A New *seco*-Abietane-Type Diterpene from the Stem Bark of *Picea glehni*

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Abstract

A new *seco*-abietane-type diterpenoid, 13*S*-hydroxy-9-oxo-9,10-*seco*-abiet-8(14)-en-18,10α-olide (**1**) along with a known lignan compound, pinoresinol (**2**) was isolated from the stem bark of *Picea glehni* (Fr. Schm.) Masters. Spectroscopic methods and chemical conversions were used to establish the structure of **1**. In order to assess their cancer chemopreventive potential, the inhibition of Epstein-Barr virus early antigen (EBV-EA) activation induced by 12-*O*-tetradecanoylphorbol 13-acetate (TPA) was examined for compound **1**, its synthetic analogue, 9,10-*seco*-8*S*,13*S*-epoxy-abiet-8(14)-en-18,10α-olide (**1a**) and **2**. The inhibitory effect of **1a** on EBV-EA induction was strong (0, 20.7, 67.1 and 89.2 % inhibition at 1000, 500, 100 and 10 mol ratio/TPA). The IC_{50} of **1a** was 226 mol ratio/32 pmol/TPA.

From the stem bark of *Picea glehni* (Fr. Schm.) Masters (Pinaceae), we isolated three new diterpenes, 19(4→3)*abeo*-8α,13*S*-epoxy-labda-4(18),14-diene, 19-*nor*-abieta-4(18),8,11,13-tetraen-7-one and 12-hydroxydehydroabietic acid along with nine known diterpenes [1], and two new triterpenes, 3α-methoxyserrat-14-en-21β-yl formate, and 24-methylcycloartanone together with three known triterpenes [2]. In order to assess usefulness of this plant as a source of natural agents for cancer chemoprevention, the extract was further examined and a new 13*S*-hydroxy-9-oxo-9,10-*seco*-abiet-8(14)-en-18,10α-olide (1) was isolated together with the known pinoresinol (2).

HR-EI-MS assigned the molecular formula of 1 as $C_{20}H_{30}O_4$. The IR spectrum of 1 showed a hydroxy group (ν_{max} = 3459 cm^{-1}), a γ-lactone (ν_{max} = 1770 cm^{-1}), and an α,β-unsaturated six-membered ring ketone (ν_{max} = 1675 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra (Table **1**) exhibited two tertiary methyl groups, an isopropyl group, seven sp^3 methylenes, an sp^3 methine, an sp^3 quaternary carbon, a tertiary carbon bearing a hydroxy group [δ_C = 72.3 (s)], a γ-lactone ring [δ_C = 85.4 (s), 180.3 (s)], a trisubstituted double bond [δ_H = 6.50 (1H, d); δ_C = 139.0 (s), 148.4 (d)], and a conjugated ketone [δ_C = 198.9 (s)]. The unsaturation of compound 1 suggested that it is a B-ring *seco*-abietane-type diterpenoid and possesses a lactone ring at positions C-10α and C-18 re-

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Table **1** ^{1}H and ^{13}C-NMR data for compounds **1, 1a**, **1b** and **1c** ($CDCl_3$).[a]

