Eupha-7,9(11),24-trien-3β-ol ("Antiquol C") and Other Triterpenes from *Euphorbia antiquorum* Latex and Their Inhibitory Effects on Epstein−Barr Virus Activation

Toshihiro Akihisa,*[,†] E. M. Kithsiri Wijeratne,[‡] Harukuni Tokuda,[§] Fumio Enjo,[§] Masakazu Toriumi,[†] Yumiko Kimura,[⊥] Kazuo Koike,[∥] Tamotsu Nikaido,[∥] Yasuhiro Tezuka,[△] and Hoyoku Nishino[§]

College of Science and Technology, Nihon University, 1-8 Kanda Surugadai, Chiyoda-ku, Tokyo 101-8308, Japan, Division of Natural Products Chemistry, Medical Research Institute, Colombo 98, Sri Lanka, Department of Biochemistry, Kyoto Prefectural University of Medicine, Kyoto 602-0841, Japan, College of Pharmacy, Nihon University, Chiba 274-8555, Japan, School of Pharmaceutical Sciences, Toho University, Chiba 274-8510, Japan, and Research Institute for Wakan-Yaku (Traditional Sino-Japanese Medicines), Toyama Medical and Pharmacetical University, Toyama 930-0194, Japan

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The structures of three triterpene alcohols isolated from the latex of *Euphorbia antiquorum* were established to be eupha-7,9(11),24-trien-3β-ol (**2**; antiquol C), 19(10→9)*abeo*-8α,9β,10α-eupha-5,24-dien-3β-ol (**3**; antiquol B), and 24-methyltirucalla-8,24(24[1])-dien-3β-ol (**4**; euphorbol) on the basis of spectroscopic methods. Compounds **3** and **4** have previously been assigned the erroneous structures of 10α-cucurbita-5,24-dien-3α-ol and 24-methyleupha-8,24(24[1])-dien-3β-ol, respectively. Compounds **2**−**4** and four other known compounds isolated from the latex, euphol (**1**), lemmaphylla-7,21-dien-3β-ol (**5**), isohelianol (**6**), and camelliol C (**7**), showed potent inhibitory effects on Epstein−Barr virus early antigen (EBV-EA) activation induced by the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA).

Euphorbia antiquorum L. (Euphorbiaceae), a plant native to India and Sri Lanka, has various medicinal uses. Its whole plant is used for cutaneous infections, and latex for dropsy, as nerve tonic, and for bronchitis.[1] The latex has been reported to contain euphol (**1**), euphol 3-*O*-cinnamate, antiquol A, antiquol B, 24-methylenecycloartanol, and cycloeucalenol as the triterpene constituents.[2] In this paper, we report the isolation and characterization of one novel triterpene alcohol, named antiquol C (**2**), and six known triterpene alcohols (**1**, **3**−**7**) from the latex of *E. antiquorum*, as well as their inhibitory effects on EBV-EA activation induced by TPA, as a primary screening for antitumor promoters. In addition, the structural revision of antiquol B (**3**) and euphorbol (**4**) as 19(10→9)*abeo*-8α,9β,10α-eupha-5,24-dien-3β-ol and 24-methyltirucalla-8,24(24[1])-dien-3β-ol, which were previously assigned erroneously as 10α-cucurbita-5,24-dien-3α-ol[2] and 24-methyleupha-8,24(24[1])-dien-3β-ol,[3] respectively, is described.

Results and Discussion

The seven triterpene alcohols were isolated from the latex as their acetyl derivatives, **1a**−**7a**, as described in the Experimental Section, and their identification was confirmed by comparison of chromatographic and spectral data with those of authentic compounds.

The molecular formula of **2a** was determined as $C_{32}H_{50}O_2$ from its HREIMS ([M]$^+$, *m*/*z* 466.3842) as well as from its ^{13}C NMR DEPT. The compound had a $\Delta^{7,9(11)}$-conjugated diene [λ_{max} 230 (log ϵ 4.07), 237 (4.09), and 246 (3.99) nm; ν_{max} 1650, 816 cm^{-1}; δ_H 5.20 (1H, t, J = 3.5 Hz) and 5.33 (1H, dd, J = 2.1, 3.1 Hz)],[4,5] a terminal isopropylidene group [δ_H 1.61 (s) and 1.69 (s)], a secondary [δ_H 0.86 (d)] and five tertiary methyls [δ_H 0.62, 0.84, 0.87, 0.94, and 0.96], and an acetoxyl group [ν_{max} 1738 cm^{-1}; δ_H 2.06 (3H, s) and 4.51 (1H, dd)]. These data, in combination with fragment ions having *m*/*z* 451 ([M]$^+$ − Me), 391 ([M]$^+$ − Me − HOAc), 313 [loss of side chain (s.c.; C_8H_{15}) − 42], 299 (313 − CH_2), 295 (loss of s.c. and HOAc), 253 (295−42), and 69 [CH_2CH═$C(Me)_2$]$^+$ (C-23-C-27)[5] suggested that **2a** had a 3β-acetoxy tetracyclic triterpene skeleton possessing a $\Delta^{7,9(11)}$-diene system and a C_8-side chain containing an isopropylidene group. The diagnostic fragment ion at *m*/*z* 185 [$C_{14}H_{17}$]$^+$ formed by the loss of ring D plus side chain due to cleavage of the C-8−C-14 and C-11−C-12 bonds with concomitant HOAc loss supported the $\Delta^{7,9(11)}$-unsaturation. The above evidence coupled with the ^{13}C and ^{1}H NMR data in addition to analysis of ^{1}H−^{1}H COSY, HMQC, and HMBC spectra indicated that **2a** possesses a 4,4,14-trimethyl-$\Delta^{7,9(11),24}$-statrien-3β-ol acetate structure. The stereochemistry of **2a** was established by phase-sensitive NOESY experiment. Compound **2a** showed significant NOE correlations between [H-29(4β-Me)−H-2β and H-6β−H-19(10β-Me)−H-30(14β-Me)−H-17β−H-21] on the β-face, [H-3α−H-28(4α-Me) and H-5α] and [H-18(13α-Me)−H-20] on the α-face, and [H-16α,β−H-21] of the molecule (Figure 1).[6] These NOE correlations were consistent with those for two euphane-type triterpenes, butyrospermol (eupha-7,24-dien-3β-ol) acetate and euphol acetate (**1a**),[8,9] thus suggesting that **2a** possesses the same stereochemistry at the C/D ring junction (13α,14β), C-17 (17β-H), and C-20 (20*R*) as that of butyrospermol acetate and **1a**. We concluded that **2a** is eupha-7,9(11),24-trien-3β-ol acetate (antiquol C acetate). Alkaline hydrolysis of **2a** yielded eupha-7,9(11),24-trien-3β-ol (**2**; antiquol C; $C_{30}H_{48}O$; [M]$^+$ *m*/*z* 424.3682).

