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21α-HYDROXY-3β-METHOXYSERRAT-14-EN-30-AL AND OTHER TRITERPENOIDS FROM THE CUTICLE OF *PICEA JEZOENSIS*

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Key Word Index—*Picea jezoensis* Carr. *jezoensis*; Pinaceae; cuticle; serratene; 21α-hydroxy-3β-methoxyserrat-14-en-30-al.

Abstract—A new methoxytriterpene aldehyde was isolated from the cuticle of the stem bark of *Picea jezoensis* Carr. *jezoensis*, together with six known serratene triterpenoids, 3α-methoxyserrat-14-en-21β-ol, 3β-methoxyserrat-14-en-21β-ol, 29-nor-3β-methoxyserrat-14-en-21-one, 21-episerratenediol, serratenediol and 3β-methoxyserrat-14-en-21α,29-diol; the structure of the new compound was established to be 21α-hydroxy-3β-methoxyserrat-14-en-30-al on the basis of spectral evidence.

INTRODUCTION

Picea jezoensis (Sieb. et Zucc.) Carr. *jezoensis* (Japanese name: Ezomatsu; Pinaceae), which has a highly developed cuticle, is distributed widely in the southeast Eurasian continent and in Hokkaido in Japan [1], while *Picea jezoensis* (Sieb. et Zucc.) Carr. *hondoensis* (Mayer) Rehder (Japanese name: Touhi), which has a thinner cuticle, grows in the subalpine belts between Tohoku and Kinki districts in Japan. The latter tree is thought to be a variety of the former on the basis of comparative plant morphology [2, 3].

Previously, we have reported that the stem bark of *P. jezoensis* Carr. *hondoensis* contained three new triterpene constituents, 21β-methoxyserrat-14-en-3-one, 21α-methoxyserrat-13-en-3-one and 21β-hydroxyserrat-14-en-3-one, together with the known 3α-methoxyserrat-14-en-21-one, 3α-methoxyserrat-14-en-21β-ol, 3β-methoxyserrat-14-en-21β-ol, 3,21-diepiserratenediol and 21-episerratenediol [4, 5]. For the purpose of a chemotaxonomical study on these trees, we examined the methylene chloride extract of the cuticle of *P. jezoensis* Carr. *jezoensis* in detail and successively isolated seven serratene triterpenes, **1**–**7**, including a new serratenal (**3**).

RESULTS AND DISCUSSION

Three of six known serratene derivatives isolated from the cuticle were shown to be 3α-methoxyserrat-14-en-21β-ol (**1**), 3β-methoxyserrat-14-en-21β-ol (**2**) [5] and 21-episerratenediol (**4**) [6], respectively, by direct comparison with the corresponding authentic samples obtained from *P. jezoensis* Carr. *hondoensis*. The remaining three were assumed to be serratenediol (**5**) [7], 29-nor-3β-methoxyserrat-14-en-21-one (**6**) [8–10] and 3β-methoxyserrat-14-en-21α,29-diol (**7**) [11], respectively, since physical and spectral data of **5**, **6** and the diacetate of **7** were similar to those previously published. Compound **2** was the most abundant triterpene constituent in the cuticle. Compounds **6** and **7** had been isolated first from the bark of *Pinus monticola* Dougl. [8, 11], as well as **6** from the bark of *Pinus armandii* Franchet *mastersiana* Hayata [9]. Detailed assignments of ^{1}H and ^{13}C NMR signals for **6**, **7** and its diacetate (**7a**) are listed in Tables 1 and 2, as no accurate data have been published for these compounds.

