Sunpollenol and Five Other Rearranged 3,4-*seco*-Tirucallane-Type Triterpenoids from Sunflower Pollen and Their Inhibitory Effects on Epstein−Barr Virus Activation

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Six new rearranged 3,4-*seco*-tirucallane-type triterpenoids (**1**−**6**) have been isolated from the diethyl ether extract of the pollen grains of sunflower (*Helianthus annuus*). These compounds were evaluated with respect to their inhibitory effects on the induction of Epstein−Barr virus early antigen (EBV-EA) induced by the tumor promoter 12-*O*-tetradecanoylphorbol 13-acetate (TPA) in Raji cells. All of the compounds tested showed potent inhibitory effects on EBV-EA activation (97−100% inhibition at 1×10^3 mol ratio/TPA).

We recently demonstrated that the terpenoid and lipid constituents isolated from the pollen extract of sunflower (*Helianthus annuus* L.; Compositae) exhibited significant inhibitory effects on Epstein−Barr virus early antigen (EBV-EA) induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA).[1] In a continuation of our study on the pollen extract of sunflower we now report six new triterpenoids (**1**−**6**) that possess a rearranged 3,4-*seco*-tirucallane skeleton. We also report in this paper their inhibitory effects on the induction of EBV-EA induced by TPA, as a preliminary screen for potential cancer chemopreventive activities.

Results and Discussion

Column chromatography on silica gel followed by reversed-phase HPLC of the diethyl ether extract of sunflower pollen afforded six compounds, **1**−**6**. The molecular formula of compound **1** was determined as $C_{30}H_{52}O_2$ from its HRCIMS ($[M + H]^+$ *m/z* 445.4039) and EIMS ($[M - Me]^+$ *m/z* 429) as well as from its ^{13}C NMR. The IR absorption bands of **1** suggested the presence of a hydroxyl group and a trisubstituted double bond. Compound **1** had seven tertiary methyl, a secondary methyl, an oxygenated methylene, an olefinic methine, and a tertiary hydroxyl group (Tables 1 and 2). The oxygenated methylene signals were assigned to the geminally coupled methylene protons α to the oxygen of the pyran ring.[2] The EIMS exhibited fragment ions at *m/z* 429 $[M - Me]^+$, 315 [M − side-chain (C_8H_{15}; C-20−C-27) − $H_2O]^+$,[3] and 69 $[CH_2CH{=}C(Me)_2]^+$ (C-23−C-27), which suggested that compound **1** (sunpollenol) possesses a C-24 unsaturated C_8 side-chain and a tetracyclic ring system with two oxygen atoms.[3] Fragment ions at *m/z* 385 [loss of isopropanyl group $(C_3H_7O)]^+$,[2] 274 ($C_{20}H_{34}$), corresponding to the loss of rings A and B due to the cleavages of the C-7−C-8 and C-9−C-10 bonds, and 163 ($C_{12}H_{19}$; 274 − side-chain) indicated the presence of an 2-propanol group, located most probably at C-5,[2] and the absence of a methyl group at C-10.[3] The above evidence coupled with the spectral comparison with helianol [3,4-*seco*-19(10→9)-*abeo*-8α,9*β*,10α-tirucalla-4,24-dien-3-ol][3] and terminalin A (4-hydroxy-3,4-*seco*-glutinan-3,5*β*-oxide)[2] allowed us to deduce that **1** was 4-hydroxy-3,4-*seco*-19(10→9)-*abeo*-8α,9*β*,10α-tirucall-24-en-3,5-oxide. This structure was supported by the ^1H (Table 1) and ^{13}C NMR data (Table 2) and analysis of ^{13}C DEPT NMR, $^1H-^1H$ COSY, HMQC, and HMBC spectra. The relative stereochemistry of **1** was determined by a phase-sensitive NOESY experiment. Compound **1** showed significant NOE correlations between [H-29−H-10α−H-8α−H-18 (13α-Me)−H-20] on the α-face of the molecule, [H-3*β*−H-19 (9*β*-Me)−H-30 (14*β*-Me)−H-17*β*−H-21] on the *β*-face of the molecule, and [H-12α−H-21] (Figure 1).[4]

