# Absolute Configuration of Sesquiterpenes from *Crossopetalum tonduzii* and Their Inhibitory Effects on Epstein-Barr Virus Early Antigen Activation in Raji Cells

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Two new sesquiterpenoids (1 and 2) with a dihydro- $\beta$ -agarofuran skeleton were isolated from *Crosso-petalum tonduzii*. Their structures were elucidated on the basis of spectral analysis, including homonuclear and heteronuclear correlation NMR experiments (COSY, ROESY, HSQC, and HMBC). Their absolute configurations were determined by CD studies on 3, the benzoylated derivative of 1. Chemical correlations have allowed the absolute configurations of 4 and 5, two previously known dihydro- $\beta$ -agarofuran analogues, to be reported for the first time. Compounds 1, 2, and 5 showed strong antitumor-promoting effects on Epstein—Barr virus early antigen (EBV-EA) activation.

Species of the family Celastraceae have a long history of use in traditional medicine and agriculture, especially in Asia and Latin America.1 Sesquiterpene esters, based on the dihydro- $\beta$ -agarofuran [5,11-epoxy- $5\beta$ ,10 $\alpha$ -eudesman-4(14)-ene] skeleton, are chemotaxonomic indicators of the family,2 and they have attracted considerable interest on account of their immunosuppressive,3 cytotoxic,4 insecticidal,5 anti-HIV,6 reversal of multidrug-resistance,7 and antitumor-promoting8 activities. These data along with their structural characteristics have permitted dihydro- $\beta$ agarofuran sesquiterpenes to be considered as "privileged structures". 9 Recently, the first enantioselective synthesis of a dihydroagarofuran triol has been reported. 10 However, the synthesis of more complex polyhydroxylated dihydroagarofurans represents a challenge for synthetic organic chemists.

Inhibition of the tumor promotion stage in the multistage of chemical carcinogenesis has been regarded as a promising strategy for cancer chemoprevention. In the search for cancer chemopreventive agents, the inhibition of Epstein—Barr virus early antigen (EBV-EA) induction by the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) has been conducted as a primary screening test, which correlates well with subsequent full-term tumor-inhibition studies in animal models. I2,13

As part of an intensive study of the bioactive metabolites from species of the Celastraceae, we have previously reported sesquiterpenes as modulators of daunomycin resistance in a multidrug-resistant *Leishmania tropica* line from *Crossopetalum tonduzii* (Loes.) Lund.,  $^{14,15}$  a species that grows in Panama. In a continuation of our work on this species, we report herein on the isolation of two new sesquiterpenoids (1 and 2) with a dihydro- $\beta$ -agarofuran skeleton. Their structures were elucidated on the basis of spectroscopic data, including  $^{1}H^{-13}C$  heteronuclear correlation (HSQC), long-range correlation with inverse detection (HMBC), and ROESY NMR experiments. The CD curve of the benzoylated derivative 3, and chemical cor-

OMeBut
OBz
OAc
$$AcO OBz$$
OBz
OAc
$$AcO OBz$$
OBz
OAc
$$AcO OBz$$
OR

relations with the known analogues **4** and **5**, <sup>15</sup> allowed the absolute configurations of **1**–**5** (Scheme 1) to be determined unequivocally. The compounds have been tested for their antitumor-promoting effects on EBV-EA activation induced by the tumor promoter TPA, as a test for potential cancer chemopreventive activity. <sup>12</sup> Compounds **1**, **2**, and **5** showed strong inhibitory activities in this assay.

### **Results and Discussion**

Repeated chromatography of the ethanolic extract of the leaves of *C. tonduzii* on Sephadex and Si gel afforded the new compounds **1** and **2**. Compound **1** was isolated as a colorless lacquer with the molecular formula  $C_{33}H_{44}O_{13}$  by HREIMS. The IR spectrum showed absorption bands for hydroxyl (3410 cm<sup>-1</sup>) and carbonyl (1730 cm<sup>-1</sup>) groups. The mass spectrum contained fragments attributable to the presence of benzoate (M<sup>+</sup> – 15–122, m/z511,  $C_6H_5$ COOH), 2-methylbutyrate (M<sup>+</sup> – 102, m/z546,  $C_4H_9$ COOH), acetate (M<sup>+</sup> – 60, m/z588, CH<sub>3</sub>COOH), and hydroxy (M<sup>+</sup> – 18, m/z630, H<sub>2</sub>O) groups. This was confirmed by the <sup>1</sup>H and

