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

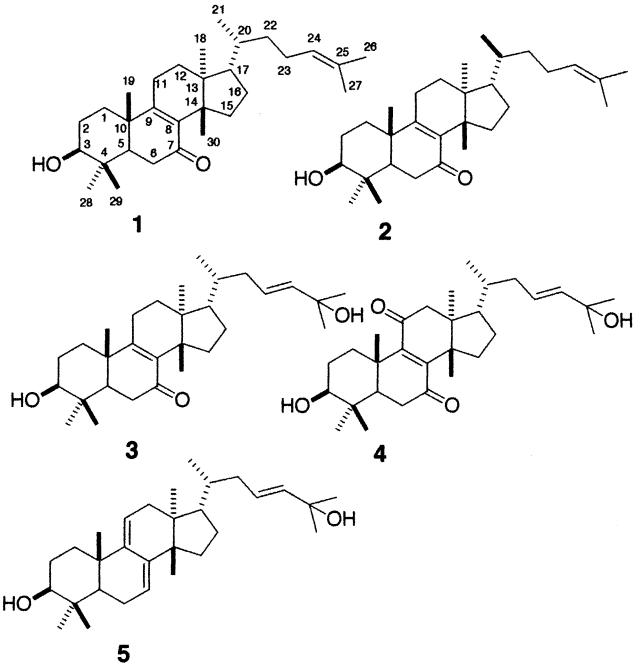
Li-Yan Wang,[†,§] Nai-Li Wang,[†] Xin-Sheng Yao,*[,†] Syohei Miyata,[‡] and Susumu Kitanaka*[,§]

Department of Natural Products Chemistry, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenhe District Shenyang 110016, People's Republic of China, Department of Chemistry, College of Humanities and Sciences, Nihon University, Sakurajosui, Setagaya-ku, Tokyo 156-8550, Japan, and College of Pharmacy, Nihon University, 7-7-1 Narasinodai, Funabashi, Chiba 274-8555, Japan

Received November 22, 2002

Four new euphane-type triterpenes, kansenone (**1**), kansenonol (**3**), 11-oxo-kansenonol (**4**), kansenol (**5**), and a new tirucallane-type triterpene, epi-kansenone (**2**), were isolated from a 60% EtOH extract of *Euphorbia kansui*, together with α-euphol. Their structures were elucidated on the basis of extensive analysis of their 1D and 2D NMR spectral data. This appears to be the first report of the natural occurrence of euphane/tirucallane-type triterpenes with a ketone at C-7. In vitro treatment of cultured individual *Xenopus laevis* cells at the blastular stage with **1**−**4** significantly arrested cleavage of the cells (10 μg/mL of each compound resulted in >50% cleavage arrest).

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 a herbal remedy for edema, ascites,[2,3] and cancer[4−6] in mainland China. In a previous paper,[7] we reported the isolation of nine ingenol esters, 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*-(decanoyl)ingenol, and 5-*O*-(2′*E*,4′*Z*-decadienoyl)ingenol, and three jatrophane diterpenes, kansuinins A, B, and C, from the 60% EtOH−40% H_2O extract of the roots of *E. kansui*. In vitro treatment of cultured individual *Xenopus* cells at the blastular stage with the ingenol esters arrested cleavage significantly. Further bioassay-directed fractionation of the 60% EtOH extract has led to the isolation of five new compounds (**1**−**5**). In this paper, we report the structure characterization and the biological evaluation of these compounds.