Compound **3a**, which showed [M]$^+$ at *m*/*z* 468.3972 ($C_{32}H_{52}O_2$) in the HREIMS, had a secondary acetoxyl group [ν_{max} 1732 cm^{-1}; δ_H 2.02 (3H, s) and 4.70 (1H, t, J = 3.1

* To whom correspondence should be addressed. Tel: 81-3-3259-0806. Fax: 81-3-3293-7572. E-mail: akihisa@chem.cst.nihon-u.ac.jp.
† College of Science and Technology, Nihon University.
‡ Medical Research Institute.
§ Kyoto Prefectural University of Medicine.
⊥ College of Pharmacy, Nihon University.
∥ Toho University.
△ Toyama Medical and Pharmaceutical University.

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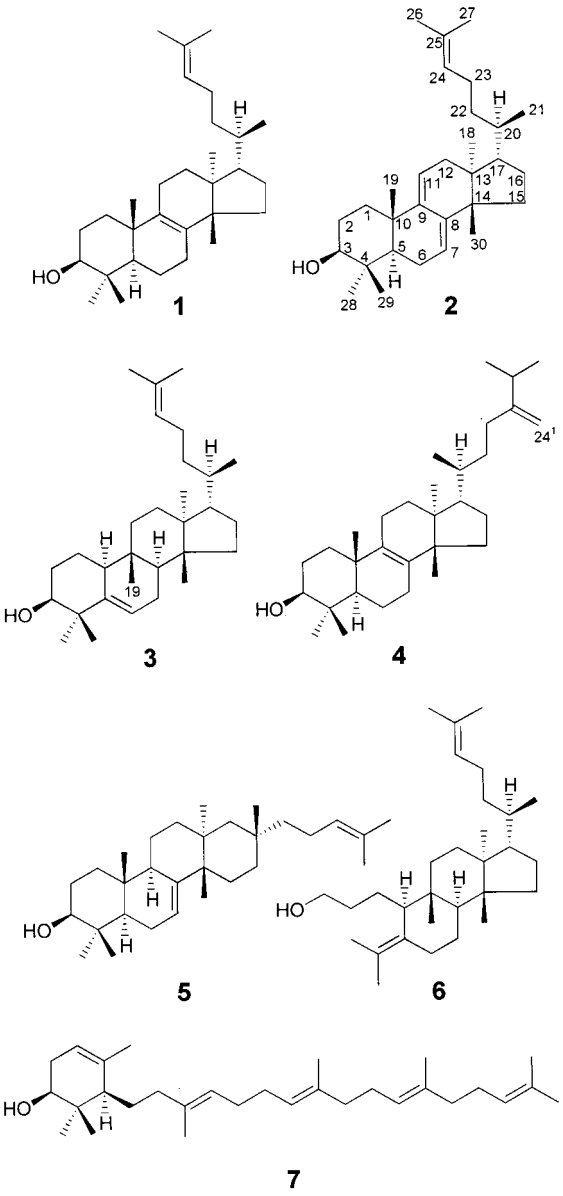

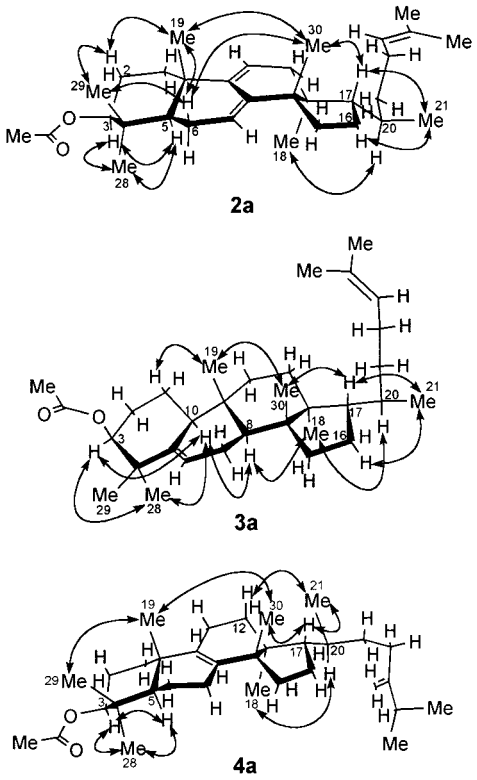

Figure 1. Major NOE correlations (← →) for **2a**, **3a**, and **4a**.

