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Jezananals A and B: two novel skeletal triterpene aldehydes from the stem bark of *Picea jezoensis* var. *jezoensis*

Reiko Tanaka,[a,*] Kazuhiro Tsujimoto,[a] Yasuko In,[a] Toshimasa Ishida,[a] Shunyo Matsunaga,[a] Harukuni Tokuda[b] and Osamu Muraoka[c]

[a]*Department of Medicinal Chemistry, Osaka University of Pharmaceutical Sciences, 4-20-1 Nasahara, Takatsuki, Osaka 569-1094, Japan*
[b]*Department of Biochemistry, Kyoto Prefectural University of Medicine, Kawaramachi Hirokoji, Kamigyo-ku, Kyoto 602-0841, Japan*
[c]*Department of Organic Chemistry, Faculty of Pharmaceutical Sciences, Kinki University, 3-4-1 Kowakae, Higashiosaka, Osaka 577-8502, Japan*

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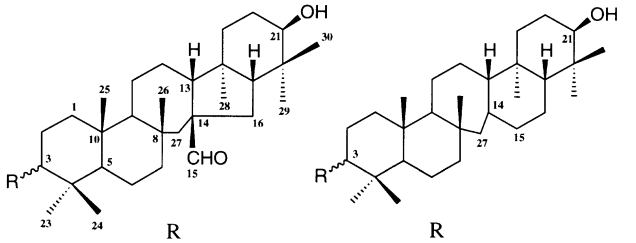
Abstract—Two novel skeletal triterpene aldehydes, jezananals A (**1**) and B (**2**) were isolated from the stem bark of *Picea jezoensis* var. *jezoensis* (Pinaceae). Their absolute stereo structures were determined to be 21β-hydroxy-3β-methoxy-16(15→14)*abeo*-13*R*,14*S*-serratan-15-al and the 3α-epimer, on the basis of spectral, single crystal X-ray analysis and chemical conversion. Compounds **1** and **2** are based on the unique 16(15→14)*abeo*-serratane skeleton. 3β-Methoxyserrat-14-en-21β-ol (**3**), 3α-methoxyserrat-14-en-21β-ol (**4**), and 14β,15β-epoxy-3β-methoxyserratan-21β-ol (**5**), which is considered to be intermediates of compounds **1** and **2**, and showed strong inhibitory effects on Epstein-Barr virus early antigen (EBV-EA) induction, while compounds **1** and **2** were inactive.

1. Introduction

Cancer chemoprevention is regarded as one of the efficient strategies for cancer prevention.[1] Inhibition of the tumor promotion stage in multistage of chemical carcinogenesis has been regarded as the most promising method for cancer chemoprevention.[2] In the search for cancer chemopreventive agents, the inhibitory effects on Epstein-Barr virus early antigen (EBV-EA) induction by the tumor promoter, 12-*O*-tetradecanoylphorbol-13-acetate (TPA), have been studied as a primary screening test.[3] In our search for naturally occurring cancer chemopreventive agents, the stem bark of *Picea jezoensis* var. *jezoensis* (Pinaceae) was selected for detailed investigation. In previous papers, we had reported a considerable number of serratane-type triterpenes from this bark.[4–7] Among them, 13α,14α-epoxy-3β-methoxyserratan-21β-ol showed significant anti-tumor promoting activity of in vivo two-stage mouse-skin carcinogenesis assay using 7,12-dimethylbenz[*a*]anthracene (DMBA) as an initiator and TPA as a tumor promoter.[8] Therefore, serratane-type triterpenes can be considered to become appropriate lead compounds to develop more potent agents with anti-tumor promoting activity for clinical employment. In our search for naturally occurring next cancer chemopreventive agents, detailed investigation of the stem bark was continued. We currently report the isolation of two unusually migrated serratane triterpenoids, jezananals A (**1**) and B (**2**) from *P. jezoensis* var. *jezoensis*, in addition to their stereochemistry and results of in vitro and in vivo anti-tumor promoting activities of compounds **1**, **2**, along with 3β-methoxyserrat-14-en-21β-ol (**3**), 3α-methoxyserrat-14-en-21β-ol (**4**), and 14β,15β-epoxy-3β-methoxyserratan-21β-ol (**5**).



	R
1	β-OMe
2	α-OMe

	R
3	β-OMe, Δ^{14}-ene
4	α-OMe, Δ^{14}-ene
5	β-OMe, 14β,15β-epoxy

2. Results and discussion

Jezananals A (**1**) and B (**2**) were confirmed to have the same molecular formula $C_{31}H_{52}O_3$ by HREIMS. Their IR spectra showed a hydroxyl (ν_{max}: **1**, 3540 cm^{-1}; **2**, 3600 cm^{-1}) and an aldehyde (ν_{max}: **1**, 1716 cm^{-1}; **2**, 1723 cm^{-1}) absorptions. The ^1H and ^{13}C NMR spectra of **1** and **2** (Table 1) exhibited seven tertiary methyls, 10 methylenes, four

Keywords: triterpene aldehyde; *Picea jezoensis* var. *jezoensis*; jezananals A and B; anti-tumor promoting activity.
* Corresponding author. Tel./fax: +81-726-90-1084; e-mail: tanakar@oysun01.oups.ac.jp

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Table 1. ^{1}H and ^{13}C NMR data for compounds **1**, **1a** and **2**