Results and Discussion

The HREIMS of kansenone (**1**) indicated a molecular ion peak at *m/z* 440.3646, which corresponded to the molecular formula $C_{30}H_{48}O_2$. The MS showed fragment ions at *m/z* 425 $[M - CH_3]^+$ and 407 $[M - CH_3 - H_2O]^+$. Fragment ions at *m/z* 273 [M − side chain − part of ring D − CH_3]$^+$, 327 [M − side chain − 2H]$^+$, and 69 $[CH_2CH{=}C(Me)_2]^+$ suggested the presence of a monounsaturated side chain of a triterpene.[8] Hydroxyl (3414 cm^{-1}) and α,β-unsaturated ketone (1655 cm^{-1}) absorptions were observed in the IR spectrum. The UV spectrum displayed an absorption maximum at 255 nm. The ^1H and ^{13}C NMR spectra (Tables 1 and 2) exhibited resonances for a trisubstituted double bond (δ_H 5.08, m; δ_C 125.1, d, and 131.0, s), an α,β-unsaturated ketone (δ_C 198.3, s, 138.9, s, and 165.4, s), a secondary alcohol methine [δ_H 3.29, dd, (J = 11.6, 4.6); δ_C 78.0, s], and one secondary [δ_H 0.88, d (J = 6.0)] and seven tertiary methyl groups [δ_H 0.72, 1.05, 1.68, 1.61, 0.99, 0.88, and 0.97 (each 3H, s)], in addition to nine methylene carbons, three methine carbons, and four quaternary carbons. The molecular formula indicated the presence of seven units of unsaturation. The compound must therefore be tetracarbocyclic since there are only two olefinic groups and one ketone. The detailed analysis of **1** using $^1H-^1H$ COSY and HMQC techniques disclosed five partial structural units (allylic couplings were observed between H-24 and H-26 and H-27 in the $^1H-^1H$ COSY spectrum). This was also supported by analysis of the HMBC spectrum, which showed two- and three-bond correlations between H-1 and C-2; between H-3 and C-1; between H-6 and C-5; between H-11 and C-12; between 3H-21 and C-17, C-20, and C-22; between H-23 and C-24 and C-25; and between H-24 and C-26, C-27, and C-22. These data confirmed the four partial structures above. Furthermore, the HMBC correlations of the five individual tertiary methyl signals

* To whom correspondence should be addressed. Tel: +81-47-465-5356. Fax: +81-47-465-5440. E-mail: kitanaka@pha.nihon-u.ac.jp.
† Shenyang Pharmaceutical University.
‡ Department of Chemistry, College of Humanities and Sciences, Nihon University.
§ College of Pharmacy, Nihon University.

10.1021/np0205396 CCC: $25.00
Published on Web 04/25/2003

Table 1. ^{1}H NMR Data for Compounds **1**, **2**, **3**, **5** (500 MHz), and **4** (400 MHz) ($CDCl_3$, TMS, δ (ppm), J = Hz)[a]

	1	2	3	4	5
1α	1.45 m	1.45 m	1.45 m	1.10 m[c]	1.56 m[b]
1β	1.86 dt (13.1, 3.3)	1.86 dt (13.1, 3.3)	1.86 dt (13.1, 3.3)	2.55 m[c]	1.82 dt (13.1, 3.3)
2	1.75 m	1.75 m	1.75 m	1.76 m	1.73 m[b]
	1.67 m[b]	1.67 m[b]	1.67 m[b]	1.67 m[b]	1.66 m[b]
3α	3.29 dd (4.6, 11.6)	3.29 dd (4.6, 11.6)	3.29 dd(4.6, 11.6)	3.30 dd (5.8, 10.5)	3.24 dd (4.0, 11.3)
5α	1.67 m[b]	1.67 m[b]	1.67 m[b]	1.62 m[b]	1.28 dd (4.6, 11.6)
6α	2.40 dd (3.9, 15.8)	2.41 dd (3.9, 15.8)	2.41 dd (3.9, 15.8)[b]	2.45 m[b]	2.21 dt (18.0, 4.9)
6β	2.38 dd (12.4, 15.8)	2.38 dd (12.4, 15.8)	2.38 dd (12.4, 15.8)[b]	2.50 m[b]	2.08 m[b]
7					5.34 m
11α	2.37 m[b]	2.37 m[b]	2.37 m[b]		5.19 brs
11β	2.24 ddd (4.2, 4.2, 20.4)	2.24 ddd (4.2, 4.2, 20.4)	2.24 ddd (4.2, 4.2, 20.4)		
12α	1.76−1.80 m	1.76−1.80 m	1.76−1.80 m	2.42 d (20.0)[b]	2.16 d (4.0, 2H)
12β				2.66 d (20.0)	
15α	1.56 m	1.56 m	1.56 m[b]	1.65 m	1.65 m[b]
15β	2.13 m	2.13 m	2.13 m	2.17 m	1.35 m[b]
16α	1.33 m	1.33 m	1.33 m	1.41 m	1.96
16β	1.93 m	1.93 m	1.93 m	2.02 m	1.31 m[b]
17	1.43 m	1.47 m	1.57 m[b]	1.65 m	1.62 m[b]
18	0.72 s	0.73 s	0.75 s	0.94 s	0.65 s
19	1.05 s	1.05 s	1.05 s	1.31 s[b]	0.92 s
20	1.43 m	1.43 m	1.55 m[b]	1.55 m	1.52 m[b]
21	0.88 d (6.0)	0.93 d (6.1)	0.84 d (6.1)	0.86 d (6.6)	0.82 d (7.0)
22	1.13 m, 1.56 m	1.06 m, 1.49 m	1.82 m, 2.32 m[b]	1.75m, 2.24 m	1.73 m, 2.33 m
23	1.90 m, 2.04 m	1.83 m, 2.04 m	5.58 m[b]	5.58 m[b]	5.59 m[b]
24	5.08 m	5.10 m	5.58 m[b]	5.58 m[b]	5.59 m[b]
26	1.68 s	1.68 s	1.32 s[b]	1.31 s[b]	1.32 s[b]
27	1.61 s	1.60 s	1.32 s[b]	1.31 s[b]	1.32 s[b]
28	0.99 s	0.99 s	1.00 s	1.03 s	0.99 s
29	0.88 s	0.88 s	0.89 s	0.90 s	0.89 s
30	0.97 s	0.96 s	0.97 s	1.08 s	0.85 s

