Pergamon

Tetrahedron Letters 40 (1999) 6415–6418

TETRAHEDRON LETTERS

Piceanonols A, and B, triterpenoids bearing a novel skeletal system isolated from the bark of *Picea jezoensis* var. *hondoensis*

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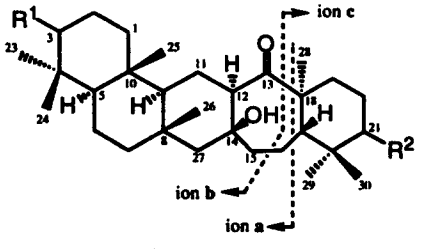
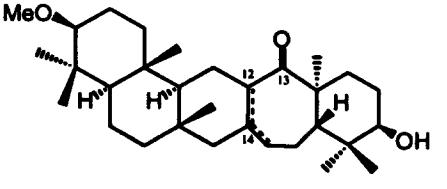
Received 22 April 1999; accepted 9 June 1999

Abstract

A $CHCl_3$ extract of the stem bark of *Picea jezoensis* var. *hondoensis* (Pinaceae) afforded two triterpenoids named piceanonols A and B with a novel 14(13→12)*abeo*-12α*H*-serratane skeleton. Extensive NMR studies, MS spectral analyses and X-ray crystallographics established their structures as 3β-methoxy-14β,21β-dihydroxy-14(13→12)*abeo*-12α*H*-serratan-13-one and 21α-methoxy-14β-hydroxy-14(13→12)*abeo*-12α*H*-serratane-3,13-dione.

From our series of studies on biologically active constituents from Pinaceae plants, we previously reported the isolation of five new serratane triterpenoids from the bark of *Picea jezoensis* var. *hondoensis*.[1–3] Continuous fractionation of the $CHCl_3$ extract of the bark further afforded two novel triterpenoids named piceanonols A (**1**) and B (**2**).





	R^1	R^2
1	β-OMe	β-OH
1a	β-OMe	β-OAc
1d	β-OMe	β-OCOPh-Br (*p*)
2	:O	α-OMe

1b 12(14)-ene

1c 14-ene

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PII: S0040-4039(99)01190-9

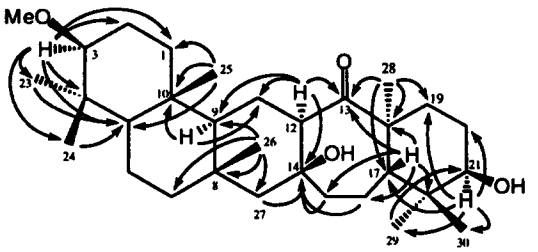


Figure 1. Selected HMBC correlations for **1**

Piceanonol A (**1**)[5] was shown to have the molecular formula $C_{31}H_{52}O_4$ by the high resolution MS spectrum. The IR spectrum showed hydroxyl absorption (ν_{max} 3540 cm^{-1}) and ketone (ν_{max} 1690 cm^{-1}). The ^1H and ^{13}C NMR spectra showed seven tertiary methyl groups, 10 methylenes, four methines of which a methine group appeared in the low magnetic field [δ_H 2.99 (1H, dd, J=12.3, 3.4 Hz)], four quaternary carbons, a methine group attached to a secondary methoxy group [δ_H 2.62 (1H, dd, J=11.2, 4.5 Hz), 3.36 (3H, s); δ_C 57.5 (q), 88.6 (d)], a methine group attached to a secondary hydroxyl group [δ_H 3.35 (1H, t, J=2.4 Hz); δ_C 75.6 (d)], a tertiary hydroxyl group [δ_C 71.8 (s)] and a ketone [δ_C 217.7 (s)]. Compound **1** was therefore a pentacyclic triterpene ketone with a secondary and a tertiary alcohol, and a secondary methoxy group. However, the ^1H and ^{13}C chemical shift values of **1** were considerably different from those of serratanes.[1–4]

In the EIMS spectrum, **1** showed fragment ion peaks at *m/z* 347.2586 (ion **a**, 17%), *m/z* 334.2504 (ion **b**, 100%), *m/z* 319.2288 (**b**-Me, 46%), *m/z* 153.1269 (ion **c**, 22%) and *m/z* 135.1176 (**c**-H_2O, 76%) and these peaks are characteristic of the cleavage of the D ring.

