Diterpenes from the Roots of *Euphorbia kansui* and Their *in Vitro* Effects on the Cell Division of *Xenopus* (2)[1,2]

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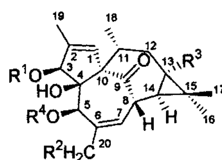
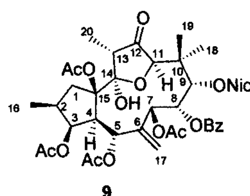
Four new ingenane-type diterpenes, 3-*O*-(2,3-dimethylbutanoyl)-13-*O*-dodecanoyl-20-*O*-acetylingenol (1), 3-*O*-(2,3-dimethylbutanoyl)-13-*O*-dodecanoyl-20-deoxyingenol (2), 3-*O*-(2*E*,4*Z*-decadienoyl)-20-deoxyingenol (3), and 3-*O*-(2*E*,4*E*-decadienoyl)-20-deoxyingenol (4), two new jatrophane-type diterpenes, kansuinins D (9) and E (10), and four known ingenane-type diterpenes were isolated from the root of *Euphorbia kansui*. Their structures were elucidated by spectroscopic and chemical analysis, and individual *Xenopus* cells at the blastular stage were cultured with the diterpenes to test for biological activity. 20-Deoxyingenol diterpenes 3 and 4 induced the greatest cell cleavage arrest (0.5 μg/ml of each compound resulted in >75% cleavage arrest), but cell cleavage inhibitory activity became weak when C-16 had an acyl residue. In contrast, the jatrophane diterpene kansuinin D (9) showed no activity.

Key words *Euphorbia kansui*; Euphorbiaceae; diterpene; ingenane; jatrophane

The dried roots of *Euphorbia kansui* L. (Euphorbiaceae) are known as *Gan Sui* in Chinese medicine and have been used as a herbal remedy for edema, ascites,[3,4] and cancer[5—7] in mainland China. In a previous paper,[1] we reported the isolation of nine ingenol diterpenes, 20-*O*-(2*E*,4*E*-decadienoyl)ingenol, 20-*O*-(2*E*,4*Z*-decadienoyl)ingenol, 3-*O*-(2*E*,4*E*-decadienoyl)ingenol, 3-*O*-(2*E*,4*Z*-decadienoyl)ingenol, 3-*O*-(2*E*,4*Z*-decadienoyl)-5-*O*-acetylingenol, 3-*O*-(2*E*,4*Z*-decadienoyl)-20-*O*-acetylingenol, 3-*O*-(2*E*,4*E*-decadienoyl)-20-*O*-acetylingenol, 20-*O*-decanoylingenol, and 5-*O*-(2*E*,4*E*-decadienoyl)ingenol; three jatrophane diterpenes, kansuinins A, B, and C; four new euphane-type triterpenes, kansenone, kansenonol, 11-oxo-kansenonol, and kansenol; a new tirucallane-type triterpene, epi-kansenone; and a known triterpene, α-euphol, from the 60% EtOH extract of *E. kansui* roots. *In vitro* treatment of cultured individual *Xenopus* cells at the blastular stage with those ingenol esters significantly prevented cell cleavage. Further bioassay-directed fractionation of the 60% ethanol extract led to the isolation of 3-*O*-(2,3-dimethylbutanoyl)-13-*O*-dodecanoyl-20-acetylingenol (**1**), 3-*O*-(2,3-dimethylbutanoyl)-13-*O*-dodecanoyl-20-deoxyingenol (**2**), 3-*O*-(2*E*,4*Z*-decadienoyl)-20-deoxyingenol (**3**), 3-*O*-(2*E*,4*E*-decadienoyl)-20-deoxyingenol (**4**), the two new jatrophane-type diterpenes kansuinin D (**9**) and kansuinin E (**10**), and four known ingenane diterpenes identified as 20-*O*-(2,3-dimethylbutanoyl)-13-*O*-dodecanoylin-



Kansuiphorin A $R^1 = COCH(Me)CH(Me)_2$, $R^2 = OCO(CH_2)_{14}Me$, $R^3 = OCO(CH_2)_{10}Me$, $R^4 = H$
1 $R^1 = COCH(Me)CH(Me)_2$, $R^2 = OAc$, $R^3 = OCO(CH_2)_{10}Me$, $R^4 = H$
2 $R^1 = COCH(Me)CH(Me)_2$, $R^2 = H$, $R^3 = OCO(CH_2)_{10}Me$, $R^4 = H$
3 $R^1 = CO\text{-}(CH\overset{E/Z}{=}CH)_2\text{-}(CH_2)_4\text{-}CH_3$, $R^2 = H$, $R^3 = H$, $R^4 = H$
4 $R^1 = CO\text{-}(CH\overset{E/E}{=}CH)_2\text{-}(CH_2)_4\text{-}CH_3$, $R^2 = H$, $R^3 = H$, $R^4 = H$
5 $R^1 = H$, $R^2 = OCOCH(Me)CH(Me)_2$, $R^3 = OCO(CH_2)_{10}Me$, $R^4 = H$
6 $R^1 = COCH(Me)CH(Me)_2$, $R^2 = OH$, $R^3 = OCO(CH_2)_{10}Me$, $R^4 = H$
7 R^1 = benzoyl, $R^2 = OH$, $R^3 = OCO(CH_2)_{10}Me$, $R^4 = H$
8 $R^1 = H$, R^2 = benzoyloxy, $R^3 = OCO(CH_2)_{10}Me$, $R^4 = H$