	1		*1a*		*1b*		*1c*	
Position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1α	27.8 t	1.59 m	27.8 t	1.61 m	27.9 t	1.61 m	27.9 t	1.62 m
1β		1.59 m		1.61 m		1.61m		1.62 m
2α	18.5 t	1.59 m	18.5 t	1.61 m	18.5 t	1.61 m	18.6 t	1.62 m
2β		1.68 m		1.69 m		1.68 m		1.74 m
3α	26.2 t	1.52 m	26.3 t	1.50 m	26.3 t	1.55 m	26.3 t	1.56 m
3β		1.39 m		1.40 m		1.42 m		1.42 m
4	46.7 s		46.7 s		46.7 s		46.6 s	
5α	54.0 d	1.80 t (6.1)	53.8 d	1.82 t (6.5)	53.6 d	1.88 t (6.1)	54.5 d	1.95 t (7.0)
6A	24.2 t	1.46 m	23.5 t	1.54 m	26.9 t	1.68 m	22.2 t	2.30 (2H) m
6B		1.53 m		1.60 m		1.78 m		
7A	29.5 t	2.20 dddd (13.5, 10.8, 5.9, 0.9)	32.2 t	2.10 ddd (14.3, 10.5, 5.5)	35.1 t	2.67 m	23.3 t	2.14 m
7B		2.28 dddd (13.5, 10.8, 5.9, 0.9)		2.40 dddd (14.3, 10.5, 5.5, 1.1)		2.67 m		2.30 m
8	139.0 s		143.6 s		141.2 s		137.7 s	
9	198.9 s		68.6 d	4.08 m	125.7 d	7.00 d (7.5)	70.7 d	4.21 dd (6.5, 3.5)
10	85.4 s		85.6 s		85.4 s		85.4 s	
11α	34.1 t	2.69 ddd (17.2, 10.3, 5.3)	29.7 t	2.03 ddt (12.3, 3.5, 5.5)	128.5 d	7.24 t (7.5)	30.9 t	1.84 m
11β		2.44 ddd (17.2, 6.4, 4.8)		1.78 m				1.78 m
12α	30.8 t	1.96 ddd (13.5, 10.3, 4.8)	28.7 t	1.66 m	124.3 d	7.09 d (7.5)	121.9 d	5.37 t (7.5)
12β		2.14 dddd (13.5, 6.4, 5.3, 1.4)		1.66 m		2.14 dddd (13.5, 6.4, 5.3, 1.4)		
13	72.3 s		72.4 s		149.3 s		149.1 s	
14	148.4 d	6.50 d (0.9)	129.4 d	5.41 s	126.4 d	7.02 s	114.3 d	6.08 s
15	37.0 d	1.92 septet (6.9)	37.6 d	1.75 septet (7.0)	34.1 d	2.89 septet (7.0)	35.5 d	2.35 septet (6.5)
16	16.4 q	1.03 d (6.9)	16.4 q	0.96 d (7.0)	24.0 q	1.25 d (7.0)	21.4 q	1.08 d (6.5)
17	17.4 q	0.99 d (6.9)	17.7 q	0.89 d (7.0)	24.0 q	1.25 d (7.0)	22.2 q	1.08 d (6.5)
18	180.3 s		180.3 s		180.3 s		180.2 s	
19	20.2 q	1.16 s	20.1 q	1.15 s	20.2 q	1.16 s	20.0 q	1.15 s
20	25.6 q	1.41 s	24.8 q	1.43 s	24.5 q	1.39 s	24.5 q	1.38 s

[a] Assignments were made by ^{1}H-^{1}H COSY, HMQC, HMBC and NOESY data.

sulting from dehydration between a C-10 hydroxy group and a C-18 carboxylic acid. The HMBC spectrum showed the following correlations: between Me-16 and C-13, C-15, C-17; Me-17 and C-13, C-15, C-16; Me-19 and C-3, C-4, C-5, C-18; Me-20 and C-1, C-5, C-10; H-14 and C-8, C-9, C-12, C-13, C-15. In the $^{1}H/^{1}H$ COSY spectrum correlations were seen between H-7α and H-6α, H-6β, H-7β; H-7β and H-6α, H-6β, H-7α, although H-14 correlated with no peak. Therefore, the tertiary hydroxy group is attached at C-13. These data suggested that the structure of 1 was a 13-hydroxy-9-oxo-9,10-*seco*-abiet-8(14)-en-18,10α-olide. Reduction of 1 with $LiAlH_4$ gave 1a, $[M]^+ m/z$ = 318, in quantitative yield and its ^{1}H- and ^{13}C-NMR spectra showed new signals at δ_H = 4.08 (1H, m) and δ_C = 68.6 (d). $NaBH_4$ reduction of 1 also gave the same compound (1a), and the following HOAc treatment gave a known compounds 1b and 1c. The NOESY spectrum of 1 (Fig. **2**) showed that isopropyl methyl groups (Me-16 and -17) were correlated not with Me-20 but with Me-19, and the relative configuration of 1 was determined as shown in Fig. **1**. Therefore, compound 1 is a new *seco*-abietane-type diterpenoid, 13*S*-hydroxy-9-oxo-9,10-*seco*-abiet-8(14)-en-18,10α-olide.