Hz)], two trisubstituted double bonds [ν_{max} 830 cm^{-1}; δ_H 5.10 (1H, t, J = 7.0 Hz) and 5.56 (1H, d, J = 5.2 Hz)], a terminal isopropylidene group [δ_H 1.61 (s) and 1.69 (s)], and five tertiary [δ_H 0.81, 0.84, 0.88, 1.05, and 1.08 (each s)] and one secondary [δ_H 0.86 (d, J = 6.1 Hz)] methyl groups. This, in combination with fragment ions having *m/z* 453 ([M]$^+$ − Me), 393 ([M]$^+$ − Me − HOAc), 355 [loss of C_8H_{15} and 2H], and 69 [$CH_2CH{=}C(Me)_2]^+$,[5] suggested that compound **3a** was a triterpene with a tetracyclic skeleton possessing one double bond, an axially oriented acetoxyl group located most likely at C-3, and a C_8-side chain containing an isopropylidene functionality. The highly deshielded methine ^{1}H signal (δ_H 5.56) due to a skeletal double bond suggested that it was located at C-5(6).[2,8,10] Further, the highly deshielded ^{13}C signal at δ_C 142.3 due to C-5 was consistent with the corresponding signal in the spectrum of 19(10→9)*abeo*-8*β*,9*β*,10α-lanost-5-ene-(10α-cucurbit-5-ene)[2,11] and 19(10→9)*abeo*-8α,9*β*,10α-euph/tirucall-5-ene skeletal structures.[10,12] The diagnostic fragment ions at *m/z* 274 [$C_{20}H_{34}]^+$ and 134 [$C_{10}H_{14}]^+$ formed by the cleavage of the C-7−C-8 and C-9−C-10 bonds, resulting from retro-Diels−Alder cleavage in ring B,[13] ruled out the possibility of the 10α-cucurbit-5-ene-skeletal structure. The above evidence coupled with the analysis of ^{1}H−^{1}H COSY, HMQC, and HMBC spectra indicated that **3a** possesses a 19(10→9)*abeo*-8α,9*β*,10α-eupha/tirucalla-5,24-diene-3*β*-ol acetate structure. The stereochemistry of **3a** was established by phase-sensitive NOESY experiment. Compound **3a** showed significant NOE correlations between [H-1*β*−H-19(10*β*-Me)−H-30(14*β*-Me)−H-17*β* -H-21] on the *β*-face, [H-3α−H-28(4α-Me)−H-10α−H-8α−H-18(13α-Me)−H-20] on the α-face, and [H-16α,*β*−H-21] of the molecule (Figure 1).[6] These NOE correlations suggest that **3a** possesses the same stereochemistry at the C/D ring junction, C-17, and C-20 as that of euphane-type triterpenes.[8,9] We concluded that **3a** is 19(10→9)*abeo*-8α,9*β*,10α-eupha-5,24-dien-3*β*-ol acetate. Alkaline hydrolysis of **3a** yielded 19(10→9)*abeo*-8α,9*β*,10α-eupha-5,24-dien-3*β*-ol (**3**, $C_{30}H_{50}O$).

Compound **4a**, which exhibited [M]$^+$ at *m/z* 482.4120 ($C_{33}H_{54}O_2$) in the HREIMS, had a secondary acetoxyl group [ν_{max} 1738 cm^{-1}; δ_H 2.05 (3H, s) and 4.50 (1H, dd, J = 4.3, 11.6 Hz)], a terminal methylene group [ν_{max} 891 cm^{-1}; δ_H 4.66 and 4.71 (each 1H and s)], and five tertiary [δ_H 0.75 (3H), 0.87 (6H), and 0.88 (6H) (each s)] and three secondary [δ_H 0.93, 1.02, and 1.03 (each 3H and d)] methyl groups. This, in combination with fragment ions having *m/z* 467 ([M]$^+$ − Me), 407 ([M]$^+$ − Me − HOAc), 297 [loss of C_9H_{17} and HOAc], 255 (297−42), and 241 (255-CH_2),[5] suggested that compound **4a** was a triterpene with a tetracyclic skeleton possessing a tetrasubstituted Δ^8-double bond, an axially oriented acetoxyl group located most likely at C-3, and a C_9-side chain containing a terminal methylene group. A further diagnostic ion at *m/z* 383 ([M]$^+$ − C_6H_{12} − Me) due to a McLafferty rearrangement involving cleavage of the C-22−C-23 bond suggested the presence of a terminal methylene group at C-24 in the side chain.[5] Furthermore, the ^{13}C and ^{1}H NMR data in addition to analysis of ^{1}H−^{1}H COSY, HMQC, and HMBC spectra enabled the structure of **4a** to be formulated as 24-methylene-4,4,14-trimethyl-Δ^8-sten-3*β*-ol acetate with as-yet-to-be-determined stereochemistry. Compound **4a** exhibited definite NOE

