Diterpenes from the Roots of *Euphorbia kansui* and Their in Vitro Effects on the Cell Division of *Xenopus*

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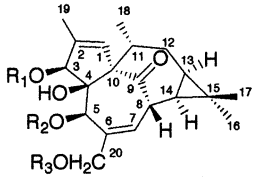
Twelve polycyclic diterpenes have been isolated from the roots of *Euphorbia kansui*. Nine were assigned with an ingenol skeleton, 20-*O*-(2′*E*,4′*E*-decadienoyl)ingenol (**1**), 20-*O*-(2′*E*,4′*Z*-decadienoyl)ingenol (**2**), 3-*O*-(2′*E*,4′*Z*-decadienoyl)ingenol (**3**), 3-*O*-(2′*E*,4′*E*-decadienoyl)ingenol (**4**), 3-*O*-(2′*E*,4′*Z*-decadienoyl)-5-*O*-acetylingenol (**5**), 3-*O*-(2′*E*,4′*Z*-decadienoyl)-20-*O*-acetylingenol (**6**), 3-*O*-(2′*E*,4′*E*-decadienoyl)-20-*O*-acetylingenol (**7**), 20-*O*-(decanoyl)ingenol (**8**), and 5-*O*-(2′*E*,4′*E*-decadienoyl)ingenol (**9**), and three with a jatrophane skeleton, kansuinins A (**12**), B (**11**), and C (**10**). Compounds **1**, **2**, **5**, **9**, and **12** are new compounds, while **4** and **7** were assigned with new geometric configurations. Their structures were elucidated by spectroscopic and chemical analysis. In vitro treatment of cultured individual *Xenopus* cells at the blastular stage with **1**−**9** arrested cleavage significantly (0.5 μg/mL of each compound resulted in >75% cleavage arrest). Of the three jatrophane diterpenes (**10**−**12**), only kansuinin B (**11**) showed any activity, resulting in 87% cleavage arrest at 50 μg/mL.

The dried roots of *Euphorbia kansui* L. (Euphorbiaceae) are known as "Kan Sui" in Chinese medicine. "Kan Sui" was recorded in *Sheng Nung's Herbal* as a low-grade drug[1] and has been used as an herbal remedy for edema, ascites,[2,3] and cancer[4−6] in mainland China. Previous investigations of *E. kansui* have yielded numerous compounds, such as tirucallol, α-euphol,[1] kansuinines A and B, 20-deoxyingenol-3-benzoate, 20-deoxyingenol-5-benzoate, 3-*O*-(2′,4′-decadienoyl)-20-*O*-acetylingenol, 13-hydroxyingenol-13-dodecanoate-20-hexanoate,[7−9] kansuiphorins A, B, C, and D,[10,11] 3-*O*-(2′*E*,4′*Z*-decadienoyl)ingenol, and 3-*O*-(2′,3′-dimethylbutyryl)-13-hydroxyingenol.[12] Recent studies have shown that diterpenes from *E. kansui* have cytotoxic activity against several human cancer cell lines,[7−11] as well as antiviral activity,[13] and stimulatory effects on the expression of the macrophage Fc receptor.[12] To identify potential anticancer components in *E. kansui*, we used an animal cap assay[14] to screen for inhibitors of cell division. Bioassay-directed fractionation of the 60% ethanol extract, which showed significant cleavage arrest activity (50 μg/mL of the extract resulted in 92% cleavage arrest), led to the isolation of 12 diterpene esters (**1**−**12**). In this article, we report the structure characterization and the biological evaluation of these compounds.



1 $R^1, R^2 = H, R^3 = CO\text{-}(\overset{E/E}{CH{=}CH})_2\text{-}(CH_2)_4\text{-}CH_3$

2 $R^1, R^2 = H, R^3 = CO\text{-}(\overset{E/Z}{CH{=}CH})_2\text{-}(CH_2)_4\text{-}CH_3$

3 $R^1 = CO\text{-}(\overset{E/Z}{CH{=}CH})_2\text{-}(CH_2)_4\text{-}CH_3, R^2, R^3 = H$

4 $R^1 = CO\text{-}(\overset{E/E}{CH{=}CH})_2\text{-}(CH_2)_4\text{-}CH_3, R^2, R^3 = H$

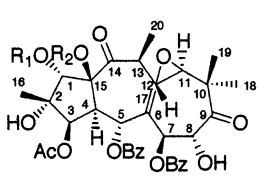
5 $R^1 = CO\text{-}(\overset{E/Z}{CH{=}CH})_2\text{-}(CH_2)_4\text{-}CH_3, R^2 = COMe, R^3 = H$

6 $R^1 = CO\text{-}(\overset{E/Z}{CH{=}CH})_2\text{-}(CH_2)_4\text{-}CH_3, R^2 = H, R^3 = COMe$

7 $R^1 = CO\text{-}(\overset{E/E}{CH{=}CH})_2\text{-}(CH_2)_4\text{-}CH_3, R^2 = H, R^3 = COMe$