The acetylation of **1** gave a monoacetate (**1a**),[6] ν_{max} 3521 (*tert* OH), and its hydroxymethine proton signal was shifted to δ 4.66. Treatment of **1** with boiling dry THF containing 0.5% H_2SO_4 gave conjugated $\Delta^{12(14)}$-en-13-one (**1b**) [δ_C 132.3 (s), 136.5 (s)] and Δ^{14}-en-13-one (**1c**) [δ_H 4.99 (1H, d, J=2.5 Hz); δ_C 131.4 (s), 133.7 (d)]. From these spectral data and chemical conversion, **1** was assumed to have a novel carbon skeleton and revealed the presence of O=C-CH-C-OH moiety in the molecule.

The selected HMBC correlations for **1** were shown in Fig. 1. The NOESY spectrum of **1** showed cross-peaks between signals of Me-25 with Me-24 and Me-26, Me-28 with H-12α and Me-29, and H-12α with H-9α. All these data indicated that **1** has a novel 14(13→12)*abeo*-12α*H*-serratane having a ketone and a tertiary hydroxyl group at the C-13 and C-14 positions.

The absolute structure was finally established by the X-ray analysis of its *p*-bromobenzoate (**1d**).[8] Fig. 2 shows the ORTEP view of **1d**, and **1** possesses a novel 6-6-6-7-6 ring system, which has not been described in the literature.

Piceanonol B (**2**)[7] was shown to have the molecular formula $C_{31}H_{52}O_4$ by the high-resolution MS spectrum. The IR spectrum showed hydroxyl absorption (ν_{max} 3550 cm^{-1}) and ketone (ν_{max} 1705 and 1698 cm^{-1}). The ^1H and ^{13}C NMR spectra resembled those of **1** except for the presence of a ketone [δ_C 217.4 (s)] instead of a secondary hydroxyl group. The EIMS spectrum of **2** showed fragment ion peaks, corresponding to those of **1**, at *m/z* 331.1992 (ion **a**, 68%), *m/z* 318.2185 (ion **b**, 100%), *m/z* 303.1992 (**b**-Me, 59%), *m/z* 167 (ion **c**, 22%) and *m/z* 135.1158 (**c**-MeOH, 63%). From the above data, compound **2** was assumed to be 21α-methoxy-14β-hydroxy-14(13→12)*abeo*-12α*H*-serratane-3,13-dione, and extensive 2D NMR studies including HMBC and NOESY spectra supported this structure. To confirm this, we conducted the X-ray analysis of **2**. Fig. 2 shows the ORTEP view of **2**,[8] and accordingly compound **2** had the same 14(13→12)*abeo*-12α*H*-serratane skeleton of **1** with a tertiary hydroxyl group at C-14β, an equatorial methoxy group at C-21, and a ketone at C-3.

