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Inhibition of Epstein-Barr Virus (EBV) Activation by Triterpenes in *Sesamum indicum* L. Callus

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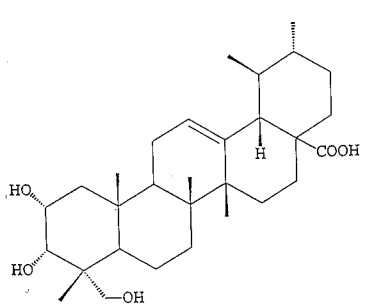
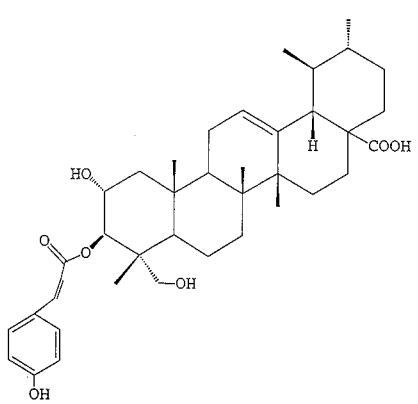
We have been searching for inhibitors for Epstein-Barr virus early antigen (EBV-EA) activation in plants and callus cells. We have induced callus cell lines from various plants and found that triterpenes contained in *Sesamum indicum* L. callus cells showed anti-tumor promoter activity. In this paper we describe isolation, characterization and anti-tumor promoter activity of triterpens in *S. indicum* L. callus.

For the induction of the callus, we used segments of the seedling of *S. indicum* L.[1]. The callus cells were cultured in modified Murasige-Skoog[1,2] liquid medium at 35°C with aeration. The cells (23.3 kg-Fresh Weight) were extracted with EtOH-H_2O (4 : 1) (72*l* × 3) and the extract was dried *in vacuo*. The dried extract was applied on an Amberlite XAD-2 (Organo) column (160 × 1300 mm) and successively eluted with H_2O, MeOH-H_2O (1 : 4), MeOH-H_2O (2 : 3), MeOH-H_2O (3 : 2), MeOH-H_2O (4 : 1), MeOH and acetone. The MeOH fraction was then fractionated on a silica gel (Merck) column, eluted with $CHCl_3$, MeOH-$CHCl_3$ (7.5 : 92.5), MeOH-$CHCl_3$ (12.5 : 87.5) and MeOH. The fraction eluted with MeOH-$CHCl_3$ (7.5 : 92.5) was applied to a column chromatography on



compound 1



compound 2

Fig. 1 Structural formula of compounds 1 and 2.

Mega Bond Elut C_{18} (Varian Associates, Inc.) eluted with MeOH-H_2O (7 : 3). The MeOH-H_2O (7 : 3) fraction was dried *in vacuo*. The dried fraction was applied to Develosil Lop ODS (Nomura Chemical) eluted with MeCN-H_2O (55 : 45). The fraction eluted with MeCN-H_2O (55 : 45) was further applied to HPLC on TSKgel ODS 80Tm (Tosoh) using MeCN-H_2O (55 : 45) to afford compound 1 (102.2 mg) and compound 2 (10.6 mg) (**Fig. 1**).

The molecular weight of compound 1 was established to be 488 by MS. The molecular formula of compound 1 was established as $C_{30}H_{48}O_5$ by the HRMS and ^{13}C-NMR spectra. Its ^{13}C-NMR spectrum (30 signals) indicated that compound 1 is a triterpenoid. The IR spectrum indicated the presence of hydroxyl (3420 cm^{-1}) and carbonyl (1690 cm^{-1}) groups. The ^{13}C-NMR spectrum showed double bonded carbon signals assignable to C-12 and C-13 at 125.4 and 139.2, respectively, and the carbon signal due to C-28 at 179.7. The ^{13}C-NMR spectrum of compound 1 was similar to that of 2α, 3α, 23-trihydroxyurs-12-en-28-oic-acid (esculentic acid)[3] (**Table 1**).

The compound 1 was identified as esculentic acid by comparison with published spectral data[3]. Esculentic acid has been isolated from *Diplazium esculentum*[4] and the fruit galls of *Actinidia polygama*[3], yet their biological activities have not been fully investigated.

Table 1. ^{13}C-NMR spectral data of compound 1.

Carbon No.	Compound 1 (Pyridine-d_5) (75.47 MHz)	2α, 3α, 23-Trihydroxyurs-12-en-28-oic acid*[1] (Pyridine-d_5) (100 MHz)
1	42.7	42.7
2	66.1	66.1
3	78.7	78.7
4	40.0	40.0
5	43.4	43.9
6	18.2	18.2
7	33.1	33.1
8	41.8	41.8
9	47.9	47.9
10	38.2	38.3
11	23.8	23.7
12	125.4	125.5
13	139.2	139.3
14	42.5	42.5
15	28.5	28.6
16	24.8	24.8
17	47.9	47.9
18	53.5	53.5
19	39.3	39.4
20	39.3	39.4
21	31.0	31.0
22	37.3	37.4
23	71.1	71.2
24	17.6	17.1
25	17.4	17.4
26	17.3	17.5
27	23.6	23.8
28	179.7	179.8
29	17.0	17.7
30	21.2	21.3