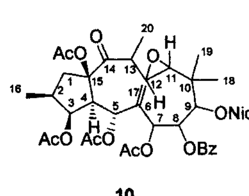


Chart 1

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Table 1. ^{1}H-NMR Data for Compounds **1**, **2**, **3**, and **4** (300 MHz, $CDCl_3$, TMS, δ (ppm) (J=Hz)[a])

	1	**2**	**3**	**4**
H-1	6.01 q (1.5)	6.03 q (1.5)	6.07 q (1.5)	6.07 q (1.5)
H-3	5.44 s	5.35 s	5.53 s	5.48 s
H-5	3.88 d (6.9)	3.69 br s	3.68 d (6.9)	3.68 br s
H-7	6.07 d (3.9)	5.71 m	5.77 m	5.76 m
H-8	4.06 dd (12.6, 3.9)	3.98 m	4.01 dd (11.7, 3.6)	4.01 dd (11.7, 3.6)
H-11	2.56 m	2.54 m	2.46 m	2.44 m
H_2-12	2.72 dd (16.8, 3.3)	2.70 dd (16.8, 3.3)	2.26 m[b]	2.26 ddd (15.9, 9.0, 3.3)
	2.19 m[b]	2.20 m[b]	1.75 m[b]	1.75 m[b]
H-13			0.67 m	0.67 m
H-14	1.23 m[b]	1.23 m[b]	0.90 m[b]	0.90 m[b]
Me-16	1.07 s	1.05 s	1.05 s	1.05 s
Me-17	1.19 s	1.18 s	1.08 s	1.08 s
Me-18	0.97 d (7.5)	0.97 d (6.6)	0.98 d (6.9)	0.98 d (6.9)
Me-19	1.78 d (1.5)	1.773 d (1.5)	1.79 d (1.5)[b]	1.79 d (1.5)[b]
H-20	4.73, 4.47 ABq (12.6)	1.777 s	1.79 s[b]	1.79 m[b]
3-R	2.31 m 1H	2.31 m 1H	2′ 5.94 d (15.3)[b]	2′ 5.85 d (15.3)
	1.92 m 1H	1.92 m 1H	3′ 7.68 dd (15.3, 11.7)	3′ 7.33 m
	0.92 d (6.9) 3H	0.92 d (6.9) 3H	4′ 6.16 dd (11.7, 10.5)	4′ 6.19 m[b]
	0.96 d (6.6) 3H	0.96 d (6.6) 3H	5′ 5.94 m[b]	5′ 6.19 m[b]
	1.14 d (7.2) 3H	1.14 d (7.2) 3H	6′ 2.33 m[b]	6′ 2.15 m
			7′ 1.43 m	7′ 1.44 m
			8′, 9′ 1.29 m	8′, 9′ 1.29 m
			10′ 0.89 t[b] (7.0)	10′ 0.89 t (7.0)[b]
13-R	2.19 t (7.5)[b] 2H	2.19 t (7.5)[b] 2H		
	1.55 m 2H	1.55 m 2H		
	1.25 s[b] –$(CH_2)_8$–	1.25 s[b] –$(CH_2)_8$–		
	0.88 t (6.9) 3H	0.88 t (6.9) 3H		
20-R	20-$COCH_3$			
	2.05 s 3H			
5-OH	3.51 d (6.9)	3.14 br s	3.02 d (6.9)	3.06 br s
3-OH	3.48 s	3.48 s	3.43 s	3.43 s

a) Assignments confirmed by decoupling, H–H COSY, HMQC, and HMBC spectra. *b*) Overlapping signal.

genol (**5**),[8] 3-*O*-(2,3-dimethylbutanoyl)-13-*O*-dodecanoylingenol (**6**),[8,9] 3-*O*-benzoyl-13-*O*-dodecanoateingenol (**7**),[10] and 20-*O*-benzoyl-13-*O*-dodecanoateingenol (**8**)[10] by comparison of their spectroscopic data with those previously described in the literature. In this paper, we report the structural characterization and biological evaluation of these compounds.

