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TWO SERRATANE TRITERPENES FROM THE STEM BARK OF *PICEA JEZOENSIS* VAR. *HONDOENSIS**

REIKO TANAKA, KAZUHIRO TSUJIMOTO, OSAMU MURAOKA† and SHUNYO MATSUNAGA‡

Osaka University of Pharmaceutical Sciences, 4-20-1 Nasahara, Takatsuki, Osaka 569-11, Japan; † Faculty of Pharmaceutical Sciences, Kinki University, 3-4-1 Kowakae, Higashiosaka, Osaka 577, Japan

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Key Word Index—*Picea jezoensis* var. *hondoensis*; Pinaceae; stem bark; triterpenes; 21α-hydroxy-3β-methoxyserrat-14-en-29-al; 29-*nor*-3α-methoxyserrat-14-en-21-one.

Abstract—Two new serratane triterpenoids were isolated from the stem bark of *Picea jezoensis* var. *hondoensis*, together with three known compounds, 3β-hydroxyserrat-14-en-21-one, 21α-hydroxy-3β-methoxyserrat-14-en-30-al and 29-*nor*-3β-methoxyserrat-14-en-21-one. The structures of the new compounds were characterized as 21α-hydroxy-3β-methoxyserrat-14-en-29-al and 29-*nor*-3α-methoxyserrat-14-en-21-one, on the basis of spectroscopic analysis. © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

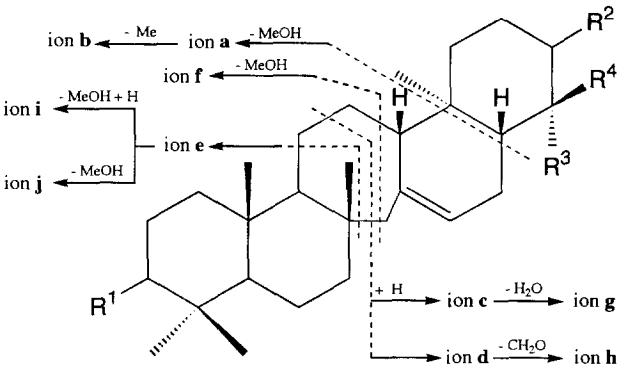
Previously, we reported that the $CHCl_3$ extract of the stem bark of *Picea jezoensis* (Sieb. et Zucc.) Carr. var. *hondoensis* Rhed. (Japanese name: Touhi, Pinaceae), a variety of *P. jezoensis* (Sieb. et Zucc.) Carr. var. *Jezoensis* (Mayr.) (Japanese name: Kuroezomatsu) [1], contained 21β-methoxyserrat-14-en-3-one, 21α-methoxyserrat-13-en-3-one and 21β-hydroxyserrat-14-en-3-one [2, 3]. In these studies, we used the stem bark of the former tree with the cuticle, as the stem of this tree is generally wrapped with a thinner cuticle than that of the latter tree. Recently, we found that the inner bark of the latter tree contains only the known 3α-methoxyserrat-14-en-21β-ol, while a number of serratenes were isolated from the highly developed cuticle [4–6]. We, therefore, decided to examine the cuticle of *P. jezoensis* var. *hondoensis*. Careful fractionation of the $CHCl_3$ extract of the cuticle of this tree led to the isolation of two new triterpenes, **4** and **5**, besides three known compounds, 3β-hydroxyserrat-14-en-21-one (**1**) [7–10], 21α-hydroxy-3β-methoxyserrat-14-en-30-al (**2**) [4] and 29-*nor*-3β-methoxyserrat-14-en-21-one (**3**) [4, 11–13]. This paper deals with the structures of **4** and **5**.

