.

The negative-ion HR-FAB-MS of compound **3**, obtained as an amorphous powder, $[\alpha]_D^{25}$ $-155.6°$ ($c=0.20$, $CHCl_3$), showed a pseudomolecular ion peak at *m*/*z* 359.2248 ($[M-H]^-$, Calcd 359.2222), consistent with the molecular formula $C_{22}H_{32}O_4$. The IR spectrum indicated the presence of hydroxyl (3447 cm^{-1}), ester (1730 cm^{-1}), and conjugated ketone (1648 cm^{-1}) functional groups. The ^{1}H- (Table 1) and ^{13}C-NMR (Table 2) spectroscopic data of **3** showed the presence of an acetyl group [δ_H 2.10 (s); δ_C 171.4, 21.1] and an oxygenated methine [δ_H 4.05 (br d, $J=4.6$ Hz), δ_C 66.1]. When compared with **2**, the occurrence of an AB system [δ_H 3.64 (d, $J=11.3$ Hz), 3.86 (d, $J=11.3$ Hz)] together with the disappearance of the singlet methyl group at C-18 were apparent for the placement of the acetoxyl group at C-18 (δ_C 72.3), meanwhile the carbon bearing the acetoxyl substituent at the 7-position was absent in the structure of **3** (δ_C 33.2 in **3** instead of δ_C 73.6 in **2**), but the chemical shift of the hydroxy-bearing methine at C-11 remained almost the same (δ_C 66.1 in **3** and δ_C 65.6 in **2**). Cross-peaks were observed between H-11 (δ 4.05) and C-8 (δ 50.4), C-9 (δ 64.1), and C-13 (δ 36.6), between H_2-18 and C-3 (δ 35.5), C-5 (δ 48.8), and the carbonyl carbon of the acetyl group (δ 171.4) in the HMBC spectrum of **3**, thus confirming the assignments of the acetoxyl and hydroxyl groups at C-18 and C-11,

* To whom correspondence should be addressed. e-mail: hotsuka@hiroshima-u.ac.jp

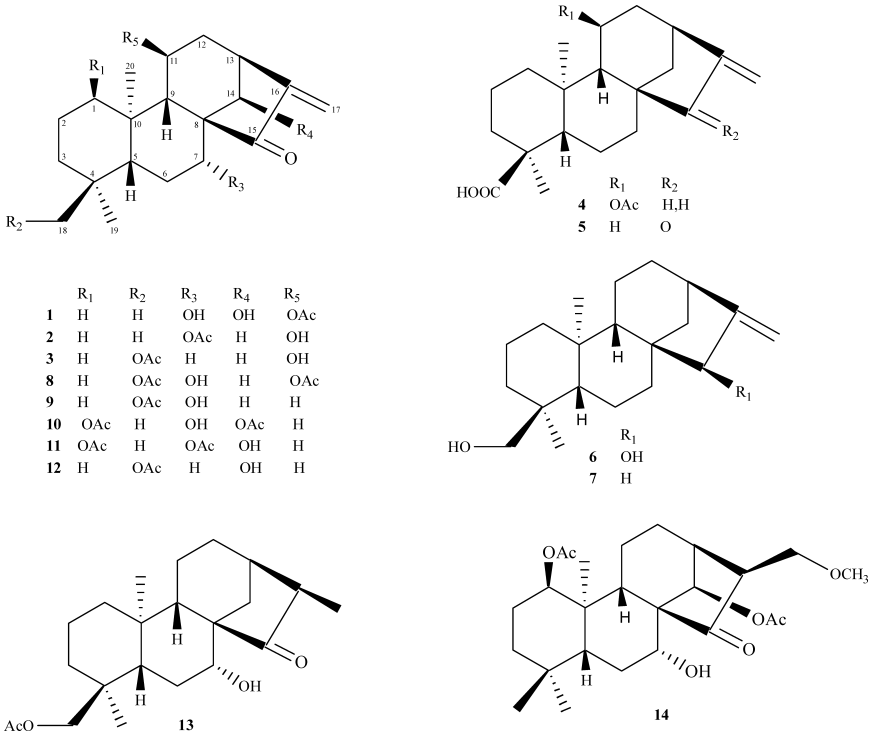

Fig. 1. Chemical Structures of *ent*-Kaurane-Type Diterpenoids from *C. tonkinensis*