Compound **1** was obtained as a gum. Hydroxyl (3440 cm^{-1}) and carbonyl (1742, 1725, 1718 cm^{-1}) absorptions were observed in the IR spectrum. The negative high resolution FAB-MS (HR-FAB-MS) of **1** indicated an $[M-H]^-$ peak at m/z 685.43168, which corresponded to the molecular formula $C_{40}H_{62}O_9$. The negative FAB-MS of **1** showed three ester groups consisting of an acetate $[M-H-60]^-$, 2,3-dimethylbutanoate $[M-H-60-116]^-$ and dodecanoate $[M-H-200]^-$ unit. Analysis of ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY), heteronuclear multiple-quantum coherence (HMQC), and heteronuclear multiple-bond connectivity (HMBC) spectra also indicated that **1** contained these three ester groups: [δ_H 2.31 m 1H, 1.92 m 1H, 0.92 d (J=6.9 Hz) 3H, 0.96 d (J=6.6 Hz) 3H, 1.14 d (J=7.2 Hz) 3H, δ_C 176.7, 46.3, 31.0, 20.6, 19.1, 14.06 (2,3-dimethylbutanoate); δ_H 2.19 t (7.5) 2H, 1.55 m 2H, 1.25 s –$(CH_2)_8$–, 0.88 t (J=6.9 Hz) 3H, δ_C 173.4, 34.3, 24.7, 29.48, 29.48, 29.34, 29.22, 29.15, 29.10, 31.79, 22.60, 13.98 (dodecanoate); δ_H 2.05 s 3H, δ_C 170.4, 21.0 (acetate)] (Tables 1, 2). The ^{1}H-NMR spectrum also contained signals at δ_H 6.07 d (J=3.9 Hz) (H-7), δ_H 6.01 d (J=1.5 Hz) (H-1), δ_H 5.44 s (H-3), δ_H 4.73, 4.47 Abq (J=12.6 Hz) (H-20), δ_H 4.06 dd (J=12.6, 3.9 Hz) (H-8), δ_H 3.88 d (J=6.9 Hz) [H-5, which had a ^{1}H–^{1}H COSY correlation with δ_H 3.51 d (J=6.9 Hz) (OH-5), H-5 changed to a singlet and OH-5 disappeared when D_2O was added in $CDCl_3$], δ_H 2.56 m (H-11), δ_H 2.72 dd (J=16.8, 3.3 Hz), and δ_H 2.19 m (H-12), δ_H 1.23 m (H-14), δ_H 0.97 d (J=7.5 Hz), 1.07 s, 1.19 s, 1.78 d (J=1.5 Hz) (Me-16-19), very similar to those in the spectrum of 13-hydroxyingenol.[9,11] After the ^{1}H- and ^{13}C-NMR data on **1** had been assigned by analysis of its ^{1}H–^{1}H COSY, HMQC, and HMBC spectra, it was obvious that **1** and a 13-hydroxyingenol-type diterpene, kansuinin A [3-*O*-(2,3-dimethylbutanoyl)-13-*O*-dodecanoyl-20-*O*-hexadecanoylingenol],[11] were based on the same parent system and differed only in the type of esterification. The absence of a hexadecanoate unit and the appearance of acetate signals indicated the replacement of one of the hexadecanoate units with an acetate group. The positions of the ester groups were confirmed by the HMBC cross-peaks between H-20 and the carbon signal at δ_C 170.4 (acetate carbonyl), and between H-3 and the carbon signal at δ_C 176.6 (2,3-dimethylbutanoate carbonyl). The dodecanoate carbonyl signal at δ_C 173.4 had no HMBC correlation with proton signals on the skeleton, indicating that the dodecanoyl is attached to the tertiary OH at C-13. Thus **1** was elucidated as 3-*O*-(2,3-dimethylbutanoyl)-13-*O*-dodecanoyl-20-*O*-acetylingenol.

High-resolution electron impact (HR-EI)-MS of **2** indicated an $[M]^+$ peak at m/z 628.43401, corresponding to the molecular formula of $C_{38}H_{60}O_7$. Hydroxyl (3460 cm^{-1}) and carbonyl (1741, 1728, 1716 cm^{-1}) absorptions were observed

Table 2. ^{13}C-NMR Data for Compounds **1**, **2**, **3** and **4** (75 MHz, $CDCl_3$, TMS)[a]

	1	2	3	4		1	2
1	131.1	131.6	132.8	132.8	13-R		
2	135.8[b]	135.3	135.8	135.8	1‴	173.4	173.5
3	82.4	82.8	83.4	83.4	2‴	34.3	34.3
4	84.3	84.4	85.2	85.2	3‴	24.7	24.7
5	74.6	76.2	77.6	77.6	4‴	29.48	29.48
6	135.9[b]	137.2	137.6	137.6	5‴	29.48	29.48
7	127.9	122.7	124.3	124.3	6‴	29.34	29.34
8	42.6	42.5	43.6	43.6	7‴	29.22	29.22
9	204.6	205.1	207.1	207.1	8‴	29.15	29.15
10	71.7	71.6	72.2	72.3	9‴	29.10	29.11
11	37.5	37.8	39.1	39.1	10‴	31.79	31.79
12	35.0	34.9	31.4	31.4	11‴	22.60	22.60
13	68.8	68.8	23.3	23.3	12‴	13.98	13.97
14	28.2	28.4	23.5	23.5			
15	30.2	30.3	24.2	24.2			
16	22.4	22.4	28.7	28.8			
17	16.6	16.7	15.7	15.7			
18	18.2	18.0	17.4	17.4			
19	15.5	15.5	15.8	15.8			
20	66.3	21.9	22.2	22.2			
3-R							
1′	176.7	176.9	168.0	168.1			
2′	46.3	46.3	120.1	118.2			
3′	31.0	31.0	141.5	146.6			
4′	20.6	20.6	126.4	128.4			
5′	19.1	19.1	143.3	147.1			
6′	14.06	14.06	28.5	33.2			
7′			29.2	28.5			
8′			31.6	31.6			
9′			22.5	22.6			
10′			14.2	14.2			
20-R							
1″	170.4						
2″	21.0						

a) Assignments confirmed by decoupling, H–H COSY, HMQC, and HMBC spectra. *b*) Assignments may be interchanged.

