Bioactive Steroids from the Whole Herb of *Euphorbia chamaesyce*

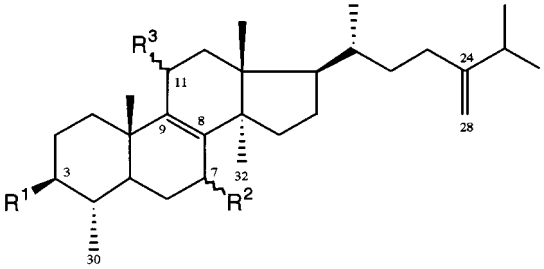
Reiko Tanaka,*,† Kazuaki Kasubuchi,† Shunji Kita,† Harukuni Tokuda,‡ Hoyoku Nishino,‡ and Shunyo Matsunaga†

Department of Medicinal Chemistry, Osaka University of Pharmaceutical Sciences, 4-20-1 Nasahara, Takatsuki, Osaka 569-1094, Japan, and Department of Biochemistry, Kyoto Prefectural University of Medicine, Kamigyo-ku, Kyoto 602-0841, Japan

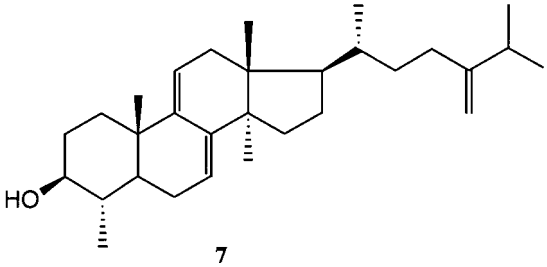
Received August 9, 1999

Three new ergostane-type steroids, 3β-hydroxy-4α,14α-dimethyl-5α-ergosta-8,24(28)-dien-11-one (**1**); 3β,11α-dihydroxy-4α,14α-dimethyl-5α-ergosta-8,24(28)-dien-7-one (**2**); and 3β,7α-dihydroxy-4α,14α-dimethyl-5α-ergosta-8,24(28)-dien-11-one (**3**), were isolated, together with two known triterpenoids, wrightial and lup-20(30)-ene-3β,29-diol from the whole herb of *Euphorbia chamaesyce*. Compound **3** showed a potent inhibitory effect on Epstein−Barr virus early antigen activation induced by the tumor promoter 12-*O*-tetradecanoylphorbol 13-acetate (TPA).

Previously, we reported the structures of 3,4-*seco*-oleana-4(23)-18-dien-3-oic acid and 3,4-*seco*-8β*H*-ferna-4(23),9(11)-dien-3-oic acid, together with seven known triterpenes, from a methylene chloride extract of the whole herb of *Euphorbia chamaesyce* L. (Euphorbiaceae).[1,2] More recently, we published the structures of two new steroids, 3β-hydroxy-4α,14α-dimethyl-5α-ergosta-8,24(28)-dien-7-one (**4**) and 3β-hydroxy-4α,14α-dimethyl-5α-ergosta-8,24-(28)-diene-7,11-dione (**5**), and two known steroids, obtusifoliol (**6**) and 4α,14α-dimethyl-5α-ergosta-7,9(11),24(28)-trien-3β-ol (**7**), from a methylene chloride extract of this herb.[3] Among them, 3,4-*seco*-8β*H*-ferna-4(23),9(11)-dien-3-oic acid and its 3-hydroxyl derivative were found to be selective inhibitors of topoisomerase II activity.[4] Further examination of the methylene chloride extract of *E. chamaesyce* has led to the isolation of three new ergostane-type steroids **1**−**3** together with two known triterpenoids, wrightial and lup-20(30)-ene-3β,29-diol. This paper deals with the structure elucidation of **1**−**3** and the inhibitory effects of these obtusifoliol analogues on Epstein−Barr virus early antigen (EBV-EA) induction in a preliminary screening of their potential antitumor-promoting activities.



	R^1	R^2	R^3
1	OH	H_2	=O
1a	OAc	H_2	=O
2	OH	=O	α-OH
3	OH	α-OH	=O
4	OH	=O	H_2
5	OH	=O	=O
6	OH	H_2	H_2



7

Results and Discussion

The two known compounds were confirmed as wrightial[5] and lup-20(30)-ene-3β,29-diol[6] because their physical and spectral data were in good agreement with those already published. Wrightial was originally isolated from the seed pods of *Wrightia tinctoria*; some differences in the ^{1}H and ^{13}C NMR data assignments were observed in the present study (see Experimental Section).