[a] Assignments confirmed by decoupling, ^{1}H−^{1}H COSY, HMQC, HMBC, NOESY, and different NOE spectra. [b] Overlapped signal.

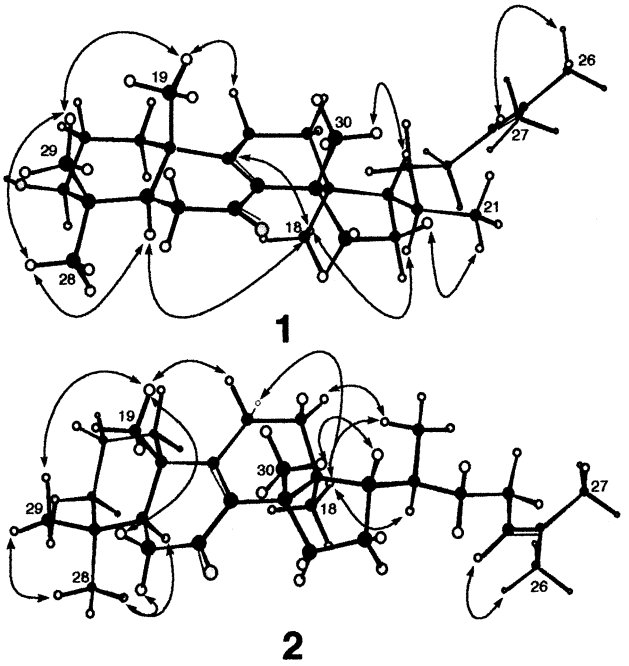


Figure 1. Key NOE correlations for **1** and **2**.