Compound **3**, obtained as a minor constituent, was assigned the molecular formula $C_{31}H_{50}O_3$ from the HR-EI-mass spectrum. It gave a purple colour with the Liebermann–Burchard reagent. The IR, ^{1}H NMR and ^{13}C NMR spectra (Tables 1 and 2) showed the presence of six tertiary methyl groups, an aldehyde group [IR ν_{max} 1727 cm^{-1}; δ_H9.41 (1H, *s*), δ_C206.60 (*d*)], an axial methine proton geminal to a methoxy group [δ_H2.63 (1H, *dd*, $J = 12.2, 4.8$ Hz) and 3.36 (3H, *s*); δ_C57.5 (OMe) and 88.4 (C-3)], an axial methine proton geminal to a hydroxy group [IR ν_{max} 3418 cm^{-1}; δ_H3.81 (1H, *dd*, $J = 11.2, 4.2$ Hz, H-21β); δ_C72.4 (*d*)], a trisubstituted double bond [IR ν_{max} 1667, 860 and 795 cm^{-1}; δ_H5.26 (1H, *m*); δ_C121.0 (–CH =) and 138.8 (=C <)]. The DEPT spectrum revealed that **3** contained six angular methyls, ten methylenes, four methines, one methoxy group, two oxymethines, a trisubstituted ethylene bond, five quaternary carbons and one aldehyde group. The presence of an aldehyde group and the absence of an angular methyl group in comparison with the quaternary methyl groups of the

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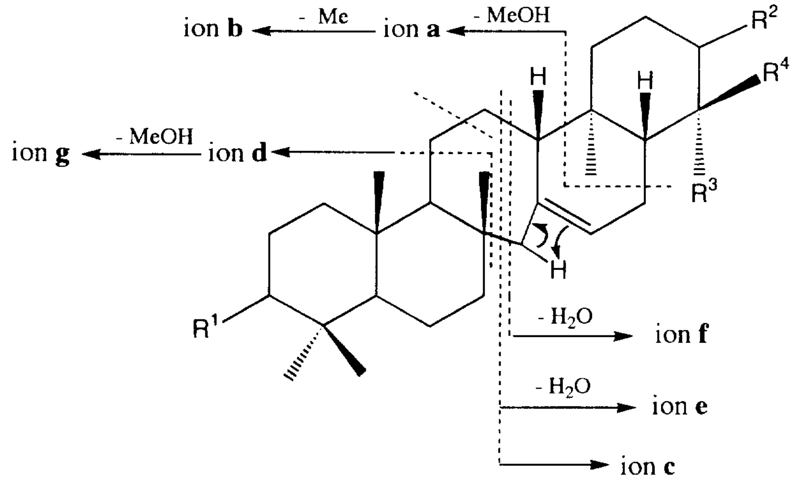

	R^1	R^2	R^3	R^4
1	α-OMe	β-OH	Me	Me
2	β-OMe	β-OH	Me	Me
3	β-OMe	β-OH	Me	CHO
4	β-OH	β-OH	Me	Me
5	β-OH	α-OH	Me	Me
6	β-OMe	: O	H	Me
7	β-OMe	α-OH	CH_2OH	Me
7a	β-OMe	α-OAc	CH_2OAc	Me

Table 1. ^1H NMR spectral data of compounds **3**, **6**, **7** and **7a** (in $CDCl_3$, TMS = 0, 500 MHz)*

H	**3**	**6**	**7**	**7a**
Me-23	0.95	0.96	0.95	0.95
Me-24	0.75	0.75	0.75	0.75
Me-25	0.80	0.80	0.80	0.80
Me-26	0.83	0.83	0.82	0.82
Me-28	0.73	0.90	0.62	0.70
Me-29	1.11	—	—	—
Me-30	—	0.98 *d* (6.6)	1.22	0.98
3	2.63 *dd* (12.2, 4.8)	2.63 *dd* (11.8, 4.4)	2.63 *dd* (12.2, 4.8)	2.62 *dd* (12.2, 4.8)
15	5.26 *m*	5.34 *m*	5.31 *m*	5.30 *m*
17	1.78 *m*	—	—	—
20	1.71 *m*	—	—	—
20	1.81 *m*	—		—
21	3.81 *dd* (11.2, 4.2)	—	3.45 *dd* (10.2, 6.1)	4.59 *dd* (10.2, 6.1)
29	—	2.49 *d* (5.5)	3.48 *d* (13.2) 4.25 *d* (13.2)	4.17 *d* (13.2) 4.46 *d* (13.2)
30	9.41	—	—	—
OMe	3.36	3.36	3.36	3.35
OAc	—	—	—	2.04
OAc	—	—	—	2.05

*Assignments were obtained by 2D ^1H–^1H COSY, 2D ^1H–^{13}C COSY and 2D long range ^1H–^{13}C COSY experiments.