*[1]Sasida, Y., *et al.* (Phytochemistry, Vol. 31, No. 8, 2801-2804, 1992)

The molecular weight of compound 2 was established to be 634 by MS. The molecular formula of compound 2 was established as $C_{39}H_{54}O_7$ by the HRMS. In ^{13}C-NMR spectrum of compound 2, signal assignable to rings B, C, D, and E resembled those of the co-occurring triterpenoid compound 1. Its ^{13}C-NMR spectrum indicated the presence of a *p*-coumarate group. The ^{13}C and ^{1}H-NMR spectral data of compound 2 were similar to those of 3β-(*trans*-*p*-coumaroyloxy)-2α, 23-dihydroxyurs-12-en-28-oic acid as has been reported[3].

Table 2. ^{13}C-NMR spectral data of compound 2.

Carbon No.	Compound 2 (Pyridine-d_5) (75.47 MHz)	2α, 23-Dihydroxy-3β-(*trans*-coumaroyloxy)-urs-12-en-28-oic acid*[1] (Pyridine-d_5) (100 MHz)
Triterpene		
1	48.6	48.5
2	66.7	66.7
3	80.1	80.0
4	43.9	43.9
5	48.1	48.0
6	18.3	18.3
7	33.2	33.1
8	40.2	40.1
9	47.3	47.2
10	38.1	38.1
11	23.8	23.8
12	125.5	125.4
13	139.4	139.2
14	42.7	42.6
15	28.7	28.7
16	25.0	24.9
17	48.1	48.0
18	53.6	53.6
19	39.5	39.4
20	39.5	39.4
21	31.1	31.1
22	37.5	37.5
23	64.9	64.8
24	14.8	14.8
25	17.5	17.5
26	17.5	17.5
27	23.9	23.9
28	179.8	180.0
29	17.5	17.5
30	21.4	21.4
p-Coumaroyl		
1	126.3	126.2
2, 6	130.6	130.6
3, 5	116.3	116.4
4	161.4	161.4
α	145.1	145.1
β	115.8	115.8
C=O	168.5	168.5

*[1]Sasida, Y., *et al.* (Phytochemistry, Vol. 31, No. 8, 2801-2804, 1992)

Table 3. Inhibitory effects on EBV activation.

Sample	Concentration ($\mu g/ml$)		
	1	2	4
	% to control		
Retinoic acid	66	56	49
Compound 1	61	55	51
Compound 2	91	61	54

Compound 2 was identified as 3β-(*trans*-*p*-coumaroyloxy)-2α, 23-dihydroxyurs-12-en-28-oic acid by comparison with published spectral data[3] (**Table 2**). This compound has been isolated from the fruit galls of *Actinidia polygama*[3].

The inhibition of EBV-EA activation[5] was assayed using the Epstein-Barr virus (EBV) genome-carrying human lymphoblastoid cells, Raji (non-virus producer), which were cultivated in RPMI 1640 medium supplemented with 10% fetal bovine serum. The indicator cells (Raji) (1×10^6/ml) were incubated at 37°C for 48h in 1.0 ml of the medium containing 4 mM of *n*-butyric acid, 8.1 pM of TPA and a test compound. The activated cells were stained by high titer EBV-positive sera from nasophayngeal carcinoma patients and detected by a conventional indirect immunofluorescence technique. In each assay, at least 200 cells were counted, and the experiments were repeated twice.

Esculentic acid (compound 1) and 3β-(*trans*-*p*-coumaroyloxy)-2α, 23-dihydroxyurs-12-en-28-oic acid (compound 2) were purified from the callus cells and they were tested using the short-term *in vitro* assay of EBV-EA activation in Raji cells induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA)[6]. Their inhibitory effects on activation are shown in **Table 3**. Inhibitory effects on EBV activation of compound 1 and 2 were nearly equal to those of retinoic acid. Viabilities of exponentially growing Raji cells were about 85%. Neither compound 1 nor 2 show any cytotoxicity at 4 $\mu g/ml$. Previously it was reported that arjunolic acid, an oleanene triterpene (2α, 3β, 23-trihydroxy-olean-12-en-28-oic acid), suppressed skin tumor promotion in mice[7]. Its structure is similar to compound 1. Compound 1 has two methyl groups at C-19 and C-20 positions of E ring but arjunolic acid has two methyl groups at C-20 position of E ring. Except for this point, compound 1 and arjunolic acid have almost the same structure.

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《和文要約》

Sesamum indicum L. カルスに含まれるトリテルペン化合物による Epstein-Barr Virus (EBV) 活性化の阻害

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ゴマ培養細胞から2種のトリテルペン化合物を単離，精製した．2種の化合物はTPAによって誘発されるRaji細胞のEBVの活性化を阻害した．EBV活性化抑制率は2種の化合物とも全-トランス型レチノイン酸と同程度であった．