respectively. The NOESY spectrum of **3**, which showed the NOEs between H-5 (δ 1.32) and H-9 (δ 1.44), H-1β (δ 1.00), between H-11 (δ 4.05) and Me-20 (δ 1.05), H-1α (δ 1.89), and between Me-20 and Me-19 (δ 0.83), H-14α (δ 2.38), indicated the stereochemistry of **3** as depicted in Fig. 1. Thus **3** was determined to be *ent*-18-acetoxy-11α-hydroxykaur-16-en-15-one. In support of the absolute configuration of **3**, the CD spectrum displayed the first negative ($\Delta\varepsilon$ -0.26 at 344 nm), the second positive ($\Delta\varepsilon$ $+0.61$ at 242 nm), and the third negative ($\Delta\varepsilon$ -1.77 at 216 nm) Cotton effects.[5]

Compound **4** was isolated as an amorphous powder, $[\alpha]_D^{25}$ $-91.2°$ (c=0.60, $CHCl_3$). Its molecular formula was determined to be $C_{22}H_{32}O_4$ by negative-ion HR-FAB-MS (*m/z* 359.2248 $[M-H]^-$, Calcd 359.2222). The IR spectrum indicated the presence of carboxylic acid (3382—2534, 1700 cm^{-1}), ester (1730 cm^{-1}) and exocyclic methylene (1660 cm^{-1}) functional groups. The ^{1}H- (Table 1) and ^{13}C-NMR (Table 2) spectroscopic data suggested that **4** was a kaurenoic acid[6] with an exocyclic double bond in the ring D [C-17: δ_H 4.68 (s), 4.82 (s); δ_C 103.3, 154.9]. In the HMBC spectrum of **4** (Fig. 3) cross-peaks involving both proton signals at δ_H 2.04 and 2.50, corresponding to a C-15 carbon signal at δ_C 47.9 [correlated from the heteronuclear single quantum correlation (HSQC) spectrum], and C-16 (δ_C 154.9), as well as both *exo*-methylene protons at δ_H 4.68, 4.82, and C-15 (δ_C 47.9), were evident for the absence of a C-15 ketone group in the ring D. The acetyl group [δ_H 1.94 (s); δ_C 170.1, 21.7] must be bonded to C-11 [δ_H 5.06 (t, J=3.2 Hz), δ_C 68.8] and was deduced to occupy a β-orientation on the basis of the HMBC and NOESY experiments (Fig. 3). Analysis of the ^{1}H-NMR spectrum indicated the presence of only two methyl groups at δ_H 1.00 (s) and 1.16 (s), corresponding to carbon signals at δ_C 17.8 and 16.2, respectively. Since the latter methyl group gave cross-peaks with the carboxyl group at δ_C 184.0, C-3 (δ 36.9), C-4 (δ 47.6), and C-5 (δ 49.9) in the HMBC spectrum of **4**, the carboxylic acid must be bonded to C-4. In this case either C-18 or C-19 was shielded and their ^{13}C signals were accordingly shifted upfield. The observed resonance of the methyl group at C-4 (δ_C 16.2) allowed the location of the carboxylic acid at C-18 [δ_C (C-19) *ca.* 16.5][4] rather than C-19 [δ_C (C-18) *ca.* 29.0].[6] In the NOESY spectrum of **4**, the methyl protons at C-19 (δ 1.16) gave cross-peaks with H-2α (δ 1.62) and H-6α (δ 1.48), but not with H-5 (δ 1.74). Thus **4** was determined to be *ent*-11α-acetoxykaur-16-en-18-oic acid.