on rings A−D [between Me-28 (δ_H 0.99) and C-29, C-4, C-3, and C-5; between Me-29 (δ_H 0.88) and C-28, C-4, C-3, and C-5; between Me-19 (δ_H 1.05) and C-1, C-5, C-9, and C-10; between Me-30 (δ_H 0.97) and C-8, C-13, C-14, and C-15; between Me-18 (δ_H 0.72) and C-12, C-13, C-14, and C-17] and HMBC correlations between H-12 (δ_H 1.78) and C-9 and between H-11 (δ_H 2.24) and C-8 and C-9 firmly established the linkages of these partial structural units. The signal of C-6 appeared at δ_C 35.8, due to the presence of a ketone group at C-7. Analysis of the HMBC spectrum established the connectivity of C-7 to C-6 and C-5. Comparing the ^{13}C NMR of **1** (Table 2) with those of a lanostane-type triterpene which has the same plane structure (7-keto-Δ^{8-24}-lanostan-3β-ol),[9] significant differences were observed in C-5, C-6, C-7, C-9, C-10, C-15, C-17, C-20, C-22, and C-30 [$\Delta\delta_C$ = −1.7, −0.8, −0.7, +0.5, −0.4, −0.6, −0.7, −0.5, −0.8, −0.6 ($\Delta\delta_C = \delta_{\mathbf{1}} - \delta_{\text{lanostane-type}}$)]. The relative configuration for **1** was determined as follows: first, the large coupling constant of H-3 indicated that the hydroxyl group was oriented equatorially (β) at C-3.[10] Next, the relative configurations of the methyl groups and other protons in the rings A−D were ascertained on the basis of the difference NOE experiments. Compound **1** showed significant NOE correlations between Me-29 (4β-Me) and Me-19 (10β-Me), between Me-19 (10β-Me) and H-6β and H-11β, between Me-30 (14β-Me) and H-17β, between Me-28 (4α-Me) and H-3α, H-5α, and H-6α, and between Me-18 (13α-Me) and H-5α, H-11α, and H-20α. Furthermore, the NOE between Me-21 and H-16α and the absence of NOE between Me-18 and Me-21 were consistent with those of euphane-type triterpenes.[10,11] The ^{1}H NMR chemical shift (δ_H 0.88, d, J = 6.0) of Me-21 and the positive optical rotation of **1** (+14.1°) also indicated that **1** belonged to the euphane rather than the tirucallane series.[12−14] Thus, kansenone (**1**) was elucidated to be eupha-8,24-diene-3β-ol-7-one.

The ^{1}H and ^{13}C NMR shifts of epi-kansenone (**2**) and kansenonol (**3**) closely resembled those of **1** (Tables 1 and 2). The ring carbon signals in the ^{13}C NMR spectra were

Table 2. ^{13}C NMR Data for Compounds **1**, **2**, **3**, **5** (125 MHz), and **4** (100 MHz) ($CDCl_3$, TMS, δ (ppm))[a]

position	1	2	3	4	5
1	34.6	34.6	34.6	33.9	35.6
2	27.4	27.4	27.4	27.4	27.7
3	78.0	78.0	78.0	77.9	79.0
4	38.8	38.8	38.8	38.5	39.0
5	48.1	48.2	48.2	48.5	48.2
6	35.8	35.8	35.8	35.7	23.6
7	198.3	198.3	196.3	199.8	118.6
8	138.9	138.9	138.9	149.7	141.2
9	165.4	165.4	165.3	154.7	145.1
10	39.3	39.3	39.3	38.0	36.14
11	23.7	23.7	23.7	201.7	115.1
12	29.9	29.9	30.0	51.3	38.4
13	44.6	44.6	44.7	45.1[b]	44.0
14	47.7	47.6	47.6	47.8[b]	49.6
15	31.4	31.4	31.3	31.7	31.1
16	28.7	28.6	28.4	27.9	27.9
17	48.2	48.7	48.9	48.9	50.6
18	15.7	15.5	15.9	18.6	16.4
19	18.6	18.6	18.6	17.7	20.7
20	35.6	36.2	35.9	36.0	36.11
21	18.8	18.7	19.6	18.7	19.0
22	35.5	36.3	38.2	37.7	38.0
23	24.7	24.9	125.5	124.8	125.7
24	125.1	125.1	139.4	139.8	139.3
25	131.0	131.0	70.8	70.7	70.7
26	25.7	25.7	29.9[b]	30.0[b]	29.88[b]
27	17.7	17.6	30.0[b]	30.1[b]	29.92[b]
28	27.3	27.3	27.2	27.6	27.6
29	15.1	15.1	15.0	15.0	15.3
30	24.4	24.3	24.4	24.0	23.1

[a] Assignments confirmed by decoupling, $^1H-^1H$ COSY, HMQC, HMBC, NOESY, and different NOE spectra. [b] Assignments within a column may be reversed.

almost identical with those of **1**, suggesting that **2** and **3** had the same tetracarbocyclic skeleton.