Compound **1** was assigned the molecular formula $C_{30}H_{48}O_2$ (HREIMS). The UV and IR spectra showed absorption bands for a hydroxyl group (ν_{max} 3421 cm^{-1}), a conjugated enone (λ_{max} 257 nm; ν_{max} 1655 cm^{-1}), and a terminal methylene group (ν_{max} 1640, 887 cm^{-1}). The ^{1}H and ^{13}C NMR and DEPT spectra (Table 1), revealed signals for three tertiary and two secondary methyl groups, an isopropyl group [δ_H 1.026, 1.032 (each 3H, d), 2.22 (1H, sept)], nine methylene groups, four methine groups, three sp^3 quaternary carbons, a hydroxymethine group [δ_H 3.11 (1H, ddd); δ_C 76.5 (d)], a terminal methylene group [δ_H 4.53 (1H, d), 4.55 (1H, s); δ_C 106.1 (t), 156.6 (s)], and a conjugated enone including a tetrasubstituted double bond [δ_C 138.5 (s), 164.4 (s), 199.4 (s)]. Acetylation of **1** afforded a monoacetate (**1a**), of which the carbinol methine proton signal was shifted to δ 4.39 (ddd). The molecular formula and ^{1}H and ^{13}C NMR spectra resembled those of 3β-hydroxy-4α,14α-dimethyl-5α-ergosta-8,24(28)-dien-7-one (**4**), except for the C-7 and C-11 chemical shifts.[3] Accordingly, compound **1** was regarded as a positional isomer of the conjugated enone of **4**. In the EIMS (Figure 1), **1** exhibited the same fragment peaks due to ions **b**−**d**, **f**, and **i**−**m** as those of **4**. A peak corresponding to ion **g**′ was observed as a predominant peak at *m*/*z* 276.2095 $[C_{18}H_{25}O_2]^+$ instead of ion **g** in **4**.[3] This conclusion was supported by the 2D

* To whom correpondence should be addressed. Tel. and Fax: (+81) 726-90-1084. E-mail: tanakar@oysun01.oups.ac.jp.
† Osaka University of Pharmaceutical Sciences.
‡ Kyoto Prefectural University of Medicine.

10.1021/np990394b CCC: $19.00
Published on Web 12/18/1999

Table 1. NMR Data for Compounds **1** and **1a** (125 and 500 MHz, $CDCl_3$)[a,b]

	1			**1a**
position	δ_C	δ_H	HMBC (C → H)	δ_C
1α	33.5 t	1.02 m	2α, 3*β*, 3α, 19	33.2 t
1*β*		2.97 dt (13.5, 3.8)		
2α	31.2 t	1.84 m	1α, 1*β*, 3α, 4*β*	27.2 t
2*β*		1.58 m		
3α	76.5 d	3.11 ddd (11.0, 9.5, 5.0)	1α, 1*β*, 2α, 2*β*, 4*β*, 5α	78.6 d
4*β*	38.3 d	1.42 m	2α, 2*β*, 3α, 5α, 6α, 6*β*	35.2 d
5α	48.5 d	0.86 m	3α, 4*β*, 6α, 7α, 7*β*	48.5 d
6α	19.4 t	1.80 m	4*β*, 5α, 7α, 7*β*	19.4 t
6*β*		1.24 m		
7α	29.0 t	2.28 m	5α, 6α, 6*β*	28.9 t
7*β*		2.31 ddd (20.3, 7.0, 2.0)		
8	164.4 s		6α, 6*β*, 7α, 7*β*, 32	164.4 s
9	138.5 s		7α, 7*β*, 12α, 12*β*	138.2 s
10	36.7 s		1α, 1*β*, 2α, 2*β*, 4*β*, 5α, 6α, 6*β*	36.5 s
11	199.4 s		12α, 12*β*	199.3 s
12α	51.8 t	2.67 dd (16.5, 1.0)	17α, 18	51.6 t
12*β*		2.50 d (16.5)		
13	47.3 s		12α, 12*β*, 17α	47.4 s
14	51.6 s		15α, 15*β*, 16α, 16*β*, 17α	51.8 s
15α	30.9 s	1.35 m	16α, 16*β*, 17α, 32	30.9 t
15*β*		1.77 m		
16α	27.0 t	2.05 m	15α, 15*β*, 17α, 20*β*	27.0 t
16*β*		1.38 m		
17α	50.1 d	1.75 m	15α, 15*β*, 16α, 16*β*, 18, 20*β*, 21	50.1 d
18	16.7 q	0.84 s	12α, 12*β*, 17α	16.7 q
19	17.5 q	1.11 s	1α, 1*β*, 5α	17.4 q
20	36.2 d	1.42 m	17α, 21	36.2 d
21	18.4 q	0.91 d (6.4)	17α, 20*β*, 22A, 22B	18.4 q
22A	34.7 t	1.16 m	20*β*, 23A, 23B	37.7 t
22B		1.45 m		
23A	31.2 t	1.89 m	20*β*, 22A, 22B	31.2 t
23B		2.12 ddd (15.0, 11.5, 5.0)		
24	156.6 s		23A, 23B, 25, 28A, 28B	156.5 s
25	33.8 d	2.23 septet (6.9)	26, 27, 28A, 28B	33.8 d
26	21.8 q[c]	1.026 d (6.9)[c]	25, 26	21.8 q[c]
27	22.0 q[c]	1.032 d (6.9)[c]	25, 27	22.0 q[c]
28A	106.1 t	4.54 d (1.5)	23A, 23B, 25	106.1 t
28B		4.55 s		
30	15.2 q	1.02 s	3α, 4*β*, 5α	15.3 q
32	25.8 q	1.14 s	15α, 15*β*	25.9 q
OAc				21.4 q
OAc				170.9 s

[a] Assignments confirmed by decoupling, H−H COSY, NOESY, HMQC, and HMBC spectra. [b] *J* values are given in Hz. [c] Assignments in the same column are interchangeable.