Position	**1**		**1a**		**2**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1α	0.86 m	38.2 t	0.87 m	38.2 t	1.42 m	33.4 t
1β	1.84 m		1.86 m		1.18 m	
2α	1.80 m	22.2 t	1.82 m	22.1 t	1.73 m	20.1 t
2β	1.42 m		1.42 m		1.73 m	
3α	2.62 dd (12.2, 4.4)	88.5 d	2.62 dd (12.2, 4.4)	88.5 d	–	86.0 d
3β	–		–		2.77 t (2.6)	
4	–	38.9 s	–	38.9 s	–	38.0 s
5α	0.71 m	55.8 d	0.71 m	55.8 d	1.20 m	49.8 d
6α	1.48 m	18.3 t	1.50 m	18.3 t	1.43 m	18.2 t
6β	1.40 m		1.39 m		1.20 m	
7α	1.15 m	44.6 t	1.13 m	44.5 t	1.23 m	44.2 t
7β	1.46 m		1.48 m		1.42 m	
8	–	39.0 s	–	39.0 s	–	39.1 s
9α	0.67 m	66.1 d	0.67 m	66.1 d	–	65.4 d
10	–	38.2 s	–	38.2 s	–	38.2 s
11α	0.90 m	21.5 t	1.13 m	21.3 t	0.87 m	21.2 t
11β	1.66 m		1.48 m		1.67 m	
12α	1.18 m	29.1 t	1.17 m	29.0 t	1.23 m	28.9 t
12β	1.93 m		1.82 m		1.82 td (6.9, 2.7)	
13β	2.44 dd (13.7, 6.8)	49.9 d	2.45 dd (13.7, 6.8)	49.7 d	2.42 dd (13.7, 6.8)	50.0 d
14	–	59.0 s	–	59.0 s	–	59.0 s
15β	9.53 s	204.2 d	9.54 s	204.2 d	9.52 s	204.3 s
16α	1.30 dd (13.7, 13.5)	34.9 t	1.31 dd (13.7,13.5)	34.7 t	1.31 dd (13.7, 6.8)	34.9 t
16β	1.61 dd (13.5, 5.0)		1.64 dd (13.5, 5.0)		1.62 dd (13.5, 5.0)	
17β	1.13 dd (13.5, 5.0)	45.6 d	1.14 dd (13.7, 5.0)	46.7 d	1.13 dd (13.7, 5.0)	45.6 d
18	–	44.6 s	–	44.6 s		44.6 s
19α	1.36 m	32.7 t	1.41 m	33.4 t	1.42 m	32.7 t
19β	1.50 m		1.41m		1.42 m	
20α	1.60 m	26.6 t	1.82 m	24.2 t	1.63 m	26.6 t
20β	1.86 m		1.65 m		1.88 tdd (13.2, 4.1, 2.9)	
21α	3.40 t (2.7)	75.1 d	4.66 t (2.7)	77.2 d	3.40 t (2.7)	75.2 d
22	–	36.8 s	–	36.0 s	–	36.7 s
23	0.94 s	28.0 q	0.94 s	28.0 q	0.92 s	28.9 s
24	0.72 s	15.9 q	0.72 s	15.9 q	0.792 s	22.2 q
25	0.69 s	16.4 q	0.70 s	16.4 q	0.70 s	16.4 q
26	0.80 s	22.8 q	0.81 s	22.8 q	0.798 s	22.8 q
27α	1.50 d (14.7)	54.7 t	1.52 d (14.7)	54.8 t	1.55 d (14.7)	54.9 t
27β	1.76 d (14.7)		1.77 d (14.7)		1.73 d (14.7)	
28	0.84 s	16.2 q	0.85 s	16.2 q	0.85 s	16.1 q
29	0.87 s	22.3 q	0.92 s	22.0 q	0.92 s	22.3 q
30	0.83 s	28.0 q	0.74 s	27.8 q	0.74 s	28.0 q
OMe	3.35 s	57.5 q	3.35 s	57.5 q	3.32 s	57.2 q
0Ac	–		2.03	21.3	–	
	–		–	170.8	–	

Assignments were made by ^{1}H–^{1}H COSY, TOCSY, HMQC, HMBC and NOESY data.

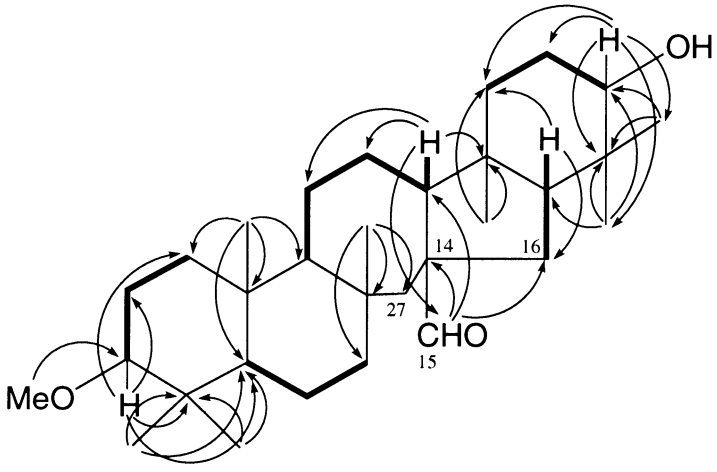


Figure 1. ^{1}H–^{1}H COSY (—) and HMBC correlations (→) of **1** and **2**.