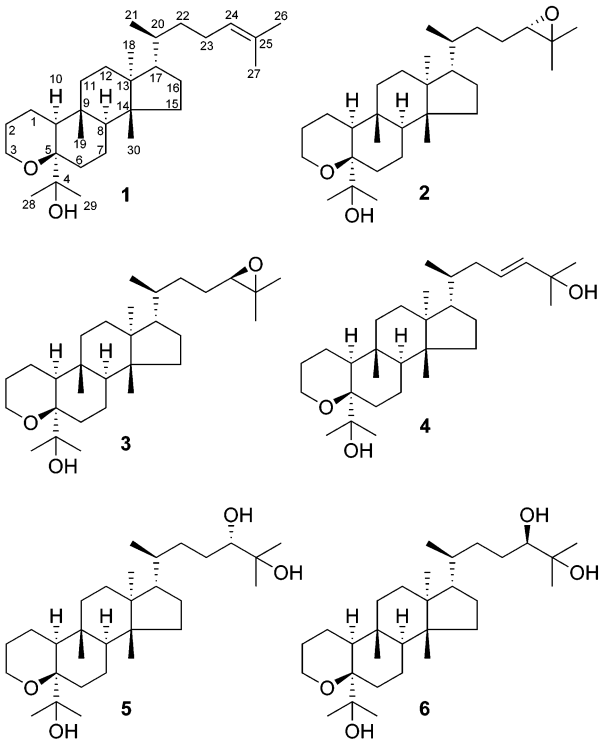

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Table 1. ^{1}H NMR Spectral Data (δ values; 500 MHz; $CDCl_3$) of Compounds **1**−**6**[a]

H no.	1	2	3	4	5	6
1	1.80 (2H)	1.80 (2H)	1.80 (2H)	1.79 (2H)	1.80 (2H)	1.80 (2H)
2	1.64 (*β*), 1.75 (α)	1.63 (*β*), 1.74 (α)	1.63 (*β*), 1.74 (α)	1.62 (*β*), 1.73 (α)	1.65 (*β*), 1.73 (α)	1.63 (*β*), 1.72 (α)
3	3.65 (*β*) (ddd) (6.4, 8.7, 11.9) 3.82 (α) (ddd) (4.1, 8.2, 11.9)	3.65 (*β*) (ddd) (6.4, 8.5, 11.9) 3.82 (α) (ddd) (4.6, 7.9, 11.9)	3.65 (*β*) (ddd) (6.4, 8.5, 11.9) 3.82 (α) (ddd) (4.6, 7.9, 11.9)	3.65 (*β*) (ddd) (6.4, 8.5, 11.6) 3.82 (α) (ddd) (4.2, 7.9, 11.9)	3.65 (*β*) (ddd) (6.4, 8.7, 11.9) 3.82 (α) (ddd) (4.6, 8.0, 12.1)	3.64 (*β*) (ddd) (6.4, 8.7, 11.9) 3.82 (α) (ddd) (4.4, 7.8, 11.9)
6	1.86 (2H)	1.85 (2H)	1.85 (2H)	1.87 (2H)	1.85 (2H)	1.85 (2H)
7	1.34 (α), 1.69 (*β*)	1.31 (α), 1.68 (*β*)	1.31 (α), 1.68 (*β*)	1.32 (α), 1.69 (*β*)	1.31 (α), 1.71 (*β*)	1.29 (α), 1.68 (*β*)
8	1.56	1.56	1.56	1.55	1.56	1.55
10	1.57	1.57	1.57	1.57	1.58	1.55
11	1.34 (α), 1.61 (*β*)	1.36 (α), 1.61 (*β*)	1.36 (α), 1.61 (*β*)	1.35 (α), 1.60 (*β*)	1.36 (α), 1.61 (*β*)	1.35 (α), 1.59 (*β*)
12	1.58 (α), 1.75 (*β*)	1.55 (α), 1.76 (*β*)	1.55 (α), 1.76 (*β*)	1.57 (α), 1.75 (*β*)	1.57 (α), 1.76 (*β*)	1.55 (α), 1.75 (*β*)
15	1.04 (α), 1.16 (*β*)	1.05 (α), 1.18 (*β*)	1.05 (α), 1.18 (*β*)	1.06 (α), 1.17 (*β*)	1.07 (α), 1.17 (*β*)	1.06 (α), 1.15 (*β*)
16	1.28 (α), 1.90 (*β*)	1.28 (α), 1.93 (*β*)	1.28 (α), 1.93 (*β*)	1.32 (α), 1.93 (*β*)	1.28 (α), 1.95 (*β*)	1.32 (α), 1.92 (*β*)
17	1.48	1.50	1.50	1.49	1.48	1.49
18 (13α)	0.79 (s)	0.80 (s)	0.80 (s)	0.79 (s)	0.80 (s)	0.79 (s)
19 (9*β*)	1.08 (s)	1.08 (s)	1.08 (s)	1.08 (s)	1.08 (s)	1.08 (s)
20	1.43	1.47	1.50	1.49	1.47	1.45
21	0.91 (d) (6.4)	0.92 (d) (6.1)	0.92 (d) (6.1)	0.89 (d) (6.1)	0.91 (d) (6.0)	0.92 (d) (6.0)
22	1.05, 1.43	1.20, 1.47	1.10, 1.62	1.75, 2.17	1.26, 1.50	1.00, 1.77
23	1.86, 2.04	1.36, 1.62	1.41, 1.55	5.59	1.38 (2H)	1.14, 1.58
24	5.09 (tt) (1.4, 6.0)	2.68 (dt) (1.5, 6.4)	2.68 (dt) (1.5, 6.4)	5.59	3.34 (t) (7.6)	3.29 (d) (9.8)
26	1.68 (s)	1.26 (s)	1.26 (s)	1.31 (s)	1.17 (s)	1.16 (s)
27	1.60 (s)	1.31 (s)	1.31 (s)	1.31 (s)	1.22 (s)	1.22 (s)
28	1.27 (s)	1.27 (s)	1.27 (s)	1.27 (s)	1.27 (s)	1.27 (s)
29	1.30 (s)	1.31 (s)	1.31 (s)	1.30 (s)	1.30 (s)	1.30 (s)
30 (14*β*)	0.85 (s)	0.86 (s)	0.86 (s)	0.85 (s)	0.86 (s)	0.86 (s)