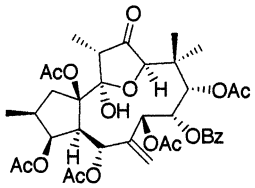
8 $R^1, R^2 = H, R^3 = CO\text{-}(CH_2)_8\text{-}CH_3$

9 $R^1 = H, R^2 = CO\text{-}(\overset{E/E}{CH{=}CH})_2\text{-}(CH_2)_4\text{-}CH_3, R^3 = H$



10 $R^1 = COCH_3, R^2 = H$

11 $R^1 = H, R^2 = COCH_3$



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Results and Discussion

Each of the isolated compounds **1**−**9** was a colorless oil. These compounds were confirmed to contain an ingenane skeleton from their ^{1}H NMR spectra (Table 1).[15,16]

The IR spectrum of **1** showed absorption bands attributable to a hydroxyl (3441 cm^{-1}) and two carbonyl groups (1732 and 1720 cm^{-1}). The noise-decoupled ^{13}C NMR spectrum of **1** showed 30 carbon atoms, which were classified as five methyls, six methylenes, 12 methines, and seven quaternary carbon atoms using distortionless enhancement by polarization transfer (DEPT) ^{13}C NMR analysis. Furthermore, the ^{13}C NMR chemical shifts suggested the presence of a carbonyl, an ester carbonyl, and four oxygen-bearing carbon atoms. From these results and the HREIMS (*m*/*z* 498.29898), the molecular formula of **1** was established as $C_{30}H_{42}O_6$. The signals for olefinic protons in the ^{1}H NMR spectrum proved the presence of an unsaturated acid moiety. The UV maximum (258 nm) and the MS fragment peaks at *m*/*z* 330 [$M^+ - C_9H_{15}COOH$]

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Table 1. ^{1}H NMR Data for Compounds **1**, **2**, **4**, **5**, **7**, and **9** [500 MHz, $CDCl_3$, TMS, δ (ppm) (J = Hz)][a]

H	1	2	4	5	7	9
1	5.93 d (1.5)	5.94 d (1.5)	6.03 d (1.5)	6.08 d (1.2)	6.03 d (1.2)	5.84 m[b]
3	4.42 s	4.44 s	5.54 s	5.03 s	5.57 s	3.88 s
5	3.69 s	3.69 s	4.04 s	5.43 s	3.87 s	5.40 s
7	6.11 d (3.6)	6.11 m[b]	6.05 (3.7)	6.20 d (3.7)	6.11 d (3.7)	6.21 m[b]
8	4.11 dd (3.6, 13.7)	4.10 dd (3.6, 13.7)	4.17−4.10 m[b]	4.25 dd (4.0, 13.7)	4.08 dd (4.0, 11.7)	4.35 dd (4.9, 11.3)
11	2.34 m	2.34 m[b]	2.52 m	2.55 m	2.51 m	2.50 m
12a	2.28 ddd (3.0, 8.8, 15.6)	2.28 m[b]	2.25 ddd (3.0, 8.8, 15.8)	2.29 m[b]	2.25 ddd (3.0, 8.8, 15.6)	2.24 ddd (3.0, 8.8, 15.6)
12b	1.76 m	1.76 m	1.75 m	1.76 m	1.77 m	1.77 m
13	0.69 m	0.69 m	0.69 m	0.71 m	0.71 m	0.72 m
14	0.95 m	0.95 m	0.93 m	0.96 m	0.97 m	1.04 m
16	1.05 s	1.05 s	1.04 s	1.05 s	1.06 s	1.07 s
17	1.12 s	1.11 s	1.08 s	1.08 s	1.08 s	1.17 s
18	0.97 d (7.0)	0.97 d (7.0)	0.98 (7.0)	1.01 d (7.3)	1.00 d (7.3)	1.01 d (7.0)
19	1.84 d (1.5)	1.85 d (1.5)	1.79 d (1.5)	1.80 d (1.5)	1.80 d (1.1)	1.83 (0.9)
20	4.78, 4.57 ABq (12.8)	4.81, 4.61 ABq (12.8)	4.17−4.10 m[b]	3.89, 3.93 ABq (13.4)	4.75, 4.50 ABq (12.5)	3.99, 3.95 ABq (13.1)
2′	5.78 d (15.2)	5.85 d (15.2)[b]	5.86 d (15.2)	5.92 d (15.3)	5.86 d (15.2)	5.84 d (15.2)[b]
3′	7.25 m	7.60 dd (15.2, 11.0)	7.30 m	7.64 dd (11.9, 15.3)	7.31 m	7.38 m
4′	6.14 m[b]	6.12 m[b]	6.20 m[b]	6.12 m	6.21 m[b]	6.21 m[b]
5′	6.14 m[b]	5.84 m[b]	6.20 m[b]	5.93 m	6.21 m[b]	6.21 m[b]
6′	2.15 m	2.29 m	2.19 m	2.29 m[b]	2.18 m	2.18 m
7′	1.42 m	1.42 m	1.44 m	1.43 m	1.43 m	1.42 m
8′	1.29 m[b]	1.29 m[b]	1.26 m[b]	1.26 m[b]	1.31 m	1.31 m
9′	1.29 m[b]	1.29 m[b]	1.26 m[b]	1.26 m[b]	1.28 m	1.28 m
10′	0.89 t (7.0)	0.89 t (7.0)	0.89 t (7.0)	0.91 t (7.0)	0.72 t (7.0)	0.72 t (7.0)
OAc				2.31 s	2.05 s	