The molecular formula of diterpenoid **6**, isolated as an amorphous powder, $[\alpha]_D^{25}$ $-76.7°$ (c=0.20, $CHCl_3$), was analyzed for $C_{20}H_{32}O_2$ by negative-ion HR-FAB-MS (*m/z* 303.2330 $[M-H]^-$, Calcd 303.2324). The IR spectrum indicated the presence of hydroxyl (3432 cm^{-1}) and exocyclic methylene (1647 cm^{-1}) functional groups. The *exo*-methylene group at C-16 was non-conjugated with the ketone group at C-15, and consequently resonated at δ 4.96 (s), 5.10 (s) in the ^{1}H-, and δ 158.3, 104.9 in the ^{13}C-NMR spectrum of **6**.[7] Of the hydroxy-bearing methylene and methine groups, the former showed an AB system at δ_H 3.11 (d, J=11.0 Hz) and 3.41 (d, J=11.0 Hz); δ_C 72.2, while the latter displayed resonances at δ_H 3.74 (t, J=2.5 Hz); δ_C 82.6. Based on the comparison of the ^{13}C-NMR spectroscopic data of **6** (Table 2) with those reported for *ent*-18-hydroxykaur-16-ene[8] and *ent*-kaur-16-en-15β-ol,[7] the hydroxyl groups were placed at C-18 and C-15. The hydroxyl group at the 15-position was deduced to be β-orientated on the basis of the significant upfield shift of C-9 (δ 46.4, $\Delta\delta$ $+3.6$ ppm) in comparison with the observed ^{13}C value at C-9 for *ent*-18-hydroxykaur-16-ene (δ 56.0)[8] due to the γ-steric compression effect between 15β-OH and C-9.[7] Therefore the structure of **6** was deter-

Table 1. ^{1}H-NMR Spectroscopic Data of **2**—**4**, **6**, **8**, and **14** (δ in ppm, 500 MHz, $CDCl_3$)

H	**2**	**3**	**4**	**6**	**8**	**14**
1α	1.87 dt (12.8, 3.7)	1.89 br d (12.9)	1.97 m	1.86 br d (13.1)	1.83 m	4.85 br s
β	1.00 ddd (12.8, 12.8, 3.5)[a]	1.00 m	1.02 m	0.84 ddd (13.1, 13.1, 3.9)	0.85 ddd (13.3, 13.3, 3.4)	
2α	1.65 m	1.53[b]	1.62 m	1.57 m	1.64 m	2.02[b]
β	1.46 m	1.53[b]	1.58[b]	1.37[b]	1.46 dquint (13.3, 3.4)	1.59 m
3α	1.41[b]	1.40 m	1.60 m	1.28[b]	1.31[b]	1.48[b]
β	1.21 ddd (14.0, 14.0, 4.6)	1.25 m	1.77[b]	1.28[b]	1.31[b]	1.44[b]
5	1.10 dd (12.4, 2.0)	1.32[b]	1.74 br d (12.0)	1.11 dd (11.7, 1.8)	1.25 br d (12.4)	1.42 dd (12.4, 2.3)
6α	1.32 q (12.4)	1.31[b]	1.48 m	1.26[b]	1.36 q (12.4)	1.74[b]
β	2.05 ddd (12.4, 4.6, 2.0)	1.53[b]	1.18 m	1.47 m	1.66 br dd (12.4, 4.4)	1.86 m
7α		1.36 m	1.51 br d (13.3)	1.30[b]		
β	5.16 dd (12.4, 4.6)	1.97[a]	1.65[b]	1.70 m	3.99 dd (11.4, 4.4)	4.06 dd (11.9, 4.6)
9	1.41 br s	1.44 br s	1.41 br s	1.38 br s	1.31 br s	1.71[b]
11α	4.07 br d (5.1)	4.05 br d (4.6)	5.06 t (3.2)	1.65[b]	5.06 d (4.6)	1.07 m
β				1.65[b]		1.52[b]
12α	2.20 ddd (15.6, 5.1, 3.2)	2.13 ddd (13.7, 4.6, 2.8)	1.87[b]	1.64[b]	2.10 ddd (14.7, 5.1, 3.2)	2.01[b]
β	2.01 br d (15.6)	1.98 br d (13.7)	1.87[b]	1.43 m	1.93 m	1.74[b]
13	3.10 br s	3.05 br s	2.63 br s	2.66 br s	3.03 br s	2.71 m
14α	2.14 d (11.9)	2.38 d (11.7)	1.92 m	2.00 d (11.9)	2.05[b]	6.09 s
β	2.04[b]	1.40[b]	1.13 m	1.02 dd (11.9, 5.1)	1.93[b]	
15α			2.04 dt (16.7, 2.5)	3.74 t (2.5)		
β			2.50 d (16.7)			
16						3.08 m
17A	5.29 s	5.27 s	4.68 s	4.96 s	5.19 s	3.44 t (9.6)
B	5.88 s	5.88 s	4.82 s	5.10 s	5.83 s	3.85 dd (9.6, 4.9)
18A	0.93 s	3.64 d (11.3)		3.11 d (11.0)	3.80 d (11.3)	0.93 s
B		3.86 d (11.3)		3.41 d (11.0)	3.57 d (11.3)	
19	0.82 s	0.83 s	1.16 s	0.77 s	0.77 s	0.89 s
20	1.04 s	1.05 s	1.00 s	1.08 s	1.04 s	1.26 s
OAc	1.94 s	2.10 s	1.94 s		2.03 s	
					1.79 s	
OMe						3.37 s
11-OH	1.58 br s					