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

Table 2. ^{13}C NMR Spectral Data (δ values; $CDCl_3$) of Compounds **1**−**6**

C no.	1	2	3	4	5	6
1	17.3	17.3	17.3	17.3	17.4	17.4
2	21.1	21.1	21.1	21.1	21.1	21.1
3	58.6	58.6	58.6	58.7	58.7	58.7
4	78.5	78.5	78.5	78.5	78.6	78.6
5	80.2	80.2	80.2	80.2	80.2	80.2
6	24.9	24.9	24.9	24.9	25.0	25.0
7	19.7	19.7	19.7	19.7	19.8	19.7
8	42.8	42.8	48.2	42.8	42.9	42.9
9	38.1	38.2	38.2	38.2	38.2	38.1
10	47.2	47.2	47.2	47.2	47.2	47.2
11	38.2	38.1	38.1	38.1	38.3	38.2
12	30.1	30.1	30.1	30.0	30.2	30.2
13	46.1	46.1	46.1	46.1	46.2	46.1
14	48.2	48.2	48.2	48.2	48.2	48.2
15	33.9	33.9	33.9	33.9	33.9	33.9
16	28.2	28.2	28.2	28.1	28.3	28.2
17	50.5	50.3	50.4	50.2	50.7	50.6
18 (13α)	15.4	15.4	15.4	15.5	15.5	15.5
19 (9*β*)	19.8	19.8	19.8	19.8	19.8	19.8
20	35.8	35.8	35.9	36.2	36.9	36.3
21	18.6	18.6	18.7	18.6	18.5	18.8
22	36.4	32.7	32.9	39.1	33.3	33.7
23	24.9	25.6	25.9	125.5	28.4	28.7
24	125.2	64.8	64.9	139.4	78.8	79.7
25	130.9	58.4	58.1	70.8	73.2	73.2
26	25.7	18.8	18.5	29.8[a]	23.3	23.2
27	17.6	24.9	24.9	29.9[a]	26.6	26.6
28	24.5	24.5	24.5	24.5	24.6	24.6
29	26.4	26.4	26.4	26.4	26.5	26.4
30 (14*β*)	18.4	18.4	18.4	18.4	18.5	18.5

[a] Values bearing the same superscript in each column are interchangeable.