Table **2** lists inhibitory effects of compounds **1**, **1a** and **2** on Epstein-Barr virus early antigen (EBV-EA) induced by the tumour promotor, TPA and the associated viability of Raji cells. The viability of Raji cells treated with the test compounds (**1**, **1a**, and **2**) was over 70% at the highest concentration of 1000 mol ratio/TPA; suggesting that these compounds had moderate cytotoxicities against *in vitro* cell lines (Table **2**). On comparison of the anti-tumour promoting activities, **1a** showed a stronger effect than **1**. It is interesting to note that the presence of an ether group in **1a** seems to enhance its activity against tumour promotion as is the case with 13α,14α-epoxy-3β-methoxyserratan-21β-ol [3].

Materials and Methods

Plant material: The stem bark of *Picea glehni* (Fr. Schm.) Masters (Pinaceae) was collected in the mountainous terrain under the control of National Hokkaido Bureau, Iwamizawa City, Japan, in October 1997. A voucher specimen (PG-9710 – 1) is deposited at

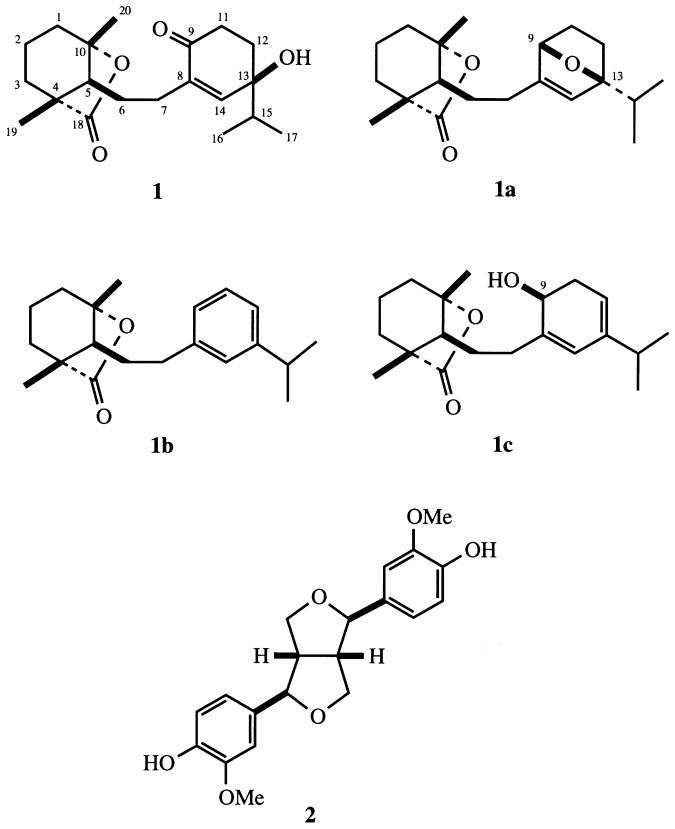

Fig. **1** Chemical structures of compounds **1**, **1a** – **c**, and **2**.

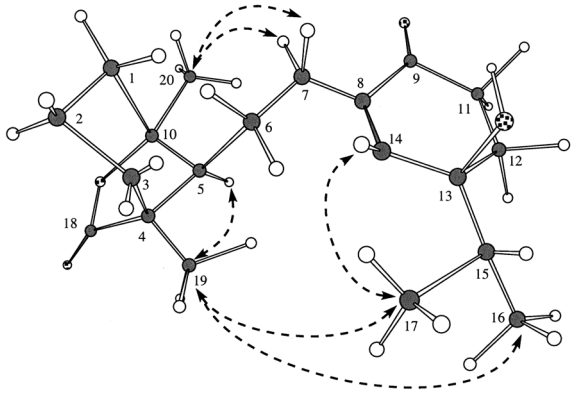

Fig. **2** NOESY correlations of **1**.