Table 1. ^{13}C, ^{1}H, and HMBC NMR Spectral Data for Triterpene Acetates **2a**−**4a** ($CDCl_3$)

	2a			**3a**			**4a**		
C no.	δ_C	δ_H[a]	HMBC (H to C)	δ_C	δ_H[a]	HMBC (H to C)	δ_C	δ_H[a]	HMBC (H to C)
1	35.3	1.66 (a), 1.80 (b)	2, 3, 5, 10, 19	19.5	1.57 (a), 1.47 (b)	2	34.9	1.30 (a), 1.77 (b)	2, 10, 19
2	24.2	1.76 (a), 1.69 (b)	1, 4	25.5	1.84 (a), 1.76 (b)	4, 5, 29	24.2	1.72 (a), 1.63 (b)	1, 3
3	80.9	4.51 (dd, 4.4, 10.8)	2, 4, 10, 28, 29	78.6	4.70 (t, 3.1)	1, 5, COMe	80.9	4.50 (dd, 4.3, 11.6)	1, 2, 4, 28, 29
4	37.9			39.2			37.9		
5	48.4	1.36 (dd, 4.8, 11.3)	3, 4, 6, 7, 10, 19, 28, 29	142.3			51.1	1.22 (br d, 11.9)	1, 6, 7, 10, 29
6	23.5	2.20 (a), 2.07 (b)	5, 7, 8, 10	119.8	5.56 (d, 5.2)	4, 7, 8, 10	18.8	1.69 (a), 1.43 (b)	5, 7, 10
7	118.1	5.33 (br s)	5, 6, 14	25.1	1.65 (a), 2.08 (b)	5, 6, 9	27.5	1.94 (a), 2.11 (b)	6, 8, 9
8	141.5			44.5	1.67 (dd, 4.4, 11.4)	7, 9, 10, 14, 19, 30	133.6		
9	144.7			35.1			133.9		
10	36.0			49.6	2.10 (br d, 11.0)	1, 5, 9, 19	37.1		
11	115.6	5.20 (br s)	10, 12, 13	35.7	1.53 (2H)	8, 9, 12, 19	21.5	2.06 (a), 1.91 (b)	8, 9, 12
12	38.4	2.18 (2H)	9, 11, 13, 14, 18	30.4	1.55 (a), 1.76 (b)	11, 13, 14, 18	30.8	1.70 (2H)	11, 13, 14, 17, 18
13	44.1			46.1			44.1		
14	49.7			47.5			49.9		
15	31.1	1.64 (a), 1.31 (b)	8, 13, 14, 17, 30	34.1	1.13 (a), 1.22 (b)	13, 14	29.8	1.55 (a), 1.19 (b)	13, 14, 16
16	28.1	1.95 (a), 1.34 (b)	13, 14, 17	28.2	1.30 (a), 1.88 (b)	13, 17	28.1	1.33 (a), 1.91 (b)	15, 14, 17, 20
17	50.8	1.6	12, 13, 14, 16, 20, 21	49.9	1.54	12, 13	50.1	1.51	12, 13, 14, 16, 18, 20
18	16.2	0.62 (s)	12, 13, 14, 17	15.4	0.81 (s)	12, 13, 14, 17	15.4	0.75 (s)	12, 13, 14, 17
19	20.8	0.94 (s)	1, 5, 9, 10	16.3	0.84 (s)	8, 9, 10, 11	20.2	0.88 (s)	1, 5, 9, 10
20	35.7	1.45	21	35.2	1.52	17, 21, 22	36.5	1.42	17, 22
21	18.8	0.86 (d, 6.0)	17, 20, 22	18.8	0.86 (d, 6.1)	17, 20, 22	18.8	0.93 (d, 6.4)	17, 20, 22, 24
22	35.3	1.05, 1.59	20, 21, 23	35.4	1.11, 1.58	21	35.0	1.11, 1.56	20, 23
23	25.0	1.87, 2.06	24, 25	24.7	1.83, 2.01	22, 24	31.3	1.89, 2.10	22, 24, 241
24	125.1	5.11 (t, 6.0)	23, 26, 27	125.2	5.10 (t, 7.0)	26, 27	156.9		
25	131.0			130.9			33.8	2.23 (sept., 6.7)	23, 24, 241, 26, 27
26	25.7	1.69 (s)	24, 25, 27	25.8	1.69 (s)	24, 25, 26	22.0	1.02 (d, 6.7)	24, 25, 27
27	17.7	1.61 (s)	24, 25, 26	17.7	1.61 (s)	24, 25, 27	21.8	1.03 (d, 7.0)	24, 25, 26
28	27.6	0.87 (s)	3, 4, 5, 29	29.1	1.08 (s)	3, 4, 5, 29	28.0	0.88 (s)	3, 4, 5, 29
29	16.4	0.96 (s)	3, 4, 5, 28	25.1	1.05 (s)	3, 4, 5, 28	16.6	0.87 (s)	3, 4, 5, 28
30	23.2	0.84 (s)	8, 13, 14, 15	19.1	0.88 (s)	8, 13, 14, 15, 17	24.4	0.87 (s)	8, 13, 14, 15
241							106.0	4.66 (s), 4.71 (s)	23, 25, 26
CO*Me*	21.3	2.06 (s)	COMe	21.3	2.02 (s)	COMe	21.3	2.05 (s)	COMe
*C*OMe	171.0			170.9			171.0		

[a] Figures in parentheses denote *J* values (Hz).

correlations between [H-29 (4*β*-Me)−H-19 (10*β*-Me)−H-30 (14*β*-Me)−H-17*β*−H-21] on the *β*-face, [H-3α−H-28 (4α-Me) and H-5α] and [H-18 (13α-Me)−H-20] on the α-face, and [H-12−H-21] of the molecule (Figure 1),[6] which were consistent with those of tirucallane-type triterpenes.[8,9,14] Thus, we propose that this compound is 24-methyltirucalla-8,24(24[1])-dien-3*β*-ol acetate. Alkaline hydrolysis of **4a** yielded 24-methyltirucalla-8,24(24[1])-dien-3*β*-ol (**4**, $C_{31}H_{52}O$).