Compounds **2** and **3** were both assigned a molecular formula of $C_{30}H_{52}O_3$, as determined from their ^{13}C DEPT NMR and MS data. These compounds showed absorption due to a hydroxyl group in the IR spectra. The ^{1}H (Table 1) and ^{13}C NMR (Table 2) data for the ring system of **2** and **3** were almost indistinguishable from those of compound **1**, whereas their side-chain NMR signals were very close to those of 4α,5α:24,25-diepoxyhelianol [4α,5α:24,25-diepoxy-3,4-*seco*-19(10→9)-*abeo*-8α,9*β*,10α-tirucallan-3-ol].[1] The presence of an epoxy group at C-24 and C-25 in compounds **2** and **3** was deduced from the HMBC experiment in which cross-correlations for H-24 (with C-22, C-23, C-25-C-27), H-26 (with C-24, C-25, C-27), and H-27 (with C-24−C-26) were observed. Thus, compounds **2** and **3** were assumed to be C-24 epimers of 4-hydroxy-3,4-*seco*-19-(10→9)-*abeo*-8α,9*β*,10α-tirucallane-3,5*β*:24,25-dioxide. The ^{13}C NMR chemical shift differences for the side-chain ^{13}C signals allowed stereochemical assignment at C-24 of **2** and **3**. On the basis of the ^{13}C NMR data (Table 2), the chemical shift differences between **2** and **3** were calculated to be C-20 ($\delta_3 - \delta_2 = 0.1$), C-21 (0.1), C-22 (0.2), C-23 (0.3), C-24 (0.1), C-25 (−0.3), C-26 (−0.3), and C-27 (0.0), consistent with the differences of $\delta_R - \delta_S$ for 4α,5α:24,25-diepoxyhelianol.[1] Therefore, compounds **2** and **3** were 24*S*- and 24*R*-4-hydroxy-3,4-*seco*-19(10→9)-*abeo*-8α,9*β*,10α-tirucallane-3,5*β*:24,25-dioxides, named (24*S*)- and (24*R*)-24,25-epoxysunpollenols, respectively. Analysis of the ^{13}C DEPT, ^{1}H−^{1}H COSY, HMQC, HMBC, and phase-sensitive NOESY spectra of **2** and **3** supported their proposed structures.

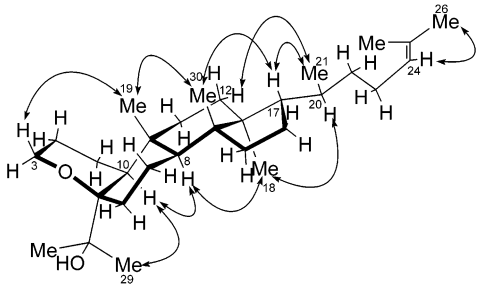

Figure 1. Energy-minimized conformation and some representative NOE correlations (←→) for **1**.

Compound **4** was assigned a molecular formula of $C_{30}H_{52}O_3$, as determined from its ^{13}C DEPT NMR data and the $[MH - H_2O]^+$ ion at *m*/*z* 443.3882 in HRCIMS. The IR absorptions suggested the presence of hydroxyl and disubstituted double bond groups. The ^{1}H (Table 1) and ^{13}C NMR (Table 2) data for the ring system of **4** were almost

indistinguishable from those of **1**, which suggested that compound **4** possesses the same ring system as **1**. Compound **4** also exhibited NMR signals of two tertiary methyls adjacent to an oxygen-bearing carbon, a secondary methyl, and two olefinic methines (see Table l and Experimental Section), arising from the side-chain moiety, which are consistent with a C-23 unsaturated 25-hydroxy C_8 side-chain.[6,7] The large coupling constant ($J \approx 15$ Hz in C_5D_5N) between H-23 and H-24 signals suggested the stereochemistry "*E*" at C-23.[7] The above evidence coupled with the ^{1}H (Table 1) and ^{13}C NMR (Table 2) data and analysis of ^{1}H−^{1}H COSY, HMQC, and HMBC spectra indicated that **4** was (23*E*)-4,25-dihydroxy-3,4-*seco*-19(10→9)-*abeo*-8α,9*β*,10α-tirucall-23-en-3,5*β*-oxide [(23*E*)-23-dehydro-25-hydroxysunpollenol].