Compound **2** showed the same M^+ at *m/z* 440.3651 ($C_{30}H_{48}O_2$) in the HREIMS as **1**. The only differences in the ^{13}C NMR between **2** and **1** were in C-17−C-23 of the C-17 side chain, viz., C-17 ($\Delta\delta_C = +0.5$; $\Delta\delta_C = \delta_2 - \delta_1$), C-20 (+0.6), and C-22 (+0.8). The negative optical rotation (−10.2°) and ^1H NMR chemical shift (δ_H 0.93, d, $J = 6.1$) of Me-21 suggested that **2** belonged to the tirucallane series.[12−14] Furthermore, **2** showed NOE correlations very similar to those of **1** except NOEs between Me-18 and H-20 and Me-21 and between Me-21 and H-12. These NOE correlations were consistent with those of tirucallane-type triterpenes.[15] Thus, epi-kansenone (**2**) was elucidated to be tirucalla-8,24-diene-3β-ol-7-one.

Compound **3** showed M^+ at *m/z* 456.3606 ($C_{30}H_{48}O_3$), which was 16 mass units (a hydroxyl group) larger than **1** and **2**. The overlapping signals at δ_H 5.58, attributed to two olefinic protons, did not offer much information as to the nature of the double bond. However, when the ^1H NMR spectrum was recorded in C_6D_6, the signal changed to an AB quartet with one member further coupled to a neighboring methylene [δ_H 5.60, ddd ($J = 15.5, 7.0, 5.8$) and δ_H 5.67, d ($J = 15.5$)], establishing the presence of a *trans*-double bond in the side chain. The deshielded nature of the methyls (δ_H 1.32, s, 6H) indicated they must be attached to the fully substituted oxygenated carbon (δ_C 70.8), which, in turn, must carry the tertiary hydroxyl group.[16] $^1H-^1H$ COSY, HMQC, and HMBC analysis confirmed the side chain to be [$CH_2-CH{=}CH-C(CH_3)_2OH$]. The NOE between Me-18 and H-20 and H-22, between Me-21 and H-16α, and between Me-30 and H-17, and the absence of NOE between Me-18 and Me-21, were consistent with those of **1.** Thus, kansenonol (**3**) was elucidated to be (23*E*)-eupha-8,23-diene-3β,25-diol-7-one.

The ^1H and ^{13}C NMR shifts of 11-oxo-kansenonol (**4**) were similar to those of **3** (Tables 1 and 2). However, a methylene signal for C-11 observed in the spectrum of **3** was absent in that of **4**. Instead, a new signal at δ_C 201.7 and lower-field shifts of C-12 by 21.3 ppm were observed, indicating that **4** has a carbonyl group at C-11. The connectivity of the carbonyl group was confirmed by HMBC correlations observed between H_2-12 [δ_H 2.45 d (20.0) and 2.66 d (20.0)] and C-11 (δ_C 201.7). The carbon signals at δ_C 149.7 and 154.7 assigned as C-8 and C-9 correlated with Me-30 and Me-19 methyl protons, respectively, in the HMBC spectrum. The configuration of a hydroxyl group at C-3 was assigned as β on the basis of multiplicity [δ_H 3.30 dd (10.5, 5.8)]. The relative configurations of the methyl groups and other protons in the rings A−D were ascertained on the basis of the difference NOE experiment. Compound **4** showed significant NOE correlations between Me-29 (4β-Me) and H-6β, between Me-19 (10β-Me) and Me-28 (4α-Me), between Me-30 (14β-Me) and H-17β, between Me-28 (4α-Me) and H-3α, H-5α, and H-6α, and between Me-18 (13α-Me) and H-5α, H-22, and H-23, and the absence of NOE between Me-18 and Me-21, which were consistent with those of euphane-type triterpenes.[10,11] Compound **4** was accordingly determined to be (23*E*)-eupha-8,23-diene-3β,25-diol-7,10-dione.