in the IR spectrum. The ^{1}H-NMR spectrum of **2** was very similar to that of **1**, but contained some differences. Signals at δ_H 4.73, 4.47 (H-20), and δ_H 2.05 (acetyl) in **1** were absent in the spectrum of **2**, but a new methyl signal at δ_H 1.777 was observed, suggesting a methyl unit was present at C-20. The EI-MS of **2** showed two ester groups consisting of a 2,3-dimethylbutanoate [$(M-116)^+$ and $(M-116-H_2O)^+$], and a dodecanoate [$(M-200)^+$ and $(M-200-116)^+$+] unit. The position of the 2,3-dimethylbutanoate linkage was deduced from further analysis of the HMBC spectrum. Correlations of the 2,3-dimethylbutanoate carbonyl (δ_C 176.9) with H-3 (δ_H 5.35) demonstrated that the 2,3-dimethylbutanoyloxy group was situated at C-3. No HMBC correlation was observed between the dodecanoate carbonyl and the proton signals of the diterpene skeleton, indicating attachment of the dodecanoate group to the tertiary hydroxyl at C-13. Thus the structure of **2** was elucidated to be 3-*O*-(2,3-dimethylbutanoyl)-13-*O*-dodecanoyl-20-deoxyingenol. Further ^{1}H–^{1}H COSY, HMQC, and HMBC analyses confirmed the structure.

The absolute stereochemistry of the 2,3-dimethylbutanoate units of **1**, **2**, **5**, and **6** was not determined.

Compound **3** displayed IR absorptions at 3456 (OH) and 1720 (C=O). The HR-EI-MS of **3** indicated an $[M]^+$ peak at *m*/*z* 482.30318, corresponding to a molecular formula of $C_{30}H_{42}O_5$. The ^{1}H-NMR data were very similar to those of 3-*O*-(2*E*,4*Z*-decadienoyl)ingenol,[9] but the absence of the methylene signal at C-20 and the appearance of a new methyl signal at δ_H 1.79 indicated that **3** was a 20-deoxyingenol-type diterpene. The ^{1}H- and ^{13}C-NMR data of the ester residue of **3** were similar to those obtained for ingenol diterpenes with a 2′*E*,4′*Z*-decadienoyl residue, indicating that **3** contained the same ester residue. The HMBC correlation between δ_H 5.53 s (3-H) and δ_C 168.0 showed a 2*E*,4*Z*-decadienoyl residue at C-3. Thus **3** was elucidated to be 3-*O*-(2*E*,4*Z*-decadienoyl)-20-deoxyingenol. Further ^{1}H–^{1}H COSY, HMQC, and HMBC analysis confirmed the structure.

The ^{1}H- and ^{13}C-NMR spectral data attributed to the diterpene moiety of **4** were almost identical to those of **3**, whereas the spectral data of the acid moiety $C_9H_{15}COOH$ were different. The olefin proton signals [δ_H 7.33 m, 6.19 m, 6.19 m, 5.85 d (J=15.3 Hz)] did not offer much information on the nature of the double bond. A previous paper[1,2] reported that, when using $CDCl_3$ as the NMR solvent, the 2,4-decadienoyl unit had an *E*/*Z* configuration and gave olefin proton and carbon signals at δ_H 7.60 dd (J=15.0, 11.0 Hz), 6.12, 5.85 d (J=15.2 Hz), 5.84 m, and δ_C 120.7, 140.2, 126.3, 142.1, while those with the *E*/*E* configuration yielded proton and carbon signals at δ_H 7.30 m, 6.20 m, 6.20 m, 5.86 d (J=15.2 Hz), and δ_C 118.2, 146.2, 128.2, 146.8. Spectral data of the 2,4-decadienoyl unit of **4** agreed well with an *E*/*E* configuration. Thus compound **4** was assigned as 3-*O*-(2*E*,4*E*-decadienoyl)-20-deoxyingenol.

The molecular weight of kansuinin D (**9**) was 793 by EI-MS (*m*/*z* 793), positive FAB-MS (*m*/*z* 794), and negative FAB-MS (*m*/*z* 792). The molecular formula was confirmed to be $C_{41}H_{47}NO_{15}$ by HR-EI-MS and NMR analyses. Positive FAB-MS also suggested the presence of benzoate (*m*/*z* 105 C_7H_5O), nicotinate (*m*/*z* 124, $C_6H_6NO_2$, and 106, C_6H_4NO), and four acetate [*m*/*z* 734 $(M+H-60)^+$, 674 $(M+H-60\times2)^+$, 614 $(M+H-60\times3)^+$, and 554 $(M+H-60\times4)^+$] units in the structure. The ^{1}H- and ^{13}C-NMR spectra indicated the presence of six ester groups, including four acetate groups [(δ_H 1.51 s, 1.95 s, 2.00 s, 2.09 s; δ_C 170.0, 168.3, 168.8, 169.5 (CO), 20.3, 20.8, 21.2, 21.9 (CH_3)), one benzoate group [δ_H 8.03 2H m, 7.55 m, 7.41 2H, m; δ_C 164.9 (CO), 129.7, 129.5×2, 127.8×2, 133.1], and one nicotinate group [δ_H 9.18 s, 8.77 d (J=4.2 Hz), 8.31 d (J=7.8 Hz), 7.41 m; δ_C 162.7 (CO), 152.7, 150.0, 137.4, 125.0, 123.2]. The proton signals attributed to the nicotinate group did not offer much information. However, when the 500-MHz ^{1}H-NMR spectrum was recorded in acetone-d_6, H-2 appeared at δ_H 9.14 d (J=1.5 Hz), H-4 at δ_H 8.85 dd (J=4.9, 1.5 Hz), H-5 at δ_H 7.57 ddd (J=7.9, 4.9, 0.6 Hz), and H-6 at δ_H 8.33 m, indicating the presence of a nicotinate group.[12] In addition to the ester groups, the ^{13}C-NMR and DEPT spectra suggested that the skeleton consisted of 20 carbons: one ketone, one exocyclic double bond, four methyls, one methylene, nine methines, and three quaternary carbons. The spectra exhibited resonances closely related to those of kansuinin A.[1,2] After the ^{1}H- and ^{13}C-NMR data on **9** had been assigned by analysis of its ^{1}H–^{1}H COSY, HMQC, and HMBC spectra, it was obvious that the structures of compound **9** and kansuinin A were based on the same parent system and differed only in the esterification. The absence of an acetate signal and the appearance of the signals of a