Table 1 shows the assigned ^{1}H and ^{13}C NMR data of **2a**−**4a**. Signal assignments were aided by DEPT, $^{1}H-^{1}H$ COSY, HMQC, HMBC, and phase-sensitive NOESY experiments.

Although triterpenes with a $\Delta^{7,9(11)}$-conjugated diene system have been reported as the natural products in various skeletal types,[4,5] compound **2** is the first example of a euphane-type triterpene alcohol with a $\Delta^{7,9(11)}$-diene system as a natural product. A triterpene alcohol isolated previously from the latex of *E. antiquorum*, the same plant material as used in this study, was assigned the structure 10α-cucurbita-5,24-dien-3α-ol (antiquol B) by Gewali et al.[2] However, there was good agreement in the MS and ^{1}H NMR spectral data reported for antiquol B[2] and our compound **3** (see Experimental Section). Gewali et al. assigned the structure of antiquol B mainly by NMR spectroscopy but without supporting evidence from NOE correlations. We conclude that antiquol B possesses the structure 19(10→9)*abeo*-8α,9*β*,10α-eupha-5,24-dien-3*β*-ol (**3**) but was erroneously assigned previously as having the cucurbitane-type structure.[2] The structure of compound **3** has been, on the other hand, given for boeticol, a triterpene alcohol isolated from the acetone extract of *Euphorbia boetica*.[11] The MS and ^{1}H and ^{13}C NMR data reported there for boeticol and its acetate were in good agreement with those of **3** and **3a**, respectively, suggesting that antiquol B and boeticol are the same compounds.[15] Euphorbol, isolated from some *Euphorbia* species and assigned the structure 24-methyleupha-8,24(24[1])-dien-3*β*-ol, and its acetate[3] exhibit melting points and optical rotations very close to those of **4** and **4a** (see Experimental Section), respectively. This strongly suggests that euphorbol possesses the tirucallane-type structure but was previously assigned erroneously the euphane-type structure.[3] There are fairly distinct differences in the physical characteristics between euphane- and tirucallane-type triterpenes, i.e., euphol acetate [**1a**; mp 117 °C; $[\alpha]_D$ + 32° ($CHCl_3$)] and tirucallol acetate [mp 163−165 °C; $[\alpha]_D$ −5° ($CHCl_3$)],[3] which supports the above supposition.

The inhibitory effects of compounds **1**−**7** on EBV-EA activation induced by TPA were examined for the primary screening of antitumor-promoting activities, and the results are shown in Table 2. All compounds, with the exception of a monocyclic compound **7**, showed potent inhibitory effects while preserving high viability of Raji cells. In this assay compounds **1**−**4** and **6** showed 100% inhibition of activation at 1000 mol ratio/TPA, of which inhibitory effects were found to be stronger than those of *β*-carotene, intensively studied in cancer prevention using animal models.[16] The inhibitory effects against EBV-EA activation have been demonstrated to be closely parallel to those against tumor promotion in vivo.[17,18] Therefore, the triterpenes **1**-**6** from *E. antiquorum* may to be useful as chemopreventive agents.

Table 2. Percentage of Epstein−Barr Virus Early Antigen Induction in the Presence of Compounds **1**−**7** with Respect to a Positive Control (100%)[a]

	concentration (mol ratio/TPA)				
compound	1000		500	100	10
1	0	(70)	29.8	75.7	93.5
2	0	(70)	28.2	75.0	92.8
3	0	(70)	34.7	78.4	96.5
4	0	(70)	32.6	77.9	95.8
5	2.2	(70)	33.8	79.4	97.2
6	0	(70)	25.8	72.4	95.7
7	20.1	(70)	51.5	86.3	100
β-carotene[b]	8.6	(70)	34.2	82.1	100

[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]Reference compound.

Experimental Section

General Experimental Procedures. Crystallizations were performed in MeOH, and melting points are uncorrected. Column chromatography was carried out on silica gel (Silica gel 60, Merck) and on octadecyl silica (Chromatorex-ODS, 100−200 mesh; Fuji Silysia Chemical, Ltd., Aichi, Japan). Reversed-phase HPLC was carried out on octadecyl silica columns (25 cm × 10 mm i.d.), on a Superiorex ODS S-5 μm column (Shiseido Co., Ltd., Tokyo, Japan), and on a TSK ODS-120A 5 μm (Toso Co., Tokyo, Japan), at 25 °C with MeOH (4 mL/min) as mobile phase. GLC was performed using a DB-17 fused-silica capillary column (30 m × 0.3 mm i.d., column temperature 275 °C). For both HPLC and GLC, cholesterol (cholest-5-en-3β-ol) acetate was the standard for the determination of t_R of acetoxy triterpenes. EIMS and HREIMS were recorded at 70 eV. NMR spectra were recorded at 500 MHz (^{1}H NMR) and 125 MHz (^{13}C NMR) in $CDCl_3$ with tetramethylsilane (TMS) (^{1}H NMR) and $CDCl_3$ at δ 77.0 (^{13}C NMR) as internal standard. Chemical shifts are δ values. IR and UV spectra were recorded in KBr disks and in EtOH, respectively. Specific rotations were measured at 25 °C in $CHCl_3$. Acetylation (Ac_2O−pyridine) and hydrolysis of acetates (5% KOH in MeOH) were performed at room temperature overnight. Triterpene acetates **1a**,[9,19] **5a**,[14] **6a**,[9] and **7a**[20] were used as reference compounds.