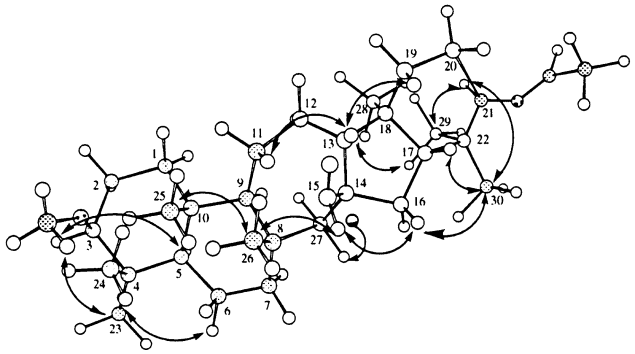
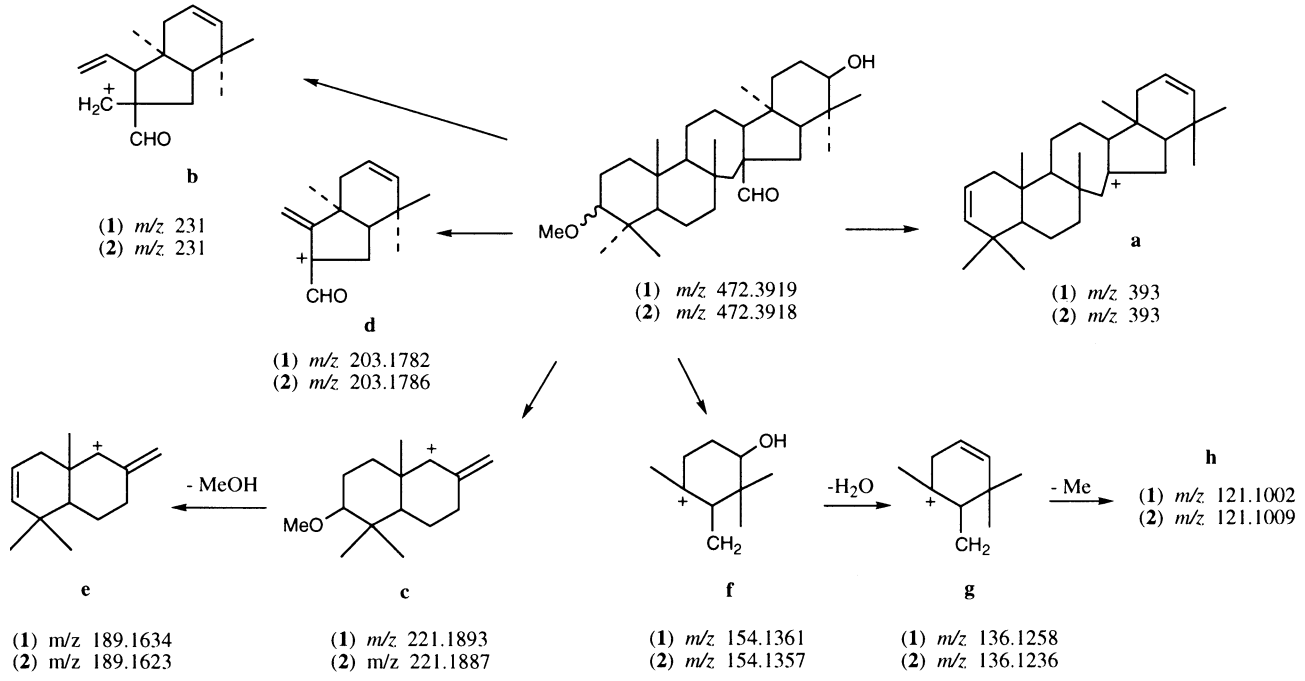


Figure 2. Selected NOESY correlations for **1a**.

methines, six quaternary carbons, a secondary hydroxyl [**1**: δ_H 3.40 (1H, t), δ_C 75.1 (d); **2**: δ_H 3.41 (1H, t), δ_C 75.2 (d)], a secondary methoxyl [**1**: δ_H 2.62 (dd), 3.35 (3H, s), δ_C 57.5 (q), 88.5 (d); **2**: δ_H 2.77 (t), 3.32 (3H, s), δ_C 57.2 (q), 86.0 (d)] and an aldehyde group [**1**: δ_H 9.53 (1H, s), δ_C 204.2 (d); **2**: δ_H 9.52 (1H, s), δ_C 204.3 (d)]. The DEPT and HMQC spectra of **1** and **2** showed the same carbon composition which had one aldehyde group and one quaternary sp^3 carbon and lacked one methylene and one methine groups in comparison with those of the serratane triterpenoids.[4–7] Except for the signals of H-3, Me-23, Me-24 and Me-25, the ^1H and ^{13}C NMR data of **1** and **2** were almost identical, therefore, **1** and **2** must be the methoxy epimer, each other. This fact suggested **1** and **2** to have an unusual novel carbon skeleton involving an aldehyde group. The acetylation of **1** and **2** gave monoacetates (**1a**) and (**2a**), in which the carbinol methine proton were shifted to δ 4.66 (t) in **1a** and 4.65 (t) in **2a**. The HMBC spectra of **1** and **2** (Fig. 1) indicated the long-range correlations between aldehyde proton and C-13, C-14, C-16 and C-27, and H-13β and C-11, C-12, C-14, C-17, C-18 and C-27, therefore, aldehyde group was attached at C-14. In addition, between H-3 proton and C-1, C-2, C-4, C-5, C-23 and C-24, between H-21 proton and C-17, C-19, C-20, C-22, C-29 and C-30. The ^1H–^1H COSY spectra (Fig. 1) of **1a** and **2**, H-16α and H-16β protons were related only H-16β and H-17β, and H-16α and H-17β, respectively. The NOESY spectra of **1a** (Fig. 2) and **2**, significant NOEs were observed between aldehyde proton and H-16β, H-27β and Me-26, suggested



Scheme 1. EIMS fragmentation of **1** and **2**.