RESULTS AND DISCUSSION

Compound **4** was assigned the molecular formula $C_{31}H_{50}O_3$ (HREI MS). The IR and ^1H and ^{13}C NMR spectra (Table 1) established the presence of six quaternary methyl groups, a methine proton [δ_H 2.62 (1H, *dd*, J = 12.2 and 4.2 Hz); δ_C 88.4 (*d*)] geminal to a methoxy group [δ_H 3.36 (3H, *s*, OMe); δ_C 57.5 (*q*)], a methine proton [δ_H 3.24 (1H, *dd*, J = 15.3 and 7.4 Hz); δ_C 77.5 (*d*)] geminal to a hydroxyl group (ν_{max} 3436 cm^{-1}), a trisubstituted double bond [ν_{max} 1665 and 800 cm^{-1}; δ_H 5.32 (1H, *m*); δ_C 120.9 (*d*) and 138.6 (*s*)] and an aldehyde group attached to a sp^3 quaternary carbon [ν_{max} 1713 cm^{-1}; δ_H 9.94 (1H); δ_C 208.3 (*d*)]. The aldehyde proton signal was observed as a shallow doublet (J = 2.0 Hz) due to long range coupling with Me-30 at δ_H 1.26 (3H, *d*, J = 2.0 Hz). Although the COLOC (Table 1) and EI mass spectral data (see Experimental) of **4** provided the carbon-carbon connectivities and fragment peaks (ions **a**–**j**) identical with those already reported for 21α-hydroxy-3β-methoxyserrat-14-en-30-al (**2**), respectively, the physical and IR spectral data of both compounds were inconsistent with each other [4]. The ^1H and ^{13}C NMR spectra indicated that the chemical shift values of signals related to E ring of **4** were considerably different from those of **2**, although the other signals of both compounds were almost identical. The signals of the aldehyde group in **4** were shifted downfield by $\Delta\delta_H$ 0.49 and $\Delta\delta_C$ 1.7, respectively, compared to those of **2**, whereas those of Me-28 in **4** were shifted $\Delta\delta_H$ 0.12 and $\Delta\delta_C$ 4.6 to higher field than those of **2**. Furthermore, the signals of H-17β and H-21β in **4** were shifted upfield by $\Delta\delta_H$ 0.24 and 0.57, respectively, compared with those resonating at the lower field of δ_H 1.78 and 3.81 in **2** due to anisotropy by the aldehyde carbonyl. These data suggested that the methoxy, hydroxyl and aldehyde groups were located at the 3β, 21α and 29

* Part 3 in the series "Serratanes from the stem bark of *Picea jezoensis* var. *hondoensis*". For Part 2 see ref. [3].

‡ Author to whom correspondence should be addressed.



	R^1	R^2	R^3	R^4
1	β-OH	: O	Me	Me
2	β-OMe	α-OH	Me	CHO
3	β-OMe	: O	H	Me
4	β-OMe	α-OH	CHO	Me
5	α-OMe	: O	H	Me

positions of the serrat-14-ene skeleton, respectively, and **4** must be the positional isomer of **2** in which Me-29 was replaced by an aldehyde at C-30. Conclusive evidence for this structure was obtained from the NOESY experiment, in which the cross correlations shown in Fig. 1 were observed for **4**. Hence, the structure of **4** was established as 21α-hydroxy-3β-methoxyserrat-14-en-29-al.

Compound **5** was determined to have the molecular formula as $C_{30}H_{48}O_2$ (HREI MS). The IR and ^1H and ^{13}C NMR spectra (Table 1) contained signals for six quaternary methyl groups, a secondary methyl group [δ_H 0.97 (3H, *d*, $J = 6.3$ Hz)], a methine proton [δ_H 2.78 (1H, *t*, $J = 2.7$ Hz, H-3β); δ_C 85.8 (*d*)] geminal to a methoxy group [δ_H 3.31 (3H, *s*); δ_C 57.1 (*q*, OMe)], a trisubstituted double bond [ν_{max} 1662 and 798 cm^{-1}; δ_H 5.32 (1H, *m*); δ_C 121.4 (*d*) and 138.8 (*s*)], a methylene group vicinal to a ketone [δ_H 2.31 (1H, *ddd*, $J = 14.7$, 4.5 and 2.9 Hz) and 2.49 (1H, *td*, $J = 14.7$ and 5.8 Hz) and a six membered ring ketone [ν_{max} 1718 cm^{-1}; δ_C 213.6 (*s*)]. Despite the inconsistency of its physical and IR spectral data with those of the known 29-*nor*-3β-methoxyserrat-14-en-3-one (**3**), the EI mass spectrum (see Experimental) exhibited the same fragment ion peaks (ions **a**–**c**, **e**, **f**, **i** and **j**) as **3** [4, 10]. The chemical shift values of signals obtained from the ^1H and ^{13}C NMR spectra of **5** were also closely similar to those of **3**, except for those arising from the A-ring. The shift values of the proton and carbon signals for the methine group geminal to a methoxy function in **5** (Table 1) were extremely different from those of **3** which appeared at δ_H 2.63 (1H, *dd*, $J = 11.8$ and 4.4 Hz) and δ_C 88.4 (*d*) [4]. Consequently, the methoxy group was located at C-3α and the structure of **5** was unambiguously proved to be that of the 3α-epimer of **3**, 29-*nor*-3α-methoxyserrat-14-en-21-one.