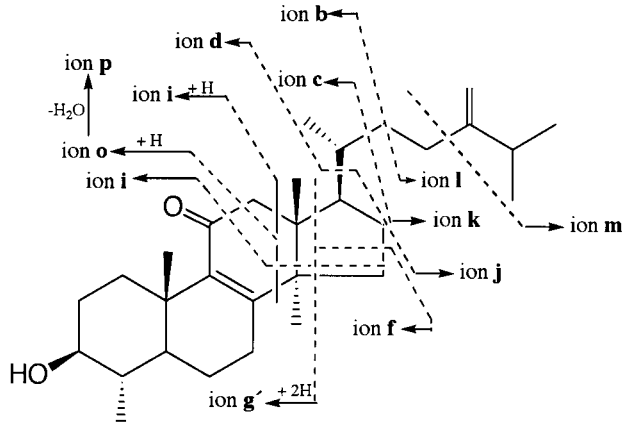


Figure 1. EIMS of compound **1**.

$^1H-^1H$ COSY, HOHAHA, and *J*-resolved NMR spectra. The NOESY data (Figure 2) exhibited cross correlations for H-3α (with H-1α and H-5α), Me-18 (with H-12*β*, H-15*β*, and Me-21), Me-19 (with H-1*β*, H-4*β*, H-6*β*, H-11*β*, and Me-18), Me-28 (with H-3α, H-5α, and H-6α), and Me-30 (with H-15α, H-16α, and H-17α). Definitive evidence of the structure of **1** was derived from its HMBC spectrum (Table 1). In the HMBC spectrum, C-11 correlated with H-12α and H-12*β*, and C-3 correlated with Me-28, H-2α, H-2*β*, and H-4*β*. Hence, **1** was assigned as 3*β*-hydroxy-4α,14α-dimethyl-5α-ergosta-8,24(28)-dien-11-one.

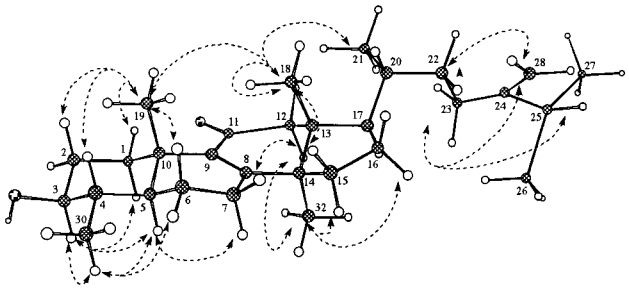


Figure 2. NOESY correlations observed for compound **1**.

Compound **2** was assigned the molecular formula $C_{30}H_{48}O_3$ (HREIMS). The UV and IR spectra showed absorption bands for a *γ*-hydroxy-α,*β*-unsaturated ketone (λ_{max} 253 nm; ν_{max} 3483−3313, 1655 cm^{-1}) and a terminal methylene group (ν_{max} 1640, 884 cm^{-1}). The ^1H and ^{13}C NMR spectra (Table 2) exhibited the presence of three tertiary and two secondary methyl groups, an isopropyl group [δ_H 1.02 and 1.03 (each 3H, d), 2.23 (1H, sept)], eight methylene groups, five methine groups, two hydroxymethine groups [δ_H 3.19 (1H, ddd), 4.53 (1H, dd); δ_C 66.1

Table 2. NMR Data for Compounds **2** and **3** (125 and 500 MHz, $CDCl_3$)[a,b]