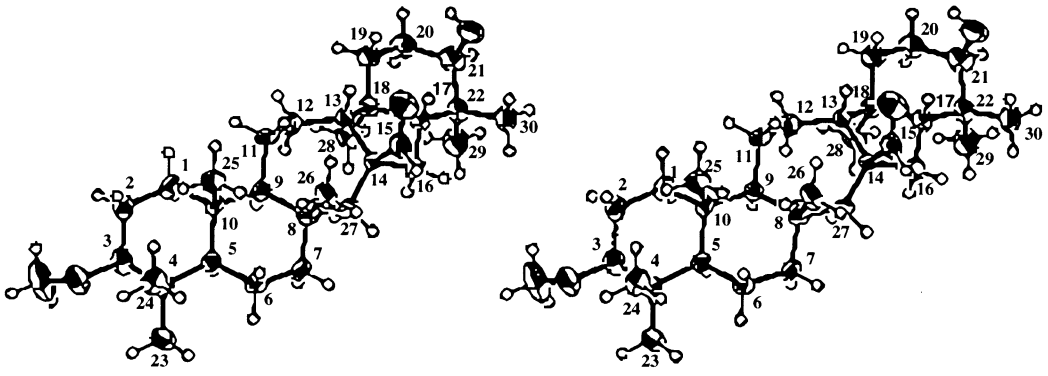
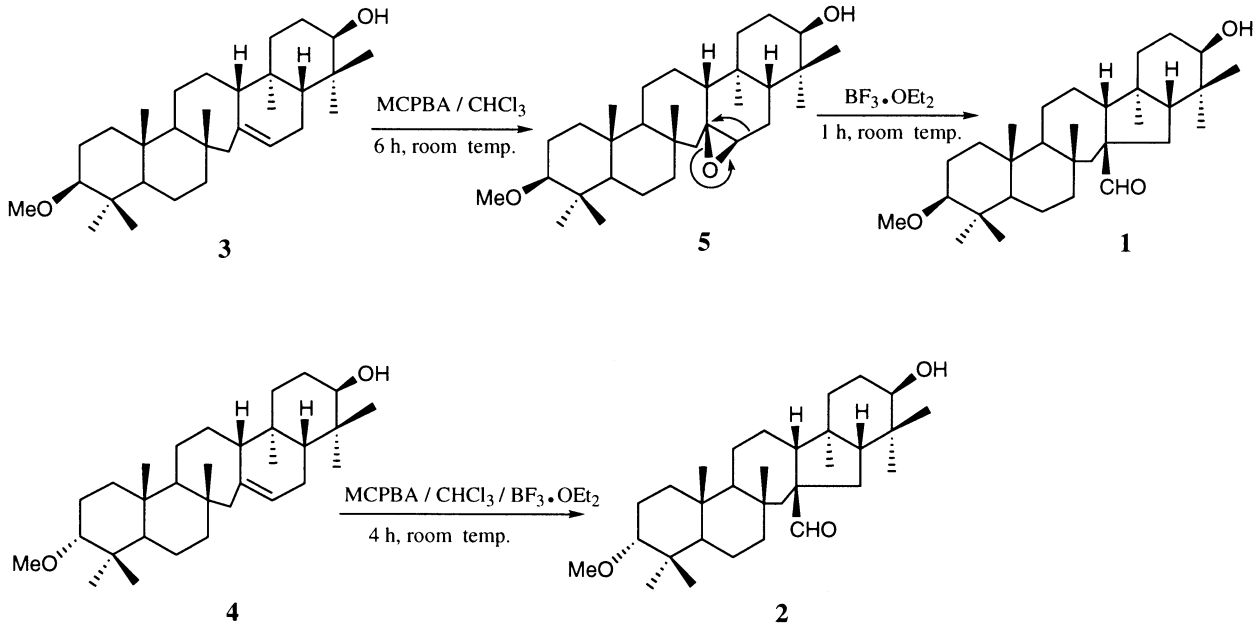


Figure 3. ORTEP view of **1**.



Scheme 2. Syntheses of jezananals A (**1**) and B (**2**) from **3** and **4**.

the aldehyde group was β configuration. NOEs were also observed between H-3α and Me-23 and H-5α for **1a**, and H-3β and Me-23 and Me-24 for **2**, and another NOEs were shown in Fig. 2. The EIMS of **1** and **2** showed eight predominant fragment ion peaks due to cleavage of the C and D-rings at *m/z* 393, 231, 221, 203, 189, 154, 136 and 121 (Scheme 1). All these data indicated that **1** and **2** have a novel 16(15→14)*abeo*-serratane skeleton (6–6–7–5–6 ring system) having a methoxy, an aldehyde and a hydroxyl groups in the 3, 15 and 21 positions. In order to confirm the proposed structure based on spectroscopic methods, a single crystal X-ray analysis of **1** was performed. The structural refinement led to a discrepancy index R=0.0555 on 1580 observed reflections and 307 variables. The ORTEP view of **1** was shown in Fig. 3.