Table 3. NMR Spectral Data of Kansuinin D (**9**) [(300 MHz and 75 MHz, $CDCl_3$, TMS, δ (ppm) (J=Hz)][a)]

Atom	^{1}H	^{13}C	^{1}H–^{1}H COSY	HMBC	NOESY
1α	2.68 dd (4.5, 12.0)	39.9	H-1β, H-2	C-2, 3, 4, 14, 15	H-1β, 2, 4, Me-16, 20
1β	2.19 m[b)]		H-1α	C-2, 14, 15	H-1α, Me-16
2	2.20 m[b)]	38.7	H-1α, H-3, H-16	C-15, Me-16	H-1α, 3, Me-16
3	5.58 br s	74.1	H-2, H-4	C-1, 15, CO (δ 169.5)	H-2, 4, Me-16
4	3.05 d (2.7)	51.4	H-3, H-5	C-3, 5, 6, 14, 15	H-3, 5, 7
5	6.19 s	69.5	H-4, H-17a	C-3, 4, 7, 15, Me-17, CO (δ 168.3)	H-4, 7
6		144.7			
7	6.43 s	69.1	H-8, H-17a, b	C-5, 6, 8, 9, Me-17, CO (δ 170.0)	H-4, 5, 8, 11
8	6.17 s	71.0	H-7, H-9	C-6, 9, 10, CO (δ 164.9)	H-7, H-9, Me-19
9	5.36 s	83.3	H-8	C-7, 8, 10, 11, Me-18, 19, CO (δ 162.7)	H-8, Me-18, 19
10		41.7			
11	4.30 s	77.4		C-9, 10, 12, Me-18, 19	H-7, Me-18
12		213.6			
13	2.33 q (6.6)	50.9	Me-20	C-12, 14, 15, Me-20	Me-19, 20
14		106.4			
15		90.5			
16	0.92 d (6.0)	13.2	H-2	C-1, 2, 3	H-1β, 2, 3
17a	5.17 s	109.9	H-7, H-5	C-5, 6, 7	
17b	4.99 s		H-7	C-5, 6, 7	
18	1.21 s	22.1		C-9, 10, 11, Me-19	H-9, 11, Me-19
19	1.40 s	18.7		C-9, 10, 11, Me-18	H-8, 9, 13, Me-18
20	1.32 d (6.5)	9.18	H-13	C-12, 13, 14	
Acetyl					
3-COMe	2.09 s	169.5			
		21.2			
5-COMe	1.95 s	168.3			
		20.8			
7-COMe	1.51 s	170.0			
		20.3			
15-COMe	2.00 s	168.8			
		21.9			
8-Benzoyl		164.9			
		129.7			
9-Nicotinoyl	8.03 m	129.5			
	7.41 m	127.8			
	7.55 m	133.1			
	9.18 s	162.7			
	8.77 d (4.2)	152.7			
	8.31 d (7.8)	150.0			
	7.39 m[b)]	137.4			
		125.0			
		123.2			

a) Assignments confirmed by decoupling, H–H COSY, HMQC, HMBC, and NOESY spectra. *b*) Assignments may be interchanged.

nicotinate group indicated the replacement of one of the acetate units with a nicotinate group. The position of this substituent was established from the HMBC cross-peak between H-9 and the carbon signal at δ_C 162.7 (nicotinate carbonyl).

The zero coupling constant between H-4 and H-5, and H-4/H-7 NOE interactions suggested that the 6,17-exomethylene group was parallel to the plane of the macrocycle as described for jatrophanes.[12)] This conformation involves a β-oriented H-5 pointing inward into the macrocycle. The relative stereochemistry of kansuinin D was studied in NOESY and NOE experiments (Table 3). NOEs from H-4 to H-7, H-7 to H-11, and H-11 to δ_H 1.21 s indicated that H-7 and H-11 were α and the resonance was Me-18. Me-19 (δ_H 1.40 s) had an NOE correlation with H-13, indicating a 20α-methyl. A combination of NOEs from H-7 to H-8, H-8 to H-9 and Me-19, H-9 to Me-18 and Me-19, and the zero ^3J (H-7 to H-8 and H-8 to H-9) coupling constant was best interpreted by designating H-8 and H-9 as β. Irradiation of H-4 also produced a NOE at H-3 and irradiation of H-3 produced a NOE at H-2, showing that H-3 and H-2 were α. All of the above data led to the structure elucidation of **9**.

