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STUDIES ON INHIBITORS OF SKIN-TUMOR PROMOTION, I. INHIBITORY EFFECTS OF TRITERPENES FROM *EUPTELEA POLYANDRA* ON EPSTEIN-BARR VIRUS ACTIVATION

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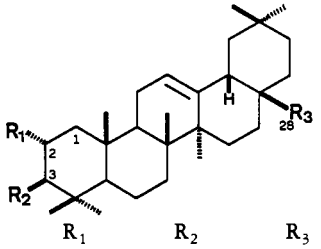
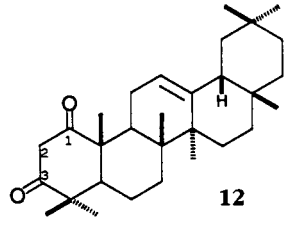
Investigations on the considerable overlap between Epstein-Barr virus associated, early antigen (EBV-EA) inducing compounds and tumor promoters of mouse skin carcinogenesis have been previously reported (1-3). Furthermore, compounds such as glycyrrhetinic acid and retinoic acid, which inhibit EBV-EA induction by tumor promoters, have been shown to act as inhibitors of in vivo tumor promotion (4-6). Koshimizu *et al.* have reported that oleanolic acid and ursolic acid, isolated from *Glechoma hederacea* L. (Labiatae), inhibited not only in-vitro EBV-EA activation but also in-vivo tumor promotion (7,8). It was also found that most antipromoters cause anti-inflammatory action in animal skin (9,10). We published earlier the isolation and identification of eight triterpenes from the bark of *Euptelea polyandra* Sieb. Zucc. (Eupteleaceae) with anti-inflammatory action (11,12).

To search for possible antitumor promoters from natural sources, we carried out a primary screening of the triterpenes **1**, **2**, **5**, **7**, **9**, **12**, **13**, and **17** isolated from *E. polyandra* and their derivatives **3**, **4**, **6**, **8**, **10**, **11**, **14**, **15**, and **16** using their possible inhibitory effects on EBV-EA activation in Raji cells.

Twelve oleanene-type triterpenes [**1**-**12**] and five lupane-type triterpenes [**13**-**17**] were tested using the short-term in-vitro assay of EBV-EA activation in Raji cells induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA). Their inhibitory effects on activation



	R_1	R_2	R_3
1	−H	−OH	−COOH
2	−H	−OAc	−COOH
3	−H	−OH	−COOMe
4	−H	−OH	−CHO
5	−H	−OAc	−CHO
6	−H	−OH	$-CH_2OH$
7	−H	−OAc	$-CH_2OH$
8	−H	−OAc	$-CH_2OAc$
9	−OH	−OH	−COOH
10	−OAc	−OAc	−COOH
11	−OH	−OH	−COOMe



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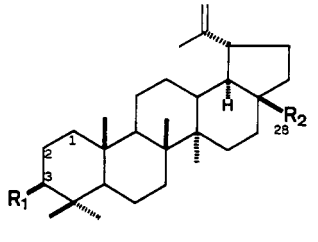

	R_1	R_2
13	−OH	−COOH
14	−OAc	−COOH
15	−OH	−COOMe
16	−OH	$-CH_2OH$
17	−OAc	−Me

TABLE 1. Inhibitory Effects of Triterpenes from *Euptelea polyandra* on EBV activation

Sample	Concentration (mol ratio compound/TPA)				
	5×10^3	1×10^3	1×10^2	1×10	
	% to control (% viability)				
TPA (32 pM)	100=positive control				35.6[b]
1	0.0±0[a](20.0)	30.0±1.5[a](60.0)	80.0±2.1[a](>80)	100.0±0[a] (100)	2.3
2	0.0±0 (50.0)	36.4±4.9 (80.0)	100.0±0.8 (100)	100.0±0 (100)	1.6
3	0.0±0 (0.0)	29.5±4.1 (50.0)	46.3±3.6 (80.0)	100.0±0.2 (100)	0.0
4	0.0±0 (40.0)	100.0±1.1 (100)	100.0±0 (100)	100.0±0 (100)	0.0
5	0.0±0 (40.0)	100.0±1.6 (100)	100.0±0 (100)	100.0±0 (100)	0.0
6	0.0±0 (10.0)	19.3±1.7 (50.0)	71.7±2.3 (>80)	100.0±0 (100)	0.0
7	0.0±0 (40.0)	32.3±3.2 (70.0)	59.4±3.3 (>80)	100.0±0.1 (100)	0.0
8	0.0±0 (50.0)	100.0±2.3 (100)	100.0±0 (100)	100.0±0 (100)	0.0
9	0.0±0 (0.0)	0.0±1.3 (0.0)	65.6±6.5 (70.0)	100.0±0.4 (100)	0.0
10	0.0±0 (0.0)	0.0±1.2 (20.0)	59.6±7.1 (70.0)	100.0±0.2 (100)	0.0
11	0.0±0 (0.0)	65.6±6.6 (80.0)	100.0±1.6 (100)	100.0±0 (100)	0.0
12	0.0±0 (0.0)	0.0±2.1 (20.0)	100.0±1.1 (100)	100.0±0 (100)	0.0
13	0.0±0 (20.0)	80.0±3.2 (>80)	100.0±0 (100)	100.0±0 (100)	0.0
14	0.0±0 (80.0)	0.0±1.1 (80.0)	55.1±4.2 (80.0)	100.0±0.1 (100)	0.0
15	0.0±0 (80.0)	71.5±6.3 (>80)	100.0±0.3 (100)	100.0±0 (100)	0.0
16	0.0±0 (80.0)	100.0±2.2 (100)	100.0±0 (100)	100.0±0 (100)	1.6
17	0.0±0 (60.0)	81.8±7.1 (80.0)	100.0±0.6 (100)	100.0±0 (100)	0.0