	2			**3**		
position	δ_C	δ_H	HMBC (C → H)	δ_C	δ_H	HMBC (C → H)
1α	33.9 t	1.90 m	2α, 2β, 3α, 19	33.4 t	1.10 m	2α, 2β, 3α, 19
1β		1.93 dt (13.2, 3.3)			2.98 dt (13.5, 3.9)	
2α	30.7 t	1.96 m	1α, 1β, 3α, 4β	31.1	1.86 m	1α, 1β, 3α, 4β
2β		1.62 m			1.58 m	
3α	75.2 t	3.19 ddd (11.1, 9.8, 4.8)	1α, 1β, 2α, 2β, 4β, 5α	76.3 t	3.17 ddd (11.0, 9,.8, 5.2)	1α, 1β, 2α, 4β, 5α
4β	39.5 d	1.47 m	2α, 2β, 3α, 5α, 6α, 6β	37.6 d	1.45 m	2α, 2β, 3α, 5α, 6α, 6β
5α	47.0 d	1.76 ddd (11.6, 13.6, 3.5)	3α, 4β, 6α, 6β	42.9 d	1.20 ddd (15.6, 13.3, 2.1)	3α, 4β, 6α, 6β, 7β
6α	39.5 t	2.56 dd (16.0, 3.5)	4β, 5α	30.1 t	1.48 m	4β, 5α, 7β
6β		2.24 dd (16.0, 14.0)			1.72 m	
7β	199.2 s		5α, 6β, 6β	67.5 d	4.35 dd (4.5, 2.2)	5α, 6α, 6β
8	142.3 s		6α, 6β, 32	160.5		6α, 6β, 7β, 32
9	160.1 s			140.6 s		7β, 12α, 12β
10	39.5 s		1α, 1β, 2α, 2β, 4α, 5α	37.3 s		1α, 1β, 2α, 2β, 4α, 5α
11β	66.1 d	4.54 dd (9.5, 5.4)	12α, 12β	200.8 s		12α, 12β
12α	44.2 t	2.50 dd (13.5, 9.5)	11β, 17α, 18	51.8 t	2.71 dd (17.2, 0.9)	17α, 18
12β		1.86 dd (13.5, 5.4)			2.50 d (17.2)	
13	47.6 s		12α, 12β, 17α, 18, 32	47.6 s		12α, 12β, 17α, 18, 32
14	48.2 s		15α, 15β, 16α, 16β, 18, 32	50.9 s		15α, 15β, 16α, 16β, 18, 32
15α	32.7 t	2.07 m	16α, 16β, 17α	30.0 t	1.70 ddd (11.7, 9.4, 1.8)	16α, 16β, 17α, 32
16β		1.65 m			1.89 m	
16α	27.9 t	1.99 m	15α, 15β, 17α, 20β	27.2 t	2.05 m	15α, 15β, 17α, 20β
16β		1.30 m			1.43 m	
17α	49.8 d	1.58 m	15α, 15β, 16α, 16β, 18, 20β	50.1 d	1.78 m	15α, 15β, 16α, 16β, 18, 20β
18	16.9 q	0.67 s	12α, 12β, 17α	16.8 q	0.81 s	12α, 12β, 17α
19	19.1 q	1.24 s	1α, 1β, 5α	16.1 q	1.04 s	1α, 1β, 5α
20	36.2 d	1.36 m	16α, 16β, 17α, 21, 22A, 22B	36.3 d	1.42 m	16α, 16β, 17α, 21, 22A, 22B
21	18.6 q	0.94 d (6.4)	17α, 20β, 22A, 22B	18.4 q	0.91 d (6.4)	17α, 20β, 22A, 22B
22A	34.7 t	1.14 m	20β, 23A, 23B	34.7 t	1.16 m	20β, 21, 23A, 23B
22B		1.57 m			1.58 m	
23A	31.2 t	1.89 m	20β, 22A, 22B, 25, 28A, 28B	31.2 t	1.88 m	22A, 22B, 25
23B		2.13 m			2.12 m	
24	156.6 s		23A, 23B, 25, 28A, 28B	156.5 s		23A, 23B, 25, 28A, 28B
25	33.7 d	2.23 septet (6.9)	26, 27, 28A, 28B	33.8 d	2.23 septet (6.9)	25, 26, 27, 28A, 28B
26	21.8 q[c]	1.02 q (6.9)[c]	25, 26	21.8 q[c]	1.028 d (6.9)[c]	25, 26
27	22.0 q[c]	1.03 q (6.9)[c]	25, 27	22.0 q[c]	1.031 d (6.9)[c]	25, 26
28A	106.1 t	4.66 d (1.4)	23A, 23B, 25	106.1 t	4.67 d (1.4)	23A, 23B, 25
28B		4.72 s			4.73 s	
30	14.6 q	1.01 q (6.4)	3α, 4β, 5α	15.2 q	1.04 d (6.2)	3α, 4β, 5α
32	25.3 q	1.13 q	15α, 15β	27.6 q	1.27 s	15α, 15β

[a] Assignments confirmed by decoupling, H−H COSY, NOESY, HMQC, and HMBC spectra. [b] *J* values are given in Hz. [c] Assignments in the same column are interchangeable.

(d), 75.2 (d)], a terminal methylene group [δ_H 4.66 (1H, d), 4.71 (1H, br s); δ_C 106.1 (t), 156.6 (s)], a tetrasubstituted double bond [δ_C 142.3 (s), 161.1 (s)], and a conjugated ketone [δ_C 199.2 (s)]. The gross structure of **2** was determined by the $^1H-^1H$ COSY, NOESY, HMQC, HMBC, and *J*-resolved 2D NMR techniques. In the HMBC spectrum (Table 2), C-11 correlated with H-12α and H-12β, C-3 correlated with Me-28, H-2α, H-2β, and H-4β, and C-7 correlated with H-6α and H-6β. The configuration of the C-11 hydroxyl group and the conformation of the C-ring were established by the NOESY spectrum. Significant NOE enhancements (Figure 3) were observed for H-11β with H-1β, Me-18, and Me-19 and for H-12α with Me-32. Accordingly, the structure of **2** was elucidated as 3β,11α-dihydroxy-4α,14α-dimethyl-5α-ergosta-8,24(28)-dien-7-one and the conformation of the C-ring adopted a half-chair form.