Compound **5** was assigned a molecular formula of $C_{30}H_{54}O_4$, as determined from its ^{13}C DEPT NMR and HREIMS data. The IR absorption indicated the presence of a hydroxyl group. The ring system ^{1}H (Table 1) and ^{13}C NMR (Table 2) signals of **5** were very similar to those of **1**, suggesting that **5** had the same ring system. On the other hand, **5** exhibited the following ^{1}H NMR signals: a secondary methyl, an oxygenated methine, and two tertiary methyls adjacent to an oxygen-bearing carbon, consistent with the signals from a 24,25-dihydroxylated C_8 side-chain.[1] The side-chain structure was supported by an HMBC experiment in which cross-correlations for H-24 (with C-22, C-23, C-25-C-27), H-26 (with C-24, C-25, C-27), and H-27 (with C-24−C-26) were observed. Thus, **5** was deduced to be 4,24,25-trihydroxy-3,4-*seco*-19(10→9)-*abeo*-8α,9*β*,10α-tirucallan-3,5*β*-oxide (24,25-dihydroxysunpollenol).

Compound **6** ($C_{30}H_{54}O_4$) exhibited ^{1}H (Table 1) and ^{13}C NMR (Table 2) data very close to those of **5**, which suggested that **6** was a stereoisomer of **5** at C-24. The ^{13}C NMR chemical shift differences for the side-chain ^{13}C signals allowed stereochemical assignments at C-24 for **5** and **6**.[1] Thus, the ^{13}C NMR chemical shift differences ($\delta_{\mathbf{6}} - \delta_{\mathbf{5}}$) for the side-chain ^{13}C signals were calculated to be C-20 (0.4), C-21 (0.3), C-22 (0.4), C-23 (0.3), C-24 (0.9), C-25 (0.0), C-26 (−0.1), and C-27 (0.0) from the ^{13}C NMR data in Table 2, which were consistent with those for the differences ($\delta_R - \delta_S$) of 24,25-dihydroxyhelianol [3,4-*seco*-19(10→9)-*abeo*-8α,9*β*,10α-tirucallane-3,24,25-triol].[1] Compounds **5** and **6** were, therefore, assigned to the 24*S*- and 24*R*-epimers of 24,25-dihydroxysunpollenol, respectively. Analysis of the ^{13}C DEPT, ^{1}H−^{1}H COSY, HMQC, HMBC, and phase-sensitive NOESY spectra for **5** and **6** supported the proposed structures.

The natural occurrence of a triterpenoid possessing a rearranged *seco*-structure with a pyran ring A and a 2-propanol moiety is extremely rare, and only one such compound, terminalin A, a glutinane-type compound isolated from the stem bark of *Terminalia glaucescens* (Combretaceae), has been reported previously as a natural product.[2] There is a possibility that the triterpenoids possessing an oxygenated side-chain (**2**−**6**) are biosynthesized via photooxygenation[8] of an olefinic precursor (**1**); however, whether these oxidation products occur during biosynthesis or are formed in the plant or upon storage remains to be clarified.

The inhibitory effects of **1**−**6** on EBV-EA activation induced by TPA were examined as a preliminary evaluation of their potential to inhibit tumor promotion, and the results are summarized in Table 3. All six compounds showed potent dose-related inhibitory effects on EBV-EA induction by TPA, while not affecting the viability of Raji cells even at 1×10^3 mol ratio/TPA (0.032 μM). The inhibitory effects (97−100% inhibition at 1×10^3 mol ratio/TPA) (Table 3) of all compounds tested were found to be almost equivalent with those of *β*-carotene, a vitamin A precursor that has been intensively studied in cancer prevention using animal models.[9] Since the inhibitory effects against EBV-EA activation have been demonstrated to closely parallel inhibition of tumor promotion in vivo,[10] the triterpenoids (**1**−**6**) from sunflower pollen are worthy of further study as potential inhibitors of tumor promotion (potential cancer chemopreventive agents).

Table 3. Percentage of Epstein−Barr Virus Early Antigen Induction in the Presence of Compounds **1**−**6** with Respect to a Positive Control (100%)[a]

	concentration (mol ratio/TPA)				
compound	1000		500	100	10
1	3.1	(70)	30.5	74.3	96.8
2	0	(70)	27.3	75.9	97.4
3	0	(60)	26.3	72.6	91.8
4	0	(70)	22.0	70.6	90.0
5	0	(70)	25.3	73.3	94.2
6	0	(70)	24.4	72.4	92.5
β-carotene[b]	8.6	(70)	34.2	82.1	100