Plant Material. The latex of *Euphorbia antiquorum* L. was collected in July of 1999 at Werellagama in the Central Province of Sri Lanka and identified by Mr. E. M. H. G. S. Ekanayake of the Department of Botany, University of Peradeniya, Sri Lanka. A voucher specimen (number) has been deposited at the Medical Research Institute of Sri Lanka.

Extraction and Isolation. The fresh latex (1250 g) was mixed with distilled water (3 L) and extracted with *n*-hexane (3 L × 3). The *n*-hexane-insoluble fraction was again extracted with EtOAc (3 L × 3). Evaporation of the EtOAc-soluble fraction under reduced pressure yielded EtOAc extract (120 g). A portion (60 g) of the EtOAc extract was subjected to chromatography on silica gel using *n*-hexane−EtOAc (1:0 → 0:1, v/v) and MeOH as eluants. The *n*-hexane−EtOAc (9:1) eluted a triterpene alcohol fraction (42.5 g). A portion (10 g) of the fraction was acetylated, and the acetate fraction was crystallized from MeOH, yielding **1a** (3.0 g) and a filtrate (3.6 g). Chromatography of the latter on an octadecyl silica column, MeOH, followed by reversed-phase HPLC eventually yielded seven triterpene acetates, **1a** (2.25 g), **2a** (2 mg), **3a** (4 mg), **4a** (23 mg), **5a** (4 mg), **6a** (2 mg), and **7a** (2 mg). On alkaline hydrolysis, these triterpene acetates yielded the corresponding free alcohols. Some physical characteristics and the spectral data of three compounds, **2**−**4**, and their acetyl derivatives are shown below.

Antiquol C (**2**): amorphous gum; $[\alpha]^{25}_D$ −35.0° (*c* 0.24); UV λ_{max} 230 (log ϵ 4.08), 237 (4.10), 246 (3.91) nm; IR ν_{max} 3404 (OH), 1639 (conjugated −CH=CH−), 816 (>C=CH−) cm^{-1}; ^{13}C and ^{1}H NMR C-1 [δ_C 35.7; δ_H 1.56 (α), 1.82 (β)], C-2 [27.8; 1.73 (α), 1.66 (β)], C-3 [79.1; 3.24 (dd, *J* = 4.4, 11.0 Hz)], C-4 [39.0], C-5 [48.3; 1.28 (dd, *J* = 4.8, 11.4 Hz)], C-6 [23.7; 2.24 (α), 2.09 (β)], C-7 [118.5; 5.34 (dd, *J* = 2.1, 3.1 Hz)], C-8 [141.4], C-9 [145.0], C-10 [36.2], C-11 [115.4; 5.19 (t, *J* = 3.5 Hz)], C-12 [38.4; 2.17 (2H)], C-13 [44.1], C-14 [49.7], C-15 [31.2; 1.65 (α), 1.31 (β)], C-16 [28.1; 1.96 (α), 1.35 (β)], C-17 [50.8; 1.62]; C-18 [16.3; 0.62 (s)], C-19 [20.7; 0.92 (s)], C-20 [35.8; 1.45], C-21 [18.8; 0.87 (d, *J* = 7.0 Hz)], C-22 [35.3; 1.06, 1.58], C-23 [25.1; 1.87, 2.07], C-24 [125.1; 5.10 (tt, *J* = 1.5, 6.6 Hz)], C-25 [131.0], C-26 [25.7; 1.68 (s)], C-27 [17.7; 1.61 (s)], C-28 [27.7; 0.99 (s)], C-29 [15.3; 0.89 (s)], C-30 [23.2; 0.84 (s)]; EIMS *m*/*z* 424 [M]$^+$ (100), 409 (17), 391 (14), 339 (5), 337 (5), 311 (70), 295 (5), 284 (3), 271 (27), 257 (9), 253 (16), 246 (14), 240 (13), 227 (13), 213 (10), 199 (11), 185 (16), 171 (21), 69 (42); HREIMS *m*/*z* 424.3682 (calcd for $C_{30}H_{48}O$, 424.3702).

Antiquol C acetate (2a): amorphous gum; $[\alpha]^{25}_D$ −23.6° (*c* 0.33); UV λ_{max} 230 (log ϵ 4.07), 237 (4.09), 240 (3.99) nm; IR ν_{max} 1738 (OAc), 1650 (conjugated −CH=CH−), 816 (>C=CH−) cm^{-1}; t_R 0.62 (HPLC I), 0.36 (HPLC II), 1.34 (GLC); ^{13}C NMR and ^{1}H NMR, see Table 1; EIMS *m*/*z* 466 [M]$^+$ (50), 451 (7), 406 (3), 391 (13), 381 (2), 353 (40), 337 (4), 313 (9), 295 (5), 288 (10), 273 (3), 253 (19), 240 (12), 227 (9), 213 (8), 199 (6), 185 (10), 171 (13), 69 (91), 43 (100); HREIMS *m*/*s* 466.3842 (calcd for $C_{32}H_{50}O_2$, 466.3809).