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**Table 1.** NMR Data for Compounds 1−3

	1				2		
position	$\delta_{\mathrm{H}^a}$	$\delta c^b$	HMBC ( <sup>13</sup> C)	$\delta_{ ext{H}^a}$	$\delta c^b$	HMBC ( <sup>13</sup> C)	$\delta_{\mathrm{H}^a}$
1	5.43 d (3.0)	78.1 d	9, 10 <sup>c</sup> , 15, CH <sub>3</sub> COO <sup>-</sup>	5.48 d (3.5)	78.9 d	9, 10 <sup>c</sup> , 15, CH <sub>3</sub> COO <sup>-</sup>	5.60 d (3.6)
2	4.14 m	67.3 d		5.37 m	67.5 d	CH <sub>3</sub> COO <sup>-</sup>	5.66 m
3	2.03 m	42.0 t	$1, 4^c, 5$	2.05 m	41.5 t		2.16 m
4 5		69.8 s			67.5 s		
5		92.5 s			91.5 s		
6	6.50 s	75.8 d	8, 10, 11, CH <sub>3</sub> <i>C</i> OO <sup>-</sup>	5.05 d (5.1)	77.2 d	5°, 10, 11	6.48 s
7	2.51 d (3.1)	52.1 d	5, 8 <sup>c</sup> , 9	2.61 d (3.3)	55.5 d	5, 9	2.54 d (3.1)
8	5.71 dd (3.1, 9.8)	74.0 d	6, 9 <sup>c</sup> ,11, CH <sub>3</sub> COO <sup>-</sup>	4.32 m	72.2 d	6, 11	5.79 dd (3.1, 9.5)
9	6.05 d (9.8)	75.7 d	1, 8 <sup>c</sup> , 15, C <sub>6</sub> H <sub>5</sub> COO <sup>-</sup>	5.88 d (9.4)	74.9 d	1, 8 <sup>c</sup> , 15, C <sub>6</sub> H <sub>5</sub> COO <sup>-</sup>	6.10 d (9.5)
10		51.6 s			50.8 s		
11		84.0 s			84.6 s		
12	1.71 s	26.5 c	7, 11 <sup>c</sup> , 13	1.75 s	26.6 c	7, 11 <sup>c</sup> , 13	1.58 s
13	1.56 s	29.7 с	7, 11 <sup>c</sup> , 12	1.62 s	30.2 c	7, 11 <sup>c</sup> , 12	1.56 s
14	1.54 s	25.1 c	$3, 4^c, 5$	1.76 s	24.0 c	$3, 4^c, 5$	1.74 s
15	4.79, 5.03	62.0 t	1, 5, 9,	4.66, 4.95	61.8 t	1, 5, 9, 10,	4.65, 5.27
	$d_{AB}$ (13.4)		$C_4H_9COO^-$	$d_{AB}$ (13.5)		$C_4H_9COO^-$	d <sub>AB</sub> (13.2)

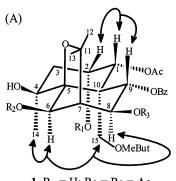
<sup>a</sup> δ, CDCl<sub>3</sub>, J values in hertz. <sup>b</sup> Data are based on DEPT and HSQC experiments. <sup>c</sup> Two-bond coupling enhancement observed.

<sup>13</sup>C NMR data (Table 1), which included signals for five aromatic protons between  $\delta$  7.42 and 7.92, signals at  $\delta$  1.01 as a triplet (3H, J= 7.4 Hz) and at  $\delta$  1.34 (3H, J= 7.0 Hz) as a doublet, two methylene protons as two multiplets at  $\delta$  1.66 and 1.90, and a multiplet at  $\delta$  2.76 (1H), characteristic of a 2-methylbutyrate moiety, and three acetate methyls as singlets at  $\delta$  1.62, 1.88, and 2.12 (3H each).