Compounds **1** and **2** seem to be biosynthesized from 3β-methoxyserrat-14-en-21β-ol (**3**) and the 3α-epimer (**4**) via corresponding 14β,15β-epoxides. Then, the biomimetic syntheses of **1** and **2** were examined. Oxidation of **3** with *m*-chloroperbenzoic acid (MCPBA) yielded 14β,15β-epoxy-3β-methoxyserratan-21β-ol (**5**), which was treated with BF_3·OEt_2 in $CHCl_3$ to afford an aldehyde identical in all respects with **1**. On the other hand, treatment of **4** with MCPBA in $CHCl_3$ containing a drop of BF_3·OEt_2 directly furnished an aldehyde identical to **2** in quantitative yield (Scheme 2).[9] Thus, the structure of **1** was established as 21β-hydroxy-3β-methoxy-16(15→14)*abeo*-13*R*,14*S*-serratan-15-al, and that of **2** was proved to be 3α-epimer of **1**.

Table 2. Percentage of Epstein-Barr virus early antigen (EBV-EA) induction in the presence of compounds **1**–**5** with respect to a positive control (100%)

Compound	Concentration (mol ratio/TPA)			
	1000	500	100	10
1	10.9 (70)	36.2	72.9	92.8
1a	18.4 (70)	42.6	77.3	100.0
2	11.2 (70)	38.3	75.5	100.0
3	0 (70)	26.4	79.1	93.4
4	0 (70)	26.0	77.8	91.6
5	0 (70)	22.5	73.1	91.6
Oleanolic acid[a]	12.7 (70)	22.4	72.5	93.7

Values represent relative percentages to the positive control value. TPA (32 pmol, 20 ng)=100%. Values in parentheses are viability percentages of Raji cells.

[a] Positive control substance.

The inhibitory effects of the above triterpenoids (**1**–**5**) on EBV-EA activation induced by 12-*O*-tetradecanoylphorbol 13-acetate (TPA), were examined as a preliminary evaluation of their potential anti-tumor-promoting activities, and the results are shown in Table 2. Thus, compounds **3**–**5** exhibited potent inhibitory effects on EBV-EA induction by TPA. Especially, the inhibitory effect of **5** was found to be more potent than that of positive control, oleanolic acid[10] at 1000 and 10 mol ratio/TPA. As shown in Table 2, jezananals A and B did not showed sensible anti-tumor promoting activities, it is therefore interesting to note that the serratane-type triterpenes (6–6–7–6–6 ring system) seems to enhance the resultant anti-tumor-promoting activity than the rearranged 16(15→14)*abeo*-serratane triterpenes. The viability percentages of Raji cells treated with the test compounds (**1**–**5**) were 70 % at the highest concentration of 1000 mol ratio/TPA, suggesting that the cytotoxicities of all compounds were considerably moderate against in vitro cell lines (Table 2). The results of the in vitro experiments prompted us to examine the effect of compound **5** on the in vivo two-stage carcinogenesis bioassay on mouse skin using DMBA as an initiator and TPA as a promotor. No significant toxic effects, such as inflammation and lesional damages, on the areas of mouse skin topically treated with the test compound was observed, except for the formation of papillomas, at the end of treatment, and also the body weight gains was not influenced during the treatment. As

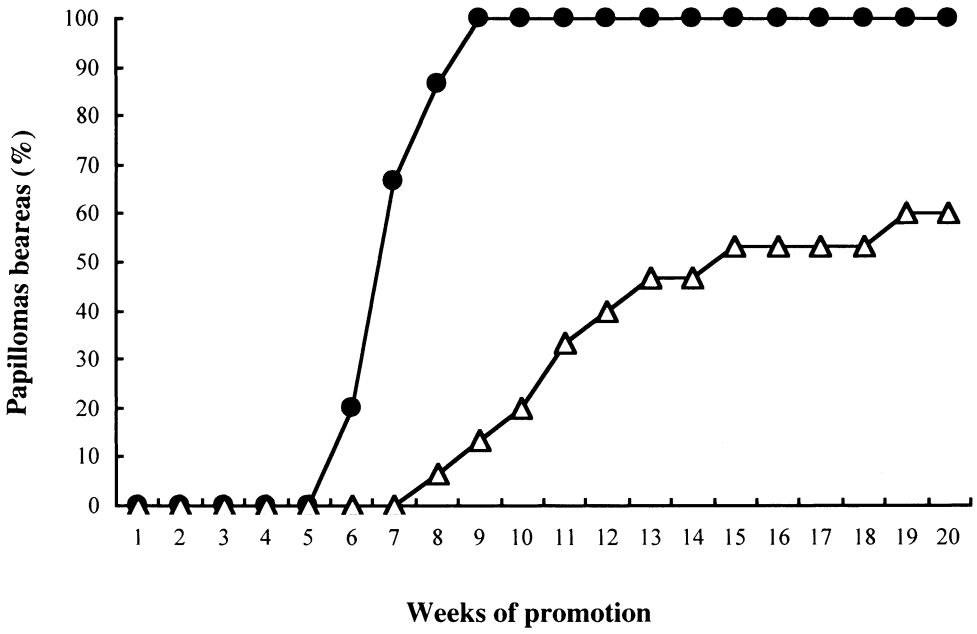


Figure 4. Inhibition of TPA-induced tumor promotion by multiple application of (**5**). All mice were initiated with DMBA (390 nmol) and promoted with 1.7 nmol of TPA, given twice weekly starting 1 week after initiation. Percentage of mice bearing papillomas. (●) control, TPA alone; (△) TPA+85 nmol of **5**.