[a] Assignments confirmed by decoupling, H−H COSY, HMQC, HMBC, and NOESY spectra. [b] Overlapped signal.

showed the ester residue was a 2,4-decadienoyloxy group.[12] The ^{1}H NMR spectral data of **1** were very similar to those of 3-*O*-(2′*E*,4′*Z*-decadienoyl)ingenol (**3**),[12] but the H-3 signal of **1** resonated at δ 4.42 (s), moving upfield by 1.16 ppm, and the H-20 signal resonated at δ 4.78, 4.57 (ABq), moving downfield by 0.70 ppm, demonstrating that the C-3 decadienoyloxy moiety of **3** was translocated at C-20 in **1**. Further HMBC analysis led to correlations between the decadienoate carbonyl C-1′ (δ 167.4) and H-20 (δ 4.78, 4.57, ABq). Thus, compound **1** was assigned as 20-*O*-(2′,4′-decadienoyl)ingenol. Most of the diterpenes that have a 2,4-decadienoyloxy group isolated from *Euphorbia* species possess an *E*,*Z*- or *Z*,*E*-configuration in the double bonds of the ester residue; however, this compound differed from the normal ones judging by the chemical shifts and coupling constants observed for protons H-2′, H-3′, H-4′, and H-5′ of the decadienoyloxy group. In compounds with the *E*,*Z*-configuration,[12,17] the coupling constants are normally $J_{2',3'} = 15$ Hz, $J_{4',5'} = 11$ Hz, and H-3′ appears at about δ 7.68 as a double doublet ($J_{2',3'} = 15$ Hz, $J_{3',4'} = 11$ Hz). In compounds with the *Z*,*E*-configuration,[18] the coupling constants are normally $J_{2',3'} = 11$ Hz, $J_{4',5'} = 15$ Hz, and the H-3′ signal resonated at about δ 7.40, moving upfield by about 0.3 ppm compared with those with the *E*,*Z*-configuration, and appearing as a double doublet ($J_{2',3'} = 15$ Hz, $J_{3',4'} = 11$ Hz). However, in the case of **1**, H-2′ appeared at δ 5.78 as a doublet ($J_{2',3'} = 15.2$ Hz), corresponding to a 2′,3′-trans double bond. The overlapping signals at δ 6.14, attributed to the H-4′, H-5′ signals, did not offer much information as to the nature of the double bond. However, when the ^{1}H NMR spectrum was recorded in C_6D_6, H-2′ appeared at δ 5.73 as a doublet ($J_{2',3'} = 15.3$ Hz), H-3′ was found at δ 7.40 as a double doublet ($J_{2',3'} = 15.3$ Hz, $J_{3',4'} = 10.8$ Hz), H-4′ appears at δ 5.83 as a double doublet ($J_{4',5'} = 15.3$ Hz, $J_{3',4'} = 10.8$ Hz), and H-5′ appeared at δ 5.63 as a double triplet ($J_{4',5'} = 15.3$ Hz, $J_{5',6'} = 6.9$ Hz), indicating that the two double bonds possess the *E*,*E*-configuration. In conclusion, the structure of **1** was established as 20-*O*-(2′*E*,4′*E*-decadienoyl)ingenol.

The mass spectral data for compounds **1**, **2**, **4**, and **9** had similar fragmentation patterns and molecular ions (see Experimental Section), which indicated the presence of isomeric compounds. Comparison of ^{1}H NMR and ^{13}C NMR data of these compounds demonstrated the differences were in the geometric configuration and in the position of the fatty acid side chain.

The ^{1}H NMR and ^{13}C NMR spectral data attributed to the diterpene moiety of **2** (Tables 1 and 2) were almost identical with those of **1**, whereas the spectral data of the acid moiety $C_9H_{15}COOH$ were in good agreement with those published for [3-*O*-(2′*E*,4′*Z*-decadienoyl)ingenol] (**3**).[12] The H-2′ signal appeared at δ 5.85 as a doublet ($J_{2',3'} = 15.2$ Hz), and the H-3′ signal was found at δ 7.60 as a double doublet ($J_{2',3'} = 15.2$ Hz, $J_{3',4'} = 11.0$ Hz) corresponding to a 2′,3′-trans double bond. The NOE difference spectra showed a relationship between H-4′ (δ 6.12, m) and H-5′ (δ 5.84, m) corresponding to a 4′,5′-cis double bond. Thus, compound **2** was concluded to be 20-*O*-(2′*E*,4′*Z*-decadienoyl)ingenol.