Antiquol B (3): needles, mp 75−76 °C; $[\alpha]^{25}_D$ +24.8 (*c* 0.21) [antiquol B, lit.[2] $[\alpha]^{25}_D$ + 13.0° ($CHCl_3$); boeticol, lit.[10] $[\alpha]^{20}_D$ + 30° ($CHCl_3$)]; IR ν_{max} 3434 (OH), 829 (>C=CH−) cm^{-1}; ^{13}C and ^{1}H NMR: C-1 [δ_C 18.8; δ_H 1.56 (α), 1.80 (β)], C-2 [27.9; 1.87 (α), 1.70 (β)], C-3 [76.4; 3.47 (t, *J* = 3.0 Hz)], C-4 [41.0], C-5 [142.0], C-6 [121.8; 5.64 (d, *J* = 5.8 Hz)], C-7 [25.2; 1.66 (α), 2.07 (β)], C-8 [44.5; 1.66], C-9 [35.1], C-10 [49.4; 2.06], C-11 [35.7; 1.55 (2H)], C-12 [30.3; 1.55 (α), 1.75 (β)], C-13 [46.1], C-14 [47.4], C-15 [34.2; 1.10 (α), 1.21 (β)], C-16 [28.2; 1.32 (α), 1.87 (β)], C-17 [49.9; 1.55]; C-18 [15.4; 0.81 (s)], C-19 [16.5; 0.84 (s)], C-20 [35.2; 1.53], C-21 [19.0; 0.86 (d, *J* = 6.0 Hz)], C-22 [35.4; 1.12, 1.57], C-23 [24.6; 1.85, 2.02], C-24 [125.2; 5.10 (t, *J* = 7.0 Hz)], C-25 [130.1], C-26 [25.8; 1.69 (s)], C-27 [17.7; 1.61 (s)], C-28 [29.0; 1.06 (s)], C-29 [25.5; 1.14 (s)], C-30 [18.9; 0.88 (s)]; EIMS *m*/*z* 426 [M]$^+$ (44), 411 (19), 408 (16), 393 (9), 313 (71), 295 (23), 274 (100), 259 (97), 231 (18), 189 (28), 173 (23), 163 (58), 134 (93); HREIMS *m*/*z* 426.3840 (calcd for $C_{30}H_{50}O$, 426.3859).

Antiquol B acetate (**3a**): needles, mp 76−77 °C; $[\alpha]^{25}_D$ +59.1 (*c* 0.22); IR ν_{max} 1732 (OAc), 830 (>C=CH−) cm^{-1}; t_R 0.62 (HPLC I), 0.35 (HPLC II), 1.52 (GLC); ^{13}C NMR and ^{1}H NMR, see Table 1; EIMS *m*/*z* 468 [M]$^+$ (7), 453 (2), 408 (9), 393 (7), 355 (29), 295 (11), 274 (40), 259 (45), 245 (6), 189 (13), 175 (20), 134 (49), 69 (93); HREIMS *m*/*s* 468.3972 (calcd for $C_{32}H_{52}O_2$, 468.3964).

Euphorbol (4): needles, mp 123−126 °C (lit.[3] mp 127−128 °C); $[\alpha]^{25}_D$ −1.0° (*c* 0.20) [lit.[3] $[\alpha]_D$ ±0° ($CHCl_3$)]; IR ν_{max} 3082 (OH), 889 (>C=CH−) cm^{-1}; ^{13}C and ^{1}H NMR C-1 [δ_C 35.3; δ_H 1.21 (α), 1.77 (β)], C-2 [27.9; 1.67 (α), 1.57 (β)], C-3 [79.0; 3.24 (dd, *J* = 4.3, 11.6 Hz)], C-4 [38.9], C-5 [51.0; 1.12 (dd, *J* = 1.8, 12.5 Hz)], C-6 [18.9; 1.69 (α), 1.42 (β)], C-7 [27.7; 2.08 (2H)], C-8 [133.5], C-9 [134.1], C-10 [37.3], C-11 [21.5; 2.06 (α), 1.92 (β)], C-12 [30.8; 1.72 (2H)], C-13 [44.1], C-14 [50.0], C-15 [29.8; 1.52 (α), 1.18 (β)], C-16 [28.1; 1.32 (α), 1.94 (β)], C-17 [50.1; 1.52]; C-18 [15.4; 0.76 (s)], C-19 [20.2; 0.96 (s)], C-20 [36.3; 1.43], C-21 [18.8; 0.93 (d, *J* = 6.4 Hz)], C-22 [35.0; 1.12, 1.55], C-23 [31.3; 1.88, 2.12], C-24 [156.9], C-25 [33.8; 2.24 (sept., *J* = 6.7 Hz)], C-26 [22.0; 1.02 (d, *J* = 6.8 Hz)], C-27 [21.9; 1.03 (d, *J* = 7.0 Hz)], C-28 [28.0; 1.00 (s)], C-29 [15.5; 0.80 (s)], C-30 [24.4; 0.88 (s)], C-24^1 [105.9; 4.66 and 4.72 (each br s)]; EIMS *m*/*z* 440 [M]$^+$ (53), 425 (100), 407 (43), 341 (11), 323 (10), 259 (33), 241 (18), 185 (18), 175 (14), 163 (22), 149 (34), 134 (55); HREIMS *m*/*s* 440.4009 (calcd for $C_{31}H_{52}O$, 440.4018).