a) Coupling constants (*J* in Hz) are given in parentheses. *b*) Overlapped signals.

Table 2. ^{13}C-NMR Spectroscopic Data of **2**—**4**, **6**, **8**, and **14** (δ in ppm, 100 MHz, $CDCl_3$)

C	**2**	**3**	**4**	**6**	**8**	**14**
1	39.3	38.9	39.2	39.9	38.7	73.2
2	18.3	17.6	17.6	18.1	17.6	22.7
3	41.5	35.5	36.9	35.2	35.2	35.1
4	33.3	36.5	47.6	37.6	36.5	32.9
5	52.1	48.8	49.9	48.7	45.9	47.7
6	24.5	18.3	23.1	19.7	27.8	27.9
7	73.6	33.2	40.4	38.5	70.8	72.6
8	54.5	50.4	43.2	45.7	58.3	61.5
9	63.6	64.1	61.9	46.4	59.0	48.5
10	38.6	38.6	37.5	38.8	39.0	42.7
11	65.6	66.1	68.8	18.0	68.5	16.8
12	41.7	41.3	39.8	33.3	38.9	25.4
13	36.4	36.6	42.4	40.1	36.4	38.7
14	29.3	36.8	39.0	36.5	27.4	76.7
15	206.5	209.6	47.9	82.6	208.1	217.4
16	149.6	150.2	154.9	158.3	149.8	49.9
17	113.5	113.1	103.3	104.9	113.5	68.5
18	33.4	72.3	184.0	72.2	71.1	33.2
19	21.6	17.5	16.2	17.6	17.4	21.5
20	17.8	17.9	17.8	18.2	18.4	18.6
1-OAc						170.2
						21.3
7-OAc	169.7					
	21.1					
11-OAc			170.1		169.3	
			21.7		21.0	
18-OAc		171.4			171.1	
		21.1			21.3	
OMe						59.1