The molecular formula of kansenol (**5**) was $C_{30}H_{48}O_2$ on the basis of HREIMS ($[M]^+$, *m/z* 440.3654), ^{13}C NMR, and DEPT. The compound had a $\Delta^{7,9(11)}$-conjugated diene [λ_{max} (log ϵ) 232 (3.89), 239 (3.93), 248 (3.74) nm; ν_{max} 1633, 808 cm^{-1}; δ_H 5.19 (1H, brs) and 5.34 (1H, m)][17−19] and a secondary [δ_H 0.82 d (7.0)] and seven tertiary methyls [δ_H 0.65, 0.92, 1.32 (6H), 0.99, 0.89, 0.85]. These data in combination with fragment ions *m/z* 422 $[M - H_2O]^+$, 407 $[M - H_2O - Me]^+$, and 127 [M − side chain − 2H]$^+$ suggested that **5** was a triterpene possessing a $\Delta^{7,9(11)}$-diene system. The ^1H and ^{13}C NMR data of the tetracyclic part of the triterpene skeleton was almost the same as antiquol C, a $\Delta^{7,9(11)}$-diene euphane-type triterpene from *E. antiquorum*, [19]but the side chain part was very similar to those of **3** and **4**. The above evidence coupled with the analysis of $^1H-^1H$ COSY, HMQC, and HMBC spectra indicated that **5** possessed a $\Delta^{7,9(11),23}$-triene-3β,25-diol structure. Compound **5** showed significant NOE correlations between Me-29 (4β-Me) and H-6β, between Me-30 (14β-Me) and H-17β, between Me-28 (4α-Me) and H-3α, H-5α, and H-6α, between Me-18 (13α-Me) and H-22 and H-20, between Me-19 (10β-Me) and H-6β, and between Me-21 and H-17 and H-16, and the absence of NOE between Me-18 and Me-21, consistent with those of euphane-type triterpenes.[10,11] Thus, kansenol (**5**) was determined to be (23*E*)-eupha-$\Delta^{7,9(11),23}$-triene-3β,25-diol.

The stereochemistry of these five compounds (**1**−**5**) is consistent with X-ray crystallographic studies reported on tirucallane and euphane-type triterpenes.[20] The structure of the known triterpene, α-euphol, was elucidated by comparison with the literature.[21]

Compounds **1**, **2**, **3**, and **4** were tested on division of isolated cells from early *Xenopus laevis* embryos. Single cells from mid-blastula stage embryos were able to divide in a non-nutritive medium. Under the standard conditions of the present study, most cells divided between 4 and 10 times.[22] The effects of **1**, **2**, **3**, and **4** on the division of these cells were studied at four different concentrations: 2, 10, 50, and 200 μg/mL. All four triterpenes induced significant cleavage arrest in the cells (10 μg/mL of each compound resulted in >50% cleavage arrest). About 18% of *Xenopus* cells incubated without these compounds (control) experi-

enced cell cycle arrest, and 47% of the cell growth was inhibited by an anticancer drug, 5-fluorourasil, at a concentration of 10 μg/mL.

As the early embryonic cell cycle in *Xenopus* consists of only S and M phases and does not include the G1 or G2 phases,[23,24] arrest of the cell cycle by *E. kansui* is unrelated to the inhibition of reactions at the G1 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 Section

General Experiment Procedures. UV spectra were obtained in MeOH on a Shimadzu UV-160 spectrophotometer, and IR spectra were recorded on a JASCO IR A-2 spectrophotometer. Optical rotations were recorded in MeOH on a JASCO DIP-360 polarimeter. 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 JEOL GCmate spectrometer. Column chromatography was carried out with silica gel (Wako gel C-300, Wako Pure Chemical Industry Ltd.). TLC was performed on Merck TLC plates (0.25 mm thickness), with compounds visualized by spraying with 5% (v/v) H_2SO_4 in EtOH and then heating on a hot plate. HPLC was performed on a JASCO PU-2089 apparatus equipped with a JASCO UV-2075 detector. A Senshu Pak PEGASIL silica 60-5 (10 × 250 mm i.d.) column was used for preparative purposes.

Plant Materials. The dried root of *Euphorbia kansui* L. was collected in Xianyang, Sannxi Province in the People's Republic of China, in October 1997 and was identified by Prof. Weichun Wu (Department of Medical Plants, Shenyang Pharmaceutical University, People's Republic of China). Voucher specimens (NK001029) 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% EtOH under reflux. Evaporation of the solvent under reduced pressure gave the 60% EtOH extract (1201 g) [inhibitory effect 50 μg/mL, 91%]. The extract was dissolved and suspended in H_2O (4.0 L) and partitioned with $CHCl_3$ (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 yielded 376 g.