By comparing the ^{1}H and ^{13}C NMR spectra data (Tables 1 and 2) of **4** with those of **1**, **2**, and 3-*O*-(2′*E*,4′*Z*-decadienoyl)ingenol (**3**),[12] the chemical shift of the H-3 proton indicated that the ester moiety is at C-3. The ^{1}H and ^{13}C NMR spectra data attributed to the diterpene of **4** were almost identical with those published for **3**, whereas the spectral data of the decadienoyloxy group were in good agreement with an *E*,*E*-configuration. The HMBC experiment showed cross-peaks between the decadienoate carbonyl (δ 168.1) and H-3 (δ 5.54 s). Thus, the structure of compound **4** was assigned as 3-*O*-(2′*E*,4′*E*-decadienoyl)-ingenol.

Comparison of the ^{1}H NMR spectra of **1** and **9** (Table 1) demonstrated that the C-20 decadienoyloxy moiety in the latter was situated at C-5, since the H-5 signal of **9**

Table 2. ^{13}C NMR Data for Compounds **1**, **2**, **4**, **5**, **7**, and **9** (125 MHz, $CDCl_3$, TMS)[a]

C	1	2	4	5	7	9
1	129.9	130.0	132.1	132.2	132.2	128.1
2	138.8	138.8	135.8	135.6	136.1	139.5
3	80.6	80.7	82.7	82.2	82.8	79.4
4	84.4	84.4	84.8	85.9	85.0	85.5
5	73.8	73.8	76.7	74.7	74.9	75.3
6	136.8	136.8	139.3	137.9	136.1	138.5
7	128.4	128.5	128.4	128.7	129.3	129.3
8	44.1	44.1	43.5	43.4	43.9	44.0
9	206.7	206.6	206.7	205.7	206.2	206.8
10	72.5	72.6	72.0	72.0	72.1	73.9
11	39.7	39.7	38.4	38.7	38.8	38.5
12	31.0	31.0	31.2	31.2	31.2	31.8
13	23.1	23.1	23.1	23.2	23.3	24.0
14	23.0	23.0	23.3	23.0	23.1	23.3
15	23.9	23.9	24.0	24.3	24.0	23.7
16	28.5	28.5	28.5	28.5	28.5	28.5
17	15.4	15.4	15.6	15.6	15.5	15.6
18	17.4	17.4	17.3	17.3	17.3	18.2
19	15.3	15.3	15.5	15.5	15.6	15.5
20	66.3	66.4	67.3	66.8	66.8	65.3
1′	167.4	167.4	168.1	168.4	167.8	167.4
2′	118.6	120.7	118.2	120.3	117.9	117.3
3′	145.8	140.2	146.2	142.8	146.3	147.7
4′	128.2	126.3	128.2	126.3	128.1	126.8
5′	145.4	142.1	146.8	143.1	146.9	143.0
6′	32.9	28.3	33.0	28.3	33.0	33.1
7′	28.4	29.0	28.3	29.0	28.3	28.3
8′	31.3	31.4	31.4	31.4	31.3	31.4
9′	22.5	22.5	22.5	22.5	22.5	22.5
10′	14.0	14.0	14.0	14.0	14.0	14.0
acetate				171.5	171.0	
				20.9	21.1	

[a] Assignments confirmed by decoupling, H−H COSY, HMQC, HMBC, and NOESY spectra.

resonated at δ 5.40, moving downfield by 1.71 ppm, and the H-20 signal of **9** resonated at δ 3.99, 3.95 (ABq), moving upfield by about 0.8 ppm, compared to **1**. Further HMBC analysis also confirmed this assignment. The geometric configuration of the 2′,4′-diene system was confirmed to be 2′*E*,4′*E* since the spectral data of the decadienoate group were identical with those of **1** and **4**. Thus, **9** was concluded to be 5-*O*-(2′*E*,4′*E*-decadienoyl)ingenol.

The EIMS of **5** exhibited a molecular ion peak at *m*/*z* 540 (M^+), a base peak at *m*/*z* 151 ($C_9H_{15}CO^+$), and three significant fragment peaks at *m*/*z* 480 ($M^+ - HOAc$), 372 ($M^+ - C_9H_{15}COOH$), and 312 ($M^+ - HOAc - C_9H_{15}COOH$). These data suggested that **5** is a diterpene diester with HOAc and $C_9H_{15}COOH$ as esterifying acids. The molecular formula of **5** was determined as $C_{32}H_{44}O_7$ (*m*/*z* 540.30965, HRMS). The ^1H and ^{13}C NMR spectral data of **5** (Tables 1 and 2) were almost identical to published data for 3-*O*-(2′*E*,4′*Z*-decadienoyl)-20-*O*-acetylingenol (**6**),[17] whereas chemical shift considerations indicated that the ester moieties were located at C-3 and C-5. Precise locations of the individual groups were deduced from further analysis of the HMBC spectrum. Correlations of the decadienoate carbonyl C-1′ (δ 168.4) with H-3 (δ 5.03 s) and acetate carbonyl C-1″ (δ 171.5) with H-5 (δ 5.43 s) demonstrated that the 2,4-decadienoyloxy group was situated at C-3 and the acetoxy group at C-5. The spectral data of the 2′,4′-diene system were in good agreement with a *E*,*Z*-configuration. Thus, the structure of compound **5** was established as 3-*O*-(2′*E*,4′*Z*-decadienoyl)-5-*O*-acetylingenol.