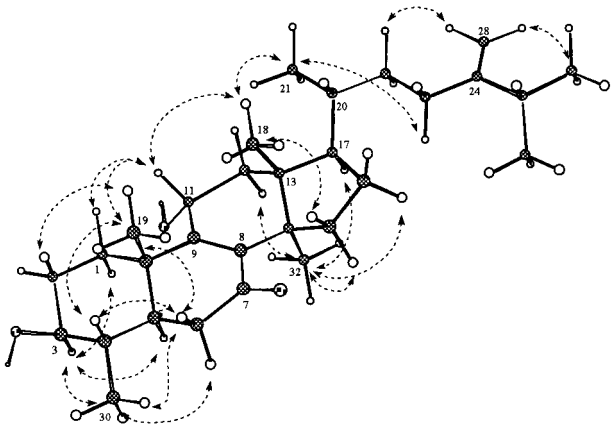


Figure 3. NOESY correlations observed for compound **2**.

Compound **3** gave the same the molecular formula $C_{30}H_{48}O_3$ (HREIMS) as that of **2**. The UV and IR spectra showed absorption bands for a γ-hydroxy-α,β-unsaturated ketone (λ_{max} 256 nm; ν_{max} 3445−3312, 1638 cm^{-1}), and a terminal methylene group (ν_{max} 1642, 885 cm^{-1}). The ^1H and ^{13}C NMR spectra (Table 2) exhibited the presence of three tertiary and two secondary methyl groups, an isopropyl group [δ_H 1.03, 1.04 (each 3H, d), 2.23 (1H, sept)], eight methylene groups, five methine groups, two hydroxymethine groups [δ_H 3.17 (1H, ddd), 4.35 (1H, dd); δ_C 67.5 (d), 76.3 (d)], a terminal methylene group [δ_H 4.67 (1H, d), 4.63 (1H, br s); δ_C 106.1 (t), 156.5 (s)], a tetrasubstituted double bond [δ_C 140.6 (s) 160.5 (s)], and a conjugated ketone [δ_C 200.8 (s)]. The EIMS of **3** showed different fragment ion peaks in comparison to **2**. In addition, the ^1H and ^{13}C NMR chemical shift values were considerably different from those of **2**, and compound **3** was deduced to be a positional isomer of **2**. This presumption was supported by the $^1H-^1H$ COSY, NOESY, HMQC, HMBC, and *J*-resolved 2D NMR spectra. In the HMBC spectrum (Table 2), C-7 correlated with the H-5α, H-6α, and H-6β, and C-11

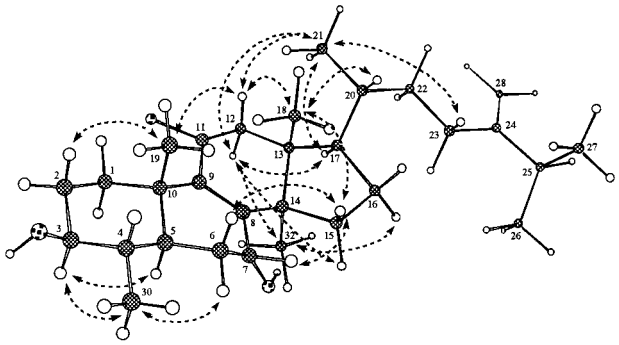


Figure 4. NOESY correlations observed for compound **3**.

Table 3. Percentage of Epstein-Barr Virus Early Antigen Induction in the Presence of Compounds **1** and **3**−**7** with Respect to a Positive Control (100%)[a]

	concentration (mol ratio/TPA)			
compound	1000	500	100	10
1	0 (70)	23.6	72.4	92.8
3	0 (70)	16.4	61.8	85.0
4	0 (70)	21.2	70.0	91.1
5	0 (70)	22.5	70.4	91.9
6	0 (70)	30.2	77.4	95.8
7	0 (70)	27.3	74.9	92.4
oleanolic acid[b]	12.7 (70)	22.4	72.5	93.7

[a] Values represent relative percentages to the positive control value. TPA (32 pmol, 20 ng) = 100%. Values in parentheses are viability percentages of Raji cells. [b] Positive control substance.

correlated with the H-12α and H-12*β* protons. The configuration of the C-7 hydroxyl group was established by the NOESY spectrum and the coupling constants. Significant NOE enhancements (Figure 4) were observed for H-15*β* with H-6*β* and H-7*β*, and the coupling constant of H-7*β* appeared as a narrow doublet of doublets (J = 4.5, 2.2 Hz). Accordingly, the C-7 hydroxyl group of **3** proved to be α, and the conformation of the B-ring adopted a half-chair form. Hence, compound **3** was assigned as 3*β*,7α-dihydroxy-4α,14α-dimethyl-5α-ergosta-8,24(28)-dien-11-one.

The inhibitory effects of compounds **1**, **3**−**7** and the control substance oleanolic acid[7] on EBV-EA activation induced by TPA were examined as a preliminary evaluation of their potential antitumor-promoting activities, and the results are shown in Table 3. Compound **2** was not tested in this assay because it was obtained only as a minor product. As shown in Table 3, all compounds exhibited potent inhibitory effects (100% inhibition of induction at 1000 mol ratio/TPA, and about 80% inhibition at 500 mol ratio/TPA, and about 30% inhibition at 100 mol ratio/TPA) on EBV-EA induction by TPA.[7,8] Among these compounds, **3** exhibited the most potent inhibitory effects in comparison to oleanolic acid on EBV-EA activation.