In the  $^1H$  NMR spectrum of 1 (Table 1) were also observed signals assignable to protons on carbons bearing four secondary ester groups at  $\delta$  5.43 (d, J=3.0 Hz, H-1), 6.50 (s, H-6), 5.71 (dd, J=3.1, 9.8 Hz, H-8), and 6.05 (d, J=9.8 Hz, H-9), two protons of a primary ester group at  $\delta$  4.79 and 5.03 (d<sub>AB</sub>, J=13.4 Hz, H-15), and a proton geminal to a secondary hydroxyl group at  $\delta$  4.14 (m, H-2). An angular methyl group at  $\delta$  1.54 attached to a carbon at  $\delta_{\rm C}$  69.8 bearing a hydroxyl group, and two angular methyls at  $\delta$  1.56 and 1.71, which were confirmed from the  $^{13}{\rm C}$  NMR spectrum (Table 1), were also observed. All these data indicated that 1 is a polyester sesquiterpene with a 1,2,6,8,9,15-hexasubstituted  $4\beta$ -hydroxydihydro- $\beta$ -agarofuran skeleton.

The regiosubstitution of 1 was established by a HMBC experiment (Table 1), showing three-bond correlations between the carboxyl signals of the acetate groups at  $\delta_C$ 169.4 ( $\times$ 2) and 169.6 and the signals at  $\delta_{\rm H}$  5.43 (H-1), 5.71 (H-8), and 6.50 (H-6); a correlation between the carboxyl signal of the benzoate group at  $\delta_{\rm C}$  166.1 and the signal at  $\delta_{\rm H}$  6.05 (H-9); and correlations between the carboxyl signal of the 2-methylbutyrate at  $\delta_{\rm C}$  176.3 and the signals at  $\delta_{\rm H}$ 4.79 and 5.03 (H-15). The relative stereochemistry of 1 was established on the basis of the coupling constants and confirmed by a ROESY experiment (Figure 1A). Thus, in the COSY experiment, the coupling constants of H<sub>1</sub>-H<sub>2</sub> and  $H_8-H_9$  ( $J_{1,2}=3.0$  Hz and  $J_{8,9}=9.8$  Hz) indicated a cisrelationship between H-1 and H-2 and a trans-relationship between H-8 and H-9. In the ROESY experiment, significant cross-peaks were observed between H-1 and H-2 and H-9, between H-15 and H-6, H-8, and Me-14, and between Me-12 and H-9.

To determine the absolute configuration of 1, it was necessary to introduce another chromophoric group. Benzoylation yielded the dibenzoate, 3 (Scheme 1, Table 1), a derivative suitable for applying the dibenzoate chirality method, an extension of the circular dichroism exciton



1  $R_1 = H$ ;  $R_2 = R_3 = Ac$ 2  $R_1 = Ac$ ;  $R_2 = R_3 = H$ 

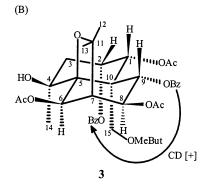


Figure 1. (A) NOE effects for compounds  ${\bf 1}$  and  ${\bf 2}$ ; (B) CD exciton coupling for compound  ${\bf 3}$ .

chirality procedure. <sup>16</sup> Its CD spectrum showed a split curve with a first positive Cotton effect at 235.4 nm ( $\Delta\epsilon=+14.3$ ) and a second negative effect at 220.2 ( $\Delta\epsilon=-1.9$ ) due to the couplings of the two chromophoric benzoates at C-2 $\alpha$  and C-9 $\alpha$  (Figure 1B). Therefore, **3** was identified as (1*R*,2*S*,4*S*,5*S*,6*R*,7*R*,8*S*,9*S*,10*S*)-1,6,8-triacetoxy-2,9-dibenzoyloxy-15-(2-methylbutyroyloxy)-4-hydroxydihydro- $\beta$ -agarofuran.

Compound 2, with the molecular formula  $C_{30}H_{39}O_{12}$  (HREIMS) and after the measurement of its IR, UV,  $^1H$  and  $^{13}C$  NMR data (Table 1), and 2D NMR experiments, was shown to be a dihydro- $\beta$ -agarofuran sesquiterpene with two acetates, one benzoate, one 2-methylbutyrate, and

**Table 2.** Percentage of Epstein−Barr Virus Early Antigen Induction in the Presence of Compounds 1−5 and with Respect to a Positive Control<sup>c</sup>

concentration (mol ratio/ TPA) <sup>a</sup>	1	2	3	4	5	$\beta$ -carotene $^d$
1000	0 <sup>b</sup> (60)	0 (60)	4.6 (60)	0 (70)	0 (70)	8.6 (70)
500	31.2	32.7	33.8	22.0	21.8	34.2
100	73.7	74.0	75.9	79.2	77.4	82.1
10	90.5	90.1	96.0	96.7	94.1	100