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Isolation procedure: Preliminary silica gel column chromatography separated the $CHCl_3$ extract (412.4 g) of the chopped stem bark (9.0 kg) of *P. glehni* into 13 (residues I – XIII) fractions as reported previously [1]. Residue XI (fraction nos 81 – 85, 1.958 g) was rechromatographed over silica gel (300 g) eluting with $CHCl_3$:EtOAc (10 : 1) to give an amorphous gum (fraction nos 50 – 68, 379.6 mg, 3.8 L), which has a UV absorption band on the TLC plate (254 nm). This material was rechromotographed over silica gel (30 g) using $CHCl_3$:EtOAc (20 : 1) to give pinoresinol (**2**) [4] (fraction no 64, 18.8 mg, 50 mL) and crude compound **1** (fraction nos 72 – 77, 75.9 mg, 300 mL), which was subjected to PTLC (*n*-hexane:EtOAc:MeOH, 50 : 50 : 2) to give pure compound **1** (57.0 mg). Compounds **1** and **2** had purities of over 98 %.

*13S-Hydroxy-9-oxo-9,10-*seco*-abiet-8(14)-en-18,10a-olide* (**1**): Colourless oil; $[\alpha]_D^{23}$: –4.8° (*c* 0.46, $CHCl_3$); HR-EI-MS: *m/z* = 334.2143 $[M]^+$ ($C_{20}H_{30}O_4$ requires 334.2142); IR (film): ν_{max} = 3459 (OH), 2960, 2933, 1770 (γ-lactone), 1675 (C = C-C = O), 923 cm^{-1}; EI-MS: *m/z* = 334 (7), 316.2058 (16) $[M - H_2O]^+$, 291.1596 (33) $[M - C_3H_7]^+$, 273.1501 (16), 245.1545 (86), 227.1437 (18), 167 (14), 150 (15), 137 (13), 123 (24), 122 (21), 109 (100); ^{1}H- and ^{13}C-NMR: see Table **1**.

Reduction of **1** *with $LiAlH_4$:* To a solution of compound **1** (16.9 mg) in absolute ether (10 mL), $LiAlH_4$ (20 mg) was added and the mixture stirred at room temperature for 6 h. The reaction mixture was worked up as usual to give **1a** (15.8 mg).

*9S,13S-Epoxy-9,10-*seco*-abiet-8(14)-en-18,10a-olide* (**1a**): M.p. 156 – 158 °C; $[\alpha]_D^{23}$: –21.7° (*c* 0.35, $CHCl_3$); EI-MS: *m/z* = 318 (2) $[M]^+$; ^{1}H- and ^{13}C-NMR: see Table **1**.

Reduction of **1** *with $NaBH_4$:* To a solution of **1** (14.1 mg) in MeOH (2 mL), $NaBH_4$ (2.0 mg) was added and the mixture was allowed to stand at room temperature for 1 h. The reaction product was confirmed as **1a** by TLC ($CHCl_3$:MeOH, 19 : 1). One drop of HOAc was added into the reaction mixture, followed by usual work-up. Evaporation of the solvent under reduced pressure afforded a residue (13.9 mg), which was subjected to PTLC ($CHCl_3$:MeOH, 19 : 1) to give compounds **1b** (5.2 mg) and **1c** (4.3 mg).

Table **2** Relative ratio[a] of EBV-EA activation with respect to positive control (100 %) in the presence of compounds **1**, **1a** and **2.**

Compounds	*EBV-EA positive cells (% viability)*				
	Compound concentration (mol ratio/32 pmol TPA)				*IC50*
	1 000	*500*	*100*	*10*	*(mol ratio/32 pmol TPA)*
1	3.7 (70)[b]	22.5	70.7	90.3	273
1a	0 (70)	20.7	67.1	89.2	226
2	11.5 (70)	40.6	72.0	100.0	398
Curcumin[c]	0 (60)	22.8	81.7	100.0	341

[a] Values represent percentages relative to the positive control value (100 %).
[b] Values in parentheses are the viability percentages of Raji cells.
[c] Positive control substance [8].