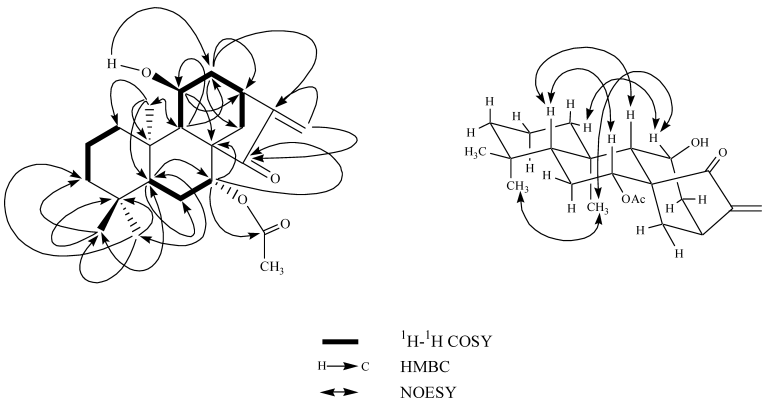

Fig. 2. ^{1}H–^{1}H COSY, HMBC, and NOESY Correlations of **2**

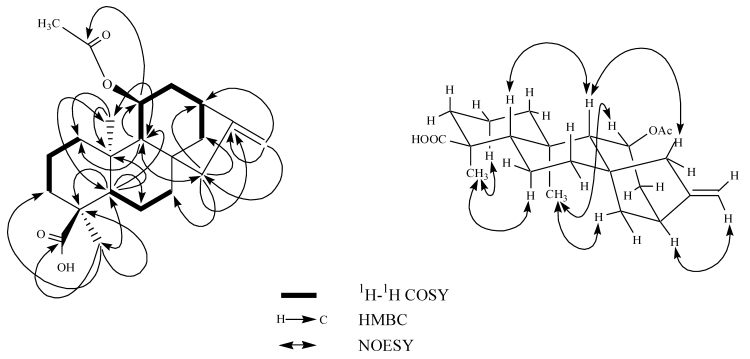

Fig. 3. ^{1}H–^{1}H COSY, HMBC, and NOESY Correlations of **4**

mined to be *ent*-15α,18-dihydroxykaur-16-ene.

Compound **8** was isolated as an amorphous powder, $[\alpha]_D^{25}$ −135.7° (c=2.24, $CHCl_3$), and found to possess the molecular formula $C_{24}H_{34}O_6$ by negative-ion HR-FAB-MS (m/z 417.2243 $[M-H]^-$ (Calcd for $C_{24}H_{33}O_6$: 417.2277). The IR spectrum indicated the presence of hydroxyl (3449 cm^{-1}), ester (1737 cm^{-1}) and conjugated ketone (1646 cm^{-1}) functional groups. The structure of **8** was reminiscent of **9**[2] with an additional acetyl group [δ_H 1.79; δ_C 169.3, 21.0] at C-11 [δ_H 5.06 (d, J=4.6 Hz), δ_C 68.5]. The stereochemistry was determined as shown by the NOE observations between H-11 and H-1α (δ 1.83), and between H-7 (δ 3.99) and H-9 (δ 1.31). Thus **8** was determined to be *ent*-11α,18-diacetoxy-7β-hydroxykaur-16-en-15-one.

Compound **14** was obtained as an amorphous powder, $[\alpha]_D^{25}$ −225.0° (c=0.16, $CHCl_3$). Its molecular formula was analyzed for $C_{25}H_{38}O_7$ by negative-ion HR-FAB-MS (m/z 449.2546 $[M-H]^-$ (Calcd for $C_{25}H_{37}O_7$: 449.2539). The IR spectrum indicated the presence of hydroxyl (3449 cm^{-1}), ester (1729 cm^{-1}), and saturated ketone (1729 cm^{-1}, overlapped) functional groups. The ^{13}C-NMR spectra of compounds **14** (Table 2) and **10**[2] were similar except for the presence of a methoxymethyl group [δ_H 3.44 (t, J=9.6 Hz), 3.85 (dd, J=9.6, 4.6 Hz); δ_C 68.5 (C-17), and δ_H 3.37 (s); δ_C 59.1 (–OMe)] instead of the C-16/C-17 double bond in the D-ring. The NOESY correlations between H_2-17 and H-11β (δ 1.52) established the β-orientation of the methoxymethyl group at C-16. Consequently **14** was determined to be *ent*-(16*S*)-1α,14α-diacetoxy-7β-hydroxy-17-methoxykauran-15-one (**14**). Compound **14** may be an artifact formed from **9**.