shown in Fig. 4, the percentage of papilloma bearers in the control group (DMBA and TPA only) increased rapidly from week 6 and reached 100% after week 9, whereas the treatment with compound **5** along with DMBA/TPA reduced the percentage of papilloma-bearing mice to approximately 20% during weeks 8–10 and thereafter 60% over the period of week 20. As shown in Fig. 5, in the control group, the number of papillomas per mouse formed increased rapidly after 6 and reached 10.0 papillomas/mouse at week 20. On the other hand, the mice treated with compound **5** bore 4.8 papillomas over the period of week 20. In comparison with the results of the triterpenes reported so far, compound **5** is considered to be a naturally occurring triterpene with stronger anti-tumor promoting activity than cucurbitane-type triterpenoids, scandenoside R6, 23,24-dihydrocucurbitacin F, cucurbitacin F,[11] and hopane-type triterpenoids, hop-17(21)-ene and neohop-13(18)-ene[12] in the in vivo assay. The serratane-type triterpenoids including compound **5**, 13α,14α-epoxy-3β-methoxyserratan-21βol and 21α-hydroxy-3β-methoxy-serrat-14-en-29-al[8] can be considered to become appropriate lead compounds to develop more potent agents with anti-tumor promoting activity for clinical employment.

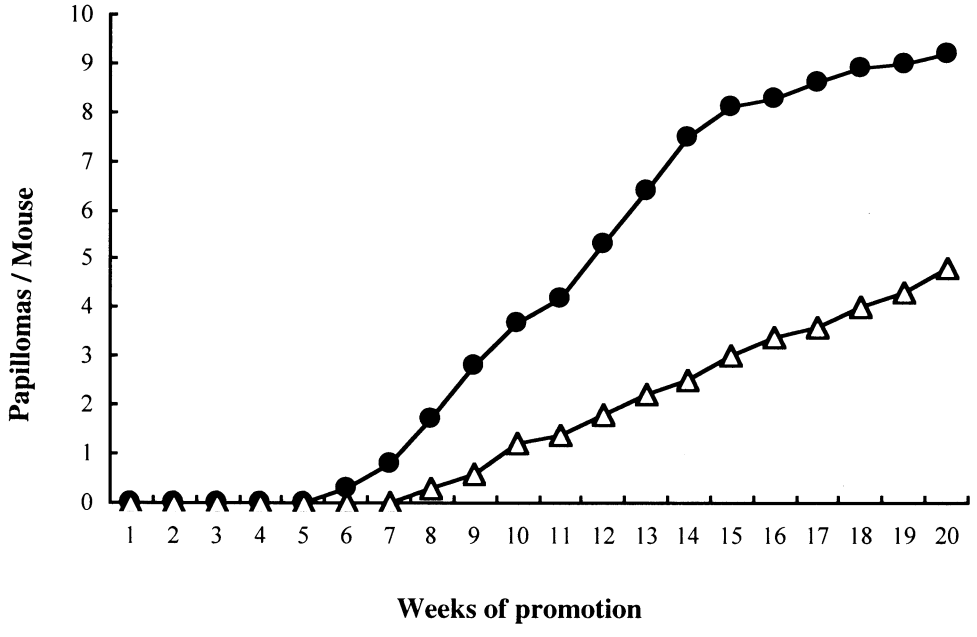


Figure 5. Inhibition of TPA-induced tumor promotion by multiple application of (**5**). All mice were initiated with DMBA (390 nmol) and promoted with 1.7 nmol of TPA, given twice weekly starting 1 week after initiation. Percentage of mice bearing papillomas. (●) control, TPA alone; (△) TPA+85 nmol of **5**.

3. Experimental

3.1. General procedures

Melting points were measured with a Yanagimoto micro-melting point apparatus without correction. Optical rotations were determined with a JASCO DIP-1000 digital polarimeter. IR spectra were recorded using a Perkin–Elmer 1720X FTIR spectrophotometer. ^{1}H and ^{13}C NMR spectra were obtained on a Varian INOVA 500 spectrometer with standard pulse sequences, operating at 500 and 125 MHz, respectively. $CDCl_3$ was used as the solvent and Me_4Si (TMS) as the internal standard. EIMS were recorded on a Hitachi 4000H double-focusing mass spectrometer (70 eV). Column chromatography was carried out over silica gel (70–230 mesh, Merck) and medium-pressure liquid chromatography (MPLC) was carried out with silica gel (230–400 mesh, Merck). Fractions obtained from column chromatography were monitored by TLC (silica gel 60 F_{254}, Merck). Preparative TLC was carried out on Merck silica gel F_{254} plates (20×20 cm^2, 0.5 mm thick).

3.2. Plant material

Cuticles of *P. jezoensis* (sieb. et Zucc.) Carr. var. *jezoensis* were collected at ca. 1000 m in the mountains of Hidaka town, Saryu district, Hokkaido, Japan, in June 2001. A voucher specimen (PJJ-01-01) is deposited at the Herbarium of the Laboratory of Medicinal Chemistry, Osaka University of Pharmaceutical Sciences.