Kansuinin E (**10**) gave a molecular ion in the HR-EI-MS at *m*/*z* 777.29972 appropriate for a molecular formula of $C_{41}H_{47}NO_{14}$. It displayed an IR absorption band characteristic of ester groups at 1738 cm^{-1}. The ^{1}H- and ^{13}C-NMR spectra of **10** indicated the presence of six ester groups, similar to **9**. In addition to the ester groups, the ^{13}C-NMR and DEPT spectra suggested that the skeleton consisted of 20 carbons: one ketone, one exocyclic double bond, four methyls, one methylene, 10 methines, and two quaternary carbons. The ^{1}H–^{1}H COSY spectrum defined three structural fragments with correlated protons: –CH_2–$CH(CH_3)$–CHR–CHR–C=CH_2–CHR–CHR–CHR–(A), –CHR–CHR–$CH(CH_3)$–(B), and –CH_2–$CH(CH_3)$CH–(C). The long-range correlations of the carbons (C-6, C-10, C-14, C-15) with proton signals of the skeleton connected these three fragments and established fragments of a jatrophane skeleton (Fig. 1, Table 4). The correlations of the benzoate carbonyl C-8 (δ_C 164.6) with H-8 (δ_H 5.98 s) demonstrated that one benzoyloxy group was situated at C-8. The locations of the C-9

nicotinoyl group and the C-3, C-5, and C-7 triacetyl groups were ascertained in the same manner. The last acetyl group was located at C-15, determined by the chemical shift of C-15 at δ_C 93.3, which was very close to that of the 15-acetyl group of kansuinin B. In kansuinin C, which has a 15-hydroxy group, the chemical shift of C-15 was shifted upfield to δ_C 84.8. The presence of an epoxy group in the molecule was postulated. The chemical shifts of H-11 and H-12 (δ_H 3.37 d and 3.27 br d), and C-11 and C-12 (δ_C 61.5, 57.9) indicated that the epoxy group was located between C-11 and C-12.[1,2]

The relative stereochemistry of kansuinin E (**10**) was studied using NOESY measurements. NOE interactions and coupling constants of H-1, H-2, H-3, and H-4 were very similar to those of **9**, suggesting the same configurations at C-2, C-3, C-4, and C-15. The stereochemistry of C-4 and C-15 was also established from the observation that all jatrophane diterpenes subjected to X-ray analysis exhibited a *trans* ring junction and no NOE was observed between H-4 and 15-OAc.[14] The zero coupling constant between H-4 and H-5 required a β configuration for H-5, similar to **9**. In contrast to kansuinins C and D (**9**), Me-18 and Me-19 of **10** produced very broad peaks in the ^{1}H-NMR spectrum, indicating greater flexibility of the macrocycle. The stereochemistry of C-7, C-8, C-9, C-11, C-12, and C-13 could not be determined on the basis of NOESY correlations.

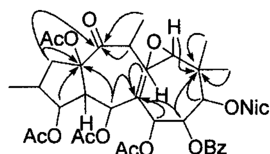

Fig. 1. Key HMBC Correlation of Kansuinin E (**10**) (H–C)

The effects of the compounds on the division of isolated cells from early *Xenopus laevis* embryos were determined. Single cells from mid-blastula stage embryos can divide in a nonnutritive medium. Under the standard conditions of the present study, most cells divided between 4 and 10 times. 20-Deoxyingenols **3** and **4** significantly prevented cell cleavage (0.5 μg/ml of each compound resulted in $>$60% cleavage arrest) in a manner similar to that caused by ingenol derivatives.[1,2] Of the six 16-hydroxyingenol diterpenes, only **6** and **7** showed some activity (10 μg/ml of each compound resulted in $>$60% cleavage arrest). 16-Hydroxyingenol diterpenes **1**, **2**, **5**, and **8** showed no activity, even at concentrations up to 50 μg/ml. These data show that, in ingenol diterpenes, cell cleavage inhibitory activity weakens when C-16 has an acyl

Table 4. NMR Spectral Data of Kasuinin E (**10**) [(500 MHz and 125 MHz, CDCl$_3$, TMS, δ (ppm) (J=Hz)][a]

Atom	^{1}H	^{13}C	^{1}H–^{1}H COSY	HMBC	NOESY
1α	2.91 dd (7.9, 14.0)	45.8	H-1β, H-2	C-2, 3, 4, 14, 15	H-1β, 2
1β	1.89 m		H-1α, H-2	C-2, 14, 15	H-1α, Me-16
2	2.29 m	38.7	H-1α, H-1β, H-3, Me-16		H-1α, 3, 4, Me-16
3	5.48 s	75.7	H-2, H-4	C-15, CO (169.6—169.9)	H-2, 4, Me-16
4	3.18 s	54.6	H-3, H-5		H-3, 5, 7
5	5.57 s	68.1	H-4, H-17a, H-17b	C-4, 6, 7, 15, 17, CO (168.8)	H-4, 7, 8, 11
6		143.2			
7	4.88 s	70.6	H-8	C-5, 6, 17, CO (169.6—169.9)	H-4, 5, 8, 11
8	5.98 s	69.1	H-7, H-9	C-6, 10, CO (164.6)	H-5, 9, 11, Me-19
9	5.38 s	79.6	H-8	C-8, 10, 11, 18, 19, CO (163.8)	H-8, 11, Me-18, 19
10		38.8			
11	3.37 d (1.8)	61.5	H-12	C-10, 12, 13, 18, 19	H-5, 8, 9, 13, Me-20
12	3.27 br d (6.8)	57.9	H-11, H-13	C-13	Me-20
13	2.75 m	43.6	H-12, Me-20	C-11, 12, 14, 20	H-5, H-11, Me-20
14		216.5			
15		93.3			
16	0.90 d (6.8)	13.3	H-2	C-1, 2, 3	H-1β, 2, 3
17a	5.11 s	111.8	H-5	C-5, 6, 7	H-3, 17b
17b	4.94 s		H-5	C-5, 6, 7	H-17a
18	0.72 br s	17.9		C-9, 10, 11, 19	H-9, Me-19
19	1.32 br s	25.5		C-9, 10, 11, 18	H-9, 11, Me-18
20	1.36 d (6.7)	17.4	H-13	C-12, 13, 14	H-9, 12
Acetyl					
3, 5, 7	2.11 s	21.4, 21.2			
15-COMe	2.09 s	20.9, 20.3			
	1.69 s	169.9, 169.8			
		169.6, 168.8			
8-Benzoyl	8.04 m	164.6			
	7.44 m	129.3			
	7.58 m	129.9			
		128.5			
		133.4			
9-Nicotyl	9.33 br s	163.8			
	8.86 br s	154.1			
	8.34 d (6.3)	151.4			
	7.53 m	143.2			
		137.3			
		123.7			