Compounds **6** and **7** had the same molecular formula of $C_{32}H_{44}O_7$ as **5** and similar fragmentation patterns (see Experimental Section). The ^1H and ^{13}C NMR spectral data (Tables 1 and 2) assigned for compound **7** were almost identical with those of 3-*O*-(2′*E*,4′*Z*-decadienoyl)-20-*O*-acetylingenol[17] (**6**) and 3-*O*-(2′*Z*,4′*E*-decadienoyl)-20-*O*-acetylingenol,[18] and the chemical shifts of H-3 and H-20 indicated that the ester moieties were at C-3 and C-20. The HMBC spectrum showed cross-peaks between the acetate carbonyl C-1″ (δ 171.0) and H-20 (δ 4.75, 4.50 ABq), and the decadienoate carbonyl C-1′ (δ 167.8) and H-3 (δ 5.57 s), and demonstrated that the acetoxy group was situated at C-20 and the 2,4-decadienoyloxy group at C-3. Spectral data of the decadienoyloxy group were in good agreement with an *E*,*E*-configuration. Thus, the structure of compound **7** was determined to be 3-*O*-(2′*E*,4′*E*-decadienoyl)-20-*O*-acetylingenol.

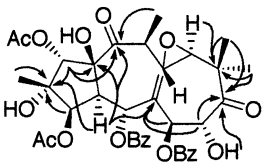

Figure 1. HMBC correlations of quaternary carbons of kansuinin C (**10**) (H−C).

The non-ingenol-based derivative **10** was crystallized from methanol. Its molecular formula ($C_{38}H_{42}O_{14}$) was derived from HREIMS and NMR analysis. The EIMS revealed fragment ions (*m*/*z* 662, 644, 600) from the molecular ion (*m*/*z* 722) produced by the sequential elimination of acetic units and ketenes of the acetoxy groups in the compound. A base peak at *m*/*z* 105 suggested that the molecule contained a benzoyl group. The ^1H and ^{13}C NMR spectra revealed the presence of two acetate groups [δ_H 2.13 s, 1.95 s; δ_C 170.6, 169.8 (CO) and 20.3, 20.6 (CH_3)] and two benzoate groups [δ_H 7.53, 7.48, 7.24, 7.09, 7.02, 6.88; δ_C 132.9, 132.6, 129.4 × 2, 129.1 × 2, 127.9 × 2, 127.7 × 2, 128.6, 128.3, 166.2 (CO), 164.8 (CO)]. The ^{13}C NMR and DEPT spectra suggested that the skeleton consisted of 20 carbons: four methyls, one methylene, nine methines, and six quaternary carbons, including two ketones (δ_C 211.4, 209.3). The ^1H NMR spectrum contained 16 signals due to the parent skeleton, which were assigned with the aid of HMQC and $^1H-^1H$ COSY experiments. The $^1H-^1H$ COSY spectrum defined three structural fragments with correlated protons: −CHR−CHR−CHR− (A), −CHR−CHR− (B), and −CH(CH_3)−CHR−CHR− (C). The long-range correlations of the quaternary carbons (C-2, C-6, C-10, and C-15) with proton signals of the skeleton connected these three fragments and established fragments [CHR−CR_2−CHR−CHR−CHR−C(=CH_2)-CHR−CHR− (C-1−C-8)] and [−CH(CH_3)−CHR−CHR− (C-11−C-13)] of a jatrophane skeleton (Figure 1). The $^3J_{CH}$ correlations between Me-20, H-1, and H-4 and the carbon signal at δ_C 211.4 placed one keto group at C-14. The $^2J_{CH}$ and $^3J_{CH}$ correlations between Me-18, Me-19, H-8, and H-7 and the carbon signal at δ_C 209.3 placed the other keto group at C-9. The correlations of the benzoate carbonyl C-5′ (δ_C 164.8) with H-5 (δ_H 5.95) demonstrated that one benzoloxy group was situated at C-5. The attachment of the C-7 benzoyl group and the C-1 and C-3 diacetyl groups was ascertained in the same manner. With regard to the molecular formula and substitutions already postulated, the presence of a further epoxy group in the molecule was concluded. Chemical shift values of H-11, H-12 (δ_H 3.65 d and 3.43 m), C-11 and C-12 (δ_C 65.0, 58.0) indicated that the epoxy group must be at positions C-11 and C-12.[19] The relative stereochemistry of **10** was assigned on the basis of NOESY and NOE difference experiments. A convenient point of reference was H-4, which was assumed to be α.[20,21] H-13 and H-4 exhibited a NOE correlation, which would be possible only with a trans-ring junction and with an α-configuration of H-13 and