3.3. Extraction and isolation

The freshly chopped cuticle (12 kg) of *P. jezoensis* was extracted with $CHCl_3$ (20 L) employing an automatic percolator for 7 days at 50°C. The $CHCl_3$ solution was evaporated under reduced pressure and the resulting dark green residue (685.0 g) was subjected to silica gel (13 kg) column chromatography. Elution of the column with $CHCl_3$ afforded residues A (39.3 g), B (67.2 g), C (28.9 g), D (71.0 g) E (51.6 g), and F (23.8 g), from fractions 1–13, 14–26, 27–30, 31–40, 41–60 and 61–74 (each 2 L). Elution was continued with $CHCl_3$–EtOAc (5:1) to give residues G (60.1 g), H (52.9 g), and I (12.6 g) from fractions 75–83, 84–90 and 91–105. Further elution with EtOAc gave a residue J (83.1 g) from fractions 106–111. Recrystallization of residues B and E gave 3α-methoxyserrat-14-en-21β-ol (**4**) (26.2 g) and 3β-methoxyserrat-14-en-21β-ol (**3**) (33.8 g), respectively. Repeated column chromatography of residue G on silica gel (2 kg) eluting with $CHCl_3$–EtOAc (10:1) afforded a crystalline solid (fractions 22–30, 1.95 g), which was recrystallized from MeOH–$CHCl_3$ to give compound **2** (488 mg). Further elution with the same column with same solvent gave a triterpene mixture (fractions 31–42, 3.01 g), which was subjected to MPLC with *n*-hexane–EtOAc (10:1) to give compounds **2** (109 mg) and **1** (1.232 g). Repeated column chromatography of residue H on silica gel (2 kg) eluting with $CHCl_3$–EtOAc (10:1) afforded a crystalline solid (fractions 83–91, 0.82 g), which was recrystallized from MeOH–$CHCl_3$ to give 14β,15β-epoxy-3β-methoxyserratan-21β-ol (**5**) (246 mg).

3.3.1. Jezananal A (1). Colourless prisms; mp 240–241.5°C (from MeOH–$CHCl_3$); $[\alpha]_D^{23}=-4.0$ (*c* 0.53, $CHCl_3$); HREIMS *m/z*: 472.3919 $[M]^+$ ($C_{31}H_{52}O_3$, calcd for 472.3913); IR (KBr) ν_{max}: 3540 (OH), 2932, 2850, 1716 (CHO), 1461, 1386 and 1366 (*gem.* dimethyl), 1104, 997 and 948; ^{1}H and ^{13}C NMR, see Table 1. EIMS *m/z* (rel. int.): 472 (11) $[M]^+$, 454 (2) $[M-H_2O]^+$, 425.3801 (38) $[M-H_2O-CHO]^+$, 393 (23) [ion **a**], 257 (20), 231 (18) [ion **b**], 221 (23) [ion **c**], 203 (16) [ion **d**], 189 (26) [ion **e**], 154 (71) [ion **f**], 136 (100) [ion **g**], 121 (46) [ion **h**].

3.3.2. Jezananal A acetate (1a). Compound **1** (25 mg) was acetylated as usual (Ac_2O–pyridine, 1:1, 2 mL) to yield a crystalline solid. Purification by PTLC ($CHCl_3$–MeOH, 30:1) afforded a corresponding monoacetate (**1a**), mp 180.5–183°C (from MeOH–$CHCl_3$); HREIMS *m/z* 514.4010 $[M]^+$ ($C_{33}H_{54}O_4$, calcd for 514.4019); IR (KBr) ν_{max} 1727 and 1246 (OAc); ^{1}H and ^{13}C NMR, see Table 1.

3.3.3. Conversion of jezananal A (1) to 3β-methoxyserrat-14-en-21β-ol (3) via 14β,15β-epoxy-3β-methoxyserratan-21β-ol (5). A solution of MCPBA (40 mg) in dry $CHCl_3$ (5 mL) was gradually added over a solution of compound **3** (40 mg) in dry $CHCl_3$ (5 mL) under stirring at room temperature for 4 h. The reaction mixture was poured into ice water, and extracted with $CHCl_3$ (50 mL×3). The $CHCl_3$ extract was washed with 5% Na_2CO_3 and H_2O, and dried over Na_2SO_4. Evaporation of $CHCl_3$ under reduced pressure yielded a crystalline mass (38 mg), which was purified by PTLC ($CHCl_3$–MeOH, 20:1) to give 14β,15β-epoxy-3β-methoxyserratan-21β-ol (**5**) (35 mg). Then, one drop of $BF_3{\cdot}OEt_2$ was gradually added over a solution of compound **5** (11.7 mg) in $CHCl_3$ (10 mL) under ice cooling, and the mixture was kept for 80 min. The $CHCl_3$ layer was neutralized with 10% $NaHCO_3$ solution, washed with H_2O, and dried over Na_2SO_4. Evaporation of $CHCl_3$ yielded a crystalline mass (9.1 mg), which was identified by direct comparison with jezananal A (**1**).

3.3.4. Crystal data of 1. $C_{31}H_{52}O_3$, M=472.73, orthorhombic, space group: $P2_12_12_1$, a=11.063 (4) Å, b=32.573 (3) Å, c=7.492 (5) Å. V=2699.9 (19) Å^3, Dx=1.163 g/cm^3, Z=4. A single crystal was used for X-ray diffraction data collection on a Rigaku AFC-5 diffractometer employing graphite-monochromated Cu Kα radiation. A total of 2475 independent reflection intensities up to 2θ=130° were collected in an $\omega-2\theta$ scan mode and were corrected for the Lorenz and polarization factors. The structure was solved by direct method using the SIR 92 program.[13] The non-hydrogen atoms were refined by a full-matrix least-squares method with anisotropic thermal parameters using the SHELXL-97 programs.[14] Hydrogen atoms were calculated assuming idealized geometries but not refined. The discrepancy indices R and Rw are 0.0555 and 0.162 for 1580 $[F_o>4\sigma(F_o)]$ reflections. All calculations were performed using teXan[15] crystallographic software package of Molecular Structure Corporation. Lists of atomic coordinates, anisotropic thermal parameters, and bond lengths and angles have been deposited at the Cambridge Crystal Crystallographic Data Centre, UK.