The $CHCl_3$ 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 isolated and further purified by column chromatography and HPLC (hexane−$CHCl_3$−EtOAc, 13:4:3, flow rate 4 mL/min; UV detector set at 254 nm) to give **1** (51 mg, t_R 15.6 min), **2** (12 mg, t_R 17.3 min), and **3** (1 mg, t_R 34.3 min). Fraction 7, eluted with hexane−EtOAc (60:40), was further purified by column chromatography and HPLC ($CHCl_3$−EtOAc, 85:15, flow rate 4 mL/min; UV detector set at 254 nm) to give **3** (10 mg, t_R 15.6 min) and **4** (2 mg, t_R 16.7 min).

Kansenone (eupha-8,24-diene-3β-ol-7-one) (1): colorless gum; $[\alpha]^{23}_D$ +14.1° (*c* 0.41, MeOH); UV (MeOH) λ_{max} (log ϵ) 255 (3.75), 202 (3.52); IR (KBr) ν_{max} 3414, 1655, 1456, 1375, 1034, 620 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 440 $[M]^+$ (81), 425 (100), 407 (10), 327 (45), 273 (15), 69 (44); HREIMS *m*/*z* 440.3646 (calcd for $C_{30}H_{48}O_2$, 440.3654).

Epi-kansenone (tirucalla-8, 24-diene-3β-ol-7-one) (2): colorless gum; $[\alpha]^{23}_D$ −10.2° (*c* 0.43, MeOH); UV (MeOH) λ_{max} (log ϵ) 264 (3.75), 205 (3.44); IR (KBr) ν_{max} 3434, 1660, 1456, 1375, 1265, 1375, 617 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 440 $[M]^+$ (100), 425 (91), 407 (10), 327 (25), 273 (16), 69 (47); HREIMS *m*/*z* 440.3651 (calcd for $C_{30}H_{48}O_2$, 440.3654).

Kansenonol ((23*E*)-eupha-8,23-diene-3β,25-diol-7-one) (3): colorless gum; $[\alpha]^{23}_D$ +14.3° (*c* 0.21, MeOH); UV (MeOH) λ_{max} (log ϵ) 255 (3.76), 202 (3.58); IR (KBr) ν_{max} 3427, 1649, 1457, 1373, 755 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 456 $[M]^+$ (41), 438 (100), 423 (99), 329 (90), 69 (10); HREIMS *m*/*z* 456.3606 (calcd for $C_{30}H_{42}O_3$, 456.3603).

11-Oxo-kansenonol ((23*E*)-eupha-8,23-diene-3β,25-diol-7,11-dione) (4): colorless gum; $[\alpha]^{23}_D$ +6.6° (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ϵ) 270 (3.67), 202 (3.64); IR (KBr) ν_{max} 3450, 1665, 1460, 1380, 1228, 1160, 640, 510 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 470 $[M]^+$ (20), 452 (100), 437 (19), 69 (10); HREIMS *m*/*z* 470.3397 (calcd for $C_{30}H_{46}O_4$, 470.3396).

Kansenol ((23*E*)-eupha-$\Delta^{7,9(11),23}$-triene-3β,25-diol) (5): colorless gum; $[\alpha]^{23}_D$ −50.2° (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ϵ) 248 (3.74), 239 (3.93), 232 (3.89); IR (KBr) ν_{max} 3430, 1633, 1460, 1380, 1092, 808, 676 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m*/*z* 440 $[M]^+$ (5), 422 (95), 407 (40), 311 (100); HREIMS *m*/*z* 440.3656 (calcd for $C_{30}H_{48}O_2$, 440.3654).

Animal Cap Assay. Animal caps were dissected from stage 8 *X. laevis* blastulae. Single cells from the inner surface of the caps were separated off by directing a gentle stream of calcium- and magnesium-free medium (50 mM phosphate buffer, 35 mM NaCl, 1mM KCl, pH 7.0) as described by Godsave and Slack.[13] Two or three cells were transferred into a well of a Terasaki plate filled with 10 mL of 2 mg/mL γ-globulin in a simple salt solution (NAM/2) and cultured for 20 h at 25 °C.

Acknowledgment. This investigation was supported in part by a Grant-in Aid for Scientific Research (No. 14572017) and a grant to promote multidisciplinary research projects from the Ministry of Education, Science, Sports and Culture of Japan, by the Japan−China Medical Association, and by Special Research Grant-in Aid for the promotion and mutual aid corporation for private school of Japan to Nihon University.

