Short Communication

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 triterpenes 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-Skoog1,2) liquid medium at 35°C with aeration. The cells (23.3 kg-Fresh Weight) were extracted with EtOH-H2O (4:1) (721×3) 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 H2O, MeOH-H2O (1:4), MeOH-H2O (2:3), MeOH-H2O (3:2), MeOH-H2O (4:1), MeOH and acetone. The MeOH fraction was then fractionated on a silica gel (Merck) column, eluted with CHCl3, MeOH-CHCl3 (7.5:92.5), MeOH-CHCl3 (12.5:87.5) and MeOH. The fraction eluted with MeOH-CHCl3 (7.5:92.5) was applied to a column chromatography on

Fig. 1 Structural formula of compounds 1 and 2.
Mega Bond Elut C_{18} (Varian Associates, Inc.) eluted with MeOH–H₂O (7:3). The MeOH–H₂O (7:3) fraction was dried *in vacuo*. The dried fraction was applied to Develosil Lop ODS (Nomura Chemical) eluted with MeCN–H₂O (55:45). The fraction eluted with MeCN–H₂O (55:45) was further applied to HPLC on TSKgel ODS 80Tm (Tosoh) using MeCN–H₂O (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 2\(_a\), 3\(_a\), 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\(^5\). 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.

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<th>Carbon No.</th>
<th>Compound 1 (Pyridine-(_d_5)) (75.47 MHz)</th>
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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 3\( \beta \)-(\( trans \)-(\( p \)-coumaroyloxy)-2\( \alpha \), 23-dihydroxyurs-12-en-28-oic acid as has been reported\(^9\).

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\( p \)-Coumaroyl

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\(^*\)Sasida, Y., \textit{et al.} (Phytochemistry, Vol. 31, No. 8, 2801-2804, 1992)
Compound 2 was identified as 3β-(trans-p-coumaroyloxy)-2α,23-dihydroxyurs-12-en-28-oic acid by comparison with published spectral data3) (Table 2). This compound has been isolated from the fruit galls of Actinidia polygama3).

The inhibition of EBV-EA activation5) 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 x 10⁶/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 nasopharyngeal 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.

Esculetinic 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 µg/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 mice7). 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.

### Acknowledgment

The authors wish to thank to Mr. T. Nakayama and Miss F. Itoh for supplying the cultivated cells and to Mr. H. Yasuda and Mr. H. Yoshida for analyzing chemical structures.

### References


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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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三村精男*・高原義昌*・徳田春邦**

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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ド酸と同程度であった。