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were measured using a JASCO DIP-1000 digital polarimeter. UV spectra were recorded using a Hitachi 150−20 spectrophotometer. IR spectra were recorded using a Perkin−Elmer 1720X FTIR spectrophotometer. ^{1}H and ^{13}C NMR spectra were obtained on a Varian INOVA 500 spectrometer with standard pulse sequences, operating at 500 and 125 MHz, respectively. $CDCl_3$ was used as the solvent and TMS as the internal standard. EIMS were recorded on a Hitachi 4000H double-focusing mass spectrometer (70 eV). Column chromatography was carried out over Si gel (70−230 mesh), and MPLC was carried out with Si gel (230−400 mesh, Merck). Fractions obtained from column chromatography were monitored by TLC (Si gel 60 HF_{254}). Preparative TLC was carried out on Merck Si gel PF_{254} plates (20 × 20 cm, 0.5 mm thick).

Plant Material. Seeds of *Euphorbia chamaesyce* were collected at the botanical garden of Osaka University of Pharmaceutical Sciences in July 1994, and the seeds were also cultivated. The cultivated whole plants were harvested in September 1995, and a voucher specimen (EC-95-01) is deposited at the Herbarium of the Laboratory of Medicinal Chemistry, Osaka University of Pharmaceutical Sciences.

Extraction and Isolation. The whole herb of *E. chamaesyce* (5 kg) was extracted with $CHCl_3$ (20 L) employing an automatic percolator for 7 days at 50 °C. The $CHCl_3$ solution was evaporated under reduced pressure, and the resulting dark green residue (560 g) was subjected to Si gel column chromatography (10 kg). Elution of the column with $CHCl_3$ afforded a dark brown residue A (33.73 g) from fractions 17−22 and a dark brown residue B (13.0 g) from fractions 23−53 (each 2 L). Repeated column chromatography of residue A on Si gel (1.2 kg) furnished a yellow gum (6.26 g) eluted with $CHCl_3$ from fractions 30−43 (each fraction, 500 mL), which was subjected to MPLC with $CHCl_3$ and $CHCl_3$−EtOAc (10:1) (Si gel, 300 g, 230−400 mesh) to afford 3*β*-hydroxy-4α,14α-dimethyl-5α-ergosta-8,24(28)-diene-7,11-dione (**5**) (122 mg) from fractions 93−96 (each fraction, 200 mL), compound **1** (118 mg) from fractions 122−132, and compound **3** (48 mg) from fractions 166−168. Subsequent column chromatography with the residue A gave a crystalline mass (1.40 g) from fractions 44−56, which was subjected to Si gel (50 g) column chromatography using $CHCl_3$ to afford wrightial [mp 97−99 °C, $[\alpha]^{23}_D$ +18° (*c* 0.43, $CHCl_3$)] from fractions 27−41. Repeated column chromatography of residue B on Si gel (150 g) eluted with $CHCl_3$ gave a crystalline mass (8.5 mg) from fractions 18−20, which was subjected to preparative TLC ($CHCl_3$−MeOH, 30:1) to yield compound **2** (3.2 mg). Subsequent column chromatography with the same solvent yielded lup-20(30)-ene-3*β*,29-diol (28 mg) [mp 236−239 °C, $[\alpha]^{23}_D$ −15° (*c* 0.33, $CHCl_3$)] from fractions 42−45.

3*β*-Hydroxy-4α,14α-dimethyl-5α-ergosta-8,24(28)-dien-11-one (1): needles, mp 134.5−136 °C (MeOH−$CHCl_3$); $[\alpha]^{23}_D$ +162° (*c* 0.30, $CHCl_3$); UV (EtOH) λ_{max} 257 (ϵ 7000) (conjugated enone) nm; IR (KBr) ν_{max} 3421 (OH), 3078 (=C−H), 2961, 2929, 1655 (>C=C−C=O), 1640 and 887 (>C=CH_2), 1457, 1418 (−CH_2CO), 1375, 1284, 1247, 1097, 1053, 1025, 977 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 440 (57) $[M]^+$, 425 (8) $[M - Me]^+$, 422.3534 (13) $[M - H_2O]^+$, 407.3291 (7) $[M - Me - H_2O]^+$, 357 (3, ion **b**) $[M - C_6H_{11}]^+$, 356.2721 (6, ion **b** − H) [$C_{24}H_{36}O_2$, calcd for 356.2713), 343 (1, ion **c**) $[M - C_7H_{13}]^+$, 341 (4, ion **c** − 2H), 315 (6, ion **d**) $[M - C_9H_{17}]^+$, 313.2179 (1, ion **d** − 2H), 288.2086 (11, ion **f**) [$C_{19}H_{28}O_2$, calcd for 288.2087], 276.2095 (70, ion **g′**) [$C_{18}H_{28}O_2$, calcd for 276.2088], 247.1682 (29, ion **i**), [$C_{16}H_{23}O_2$, calcd for 247.1697], 221.1528 (100, ion **n**) [$C_{14}H_{21}O_2$, calcd for 221.1540], 207.1388 (25, ion **o**) [$C_{13}H_{19}O_2$, calcd for 207.1384], 189.1291 (18, ion **p**) [$C_{13}H_{17}O$, calcd for 189.1278], 187 (11, ion **k** − 2H), 175 (29), 135 (18), 125 (1, ion **j**) [side chain, $C_9H_{17}]^+$, 123 (7, ion **j** − 2H), 97.1024 (5, ion **k**) [C_7H_{13}, calcd for 97.1016], 95.0844 (15, ion **k** − 2H)[C_7H_{11}, calcd for 95.0860], 83.0869 (9, ion **l**) [C_6H_{11}, calcd for 83.0861], 81 (13, ion **l** − 2H), 69.0704 (19, ion **m**) [C_5H_9, calcd for 69.0703], 67 (7, ion **m** − 2H); HREIMS *m*/*z* 440.3647 ($C_{30}H_{48}O_2$ requires 440.3652).