It is of biogenetical interest that the stem bark of *P. jezoensis* var. *hondoensis* contained a pair of serratenals, **2** and **4**, together with a pair of 29-*nor*-serratenes, **3** and **5**, although 3β-methoxy-21-oxo-serrat-14-en-29-al and compound **4** acetate had previously been isolated from the bark of *Pinus luthunensis* var. *mastersiana* [13].

EXPERIMENTAL

General

Mps: uncorr.; Optical rotations: $CHCl_3$ at 23°; IR: KBr discs; ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz): $CDCl_3$ with TMS as int. standard; EIMS: 70 eV (probe); CC: silica gel 60 and alumina 90 (each 70–230 mesh, Merck); TLC: silica gel HF_{254} and PF_{254} (Merck).

Plant material

The stem bark of *P. jezoensis* var. *hondoensis* was collected at *ca* 1000 m height in a mountain close to Takane Village, Gifu Prefecture, under the management of the National Kosaka-Takayama Forestry Office, Takayama City, Japan, in August 1994. The plant material was identified by Dr G. Murata, ex-Lecturer of the Department of Botany, Faculty of Science, Kyoto University, Kitashirakawaoiwakechyo, Sakyoku, Kyoto, Japan. The voucher speci-

Table 1. NMR data for compounds **4** and **5**

	δ_H		δ_C		
Position	**4**	**5**	**4**	**5**	COLOC data for **4** (C to H)
1α	0.90 *m*		38.5 *t*	33.5 *t*	Me-25
1β	1.82 *m*				
2α	1.81 *m*		22.3 *t*	20.2 *t*	
2β	1.44 *m*				
3α	2.62 *dd* (12.2, 4.2)		88.4 *d*	85.8 *d*	Me-23, Me-24, OMe
3β	—	2.78 *t* (2.7)			
4	—	—	38.9 *s*	38.1 *s*	Me-23, Me-24
5α	0.74 *dd* (13.7, 2.1)	1.26 *m*	56.2 *d*	50.1 *d*	Me-23, Me-24, Me-25
6α	1.48 *m*		18.7 *t*	18.7 *t*	H-5α
6β	1.48 *m*				
7α	1.21 *m*		45.1 *t*	44.7 *t*	Me-26, H-27
7β	1.39 *dt* (12.8, 3.3)				
8	—		37.1 *s*	37.2 *s*	Me-26
9	0.77 *dd* (13.1, 2.2)	1.14 *dd* (12.2, 2.2)	62.7 *d*	62.2 *d*	Me-25, Me-26, H-11
10	—		38.2 *s*	38.0 *s*	Me-25
11α	1.74 *dd* (13.1, 8.8)		25.6 *t*	25.6 *t*	H-13β
11β	1.07 *m*				
12α	2.03 *ddd* (13.1, 7.8, 3.0)		27.2 *t*	27.2 *t*	H-9α
12β	1.15 *m*				
13	1.79 *m*		55.5 *d*	54.5 *d*	Me-28
14	—		138.6 *s*	138.8 *s*	H-27
15	5.32 *dd* (4.2, 2.0)	5.32 *dd* (4.3, 2.1)	120.9 *d*	121.4 *d*	H-27, H-16
16α	2.13 *m*		23.9 *t*	29.5 *t*	H-15
16β	2.33 *m*				
17β	1.54 *dd* (12.0, 5.7)		51.0 *d*	45.7 *d*	Me-28, Me-30
18	—		36.3 *s*	36.1 *s*	Me-28
19α	1.98 *dt* (13.2, 3.4)		37.1 *t*	38.0 *t*	Me-28
19β	1.18 *m*				
20α	1.92 *ddd* (14.0, 7.4, 3.6)	2.49 *td* (14.7, 5.8)	28.4 *t*	39.1 *t*	H-21β
20β	2.88 *m*	2.31 *ddd* (14.7, 4.5, 2.9)			
21	3.24 *dd* (15.3, 7.4)		77.5 *d*	213.6 *s*	Me-30, H-29
22	—		52.4 *s*	49.4 *d*	Me-30, H-29
23	0.95 *s*	0.92 *s*	28.1 *q*	28.5 *q*	Me-24
24	0.75 *s*	0.82 *s*	16.2 *q*	22.5 *q*	Me-23
25	0.80 *s*	0.82 *s*	15.7 *q*	15.7 *q*	H-1, H-9α
26	0.83 *s*	0.83 *s*	19.8 *q*	19.9 *q*	H-9α, H-27α
27α	2.22 *d* (14.8)	2.21 *d* (14.8)	55.9 *t*	56.1 *t*	Me-26
27β	1.76 *d* (14.8)	1.76 *d* (14.8)			
28	0.61 *s*	0.89 *s*	13.1 *q*	11.1 *q*	H-13β, H-19β
29	9.94 *d* (2.0)		208.27 *d*	—	Me-30
30	1.26 *d* (2.0)	0.97 *d* (6.3)	19.0 *q*	11.5 *q*	H-29
OMe	3.36 *s*	3.31 *s*	57.5 *q*	57.1 *q*	