Compounds **2**—**4** and **6**, together with **9**—**13**, which were isolated by us from the same plant extract,[1,2] were tested for cytotoxicity in the brine shrimp toxicity test.[9] Compounds **4** and **13** were shown to be inactive, compounds **9**, **10**, and **12** showed significant activity (the percentage of deaths after 24 h was 100%, 96.7%, and 96.7% at 100 μg/ml, respectively), while compounds **2**, **3**, **6**, and **11** showed weak activity (the percentage of deaths after 24 h was 26.7% at 85 μg/ml, 30% at 100 μg/ml, 46.7% at 71.4 μg/ml, and 16.7% at 100 μg/ml, respectively). The fact that compounds **6**, **9**, **12** and **13** were not completely dissolved in 10% DMSO in H_2O may have been the reason for the observed lower toxicity of these compounds.

Experimental

General Procedure Optical rotations were measured on a Union Giken PM-101 digital polarimeter. FT-IR spectra were recorded on a Horiba FT-710 spectrophotometer. ^{1}H- (500 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded using JEOL JNM-ECP 500 and JEOL JNM-α 400 NMR spectrometers, respectively, in $CDCl_3$ with tetramethylsilane as an internal standard. Negative-ion HR-FAB-MS were measured on a JEOL SX-102 mass spectrometer with PEG-400 as a calibration matrix. CD spectra were obtained on a JASCO J-720 spectropolarimeter. HPLC was carried out with a JASCO PU-1580 pump and UV-2075 Plus detector (set at 210 nm) on YMC ODS columns (150×4.6 mm i.d. in analytical and 150×20 mm i.d. in preparative scales) at the corresponding flow rates of 0.5 and 5 ml/min. Silica gel 60 (0.063—0.200 mm, Merck, Germany) and reversed-phase octadecyl silica (ODS) gel (YMC, Japan) were used for open column chromatography. TLC was carried out on Merck precoated TLC sheets (silica gel 60 F_{254}), and detected by spraying with 10% H_2SO_4 in 50% EtOH, followed by heating on a hot plate at 200 °C.

Plant Material The air-dried leaves of *C. tonkinensis* were collected in the suburbs of Hanoi, Vietnam, and identified by Professor Vu Van Chuyen at Hanoi College of Pharmacy (Hanoi, Vietnam) in September 2003. A voucher specimen (No. DHD 2002-5) is deposited in the Herbarium of the Hanoi College of Pharmacy.

Extraction and Isolation of Diterpenoids 1—8, and 14 The powdered air-dried leaves of *C. tonkinensis* (2.0 kg) were extracted with MeOH and fractionated with solvents of increasing polarity (*n*-hexane, CH_2Cl_2, EtOAc and *n*-BuOH) by partition with H_2O. The combined *n*-hexane- and CH_2Cl_2-soluble fractions (59.2 g) were chromatographed on a silica gel column using *n*-hexane with increasing amounts of EtOAc as solvent systems (0—100%) to afford five fractions. Fraction 4 (9.5 g) eluted with 50% EtOAc in *n*-hexane was further chromatographed successively by gravity column chromatography on silica gel (*n*-hexane–EtOAc, 2 : 1), on ODS gel (MeOH–H_2O 7 : 3), and finally by ODS HPLC (MeOH–H_2O 7 : 3) to yield compounds **1** (1.6 mg), **2** (2.0 mg), **3** (3.0 mg), **4** (7.0 mg), **6** (2.0 mg), a mixture of **5** and **7** (3.2 mg), **8** (22.4 mg) and **14** (1.6 mg).

ent-7β-Acetoxy-11α-hydroxykaur-16-en-15-one (**2**): White amorphous powder, $[\alpha]_D^{25}$ −127.3° (c=0.20, $CHCl_3$). IR ν_{max} (film) cm^{-1}: 3446, 2927, 2868, 1734, 1701, 1649, 1238, 1032. CD (MeOH): $\Delta\varepsilon$ (nm): −0.19 (340), +0.62 (235), −2.11 (206) (c=5.27×10^{-4} M). ^{1}H- and ^{13}C-NMR: see Tables 1 and 2. Negative-ion HR-FAB-MS: m/z 359.2183 $[M-H]^-$ (Calcd for $C_{22}H_{31}O_4$: 359.2222).