3.3.5. Jezananal B (2). Colorless prisms; mp 221–224.5°C (from MeOH–$CHCl_3$); $[\alpha]_D^{23}=-22.2$ (*c* 0.52, $CHCl_3$);

HREIMS *m/z*: 472.3918 $[M]^+$ ($C_{31}H_{52}O_3$, calcd for 472.3913); IR (KBr) ν_{max}: 3600 (OH), 2931, 2873, 1723 (CHO), 1460, 1387 and 1365 (*gem.* dimethyl), 1211, 1092, 994 and 908; ^1H and ^{13}C NMR, see Table 1. EIMS *m/z* (rel. int.): 472 (3) $[M]^+$, 454 (1) $[M-H_2O]^+$, 443.3874 (1) $[M-CHO]^+$, 425.3735 (10) $[M-H_2O-CHO]^+$, 393 (16) [ion **a**], 231 (16) [ion **b**], 221 (20) [ion **c**], 203 (12) [ion **d**], 189 (26) [ion **e**], 154 (59) [ion **f**], 136 (100) [ion **g**], 121 (36) [ion **h**].

3.3.6. Jezananal B acetate (2a). Compound **2** (15 mg) was acetylated as usual (Ac_2O–pyridine, 1:1, 2 mL) to yield a crystalline solid. Purification by PTLC ($CHCl_3$–MeOH, 30:1) afforded a corresponding monoacetate (**1a**), HREIMS *m/z*: 514.4010 $[M]^+$ ($C_{33}H_{54}O_4$, calcd for 514.4019); IR (KBr) ν_{max}: 1728 and 1244 (OAc).

3.3.7. Conversion of jezananal B (2) to 3α-methoxy-serrat-14-en-21β-ol (4). A solution of MCPBA (85 mg) in dry $CHCl_3$ (10 mL) was gradually added over a solution of compound **4** (85 mg) in dry $CHCl_3$ (10 mL) under stirring at −10°C. Then, one drop of $BF_3{\cdot}OEt_2$ was added to the mixture and kept at room temperature for 4 h. The reaction mixture was extracted with $CHCl_3$ (50 mL×3) and 10% $NaHCO_3$ solution, H_2O, and dried over Na_2SO_4. Evaporation of $CHCl_3$ under reduced pressure yielded a crystalline mass (58.0 mg), which was identified by direct comparison with jezananal B (**2**).

3.4. In vitro EBV-EA induction effect

EBV-EA positive serum from a patient with nasopharyngeal carcinoma (NPC) was a gift from Dr Y. Zaizen, the Department of Biochemistry, Oita Medicinal University. The EBV genome-carrying lymphoblastoid cells (Raji cells derived from Burkitts lymphoma) were cultured in 10% fetal bovine serum (FBS) in RPMI-1640 medium (Nissui). Spontaneous activation of EBV-EA in our sub-line Raji cells was less than 0.1%. The inhibition of EBV-EA activation was assayed using Raji cells (virus non-producer type) as described previously.[16] The indicator cells (Raji cells, 1×10^6/mL) were incubated at 37°C for 48 h in 1 mL of a medium containing *n*-butyric acid (4 mmol), TPA (32 pmol=20 ng in dimethylsulfoxide, DMSO), as inducer and various amounts of test compound in 5 μL DMSO. Smears were made from the cell suspension, and the activated cells that were stained by EBV-EA positive serum from NPC patients were detected by an indirect immunofluorescence technique.[17] In each assay, at least 500 cells were counted, the number of stained cells were counted, and the number of stained cells (positive cells) present was recorded. Triplicate assays were performed for each compound. The average EBV-EA induction of the test compounds was expressed as a relative ratio to the control experiment (100%) which was carried out only with *n*-butylic acid (4 mmol) plus TPA (32 pmol). EBV-EA induction was ordinarily around 35%. The viability of treated Raji cells was assayed by Tripan Blue staining methods.

3.5. In vivo two-stage carcinogenesis test for mouse skin papillomas promoted by TPA[18]

Specific pathogen-free female ICR mice (6 weeks old body weight approx. 30 g) were obtained from Japan SLC Inc., Shizuoka, Japan, and the animals were housed, five per polycarbonate cage, in a temperature-controlled room at 24±2°C and given food and water ad libitum throughout the experiment. Animals were divided into three experimental groups containing 15 mice each. The back (2×8 cm^2) of each mouse was shaved with surgical clippers, and the mice were topically treated with DMBA (100 g, 390 nmol) in acetone (0.1 mL) as an initiating treatment. One week after the initiation, papilloma formation was promoted twice weekly by the application of TPA (1 μg, 1.7 nmol) in acetone (0.1 mL) to the skin. Group I received the TPA treatment alone, and groups II received a topical application of compound **5** (85 nmol), in acetone (0.1 mL), respectively, 1 h before the TPA treatment. The incidence and numbers of papillomas were monitored weekly for 20 weeks.

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