Table 3. ^{1}H and ^{13}C NMR Data for Compounds **10**−**12** [(500 and 125 MHz, $CDCl_3$, TMS, δ (ppm) (J = Hz)][a]

	10		**11**		**12**	
position	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1	4.93 s	83.4	4.32 d (3.9)	87.1	2.65 dd (6.4,13.9)	40.3
					2.20 m	
2		80.0		78.3	2.12 m	38.8
3	5.38 d (4.9)	79.7	5.54 d (4.9)	76.7	5.58 m	74.4
4	3.48 m	46.4	3.61 dd (11.3, 4.8)	45.1	2.97 brs	51.4
5	5.95 s	74.3	5.91 m	73.8	6.13 s	70.1
6		135.8		135.8		145.4
7	5.87 s	65.1	5.89 m	64.6	6.39 s	69.1
8	4.70 d (9.4)	72.5	4.65 d (9.2)	72.7	6.05 s	71.0
9		209.3		209.5	5.07 s	82.4
10		47.9		48.1		41.5
11	3.65 d (2.1)	61.2	3.69 d (2.2)	60.8	4.13 s	77.5
12	3.43 m	58.0	3.33 dd (2.4, 9.4)	59.0		214.0
13	3.26 m	41.6	3.93 m	42.6	2.28 q (6.5)	50.7
14		211.4		204.9		106.3
15		84.8		96.1		90.6
16	1.32 s	19.4	1.31 s	20.2	0.92 d (6.3)	13.3
17	6.31 s	127.9	6.52 brs	128.3	5.24 s	106.3
	5.91 s		5.94 brs		5.14 s	
18	1.33 s	21.6	1.34 s	21.6	1.29 s	18.6
19	0.85 s	18.9	0.85 s	19.0	1.14 s	22.1
20	1.66 d (6.4)	19.2	1.52 d (6.4)	17.0	1.30 d (6.5)	9.21
Acetyls			Acetyls		Acetyls	
CO-1		170.6	CO-15	172.4	CO-3, 15	169.5
						170.2
COMe-1	2.13 s	20.3	COMe-15	2.31 s	21.3	COMe-	1.98 s	22.0
						3,15	2.09 s	21.3
CO-3		169.8	CO-3		168.8	CO-5		168.8
COMe-3	1.95 s	20.6	COMe-3	1.89 s	20.4	COMe-5	1.91 s	20.9
						CO-7		170.3
						COMe-7	2.18 s	21.1
						CO-9		169.2
						COMe-9	2.07 s	20.4
Benzoyls			Benzoyls			Benzoyls		
CO-5		164.8			164.9	CO-8		165.4
COPh-5 1		128.3			128.3	COPh-8 1		130.1
2,6	7.48 m	129.1		7.55 m	129.3	2,6	8.03 m	129.9
3,5	6.88 m	127.7		7.02 m	127.7	3,5	7.42 m	128.3
4	7.09 m	132.6		7.22 m	132.8	4	7.53 m	132.9
CO-7		166.2			166.0			
COPh-7 1		128.6			128.4			
2,6	7.53 m	129.4		7.50 m	129.5			
3,5	7.02 m	127.9		6.92 m	127.8			
4	7.24 m	132.9		7.11 m	132.8			
OH-2	2.32 s		OH-2	2.52 s				
OH-8	3.53 d (9.4)		OH-8	3.54 d (9.4)				
OH-15	4.11 s		OH-1	3.92 d (3.9)				

[a] Assignments confirmed by decoupling, H−H COSY, HMQC, HMBC, and NOESY spectra.

a β-configuration of Me-20.[14] H-13 exhibited a NOE correlation with H-11, and, in turn, H-11 had a NOE correlation with the signal at δ_H 1.33, which indicated H-11 was α. In addition, Me-20 had a NOE correlation with the H-12 signal, showing that H-12 is β. Irradiation of the signal at δ_H 0.85 caused an NOE of H-12, demonstrating that the former resonance was Me-19. The large coupling constant (J = 11.0 Hz) between H-4 and H-5, taken in conjunction with the observation of NOEs from H-5 to OH-15, required that H-5 be β. A combination of NOEs from H-4 to H-7 and from Me-19 to H-8 and the zero ^3J (H-7 to H-8) coupling constant was best accommodated by designating H-7 as α and H-8 as β. Irradiation of H-4 also produced a NOE at H-3, which showed that H-3 is α. The OH-15 signal had a NOE correlation with H-1 and Me-2, indicating H-1 and Me-2 are both β. The absolute configuration of **10** was elucidated by the CD exciton chirality method.[22] The CD spectrum of **10** showed well-split intense Cotton effects ($\Delta\epsilon_{242}$ −7.3 and $\Delta\epsilon_{210}$ +11.7), indicating that the benzoate at C-5 and the benzoate at C-7 should be anticlockwise, which clearly specified the 7*S* configuration of the diterpene. In conclusion, the structure of compound **10** was established as (7*S*)-72α,8α,15β-trihydroxy-1α,3β-diacetoxy-5α,7β-dibenzoloxyjatropha-6(17)-en-9,14-dione, which has been given the name kansuinin C.