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

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-370 polarimeter in $CHCl_3$ at 25 °C. IR spectra were recorded on a JASCO IR-300 IR spectrometer in KBr disks or as liquid films. Nuclear magnetic resonance (NMR) spectra were recorded with a JEOL JNM LA-500 spectrometer at 500 MHz (^{1}H NMR) and 125 MHz (^{13}C NMR), if not otherwise specified, in $CDCl_3$ with tetramethylsilane (TMS; ^{1}H NMR) and $CDCl_3$ at δ 77.0 (^{13}C NMR) as internal standard. Electron-impact mass spectra (EIMS), chemical ionization MS (CIMS), and high-resolution MS (HRMS) were recorded on a JEOL JMS-GC mate spectrometer (70 eV) using a direct inlet system. Thin-layer chromatography (TLC) on silica gel (Kieselgel 60G, Merck; 0.5 mm thick; 20 × 20 cm) was developed using *n*-hexanes−ethyl acetate (EtOAc) (6:1, v/v). Silica gel (Kieselgel 60, 230−400 mesh, Merck) and octadecyl silica (Chromatorex-ODS, 100−200 mesh; Fuji Silysia Chemical, Ltd., Aichi, Japan) were used for open column chromatography. Reversed-phase preparative HPLC was carried out on an octadecyl silica column (Pegasil ODS II column, 25 cm × 10 mm i.d.; Senshu Scientific Co., Ltd., Tokyo, Japan) at 25 °C with MeOH [HPLC(A)] and MeOH−H_2O [85:15, v/v; HPLC(B)] as mobile phase at 4 mL/min. Normal-phase HPLC was carried out on silica columns: Senshu Pak Silica-1301N column (25 cm × 4.6 mm i.d.; Senshu Scientific Co., Ltd.) with *n*-hexane−EtOAc [92:8, v/v, 1 mL/min; HPLC(C)] and Sehnshu Pak Silica 4251-N (25 cm × 10 mm i.d.; Senshu Scientific Co., Ltd.) with *n*-hexane−EtOAc [7:3, v/v, 4 mL/min; HPLC(D)] as mobile phase at 25 °C.

Materials. *Helianthus annuus* L. (cultivar: Russian sunflower) was cultivated at an herbal garden of Toho University (Chiba, Japan) in 1997, and pollen grains were collected from the flowers.[1] A voucher specimen (registry no. COM-HA-970724C) was deposited in the herbarium of the School of Pharmaceutical Sciences, Toho University. The pollen grains were stored in a freezer prior to extraction.

Isolation. Column chromatography on silica gel (600 g) of an Et_2O extract (28 g) of *H. annuus* pollen grains (336 g) yielded fractions I (R_f 0.53; 2.0 g) and II (R_f 0.10; 1.3 g) along with five other fractions.[1] Fraction I, upon chromatography on an ODS column with MeOH as eluant followed by preparative HPLC (A), eventually yielded compounds **1** (116.7 mg; retention time (t_R) 17.1 min) and **4** (17.5 mg; t_R 6.9 min) and

a mixture of **2** and **3** (t_R 7.8 min). Isolation of **2** and **3** from the mixture was undertaken by normal-phase HPLC (C): **2** (5.2 mg; t_R 48.8 min) and **3** (11.3 mg; t_R 51.4 min). Fraction II upon chromatography on an ODS column with MeOH−H_2O (85:15) as eluant followed by preparative HPLC (B) yielded a mixture of **5** and **6** (t_R 14.7 min). The mixture of **5** and **6** was subjected to normal-phase HPLC (D) to yield **5** (2.7 mg; t_R 36.0 min) and **6** (4.4 mg; t_R 38.0 min).

Sunpollenol (1): amorphous solid; $[\alpha]^{25}_D$ −10.4° ($CHCl_3$; *c* 0.56); IR (KBr) ν_{max} 3465 (OH), 1079 (C−O), 829 (>C═CH−) cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 429 $[M - Me]^+$ (1), 411 (6), 385 (100), 315 (1), 287 (6), 274 (2), 273 (2), 205 (13), 179 (6), 163 (3), 149 (13), 111 (32), 69 (20); CIMS *m/z* 445 $[MH]^+$ (13), 427 (100), 385 (41), 369 (4); HRCIMS *m/z* 445.4040 (calcd for $C_{30}H_{53}O_2$ $[MH]^+$, 445.4045).