Acetylation of 1. A solution of compound **1** (15.0 mg) in Ac_2O−C_5H_5N (1:1, 2 mL) was kept at room temperature overnight. Workup as usual afforded a crude solid, which was purified by preparative TLC (plate, 20 × 20 cm; solvent, $CHCl_3$−MeOH, 100:1) to afford the corresponding acetate **1a**, 15.2 mg, mp 78−80 °C (MeOH−$CHCl_3$), $[\alpha]^{23}_D$ +95° (*c* 0.31, $CHCl_3$); UV (EtOH) λ_{max} 257 (ϵ 7000) nm; IR (KBr) ν_{max} 3084 (=C−H), 1737 and 1249 (OAc), 1661 (>C=C−C=O), 1640 and 889 (>C=CH_2), cm^{-1}; ^{1}H NMR δ 0.83 (3H, s, Me-18), 0.88 (3H, d, J = 6.4 Hz, Me-30), 0.91 (3H, d, J = 6.4 Hz, Me-21), 1.026 (3H, d, J = 6.9 Hz, Me-26), 1.032 (3H, d, J = 6.9 Hz, Me-27), 1.12 (3H, s, Me-19), 1.14 (3H, s, Me-32), 2.01 (3H, s, OAc), 4.39

(1H, ddd, J = 11.5, 11.5, 5.0 Hz, H-3α), 2.50 (1H, d, J = 16.5 Hz, H-12β), 2.68 (1H, dd, J = 16.5, 1.0 Hz, H-12α), 4.66 (1H, d, J = 1.5 Hz, H-28), 4.73 (1H, s, H-28); ^{13}C NMR, see Table 1; EIMS m/z 482 (30) $[M]^+$, 467 (3), 422 (18), 407 (9), 399 (2, ion **b**), 398 (6, ion **b** − H), 385 (1, ion **c**), 330.2183 (9, ion **f**) [$C_{21}H_{30}O_3$, calcd for 330.2193], 318.2197 (61, ion **g′**) [$C_{21}H_{30}O_3$, calcd for 318.2194], 290 (24), 289.1820 (23, ion **i**) [$C_{18}H_{25}O_3$, calcd for 289.1803], 263.1631 (100, ion **n**) [$C_{16}H_{23}O_3$, calcd for 263.1616], 249 (10, ion **o**), 189 (23, ion **p**), 187 (16, ion **p** − 2H), 125 (1, ion **j**), 123 (3, ion **j** − 2H), 121 (5), 97 (2, ion **k**), 95 (8, ion **k** − 2H), 83 (3, ion **l**), 81 (5, ion **l** − 2H), 69 (12, ion **m**), 67 (3, ion **m** − 2H).

3β,11α-Dihydroxy-4α,14α-dimethyl-5α-ergosta-8,24(28)-dien-7-one (2): needles, mp 148−150 °C (MeOH−$CHCl_3$); $[\alpha]^{23}_D$ +11° (c 0.23, $CHCl_3$); UV (EtOH) λ_{max} 270 (ϵ 6200) (γ-hydroxy-α,β-unsaturated C=O) nm; IR (KBr) ν_{max} 3483−3313 (OH), 3033 (=C−H), 2963, 2930, 2870, 1655 (>C=C−C=O), 1643 and 887 (>C=CH_2), 1459, 1421 (−CH_2CO), 1375, 1344, 1243, 1212, 1026 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 2; EIMS m/z 456 (89) $[M]^+$, 441 (18) $[M - Me]^+$, 438 (19) $[M - H_2O]^+$, 423 (30) $[M - Me - H_2O]^+$, 405 (7) $[M - Me - 2H_2O]^+$, 357 (5), 331 (7), 329 (24), 316 (100), 313 (19), 304 (24), 299 (22), 283 (30), 247 (43), 238 (43), 227 (29), 125 (13), 123 (34), 121 (50), 97 (20), 95 (50), 83 (29), 81 (37), 69 (77); HREIMS m/z 456.3626 ($C_{30}H_{48}O_3$ requires 456.3601).