[a]Each value represents the average of three determinations ±S.D.

[b]Each value of this column represents the % of EA activation by each compound alone (1 μM).

and viabilities of Raji cells are shown in Table 1.

In the series of oleanene-type triterpenes, 3-*O*-acetyloleanolic acid [**2**] and 3-*O*-acetylerythrodiol [**7**] showed remarkable inhibitory effects and preserved higher viabilities of Raji cells than oleanolic acid [**1**] and erythrodiol [**6**]. Whereas maslinic acid [**9**] having a 2α-OH group, 2,3-di-*O*-acetylmaslinic acid [**10**], and 1,3-dioxo-olean-12-ene [**12**] exhibited higher cytotoxicity on Raji cells than triterpenes having a 3β-OH group. On the other hand, these remarkable inhibitory effects were not found with oleanolic aldehyde [**4**] and its acetate [**5**]. In the series of lupane-type triterpenes only 3-*O*-acetylbetulinic acid [**14**] showed significant inhibitory activity (complete inhibition of activation even at 1×10^3 mol ratio) and preserved high viability even at high doses (5×10^3 mol ratio), whereas the other lupane-type triterpenes [**13**, **15**, **16**, and **17**] showed no activity at all. It has been reported that retinoic acid and glycyrrhetinic acid not only exhibited lower inhibitory effects than **1** but also influenced the viabilities of the cells more than **1** (7). In our experiments the degree of inhibitory activity of **14** was more than 10 times higher than that obtained with **1**, whereas the inhibitions obtained with **6** and **7** were almost comparable to that found with **1**.

These results strongly suggest that **2**, **6**, **7**, and **14** may be valuable antipromoters.

Initiation-promotion tests in vivo are now in progress.

EXPERIMENTAL

PLANT MATERIAL, EXTRACTION, AND ISOLATION.—*E. polyandra* was collected in Kyoto, Japan, in June 1984, and the details of the extraction and isolation of **2**, **5**, **7**, **9**, **12**, **13**, and **17** were reported in a previous paper (12).

GENERAL PROCEDURE FOR ACETYLATION.—The acetates **8**, **10**, and **14** were prepared by acetylation of **7**, **9**, and **13**, respectively, with Ac_2O in pyridine at room temperature for 18 h followed by usual work-up and purification by either column chromatography or recrystallization to yield the pure compounds. Identification of these compounds was achieved by comparison with the reported data (13-18).

GENERAL PROCEDURE FOR METHYLATION.—The methyl esters **3**, **11**, and **15** were prepared by methylation of **1**, **9**, and **13**, respectively, with CH_2N_2 in Et_2O and MeOH followed by usual work-up and purification by either column chromatography or recrystallization to yield the pure compounds. Identification of these compounds was achieved by comparison with the reported data (15-18).

GENERAL PROCEDURE FOR REDUCTION.—Compounds **6** and **16** were prepared by reduction of **3** and **15**, respectively, with $LiAlH_4$ in Et_2O followed by usual work-up and recrystallization to yield the pure compounds. Identification of these compounds was achieved by direct comparison with commercially available samples (Tokyo Kasei Co., Ltd., Japan).

BIOLOGICAL ACTIVITIES.—The inhibition of EBV-EA activation was assayed using the EBV genome-carrying human lymphoblastoid cells, Raji (non-virus producer), which were cultivated in 8% FBS RPMI 1640 medium (Nissui). The indicator cells (Raji) (1×10^6/ml) were incubated at 37° for 48 h in 1 ml of the medium containing *n*-butyric acid (4 mM, inducer) (19), 2 μl of TPA (20 ng/ml in 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-positive sera from nasopharyngeal carcinoma (NPC) patients and detected by a conventional indirect immunofluorescence technique (20,21). In each assay, at least 500 cells were counted, and the experiments were repeated twice. The average EA induction was compared to that of positive control experiments with *n*-butyric acid (4 mM) plus TPA (20 ng/ml) in which EA induction was ordinarily around 30%.

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