Euphorbol acetate (4a): needles, mp 125−127 °C (lit.[3] mp 124−125 °C); $[\alpha]^{25}_D$ ±0.0° (*c* 0.21) [lit.[3] $[\alpha]_D$ ±0° ($CHCl_3$)]; IR ν_{max} 1738 (OAc), 891 (>C=CH−) cm^{-1}; t_R 0.84 (HPLC I), 0.56 (HPLC II), 1.58 (GLC); ^{13}C NMR and ^{1}H NMR, see Table 1; EIMS *m*/*z* 482 [M]$^+$ (32), 467 (73), 407 (40), 383 (4), 369 (3), 323 (9), 301 (15), 283 (7), 255 (8), 247 (3), 241 (17), 229 (11), 215 (12), 189 (16), 69 (96), 43 (100); HREIMS *m*/*s* 482.4120 (calcd for $C_{33}H_{54}O_2$, 482.4120).

Method of EBV-EA Induction Tests. 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% fetal bovine serum, Roswell Park Memorial Institute (FBS RPMI) 1640 medium solution (Nacalai Tesque, Inc., Kyoto, Japan). 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) and 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%.

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References and Notes

(1) Jain, S. K.; DeFilipps, R. A. *Medicinal Plants of India*, Vol. 1; Reference Publ. Inc.: Algonac, MI, 1991; p 298.
(2) Gewali, M. B.; Hattori, M.; Tezuka, Y.; Kikuchi, T.; Namba, T. *Phytochemistry* **1990**, *29*, 1625−1628.
(3) Connolly, J. D.; Hill, R. A. *Dictionary of Terpenoids*, Vol. 2; Chapmann & Hall: London, 1991; p 1197.
(4) Akihisa, T.; Tamura, T.; Matsumoto, T.; Eggleston, D. S.; Kokke, W. C. M. C.; Shimizu, N. *J. Chem. Soc., Perkin Trans. 1* **1988**, 439−443.
(5) Goad, L. J.; Akihisa, T. *Analysis of Sterols*; Blackie Academic & Professional: London, 1997.
(6) Drawings correspond to energy-minimized conformations of triterpene alcohols. Calculations were performed using MacroModel Ver. 6.0 with extended MM3 parameters. The conformation with minimum steric energy was obtained through a Metropolis Monte Carlo procedure.[7]
(7) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440−467.
(8) Akihisa, T.; Kimura, Y.; Kokke, W. C. M. C.; Takase, S.; Yasukawa, K.; Tamura, T. *J. Chem. Soc., Perkin Trans. 1* **1996**, 2379−2384.
(9) Akihisa, T.; Kimura, Y.; Koike, K.; Shibata, T.; Yoshida, Z.; Nikaido, T.; Tamura, T. *J. Nat. Prod.* **1998**, *61*, 409−412.
(10) Ferreira, M. J. U.; Ascenso, J. R.; Tavares, O. S. *J. Nat. Prod.* **1995**, *58*, 275−279.
(11) Nes, W. D.; Wong, R. Y.; Benson, M.; Akihisa, T. *J. Chem. Soc., Chem. Commun.* **1991**, 1272−1274.
(12) Ferreira, M. J. U.; Lobo, A. M.; O'Mahoney, C. A.; Williams, D. J.; Wyler, H. *J. Chem. Soc., Perkin Trans. 1* **1990**, 185−187.
(13) Gamalath, C. B.; Gunatilaka, A. A. L.; Schlemper, E. O. J. *Chem. Soc., Chem. Commun.* **1988**, 249−250.
(14) Akihisa, T.; Yasukawa, K.; Kimura, Y.; Takase, S.; Yamanouchi, S.; Tamura, T. *Chem. Pharm. Bull.* **1997**, *45*, 2016−2023.
(15) The ^{13}C NMR signal assignment done for C-28, C-29, and C-30 of boeticol and its acetate[10] should be revised to be those for the corresponding signals of compounds **3** and **3a**, respectively.
(16) Murakami, A.; Ohigashi, H.; Koshimizu, K. *Biosci. Biotech. Biochem.* **1996**, *60*, 1−8.
(17) Konoshima, T.; Takasaki, M.; Tokuda, H.; Masuda, M.; Arai, Y.; Shiojima, K.; Ageta, H. *Biol. Pharm. Bull.* **1996**, *19*, 962−965.
(18) Takasaki, M.; Konoshima, T.; Tokuda, H.; Masuda, K.; Arai, Y.; Shiojima, K.; Ageta, H. *Biol. Pharm. Bull.* **1999**, *22*, 606−610.
(19) Yasukawa, K.; Akihisa, T.; Yoshida, Z.; Takido, M. *J. Pharm. Pharmacol.* **2000**, *52*, 119−124.
(20) Akihisa, T.; Koike, K.; Kimura, Y.; Sashida, N.; Matsumoto, T.; Ukiya, M.; Nikaido, T. *Lipids* **1999**, *34*, 1151−1157.

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