9,10-seco-*Abieta-8,11,13-trien-18,10a-olide* (**1b**): Colourless oil; $[\alpha]_D^{20}$: +8.5° (*c* 0.41, $CHCl_3$); IR (film): ν_{max} = 3459 (OH), 1675 cm^{-1}; EI-MS: *m/z* = 300 $[M]^+$; ^1H- and ^{13}C-NMR: see Table **1**. Compound **1b** was identified from published data including EI-MS, ^1H- and ^{13}C-NMR chemical shift values and its $[\alpha]_D$ value {**1b**: $[\alpha]_D^{20}$: +8.5° (*c* 0.41, $CHCl_3$), synthetic sample: $[\alpha]_D^{20}$: +9.5° (*c* 0.11, $CHCl_3$) [5]; $[\alpha]_D^{20}$: +9.2° (*c* 1.85, $CHCl_3$) [6]}.

9S-Hydroxy-9,10-seco-*abieta-11,13-dien-18,10a-olide* (**1c**): Colourless oil; $[\alpha]_D^{23}$: +8.7° (*c* 0.10, $CHCl_3$); EI-MS: *m/z* = 318 $[M]^+$; ^1H- and ^{13}C-NMR: see Table **1**.

Pinoresinol (**2**): Colourless oil; $[\alpha]_D^{23}$: +53.1° (*c* 0.53, $CHCl_3$).

Inhibition of EBV-EA activation assay: The inhibition of EBV-EA was assayed according to Ito et al. [7]. The inhibiting activities of the test compounds were estimated on the basis of the percentage of the number of positive cells compared with that of a control without the test compound. The viability of the cells was assayed by the trypan-blue staining method.

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References

[1] Kinouchi Y, Ohtsu H, Tokuda H, Nishino H, Matsunaga S, Tanaka R. Potential antitumor-promoting diterpenoids from the stem bark of *Picea glehni*. Journal of Natural Products 2000; 63: 817 – 20

[2] Tanaka R, Kinouchi Y, Tokuda H, Nishino H, Matsunaga S. Bioactive triterpenoids from the stem bark of *Picea glehni*. Planta Medica 2000; 66: 630 – 4

[3] Tanaka R, Minami T, Tsujimoto K, Matsunaga S, Tokuda H, Nishino H, Terada Y, Yoshitake A. Cancer chemopreventive agents, serratane-type triterpenoids from *Picea jezoensis*. Cancer Letters 2001; 172: 119 – 26

[4] Ludwig CH, Nist BJ, McCarthy JL. The high resolution nuclear magnetic resonance spectroscopy of protons in compounds related to lignan. Journal of American Chemical Society 1964; 86: 1186 – 96

[5] Herz W, Mohanraj S. Structure proof by synthesis of unusual secodehydroabietanes from Tall oil. Journal of Organic Chemistry 1980; 45: 5417 – 9

[6] Fuji K, Zheng SZ, Node M, Hao XJ. A chiral synthesis of a unique secodehydroabietane from Tall oil. Chemical and Pharmaceutical Bulletin 1991; 39: 202 – 3

[7] Ito M, Kawanishi M, Harayama T, Takabayashi S. Combined effect of the extracts from *Croton tiglium*, *Euphorbia lathyris* or *Euphorbia tirucalli* and *n*-butyrate on Epstein-Barr virus expression in human lymphoblastoid P3HR-1 and Raji cells. Cancer Letters 1981; 12: 175 – 80

[8] Huang M-T, Smart R-C, Wong C-Q, Conney A-H. Inhibition effect of curcumin, chlorogenic acid, caffeic acid, and ferulic acid on tumor promotion in mouse skin by 12-*O*-tetradecanoylphorbol 13-acetate. Cancer Research 1988; 48: 5941 – 6