usual serratenes [4, 5, 8] suggested **3** to be a novel serratene aldehyde. The chemical shift value of the axial hydroxymethine proton signal of **3** observed at δ3.81 (*dd*) was *ca* 0.6 ppm lower in comparison with that of serratenediol (**5**), indicating the proton to suffer from deshielding by the aldehyde carbonyl. In the HR-EI-mass spectrum, **3** showed fragment ion peaks characteristic for cleavage of serrat-14-enes [4, 5, 10] at *m/z* 284.2489 $[C_{21}H_{32}]^+$ (ion **a**), 269.2275 $[C_{20}H_{29}]^+$ (ion **b**), 234.1623 $[C_{15}H_{22}O_2]^+$ (ion **c**), 221.1903 $[C_{15}H_{25}O]^+$ (ion **d**), 217 $[C_{15}H_{21}O]^+$ (ion **e**), 203.1800 $[C_{14}H_{19}O]^+$ (ion **f**), and 189.1651 $[C_{14}H_{21}]^+$ (ion **g**), together with ions at *m/z* 452.3641 $[M - H_2O]^+$, 442.3755 $[M - CO]^+$, 424.3691 $[M - CO - H_2O]^+$, 423 $[M - Me - MeOH]^+$ and 420 $[M - H_2O - MeOH]^+$. All the above data suggest that **3** is probably a new serrat-14-ene derivative bearing an equatorial methoxyl group at C-3 and both the aldehyde and the equatorial secondary hydroxyl groups in the E-ring. This assumption was supported by analysing the 2D ^1H–^1H COSY, 2D ^1H–^{13}C COSY and 2D long-range ^1H–^{13}C COSY data. Figure 1 shows ^2J and ^3J C–H correlations obtained from the 2D long-range ^1H–^{13}C COSY experiment. Therefore, **3** was assumed to be either 21α-hydroxy-3β-methoxyserrat-14-en-29-al or -30-al. The structure was finally determined by the NOESY experiment of **3** (Fig. 2), in which characteristic cross-peaks were observed among the signals of H-17β (with H-21β and H-30), Me-28 (with H-20α and Me-29), Me-29 (with H-20α, Me-28 and H-30) and H-30 (with

Table 2. ^{13}C NMR spectral data of compounds **3**, **6**, **7** and **7a** (in $CDCl_3$, TMS = 0, 125 MHz)*

C	3	6	7	7a
1	38.50	39.00	38.49	38.48
2	22.36	22.33	22.36	22.35
3	88.43	88.43	88.45	88.45
4	38.89	38.88	38.89	38.89
5	56.26	56.26	56.25	56.28
6	18.75	18.75	18.77	18.77
7	45.13	45.16	45.14	45.15
8	37.14	37.09	37.11	37.14
9	62.78	62.71	62.81	62.82
10	38.20	38.18	38.20	38.21
11	25.31	25.68	25.46	25.45
12	27.24	27.28	27.22	27.18
13	56.68	54.41	57.09	56.95
14	138.83	138.62	138.25	138.22
15	120.96	121.58	121.80	121.64
16	25.31	29.50	23.90	24.22[a]
17	42.74	45.66	50.37	50.46
18	35.09	36.06	35.77	35.82
19	36.64	37.99	37.11	36.86
20	26.83	38.47	28.14	24.42[a]
21	72.40	213.43	81.14	80.25
22	55.04	49.33	42.26	40.99
23	28.19	28.13	28.14	28.12
24	16.18	16.19	16.19	16.19
25	15.69	15.68	15.70	15.71
26	19.79	19.78	19.81	19.81
27	55.91	56.03	55.96	55.94
28	17.72	11.04	14.18	13.74
29	8.09	—	63.92	63.98
30	206.60	11.47	21.80	21.71
OMe	57.51	57.49	57.51	57.49
OCOMe	—	—	—	21.12
OCOMe	—	—	—	21.24
OCOMe	—	—	—	170.62
OCOMe	—	—	—	170.95

*Assignments were obtained by 2D ^{1}H–^{1}H COSY, 2D ^{1}H–^{13}C COSY and 2D long range ^{1}H–^{13}C COSY experiments.

[a] Assignments may be interchangeable vertically.