a) Assignments confirmed by decoupling, H–H COSY, HMQC, HMBC, and NOESY spectra.

residue. Jatrophane diterpene **9** showed no activity at 50 μg/ml. About 18% of the cells incubated without these compounds (control) experienced cell cycle arrest.

As the early embryonic cell cycle in *Xenopus* consists of only S and M phases and does not include the G_1 or G_2 phases,[15,16] arrest of the cell cycle by *E. kansui* is unrelated to the inhibition of reactions at the G_1 to S phase transition. Arrest of the cell cycle of *Xenopus* embryos by triterpenes from *E. kansui* may be related to the preservation or progression of the M phase.

Experimental

General Procedures Melting points were determined on a Yanagimoto micro-melting point apparatus, which were uncorrected. The UV spectrum was obtained in MeOH on a Shimadzu UV-160 spectrophotometer, and the IR spectrum was recorded on a JASCO IR A-2 spectrophotometer. Optical rotations were measured in MeOH on a JASCO DIP-360 polarimeter. The CD spectrum was obtained in MeOH with a JASCO J-600 spectrophotometer. The NMR spectra were recorded on a JEOL GL-500 spectrometer and a VARIAN MERCURY 300 spectrometrer, with TMS as an internal standard. The mass spectra (MS) were obtained on a JEOL GCmate spectrometer. Column chromatography was carried out with silica gel (Wako gel C-300, Wako Pure Chemical Industry Ltd.). Thin-layer chromatography (TLC) was performed on Merck TLC plates (0.25 mm thickness), with compounds visualized by spraying with 5% (v/v) H_2SO_4 in ethanol solution and then heating on a hot plate. HPLC was performed on a JASCO PU-2089 apparatus equipped with a JASCO UV-2075 and a SHODEX RI-101 detector. A Senshu Pak PEGASIL Silica 60-5 (10×250 mm i.d.) column and a Senshu Pak PEGASIL ODS 2 were used for preparative purposes.

Plant Materials The dried root of *E. kansui* L. was collected in Xianyang, Sannxi Province, People's Republic of China, in October 1997 and was identified by Professor Weichun Wu (Department of Medical Plants, Shenyang Pharmaceutical University, People's Republic of China). Voucher specimens have been deposited at the Department of Natural Products Chemistry of Shenyang Pharmaceutical University and College of Pharmacy, Nihon University.

Extraction and Isolation The dried roots of *E. kansui* (15.1 kg) were extracted twice with 60% ethanol under reflux. Evaporation of the solvent under reduced pressure from the combined extract gave the 60% EtOH extract (1201 g, inhibitory effect 50 μg/ml, 91%). The extract was dissolved and suspended in water (4.0 l) and partitioned with chloroform (3×4 l), ethyl acetate (3×4 l), and *n*-butanol (3×4 l). The amounts extracted were 165 g, 23 g, and 64 g, respectively, and the residual aqueous extract yielded 376 g.

The chloroform fraction was subjected to silica gel column chromatography (13×22 cm, eluted with hexane and ethyl acetate in increasing polarity). The column chromatographic fractions (200 ml each) were combined according to TLC monitoring into nine portions. Fraction 6, eluted with hexane–EtOAc (70 : 30), was subjected to ODS C-18 column chromatography (10×15 cm, eluted with MeOH : H_2O 30 : 70—90 : 10). The column chromatographic fractions (100 ml each) were combined according to TLC monitoring into eight portions. Portion six was isolated and further purified by HPLC (Senshu pak PWGASIL Silica 60-5, 10×250 mm, hexane–$CHCl_3$–EtOAc, 75 : 20 : 5) to give **3** (15 mg) and **4** (10 mg). Portion eight was further purified by HPLC (Senshu pak PEGASIL ODS, 10×250 mm, CH_3CN : H_2O, 10 : 1) to give **1** (10 mg) and **2** (21 mg). Fraction 7, eluted with hexane–EtOAc (60 : 40), was subjected to ODS C-18 column chromatography (10×15 cm, eluted with MeOH : H_2O 30 : 70—90 : 10). The column chromatographic fractions (100 ml each) were combined according to TLC monitoring into nine portions. Portion nine was isolated and further purified by HPLC (Senshu pak PEGASIL Silica 60-5, 10×250 mm, hexane–EtOAc, 80 : 20) to give **5** (10 mg), **6** (20 mg), **7** (11 mg), **8** (9 mg), and **10** (25 mg). Fraction 8, eluted with EtOAc, was isolated and further purified by column chromatography and HPLC (Senshu pak PWGASIL ODS, 10×250 mm, MeOH : H_2O, 70 : 30) to give **9** (40 mg).