mens (PJH-940803) are deposited at the Herbarium of the Department of Medicinal Chemistry, Osaka University of Pharmaceutical Sciences.

Extraction and isolation of compounds

The cuticle of *P. jezoensis* var. *hondoensis*, peeled off from the stem bark (8.37 kg), was chopped and extracted with $CHCl_3$ (3 × 10 l) employing an automatic glass percolator for 20 hr at 61°. The $CHCl_3$ soln was then evapd *in vacuo* and the resulting dark brown residue (407 g) was subjected to CC on silica gel (6 kg). Elution of the column with $CHCl_3$ afforded a yellow residue A (20.48 g) from frs 40–56 (each fr. 1 l). Further elution with $CHCl_3$–EtOAc (10:1) yielded a pale yellow reside B (16.05 g) from frs 57–66. Repeated CC of residue A on silica gel (1 kg) furnished a crystalline solid (238 mg) from frs 87–103 (each fr.: 200 ml) eluted with $CHCl_3$. Recrystallization from MeOH–$CHCl_3$ yielded the known compound, 3β-hydroxyserrat-14-en-21-one (**1**), 115 mg, mp 267–268°, $[\alpha]_D$ −40 (*c* 0.46) (lit. [8] mp 267–268.5°, $[\alpha]_D$ −40), which was identified (co-TLC, mp, $[\alpha]_D$, IR, ^1H and ^{13}C NMR and EIMS) by direct comparison with an authentic specimen. Continuous elution with $CHCl_3$–EtOAc (10:1) yielded a crystalline solid, 34 mg, from frs 125–130. Purification of the solid by prep. TLC [plate: 0.5 mm thick, 20 × 20 cm; solvent: $CHCl_3$–

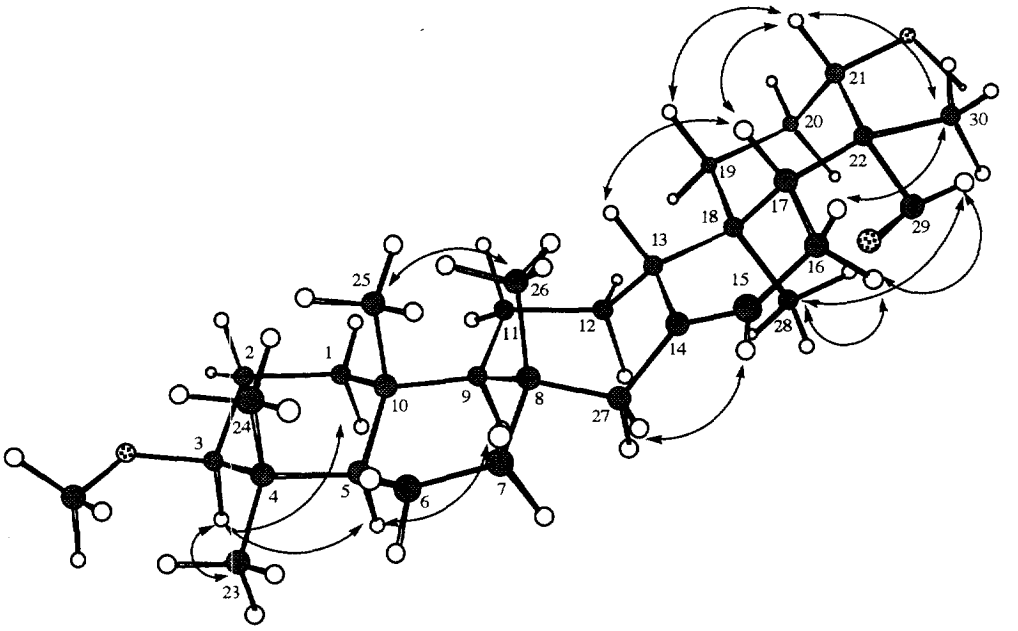


Fig. 1. NOESY experiment on compound **4**.