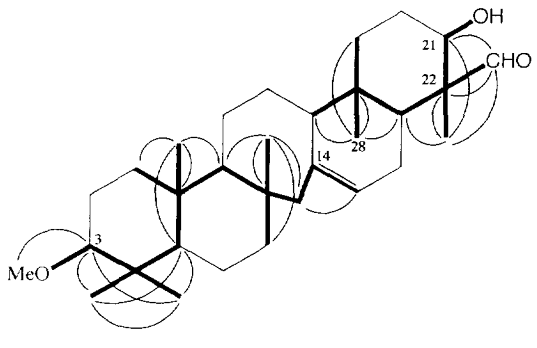

Fig. 1. 2D long range correlation of **3**.

H-17β, H-21β and Me-29). Consequently, the aldehyde group was located at C-30 and the structure of **3** was unequivocally established as 21α-hydroxy-3β-methoxy-serrat-14-en-30-al, which has not yet been described in the literature.

The systematic name used for **3** depended on the convincing numbering for the serratane skeleton presented by Ageta *et al.* [12], as the literature survey revealed that confusing numberings have been used for some natural serratenes oxygenated at either C-29 or C-30 [8, 9, 11].

EXPERIMENTAL

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

Plant material. The cuticle of *P. jezoensis* Carr. *jezoensis* was collected at *ca* 1000 m height in the mountains of Okujozankei district under the management of National Hokkaido Forestry Bureau, Sapporo City, Japan, in August 1992. The plant materials including the leaves, the cone and the tip of the twig were identified by Dr G.

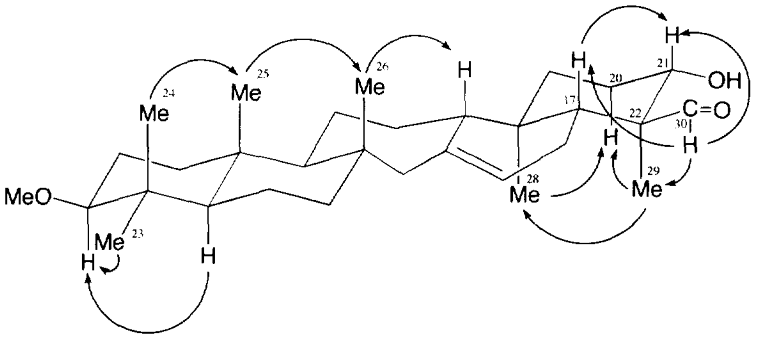

Fig. 2. NOESY experiment of **3**.

Murata, Department of Botany, Faculty of Science, Kyoto University, Kitashirakawa-oiwakecho, Sakyo-ku, Kyoto, Japan. Voucher specimens (PJJ-H-92-08) are deposited at the Herbarium of the Laboratory of Medicinal Chemistry, Osaka University of Pharmaceutical Sciences.