 $^a$  Mol ratio/TPA (32 pmol = 20 ng/mL), 1000 mol ratio = 32 nmol, 500 mol ratio = 16 nmol, 100 mol ratio = 3.2 nmol, and 10 mol ratio = 0.32 nmol.  $^b$  Values in parentheses represent viability percentages of Raji cells; unless otherwise stated, the viability percentages of Raji cells were more than 80%.  $^c$  Values represent percentages of EBV-EA induction to the positive control values (100%) (n=3).  $^d$  Internal standard control substance.

three hydroxyl groups, located at positions C-1 $\alpha$ , C-2 $\alpha$ ,  $C-4\beta$ ,  $C-6\beta$ ,  $C-8\beta$ ,  $C-9\alpha$ , and C-15. A HMBC experiment (Table 1) established the regiosubstitution patterns, and the relative stereochemistry was solved by analysis of a ROESY experiment (Figure 1A). Its absolute configuration was established by chemical correlation with 1; thus, acetylation of 1 and 2 yielded the previously described compound 415 (Scheme 1). Therefore, the structure of 2 was established as (1R,2S,4S,5S,6R,7R,8S,9S,10S)-1,2-diacetoxy-9-benzoyloxy-15-(2-methylbutyroyloxy)-4,6,8-trihydroxydihydro- $\beta$ -agarofuran. In the same way, the absolute configurations of the known compounds 4 and 5,15 which have not been reported previously, were accordingly established by chemical correlations (Scheme 1) as (1R,2S,4S,-5S,6R,7R,8S,9S,10S)-1,2,6,8-tetraacetoxy-9-benzoyloxy-15-(2-methylbutyroyloxy)-4-dihydro-β-agarofuran and (1R,2S,-4S,5S,6R,7R,8S,9S,10S)-1,2,8-triacetoxy-9-benzoyloxy-15-(2-methylbutyroyloxy)-4,6-dihydroxydihydro-β-agarofuran, respectively. All compounds in Scheme 1 have the basic polyhydroxylated skeleton of 8-epi-4β-hydroxyalatol.<sup>17</sup>

Compounds 1−5 were tested for their inhibitory effects on Epstein-Barr virus early antigen (EBV-EA), induced by the tumor promoter 12-O-tetradecanoylphorbol-13acetate (TPA) in Raji cells (Table 2), which was conducted as a primary screening test in the search for cancer chemopreventive agents. 12,13 Compounds 2, 1, and 5 exhibited strong antitumor-promoting activity in decreased order of inhibitory potency (9.9%, 9.5%, and 5.9% at 10 mol ratio/ATP, respectively), and all preserved a high viability of Raji cells (more than 60% at 10-1000 mol ratio/TPA). Furthermore, the inhibitory activities of these compounds were greater than those of glycyrrhetinic acid, a known active compound in this test system, and were also more potent than other dihydro- $\beta$ -agarofuran sesquiterpenes previously evaluated.8 From these results, it was concluded that sesquiterpenes 1, 2, and 5 might be valuable cancer chemopreventive agents and should be considered for additional biological testing.

# **Experimental Section**

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 automatic polarimeter, and  $[\alpha]_D$  values are given in  $10^{-1}$  deg cm² g $^{-1}$ . CD spectra were run on a JASCO J-600 spectropolarimeter. IR spectra were recorded in CHCl $_3$  on a Bruker IFS 55 spectrophotometer, and UV spectra were collected in absolute EtOH on a JASCO V-560 instrument.  $^1H$  and  $^{13}C$  NMR spectra were recorded on a Bruker Avance 400 at 400 and 100 MHz, respectively. EIMS and HREIMS were recorded on a Micromass Autospec spectrometer. Schleicher and Schuell TLC 1500/LS 25 foils were used for thin-layer chromatography, while Si gel (0.2–0.63 mm) and Sephadex LH-20 were used for column chromatog-

raphy. The cell culture reagent and *n*-butyric acid were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). 12-*O*-Tetradecanoylphorbol-13-acetate (TPA) was obtained from Sigma Chemical Co. (St. Louis, MO).