3-*O*-(2,3-Dimethylbutanoyl)-13-*O*-dodecanoyl-20-*O*-acetylingenol (**1**): Colorless gum; $[\alpha]_D^{23}$ 11.5° (c=0.69, MeOH); UV (MeOH) λ_{max} (log ε) 206 (2.88); IR (KBr) ν_{max} 3440, 1742, 1725, 1718, 1650 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; negative HR-FAB-MS m/z 685.43168 (Calcd for $C_{40}H_{61}O_9$, 685.43152); negative FAB-MS m/z 685 $[M-H]^-$ (3), 625 (10), 509 (10), 425 (2), 199 (75), 115 (100).

3-*O*-(2,3-Dimethylbutanoyl)-13-*O*-dodecanoyl-20-deoxyingenol (**2**): Colorless gum; $[\alpha]_D^{23}$ −4.4° (c=0.73, MeOH); UV (MeOH) λ_{max} (log ε) 206 (2.88); IR (KBr) ν_{max} 3460, 1741, 1728, 1716, 1650 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; HR-EI-MS m/z 628.43401 (Calcd for $C_{38}H_60O_7$, 628.43390); EI-MS m/z 628 $[M-H]^+$ (3), 512 (5), 494 (5), 428 (5), 312 (70), 71 (100).

3-*O*-(2*E*,4*Z*-Decadienoyl)-20-deoxyingenol (**3**): Colorless gum; $[\alpha]_D^{23}$ +42.1° (c=0.28, MeOH); UV MeOH) λ_{max} (log ε) 206 (4.11), 260 (3.48); IR (KBr) ν_{max} 3456, 1720, 1635, 1456, 1379, 1269, 1169, 999, 755, 687 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; HR-EI-MS m/z 482.30318 (Calcd for $C_{30}H_{42}O_5$, 482.30320); EI-MS m/z 482 $[M]^-$ (1), 314 (72), 296 (60), 286 (58), 253 (50), 163 (82), 151 (95), 122 (100), 109 (68), 95 (65).

3-*O*-(2*E*,4*E*-Decadienoyl)-20-deoxyingenol (**4**): Colorless gum; $[\alpha]_D^{23}$ +57.5° (c=0.16, MeOH); UV (MeOH) λ_{max} (log ε) 205 (4.01), 260 (3.87); IR (KBr) ν_{max} 3443, 1719, 1642, 1457, 1380, 1265, 1174, 755, 688 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; positive HR-FAB-MS m/z 505.29259 (Calcd for $C_{30}H_{42}O_5Na$, 505.29300); positive FAB-MS m/z 505 $[M+Na]^+$ (3), 353 (5), 315 (25), 297 (24), 123 (65), 89 (85), 69 (65).

Kansuinin D (**9**): Colorless crystals (MeOH), mp 175—177 °C; $[\alpha]_D^{23}$ +76.5° (c=0.40, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.99), 225 (4.19), 260 (3.47); IR (KBr) ν_{max} 3452, 1739, 1652, 1592, 1455, 1375, 1283, 1264, 1241, 1113, 1031, 741, 712 cm^{-1}; CD (MeOH) λ 226 nm ($\Delta\varepsilon$ +9.6), 294 nm ($\Delta\varepsilon$ +7.3); ^{1}H- and ^{13}C-NMR data, see Table 3; HR-EI-MS m/z 793.29422 (Calcd for $C_{41}H_{47}NO_{15}$, 793.29454); positive FAB-MS m/z 794 $[M+H]^+$ (5), 734 (40), 674 (15), 632 (1), 614 (4), 572 (3), 554 (3), 124 (100), 105 (100).

Kansuinin E (**10**): White crystals (MeOH), mp 126—128 °C; $[\alpha]_D^{23}$ +43.6° (c=0.48, MeOH); UV (MeOH) λ_{max} (log ε) 201 (3.92), 227 (4.15), 260 (3.42); IR (KBr) ν_{max} 3450, 1738, 1590, 1454, 1374, 1263, 1234, 1177, 1041, 740, 712 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Table 4; HR-EI-MS m/z 777.29972 (Calcd for $C_{41}H_{47}NO_{14}$, 777.29954); EI-MS m/z 777 $[M]^+$ (18), 735 (15), 718 (12), 620 (25), 578 (40), 230 (20), 189 (18), 166 (85), 124 (86), 105 (100), 77 (20).

Animal Cap Assay Animal caps were dissected from stage 8 *Xenopus laevis* blastulae. Single cells from the inner surface of the caps were separated by directing a gentle stream of calcium- and magnesium-free medium (50 mM phosphate buffer, 35 mM NaCl, 1 mM KCl, pH 7.0) as described by Godsave and Slack.[17] Two or three cells were transferred into a well of a Terasaki plate filled with 10 μl of 2 mg/ml γ-globulin in a simple salt solution (NAM/2) and cultured for 20 h at 25 °C.

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