Isolates **3**, **6**, and **8** were known compounds, whose structures were elucidated by comparisons with the literature.[12,15,23] Compounds **12** and **11** are known as kansuinins A and B, respectively. However, unambiguous NMR data for these molecules were not all provided in earlier reports.[7] Therefore, we report herein the ^{1}H and ^{13}C NMR data for these two compounds (Table 3).

In an attempt to determine whether cell growth was inhibited by the diterpenes from the roots of *Euphorbia kansui*, we tested the effects on division of isolated cells from the early *Xenopus laevis* embryo. Single cells from mid-blastula-stage embryos are able to divide in a non-nutritive medium. Under the standard conditions of the present study, most cells divided between 4 and 10 times. Six different concentrations of **1**−**9** were tested, namely, 0.1, 0.5, 2, 10, 50, and 200 μg/mL. These ingenol derivatives showed significant cleavage arrest activity (0.5 μg/mL of each compound resulted in >75% cleavage arrest). Diterpenes with a jatrophane skeleton (**10** and **12**) did not inhibit

cell division of the isolated cells. Treatment of cells with 10, 50, or 200 μg/mL kansuinin B (**11**) whose structure is very similar to **12** resulted in cleavage arrest in 57%, 87%, and 98% of cells, respectively. About 18% of the cells incubated without these compounds were cleavage-arrested.

As the early embryonic cell cycle in *X. laevis* consists of only S and M phases and does not include the G1 or G2 phases,[24,25] arrest of the cell cycle by *E. kansui* is unrelated to the inhibition of reactions at the G1 → S phase transition. Arrest of the cell cycle of *X. laevis* embryos by diterpenes from *E. kansui* may be related to the preservation or progression of the M phase.

Experimental Section

General Experiment Procedures. Melting points were determined on the Yanagimoto micro-melting-point apparatus and are uncorrected. Optical rotations were taken in MeOH on a JASCO DIP-360 polarimeter. The UV spectra were obtained in MeOH on a Hitachi 200-10 spectrophotometer, and the IR spectra were recorded on a JASCO IR A-2 spectrophotometer. CD spectra were obtained in MeOH with a JASCO J-600 spectrophotometer. The NMR spectra were taken on a JEOL GL-500 spectrometer, with TMS as an internal standard. The mass spectra (MS) were obtained on a Hitachi M-80B 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 detector. Senshu Pak PEGASIL silica 60-5 (10 × 250 mm i.d.) and Senshu pak PWGASIL ODS (10 × 250 mm i.d.) columns were used for preparative purposes.

Plant Material. The dried roots of *Euphorbia kansui* L. were collected in Xianyang, Sannxi Province, People's Republic of China, in October 1997, and identified by Prof. Weichun Wu (Department of Medical Plants, Shenyang Pharmaceutical University, People's Republic of China). A voucher specimen has been deposited at the Department of Natural Products Chemistry of Shenyang Pharmaceutical 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, 23, and 64 g, respectively, and the residual aqueous extract weighed 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 7, eluted with hexane−EtOAc (60:40), was isolated and further purified by column chromatography and HPLC (Senshu Pak PWGASIL silica 60-5, 10 × 250 mm, hexane−EtOAc, 7:3, flow rate 4 mL/min; UV detector set at 254 nm) to give **7** (9 mg, t_R 11.23 min), **1** (12 mg, t_R 14.12 min), and **2** (14 mg, t_R 17.56 min), by HPLC (Senshu Pak PWGASIL silica 60−5, 10 × 250 mm, hexane−EtOAc, 6:4, flow rate 4 mL/min; UV detector set at 254 nm) to give **3** (17 mg, t_R 14.21 min) and **4** (14 mg, t_R 16.26 min), by HPLC (Senshu Pak PWGASIL silica 60-5, 10 × 250 mm, hexane−EtOAc, 8:2, flow rate 4 mL/min; UV detector set at 254 nm) to give **5** (8 mg, t_R 14.12 min) and **6** (11 mg, t_R 17.56 min), by HPLC (Senshu Pak PWGASIL silica 60-5, 10 × 250 mm, $CHCl_3$−EtOAc, 97:3, flow rate 4 mL/min; UV detector set at 210 nm) to give **8** (11 mg, t_R 11.59 min), by HPLC (Senshu Pak PWGASIL silica 60−5, 10 × 250 mm, hexane−EtOAc−$CHCl_3$, 7:3:10, flow rate 4 mL/min; UV detector set at 254 nm) to give **9** (234 mg, t_R 14.12 min), and by reversed-phase HPLC (Senshu Pak PWGASIL ODS column, 10 × 250 mm, CH_3CN−H_2O, 1:1, flow rate 4 mL/min; UV detector set at 254 nm) to give **10** (14 mg, t_R 6.42 min), **11** (12 mg, t_R 11.12 min), and **12** (56 mg, t_R 12.45 min).