Plant Material. Crossopetalum tonduzii was collected at Boquete, Chiriquí, Panamá, in August 1991. A voucher specimen (FLORPAN 882) is deposited at the Herbarium of the University of Panamá. The leaves (1.5 kg) of *C. tonduzii* were extracted with ethanol in a Soxhlet apparatus, yielding 190 g of residue, which was chromatographed on Si gel, using mixtures of n-hexane–EtOAc of increasing polarity as eluent. The n-hexane–EtOAc (1:1) eluting fraction was then chromatographed on Sephadex LH-20 (n-hexane–CHCl<sub>3</sub>–MeOH, 2:1:1) and Si gel (n-hexane–1,4-dioxane, 3:2) to yield compounds 1 (8.0 mg,  $R_f$  = 0.46) and 2 (3.4 mg,  $R_f$  = 0.39). Compound 3 used for CD was purified by HPLC using a semipreparative  $\mu$ -Porasil column and eluted with a mixture of n-hexane–EtOAc (1:1).

1*R*,2*S*,4*S*,5*S*,6*R*,7*R*,8*S*,9*S*,10*S*)-1,6,8-Triacetoxy-9-benzoyloxy-15-(2-methylbutyroyloxy)-2,4-dihydroxydihydro**β-agarofuran (1):** colorless lacquer;  $[\alpha]_D^{25}$  +10.7° (c 0.3, CHCl3); UV (EtOH)  $\lambda_{max}$  (log  $\epsilon) 273$  (3.01), 228 (4.05) nm; IR (CHCl<sub>3</sub>)  $\nu_{\text{max}}$  3410, 2924, 1750, 1730, 1278, 1221, 1091, 712 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.01 (3H, t, J = 7.4 Hz), 1.34 (3H, d, J = 7.0 Hz), 1.62 (3H, s), 1.66 (1H, m), 1.88 (3H, s), 1.90 (1H, m), 2.12 (3H, s), 2.76 (1H, m), 2.77 (1H, s, OH-4), 7.42 (2H, m), 7.56 (1H, m), 7.92 (2H, m), for other signals, see Table 1;  $^{13}\text{C}$  NMR (CDCl<sub>3</sub>)  $\delta$  11.6 (q), 16.5 (q), 20.8 (q), 20.9 (q), 21.4 (q), 25.8 (t), 41.4 (d), 128.6 (2 x d), 129.4 (s), 129.6 (2  $\times$  d), 133.5 (d), 166.1 (s), 169.4 (2  $\times$  s), 169.6 (s), 176.3 (s), for other signals, see Table 1; EIMS m/z 648 [M]+ (1), 633 (2), 630 (1), 588 (3), 570 (4), 546 (1), 528 (2), 511 (1), 510 (3), 486 (1), 468 (1), 336 (9), 202 (17), 149 (27), 123 (8), 105 (100), 85 (18); HREIMS m/z 648.2795 (calcd for  $C_{33}H_{44}O_{13}$ , 648.2782).

(1*R*,2*S*,4*S*,5*S*,6*R*,7*R*,8*S*,9*S*,10*S*)-1,2-Diacetoxy-9-benzoyloxy-15-(2-methylbutyroyloxy)-4,6,8-trihydroxydihydro- $\beta$ -agarofuran (2): colorless lacquer; [α]<sub>D</sub><sup>25</sup> +5.3° (c 0.19, CHCl<sub>3</sub>); UV (EtOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 273 (3.03), 229 (4.02) nm; IR (CHCl<sub>3</sub>)  $\nu_{\text{max}}$  3427, 2926, 2854, 1731, 1745, 1368, 1278, 1142, 712 cm<sup>-1</sup>; <sup>1</sup>H NMR δ 0.99 (3H, t, J = 7.4 Hz), 1.31 (3H, d, J = 7.2 Hz), 1.48 (3H, s), 1.60 (1H, m), 1.79 (1H, m), 2.13 (3H, s), 2.54 (1H, m), 3.12 (1H, s, OH-4), 5.17 (1H, d, J = 5.1 Hz, OH-6), 7.44 (2H, m), 7.57 (1H, m), 7.95 (2H, m), for other signals, see Table 1; <sup>13</sup>C NMR δ 11.7 (q), 16.9 (q), 20.1 (q), 21.1 (q), 26.4 (t), 41.1 (d), 128.7 (2 × d), 129.4 (s), 129.7 (2 × d), 133.6 (d), 167.9 (s), 169.4 (s), 169.5 (s), 176.1 (s), for other signals, see Table 1; EIMS m/z591 [M<sup>+</sup> – 15] (9), 573 (2), 528 (2), 513 (2), 471 (2), 451 (2), 435 (1), 202 (6), 105 (100), 85 (17); HREIMS 591.2442 (calcd for C<sub>30</sub>H<sub>39</sub>O<sub>12</sub>, 591.2518).