3β,7α-Dihydroxy-4α,14α-dimethyl-5α-ergosta-8,24(28)-dien-11-one (3): needles, mp 161.5−164 °C (n-hexane−$CHCl_3$); $[\alpha]^{25}_D$ +82.8° (c 0.58, $CHCl_3$); UV (EtOH) λ_{max} 256.2 (ϵ 6700) (γ-hydroxy-α,β-unsaturated C=O) nm; IR (KBr) ν_{max} 3445−3312 (OH), 2965, 2930, 2869, 1642 and 885 (>C=CH_2), 1638 (γ-hydroxy-α,β-unsaturated C=O), 1459, 1420, 1376, 1246, 1030, 977 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 2; EIMS m/z 456 (80) $[M]^+$, 441 (34) $[M - Me]^+$, 438 (30) $[M - H_2O]^+$, 372 (9), 263 (43), 237 (100), 222 (19), 123 (20), 121 (27), 95 (35), 83 (26), 81 (41), 69 (66); HREIMS m/z 456.3598 ($C_{30}H_{48}O_3$ requires 456.3601).

Wrightial: ^{1}H NMR δ 0.34 (1H, d, J = 4.1 Hz, H-19), 0.55 (1H, d, J = 4.1 Hz, H-19), 0.81 (3H, s, Me-28), 0.88 (3H, d, J = 6.5 Hz, Me-21), 0.90 (3H, s, Me-30), 0.97 (6H, s, Me-18, Me-29), 3.29 (1H, dd, J = 11.2, 4.6 Hz, H-3α), 9.28 (1H, t, J = 1.8 Hz, H-24); ^{13}C NMR δ 14.0 (q, C-28), 18.0 (q, C-21), 18.1 (q, C-18), 19.3 (q, C-30), 19.9 (s, C-9), 21.1 (t, C-6), 25.4 (q, C-29), 25.98 (t, C-7), 26.04 (s, C-10), 26.4 (t, C-11), 28.1 (t, C-1), 28.2 (t, C-2), 29.9 (t, C-19), 30.3 (t, C-2), 31.9 (t, C-1), 32.8 (t, C-12), 35.5 (t, C-15), 40.5 (s, C-4), 41.1 (t, C-23), 45.3 (s, C-13), 47.1 (d, C-5), 48.0 (d, C-8), 48.8 (s, C-14), 52.1 (d, C-17), 78.8 (d, C-3), 203.3 (d, C-24).

Method of EBV-EA Induction Tests of Compounds 1, 3−7. The inhibition of EBV-EA activation was assayed using Raji cells (virus nonproducer type), the EBV genome-carrying human lymphoblastoid cells, which were cultivated in 10% FBS RPMI 1640 medium solution (Nacalai Tesque). The indicator cells (Raji) (1×10^6/mL) were incubated at 37 °C for 48 h in 1 mL of the medium containing n-butyric acid (4 mM, inducer), 32 pmol of TPA (20 ng/mL) in dimethyl sulfoxide (DMSO), and a known amount of test compound in DMSO. Smears were made from the cell suspension. The activated cells were stained by high titer EBV-EA-positive sera from nasopharyngeal carcinoma patients and were detected by a conventional indirect immunofluorescence technique. In each assay, at least 500 cells were counted, and the experiments were repeated twice. The average EA induction was compared with that of positive control experiments with n-butyric acid plus TPA, in which EA induction was ordinarily around 30%.

Acknowledgment. The authors are grateful to Mr. Katsuhiko Minoura and Mrs. Mihoyo Fujitake of Osaka University of Pharmaceutical Sciences for NMR and MS measurements.

References and Notes

(1) Tanaka, R.; Ida, T.; Takaoka, Y.; Kita, S.; Kamisako, W.; Matsunaga, S. *Phytochemistry* **1994**, *36*, 129−132.
(2) Tanaka, R.; Ida, T.; Kita, S.; Kamisako, W.; Matsunaga, S. *Phytochemistry* **1996**, *41*, 1163−1168.
(3) Tanaka, R.; Kasubuchi, K.; Kita, S.; Matsunaga, S. *Phytochemistry* **1999**, *41*, 457−463.
(4) Wada, S.; Tanaka, R.; Iida, A.; Matsunaga, S. *Bioorg. Med. Chem. Lett.* **1998**, *28*, 1219−1223.
(5) Pamchandra, P.; Basheermiya, M.; Krupadanam, G. L. D.; Srimannarayana, G. *J. Nat. Prod.* **1993**, *56*, 1181−1182.
(6) Corbett, R. E.; Cong, A.-N. T.; Holland, P.-T.; Wilkins, A.-L. *Aust. J. Chem.* **1987**, *40*, 461−468.
(7) Konoshima, T.; Takasaki, M.; Kozuka, M.; Tokuda, H. *J. Nat. Prod.* **1987**, *50*, 1167−1170.
(8) Konoshima, T.; Takasaki, M.; Tokuda, H.; Minh Duc, N.; Yamasaki, K. *Biol. Pharm. Bull.* **1998**, *21*, 834−838.

NP990394B