MeOH, 20:1] gave compound **4**, 29 mg. Subsequent CC with the same solvent yielded a crystalline mass, 48 mg, from frs 142–145, which was purified by prep. TLC [plate: 0.5 mm thick, 20 × 20 cm, solvent: $CHCl_3$–MeOH, 20:1] to afford the known compound, 21α-hydroxy-3β-methoxyserrat-14-en-30-al (**2**), 21 mg, mp 266–268.5° (MeOH–$CHCl_3$). $[\alpha]_D$ + 37 (*c* 0.21) (lit. [4] mp 266.5–269°, $[\alpha]_D$ + 37), identical in all respects (co-TLC, mp, $[\alpha]_D$, IR, ^1H and ^{13}C NMR and EIMS) with an authentic sample. Repeated CC of residue B on silica gel (800 g) with $CHCl_3$ successively furnished compound **5**, 3 mg, from frs 15–16, and the known compound, 29-*nor*-3β-methoxyserrat-14-en-21-one (**3**), 3 mg, mp 277–279° (MeOH–$CHCl_3$), $[\alpha]_D$ − 1 (lit. [4] mp 277–278.5°, $[\alpha]_D$ − 1), from frs 19–22, which was identified by direct comparison (co-TLC, mp $[\alpha]_D$, IR, ^1H and ^{13}C NMR and EIMS) with an authentic sample.

21α-*Hydroxy*-3β-*methoxyserrat*-14-*en*-29-*al* (**4**)

Prisms, mp 230–233° (MeOH–$CHCl_3$), $[\alpha]_D$ − 15 (*c* 0.24). HREIMS *m/z* 470.3758 $[M]^+$ ($C_{31}H_{50}O_3$ requires 470.3758); IR ν_{max} cm^{-1}: 3436 (OH), 2934, 2851, 1713 (CHO), 1665 (>C=C<), 1458, 1385 and 1363 (gem.-dimethyl), 1184, 1107, 861 and 800 (>C=C<H); ^1H and ^{13}C NMR: Table 1; EIMS *m/z* (rel. int.): 470 $[M]^+$ (5), 452 $[M-H_2O]^+$ (19), 438 $[M-MeOH]^+$ (5), 420 $[M-H_2O-MeOH]^-$ (19), 405 $[M-H_2O-MeOH-Me]^+$ (7), 357 (15), 323 (10), 284.2389 $[C_{21}H_{32}]^+$ (ion **a**, 5), 269 [ion **b**] (9), 257 (7), 235 $[C_{15}H_{23}O_2]^+$ (ion **c**, 9) 234.1626 $[C_{15}H_{22}O_2]^+$ (ion **d**, 10), 221.1910 $[C_{15}H_{25}O]^+$ (ion **e**, 84), 217 (ions **f** and **g**, 36), 204 $[234-CH_2O]^+$ (ion **h**, 45), 203 (35), 190.1739 $[C_{14}H_{22}]^+$ (ion **i**, 69), 189.1646 $[C_{14}H_{21}]^+$ (ion **j**, 95) and 135 (100).

29-Nor-3α-*methoxyserrat*-14-*en*-21-*one* (**5**)

Needles, mp 213–216° (MeOH–$CHCl_3$), $[\alpha]_D$ − 42 (*c* 0.2). HREIMS: *m/z* 440.3651 ($C_{30}H_{48}O_2$ requires 440.3652); IR ν_{max} cm^{-1}: 2959, 2920, 2851, 1718 (C=O), 1662 (>C=C<), 1458, 1385 and 1363 (gem.-dimethyl), 1227, 1103, 997, 973, 843 and 798 (>C=C<H); ^1H and ^{13}C NMR: Table 1; EIMS *m/z* (rel. int.): 440 $[M]^+$ (15), 408.3381 $[M-MeOH]^+$ (13), 393 (6), 365 (5), 284 (ion **a**, 3), 269 (ion **b**, 13) 257 (2), 221.1896 $[C_{15}H_{25}O]^+$ (ion **e**, 100), 217.1952 $[C_{16}H_{25}]^+$ (ion **f**, 15), 204 $[C_{14}H_{20}O]^+$ (ion **c**, 53), 190.1727 $[C_{14}H_{22}]^+$ (ion **i**, 70), 189.1638 $[C_{14}H_{21}]^+$ (ion **j**, 36) and 135 (50).

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