**Benzoylation of 1.** Compound **1** (4.0 mg) was dissolved in dry pyridine (0.5 mL) and benzoyl chloride (6 drops), and some crystals of 4-(dimethylamino)pyridine were added under an argon atmosphere. The mixture was heated at 60 °C for 15 h, poured over  $H_2O$  extracted with EtOAc, and purified by preparative TLC with a mixture of n-hexane—EtOAc (1:1) to give **3** (3.0 mg).

(1*R*,2*S*,4*S*,5*S*,6*R*,7*R*,8*S*,9*S*,10*S*)-1,6,8-Triacetoxy-2,9-dibenzoyloxy-15-(2-methylbutyroyloxy)-4-hydroxydihydro-β-agarofuran (3): colorless lacquer;  $[\alpha]_D^{25}$  +33.3° (c0.1, CHCl<sub>3</sub>); CD  $\lambda_{\rm ext}$  (MeCN) 235.4 ( $\Delta\epsilon$  = +14.3), 220.2 ( $\Delta\epsilon$  = -1.9) nm; UV (EtOH)  $\lambda_{\rm max}$  ( $\log\epsilon$ ) 274 (3.12), 230 (4.22) nm; IR (CHCl<sub>3</sub>)  $\nu_{\rm max}$  3411, 2923, 2853, 1728, 1459, 1263, 1095, 712 cm<sup>-1</sup>;  $^1$ H NMR δ 1.03 (3H, t, J = 7.4 Hz), 1.37 (3H, d, J = 7.0 Hz), 1.51 (3H, s), 1.65 (1H, m), 1.88 (3H, s), 1.90 (1H, m), 2.15 (3H, s), 2.81 (1H, m), 7.38 (2H, m), 7.51 (4H, m), 7.89 (2H, m), 8.08 (2H, m); EIMS m/z 737 [M<sup>+</sup> – 15] (1), 692 (1), 630 (1), 588 (1), 570 (9), 368 (3), 336 (10), 202 (22), 164 (3), 149 (35), 105 (100); HREIMS m/z 737.2809 (calcd for  $C_{39}$ H<sub>45</sub>O<sub>14</sub>, 737.2810).

**Acetylation of 1.**  $Ac_2O$  (4 drops) was added to compound 1 (2.0 mg) dissolved in pyridine (2 drops), and the mixture left at room temperature for 16 h. EtOH (3  $\times$  2 mL) was added and carried almost to dryness in a rotavapor, and this process was repeated with CHCl<sub>3</sub> (3  $\times$  2.0 mL) and purified by

preparative TLC with a mixture of n-hexane—EtOAc (1:1) to give a product (1.5 mg) for which the spectroscopic data were identical to those of 4.15

**Acetylation of 2 and 5.** Compounds **2** (1.0 mg) and **5** (3.0 mg) were treated under the same conditions as described above to give a common product (0.9 mg and 2.5 mg, respectively) for which the spectroscopic data were identical to those of 4.15

In Vitro EBV-EA Induction Assay. The EBV genome-carrying lymphoblastoid cells, Raji cells, derived from Burkitt's lymphoma, were cultivated in RPMI-1640 medium. The Raji cells were incubated for 48 h at 37 °C in a medium containing n-butyric acid (4 mmol), TPA (32 pmol), and various amounts of test compounds. Smears were made from the cell suspensions, and the EBV-EA-inducing cells were stained by means of an indirect immunofluorescence technique. The details of the in vitro assay on EBV-EA induction have been reported previously.  $^{13}$   $\beta$ -Carotene, a vitamin A precursor that has been intensively studied in cancer prevention using animal models,  $^{11}$  was used as positive control.

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