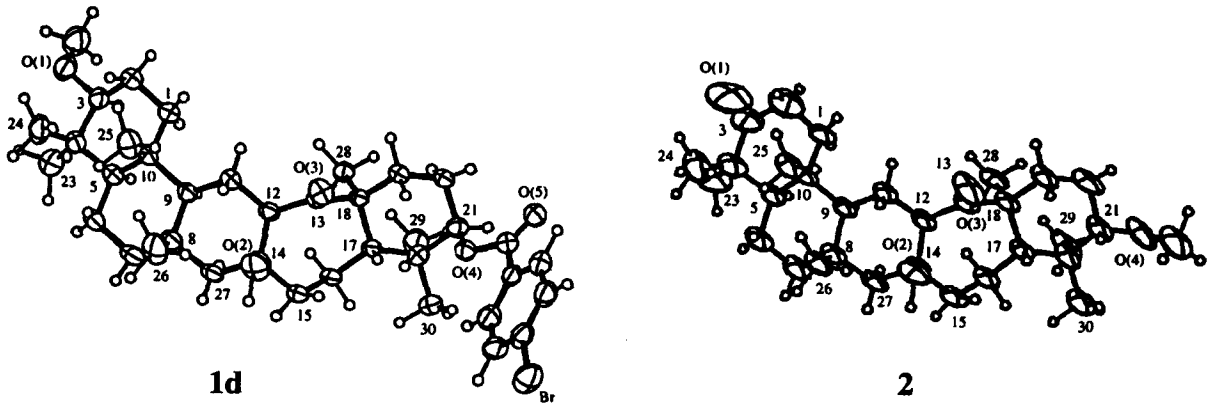
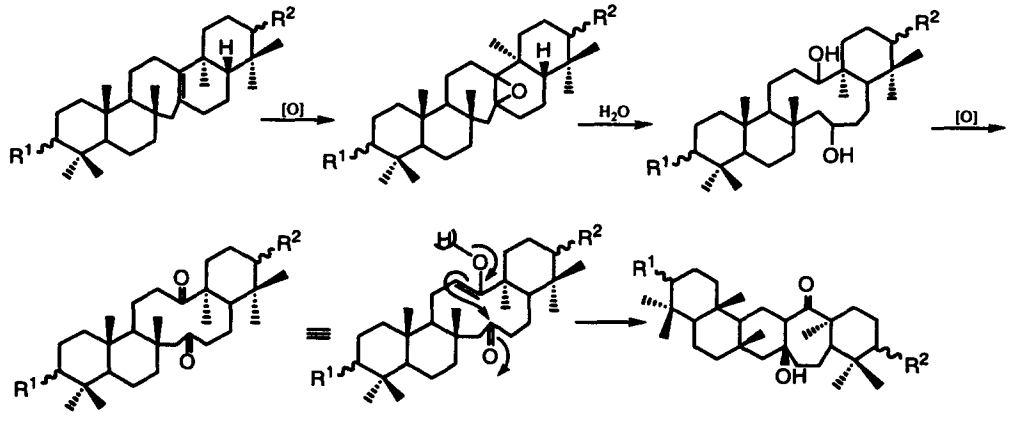


Figure 2. The ORTEP view of piceanonol A *p*-bromobenzoate (**1d**) and piceanonol B (**2**)

Plausible biogenetical routes of these compounds from Δ^{13}-serratenes are shown in Scheme 1. These compounds are particularly interesting since this is the first report of *abeo*-serratane carbon skeletons.



Scheme 1. Plausible biogenetical routes of compounds **1** and **2**

In the anti-tumor-promoting assay (Table 1),[9] compound **2** exhibited 100 and 20.4% inhibition in the 100 and 25 μg/ml concentrations, respectively, against the 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-enhanced incorporation of ^{32}Pi into phospholipids of cultured HeLa cells. Although no conspicuous

Table 1
Inhibitory effects of **1**, **1b**, **1c** and **2** on TPA-enhanced ^{32}Pi-incorporation into phospholipids of HeLa cells

	Inhibition %			
Compounds	100 μg/ml	50 μg/ml	25 μg/ml	10 μg/ml
1	0	0	0	0
1b	–	–	2.6	–
1c	–	–	10.6	–
2	100	26.8	20.4	14.5

inhibitory effects were observed for **1** (0%) in the 25 μg/ml concentration, **1b** and **1c**, the dehydration products of **1** increased the effects up to 2.6 and 10.6%, respectively.

Acknowledgements

The authors are grateful to Mrs. Michiko Yoneda and Mrs. Mihoyo Fujitake of this University for NMR and MS measurements.