References and Notes

(1) Hsu, H. Y. *Formosan Sci.* **1986**, *40*, 1−10.
(2) *Zhong Guo Yao Dian* (Chinese Pharmacopoeia); People's Health Press: Beijing, 1985; p 68.
(3) *Zhong Yao Da Zhi Dian* (Dictionary of Chinese Drugs); Shanghai Science and Technology Press: Shanghai, 1977; Vol. 1; p 573.
(4) Kondo, K.; Sugi, M. *Cancer Therapy in Modern China*; Shi Zen Sha; Tokyo, 1977; p 254.
(5) Xia, G. C.; Li, D. H. *Color Atlas of Anticancer Animals, Plants and Mineral Preparations and Their Application*; Tianjin Science, Technology and Translation Publishing Corp: Tianjin, 1999; p 289.
(6) Xu, G. J.; Wang, Q.; Yu, B. Y.; Pan, M. G. *Coloured Illustrations of Antitumour Chinese Traditional and Herbal Drugs*; Fujian Science and Technology Press: Fujian, 1997; p 218.
(7) Part 2 in the series Studies on the bioactive constituents in Euphorbiaceae. For part 1, see: Wang, L. Y.; Wang, N. L.; Yao, X. S.; Miyata, S.; Kitanaka, S. *J. Nat. Prod.* **2002**, *65*, 1246−1251
(8) Deng, J. Z.; Starck, S. R.; Sun, D. A.; Sabat, M.; Hecht, S. M. *J. Nat. Prod.* **2000**, *63*, 1356−1360.
(9) Grey, T. E.; William, K. W.; George, J. S. *Magn. Resonan. Chem.* **1989**, *27*, 1012−1024.
(10) Jiang, Z. H.; Tanaka, T.; Hirata, H.; Fukuoka, R.; Kouno, I. *Tetrahedron* **1997**, 16999−17008.
(11) Akihisa, T.; Kimura. Y.; Kokke, W. C. M. C.; Takase, S.; Yasukawa, K.; Tamura, T. *J. Chem. Soc., Perkin Trans. 1* **1996**, 2379−2384.
(12) Arai, Y.; Hirohara, M.; Ageta, H. *Tetrahedron Lett.* **1989**, *30*, 7209−7212.
(13) Abe, I.; Rohmer, M. *J. Chem. Soc., Perkin Trans. 1* **1994**, 783−791.
(14) Mamta, M.; Yogendra, N.; Sushil. K. *Phytochemistry* **2000**, *54*, 835−838.
(15) Akihisa, T.; Yasukawa, K.; Kimura. Y.; Takase, S. I.; Yamanouchi, S.; Tamura, T. *Chem. Pharm. Bull.* **1997**, *45*, 2016−2023.
(16) Leong, Y. W.; Harrison, L. J. *Phytochemistry* **1999**, *50*, 849−857.
(17) Akihihsa, T.; Tamura, T.; Matsummoto, T.; Eggleston, D. S.; Kokke, W.; Shimizu, N. *J. Chem. Soc., Perkin Trans. 1* **1988**, 439−443.
(18) Goad, L. J.; Akihisa, T. *Analysis of Sterols*; Blackie Academic & Professional: London, 1997.
(19) Akihisa, T.; Wijeratne, E. M.; Tokuda, H.; Enjo, F.; Toriumi, M.; Kimura, Y.; Koike, K.; Nikaido, T.; Tezuka, Y.; Nishino, H. *J. Nat. Prod.* **2002**, *65*, 158−162.
(20) David Nes, W.; Wong, R. Y. Benson, M.; Landery, J. R.; Nes, W. R. *Proc. Natl. Acad. Sci.* **1984**, *81*, 5896−5900.
(21) Gewall, M. B.; Hattori, M. Tezuka, Y.; Kikuchi, T.; Namba, T. *Phytochemistry* **1990**, *29*, 1625−1628.
(22) Godsave, S. F.; Slack, W. J. M. *Dev. Biol.* **1989**, *134*, 486−490.
(23) Newport, J.; Kirschner. M. *Cell* **1982a**, *30*, 675−686.
(24) Newport, J.; Kirschner. M. *Cell* **1982b**, *30*, 687−696.

NP0205396