(24*S*)-24,25-Epoxysunpollenol (2): amorphous solid; $[\alpha]^{25}_D$ −27.0° ($CHCl_3$; *c* 0.20); IR (KBr) ν_{max} 3445 (OH), 1076 (C−O) cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; CIMS *m/z* 443 $[MH - H_2O]^+$ (100), 425 (30), 401 (45); HRCIMS *m/z* 443.3889 (calcd for $C_{30}H_{51}O_2$ $[MH - H_2O]^+$, 443.3889).

(24*R*)-24,25-Epoxysunpollenol (3): amorphous solid; $[\alpha]^{25}_D$ −28.1° ($CHCl_3$; *c* 0.52); IR (KBr) ν_{max} 3480 (OH), 1076 (C−O) cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 442 $[M - H_2O]^+$ (1), 401 (75), 385 (26), 203 (13), 179 (21), 165 (23), 149 (28), 135 (26), 121 (26), 111 (100); HREIMS *m/z* 442.3812 (calcd for $C_{30}H_{50}O_2$ $[M - H_2O]^+$, 442.3811).

(23*E*)-23-Dehydro-25-hydroxysunpollenol (4): amorphous solid; $[\alpha]^{25}_D$ −22.0° ($CHCl_3$; *c* 0.2); IR (KBr) ν_{max} 3443 (OH), 1075 (C−O), 970 (−C═CH−) cm^{-1}; ^1H ($CDCl_3$) and ^{13}C NMR data, see Tables 1 and 2; ^1H NMR (C_5D_5N) δ 0.83, 0.84, 1.21, 1.53, 1.55, 1.56, 1.57 (each 3H and s), 0.98 (3H, d, J = 5.1 Hz, H-21), 3.78 (1H, ddd, J = 4.6, 5.6, 11.2 Hz, H-3), 4.47 (1H, ddd, J = 4.6, 6.5, 11.2 Hz, H-3), 5.95 (1H, d, J = 15.0 Hz, H-24), 5.99 (1H, ddd, J = 5.3, 7.0, 15.0 Hz, H-23); CIMS *m/z* 443 $[MH - H_2O]^+$ (6), 425 (23), 401 (85), 383 (100); HRCIMS *m/z* 443.3882 (calcd for $C_{30}H_{51}O_2$ $[MH - H_2O]^+$, 443.3889).

(24*S*)-24,25-Dihydroxysunpollenol (5): amorphous solid; $[\alpha]^{25}_D$ −23.4° ($CHCl_3$; *c* 0.6); IR (KBr) ν_{max} 3435 (OH), 1075 (C−O) cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 460 $[M - H_2O]^+$ (3), 419 (100), 401 (90), 111 (97); HREIMS *m/z* 460.3919 (calcd for $C_{30}H_{52}O_3$ $[M - H_2O]^+$, 460.3916).

(24*R*)-24,25-Dihydroxysunpollenol (6): amorphous solid; $[\alpha]^{25}_D$ −12.9° ($CHCl_3$; *c* 1.1); IR (KBr) ν_{max} 3434 (OH), 1075 (C−O) cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 460 $[M - H_2O]^+$ (3), 445 (7), 419 (100), 401 (84), 385 (26), 359 (20), 111 (90); HREIMS *m/z* 460.3911 (calcd for $C_{30}H_{52}O_3$ $[M - H_2O]^+$, 460.3916).

In Vitro EBV-EA Activation Experiment. The inhibition of EBV-EA activation was assayed using Raji cells (EBV genome-carrying human lymphoblastoid cells; nonproducer type), cultivated in 10% fetal bovine serum (FBS) RPMI-1640 medium (Sigma, St. Louis, MO). The indicator cells (Raji cells; 1×10^6 cells/mL) were incubated in 1 mL of the medium containing 4 mM *n*-butyric acid as an inducer, 32 pM of TPA [20 ng/mL in dimethyl sulfoxide (DMSO)], and a known amount (32, 16, 3.2, 0.32 nmol) of the test compound at 37 °C in a CO_2 incubator. After 48 h, the cell suspensions were centrifuged at 1000 rpm for 10 min, and the supernatant was removed. The activated cells were stained with high-titer EBV-EA-positive sera from nasopharyngeal carcinoma patients, and the conventional indirect immunofluorescence technique was employed for detection. In each assay, at least 500 cells were counted and the experiments were repeated three times. The average extent of EA induction was determined and compared with that on positive control experiments in which the cells were treated with *n*-butyric acid plus TPA, where the extent of EA induction was ordinarily more than around 40%. The viability of treated Raji cells was assayed by the Trypan Blue staining method.[11]

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