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5. Compound **1**, prisms, mp 241–243°C (MeOH–$CHCl_3$); $[\alpha]_D^{23}$ +7.6 (*c* 0.80, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 3540 (OH), 2939, 2874, 1690 (C=O), 1463, 1388, 1106; ^1H NMR ($CDCl_3$): δ 0.76 (s, Me-24), 0.84 (s, Me-25), 0.87 (s, Me-29), 0.95 (s, Me-23), 0.98 (s, Me-30), 1.13 (s, Me-26), 1.24 (s, Me-28), 2.62 (1H, dd, *J*=11.2, 4.5 Hz, H-3α), 2.99 (1H, dd, *J*=12.3, 3.4 Hz, H-12α), 3.36 (s, MeO), and 3.35 (1H, t, *J*=2.4 Hz, H-21α); EIMS: *m/z* 488.3883 (M^+, 2%, $C_{31}H_{52}O_4$ requires 488.3865), 470 (17%), 347 (17%), 334 (100%), 319 (46%), 221 (6%), 189 (19%), 153 (22%), 135 (76%).
6. Compound **1a**, mp 250–253°C (MeOH–$CHCl_3$), $[\alpha]_D^{23}$ +34.0 (*c* 0.46, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 3521 (OH), 1741 and 1242 (OAc), 1705 (C=O); M^+: *m/z* 530.3968 ($C_{33}H_{56}O_5$, requires 530.3968). Compound **1b**, δ_C 132.3 (s, C-12), 136.5 (s, C-14); M^+: *m/z* 470. Compound **1c**, δ_H 4.99 (1H, d, *J*=2.5 Hz, H-12α); δ_C 131.4 (s, C-14), 133.7 (d, C-15); M^+: *m/z* 470. Compound **1d**, mp >300°C (MeOH–$CHCl_3$); M^+: *m/z* 671.
7. Compound **2**, prisms, mp 266–268°C (MeOH–$CHCl_3$); $[\alpha]_D^{23}$ +34.0 (*c* 0.46, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 3550 (OH), 2938, 2871, 1705 and 1698 (C=O), 1457, 1418 (CH_2C=O), 1387, 1102; ^1H NMR ($CDCl_3$): δ 0.79 (s, Me-29), 0.95 (s, Me-25), 0.99 (s, Me-30), 1.04 (s, Me-24), 1.08 (s, Me-23), 1.17 (s, Me-26), 1.23 (s, Me-28), 2.66 (1H, dd, *J*=12.2, 4.8 Hz, H-21β), 3.04 (1H, dd, *J*=12.3, 3.5 Hz, H-12α) and 3.36 (s, MeO); EIMS: *m/z* 486.3715 (M^+, 2%, $C_{31}H_{50}O_4$ requires 486.3706), 331 (68%), 318 (100%), 303 (59%), 286 (16%), 205 (9%), 189 (6%), 167 (22%), 135 (63%).
8. Crystal data of **1d** and **2**. **1d**: $C_{38}H_{55}O_5Br$, M=671.757, monoclinic, space group $P2_1$, *a*=7.225 (3), *b*=37.268 (12), *c*=7.011 (1) Å; β=111.94 (2); *U*=1750.9 (10) $Å^3$, *Dc*=1.2741 g cm^{-3}, *Z*=2. **2**: $C_{31}H_{52}O_4$, M=486.737, monoclinic, space group $P2_12_12_1$, *a*=17.146 (3), *b*=21.610 (7), *c*=7.393 (1) Å; *V*=2739.3 (10) $Å^3$, *Dc*=1.180 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 CuKα radiation. A total of 3047 (**1d**) and 2682 (**2**) independent reflections within 2θ=130° were collected in an ω-2θ scan mode and were corrected for the Lorenz and polarization factors. The structures were solved by direct methods and refined by least-squares analysis with use of the anisotropic temperature factors for non-H-atoms and isotropic factors for H-atoms, where H-atoms were observed on a final difference Fourier map. The discrepancy indices *R* and R_W are 0.0568 and 0.0707 for 2978 [F_O>0.0] reflections for **1d**, and 0.0757 and 0.0502 for 2549 [F_O>0.0] reflections for **2**, respectively. The atomic coordinates, anisotropic thermal parameters, and bond lengths and angles have been deposited at the Cambridge Crystallographic Data Center. See Notice to Authors, Issue No. 1.
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