ent-18-Acetoxy-11α-hydroxykaur-16-en-15-one (**3**): White amorphous powder, $[\alpha]_D^{25}$ −155.6° (c=0.20, $CHCl_3$). IR ν_{max} (film) cm^{-1}: 3447, 2931, 2866, 1730, 1648, 1239, 1036. CD (MeOH): $\Delta\varepsilon$ (nm): −0.26 (344), +0.61 (242), −1.77 (216) (c=6.66×10^{-4} M). ^{1}H- and ^{13}C-NMR: see Tables 1 and 2. Negative-ion HR-FAB-MS: m/z 359.2248 $[M-H]^-$ (Calcd for $C_{22}H_{31}O_4$: 359.2222).

ent-11α-Acetoxykaur-16-en-18-oic acid (**4**): White amorphous powder, $[\alpha]_D^{25}$ −91.2° (c=0.60, $CHCl_3$). IR ν_{max} (film) cm^{-1}: 3382—2534, 2935, 2851, 1730, 1700, 1660, 1241, 1033, 1017. CD (MeOH): $\Delta\varepsilon$ (nm): −1.68 (221) (c=17.50×10^{-4} M). ^{1}H- and ^{13}C-NMR: see Tables 1 and 2. Negative-ion HR-FAB-MS: m/z 359.2248 $[M-H]^-$ (Calcd for $C_{22}H_{31}O_4$: 359.2222).

ent-15α,18-Dihydroxykaur-16-ene (**6**): White amorphous powder, $[\alpha]_D^{25}$ −76.7° (c=0.20, $CHCl_3$). IR ν_{max} (film) cm^{-1}: 3432, 2930, 2862, 1647, 1246, 1021. CD (MeOH): $\Delta\varepsilon$ (nm): +1.07 (221) (c=5.78×10^{-4} M). ^{1}H- and ^{13}C-NMR: see Tables 1 and 2. Negative-ion HR-FAB-MS: m/z 303.2330 $[M-H]^-$ (Calcd for $C_{20}H_{31}O_2$: 303.2324).

ent-11α,18-Diacetoxy-7β-hydroxykaur-16-en-15-one (**8**): White amorphous powder, $[\alpha]_D^{25}$ −135.7° (c=2.24, $CHCl_3$). IR ν_{max} (film) cm^{-1}: 3449, 2937, 1737, 1646, 1239, 1037. ^{1}H- and ^{13}C-NMR: see Tables 1 and 2. Negative-ion HR-FAB-MS: m/z 417.2243 $[M-H]^-$ (Calcd for $C_{24}H_{33}O_6$: 417.2277).

ent-(16*S*)-1α,14α-Diacetoxy-7β-hydroxy-17-methoxykauran-15-one (**14**): White amorphous powder, $[\alpha]_D^{25}$ −225.0° (c=0.16, $CHCl_3$). IR ν_{max} (film) cm^{-1}: 3449, 2944, 2873, 1729, 1240, 1030. ^{1}H- and ^{13}C-NMR: see Tables 1 and 2. Negative-ion HR-FAB-MS: m/z 449.2546 $[M-H]^-$ (Calcd for $C_{25}H_{37}O_7$: 449.2539).

Brine Shrimp Lethality Assay The cytotoxicity assay was performed according to the method described in the literature.[9]

Acknowledgments This work was supported by a Grant-in-Aid from the Japan Society for the Promotion of Science (JSPS). P.M.G. is grateful to acknowledge JSPS for a Postdoctoral Research Fellowship at Hiroshima University and the International Foundation for Science (Stockholm, Sweden) for the financial support to collect the medicinal plants in Vietnam. We thank the Research Center of the Hiroshima University Faculty of Medicine, Japan, for providing access to the 400 MHz NMR instrument.

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