20-*O*-(2′*E*,4′*E*-Decadienoyl)ingenol (1): colorless oil; $[\alpha]^{23}_D$ +3.15° (*c* 0.19, MeOH); UV (MeOH) λ_{max} (log ϵ) 258 (3.41), 204 (3.92); IR (KBr) ν_{max} 3441, 2956, 2930, 2869, 1718, 1642, 1269, 1177, 1064, 877 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 498 $[M]^+$ (1), 480 (2), 402 (2), 330 (15), 312 (21), 151 (100); HREIMS *m/z* 498.29898 (calcd for $C_{30}H_{42}O_6$, 498.29811).

20-*O*-(2′*E*,4′*Z*-Decadienoyl)ingenol (2): colorless oil; $[\alpha]^{23}_D$ +2.50° (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ϵ) 264 (3.82), 205 (3.91); IR (KBr) ν_{max} 3434, 2956, 2929, 2869, 1716, 1636, 1268, 1175, 1067, 751 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 498 $[M]^+$ (1), 480 (1), 402 (2), 330 (15), 312 (21), 151 (100); HREIMS *m/z* 498.29826 (calcd for $C_{30}H_{42}O_6$, 498.29811).

3-*O*-(2′*E*,4′*E*-Decadienoyl)ingenol (4): colorless oil; $[\alpha]^{23}_D$ +89.09° (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 268 (4.31), 206 (4.02); IR (KBr) ν_{max} 3439, 2956, 2926, 2862, 1710, 1640, 1269, 1177, 1064, 877 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 498 $[M]^+$ (1), 480 (2), 402 (2), 330 (15), 312 (21), 151 (100); HREIMS *m/z* 498.29730 (calcd for $C_{30}H_{42}O_6$, 498.29811).

3-*O*-(2′*E*,4′*Z*-decadienoyl)-5-*O*-acetylingenol (5): colorless oil; $[\alpha]^{23}_D$ +61.73° (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 261 (3.74), 203 (4.23); IR (KBr) ν_{max} 3477, 2957, 2928, 1722, 1633, 1267, 1168, 1072, 870 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 540 $[M]^+$ (1), 480 (2), 372 (15), 151 (100); HREIMS *m/z* 540.30965 (calcd for $C_{32}H_{44}O_7$, 540.30870).

3-*O*-(2′*E*,4′*E*-decadienoyl)-20-*O*-acetylingenol (7): colorless oil; $[\alpha]^{23}_D$ +84.1° (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 266 (4.11), 203 (4.23); IR (KBr) ν_{max} 3478, 2950, 2927, 1725, 1633, 1263, 1168, 1071, 871 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 540 $[M]^+$ (1), 480 (2), 372 (15), 151 (100); HREIMS *m/z* 540.30799 (calcd for $C_{32}H_{44}O_7$, 540.30870).

5-*O*-(2′*E*,4′*E*-Decadienoyl)ingenol (9): colorless oil; $[\alpha]^{23}_D$ −7.69° (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ϵ) 264 (4.31), 202 (4.12); IR (KBr) ν_{max} 3440, 2961, 2927, 2858, 1713, 1640, 1261, 1197, 1099, 875 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 498 $[M]^+$ (1), 480 (2), 402 (2), 330 (15), 312 (21), 151 (100); HREIMS *m/z* 498.29650 (calcd for $C_{30}H_{42}O_6$, 498.29811).

Kansuinin C (10): colorless crystals (MeOH); mp 287−289 °C; $[\alpha]^{23}_D$ 37.0° (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 230 (4.21); IR (KBr) $_{max}$ 3509, 1741, 1711, 1651 cm^{-1}; CD (MeOH) λ_{ext} 210 nm ($\Delta\epsilon$ +11.7), 242 nm ($\Delta\epsilon$ −7.3); ^1H and ^{13}C NMR data, see Table 3; EIMS *m/z* 722 $[M^+]$ (1), 704 (0.7), 662 (0.5), 582 (1), 105 (100), 77 (65); HREIMS *m/z* 722.25154 (calcd for $C_{30}H_{42}O_{14}$, 722.25135).

Animal Cap Assay. Animal caps from *X. laevis* pieces were dissected stage 8 blastulae. Single cells from the inner surface of the pieces were separated off by directing a gentle stream of calcium- and magnesium-free medium (50 mM phosphate buffer containing 35 mM NaCl and 1 mM KCl, pH 7.0) as described by Godsave and Slack.[14] 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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