Extraction and isolation of compounds. The air-dried and chopped cuticle of *P. jezoensis* Carr. *jezoensis* (6.0 kg) was extracted with CH_2Cl_2 (10 l) employing an automatic glass percolator for 10 hr at 40°. The CH_2Cl_2 soln was then evapd *in vacuo* and the resulting dark green residue (365.1 g) was subjected to CC on silica gel (3.6 kg). Elution of the column with $CHCl_3$ successively afforded yellow residues A (53.8 g), B (54.6 g) and a crude crystalline mass (C), 9.57 g, from fr. nos 6–21, 38–56 and 87–107 (each fr.: 1 l). Elution was continued to give yellow residues D (12.11 g) and E (35.33 g), from the frs eluted with $CHCl_3$–EtOAc (10:1, fr. nos 108–151) and $CHCl_3$–EtOAc (5:1, fr. nos 152–178), respectively. Repeated CC of residue A over silica gel (800 g) afforded a crystalline solid (3.225 g) from fr. nos 10–18 eluted with $CHCl_3$ (each fr.: 100 ml), which was recrystallized to give the known 3α-methoxyserrat-14-en-21β-ol (**1**), 1.008 g, mp 277–279° (MeOH–$CHCl_3$), $[\alpha]_D^{23}$ − 57 (*c* 0.89) (lit. [3] mp 277–278.5°, $[\alpha]_D$ − 57). Repeated CC of residue B on alumina (1 kg) afforded a crystalline mass (5.265 g) from fr. nos 16–21 (each fr.: 100 ml) eluted with C_6H_6, which was recrystallized to give the known 3β-methoxyserrat-14-en-21β-ol (**2**), 2.925 g, mp 305–307° (MeOH–$CHCl_3$), $[\alpha]_D^{23}$ − 2 (*c* 0.55) (lit. [3] mp 305–307.5°, $[\alpha]_D^{23}$ − 2). Subsequent elution with C_6H_6–$CHCl_3$ (2:1) yielded a crystalline solid (0.136 g) from fr. nos 106–138 (each fr.: 100 ml), which was purified by prep. TLC (plate: 1 mm thick, 20 × 20 cm; solvent: $CHCl_3$–MeOH, 20:1) to give **3**, 8 mg. Rechromatography of residue C on alumina (500 g) furnished the known 21-episerratenediol (**4**), 0.385 g, mp 304–306.5° (MeOH–$CHCl_3$), $[\alpha]_D^{23}$ − 20 (*c* 0.55) (lit. [6] mp 303–308°, $[\alpha]_D$ − 19), and serratenediol (**5**), 0.441 g, mp 300–301° (MeOH–$CHCl_3$), $[\alpha]_D^{23}$ − 18 (*c* 0.21) (lit. [7] mp 302.5–304.5°, $[\alpha]_D$ − 19 (*c* 0.9)), from the fr. nos 42–53 and 64–68 (each fr.: 100 ml) eluted with C_6H_6–$CHCl_3$ (1:1), respectively. Repeated CC of residue D on silica gel (400 g) yielded a solid (65 mg) from the frs eluted with C_6H_6–$CHCl_3$ (1:1) (fr. nos 78–79, each fr.: 100 ml), which was recrystallized to give **6**, 0.025 g, mp 275–277° (MeOH–$CHCl_3$), $[\alpha]_D^{23}$ − 1 (*c* 0.22) (lit. [8] mp 277–278.5°, $[\alpha]_D^{22}$ − 1); IR ν_{max} cm^{-1}: 2852, 1711, 1665, 1186, 1132, 1112, 1009, 842 and 799; ^{1}H and ^{13}C NMR: see Tables 1 and 2. Physical and IR and EI-MS spectral data of **6** were in good agreement with those of the known 29-nor-3β-methoxyserrat-14-en-21-one. Residue E on CC over silica gel (800 g) afforded a crystalline solid, 0.112 g, from the frs eluted with $CHCl_3$–EtOAc (10:1) (fr. nos 40–49; each fr.: 100 ml), which was crystallized from MeOH–$CHCl_3$ to give **7** (21 mg).

Compound **3**. Prisms, mp 266.5–269° (MeOH–$CHCl_3$), $[\alpha]_D^{23}$ + 37 (*c* 0.10); HR-EI-MS: *m/z* 470.3755 $[M]^+$ ($C_{31}H_{50}O_3$ requires 470.3756); IR ν_{max} cm^{-1}: 3418 br (OH), 2963, 2932, 2869, 2847, 1727 (CHO), 1667 (C=C), 1458, 1385 and 1365 (*gem* dimethyl), 1245, 1184, 1132, 1107, 1090, 989 and 860 and 795 (H> C=C <); ^{1}H and ^{13}C NMR: see Tables 1 and 2; EI-MS: *m/z* (rel. int.) 470 $[M]^+$ (11), 452 $[M - H_2O]^+$ (26), 442 $[M - CO]^+$ (1), 424 $[M - CO - H_2O]^+$ (3), 423 $[M - Me - MeOH]^+$ (3), 420 $[M - H_2O - MeOH]^+$ (3), 405 $[420 - Me]^+$ (3), 357 (8), 323 (5), 284 [ion **a**] (3), 269 [ion **b**] (4), 234 [ion **c**] (10), 221 [ion **d**] (32), 217 [ion **e**] (12), 203 [ion **f**] (17), 201 (11), 189 [ion **g**] (31), 185 (13), 135 (42) and 98 (100).

3β-Methoxyserrat-14-en-21α,29-diol (**7**). Prisms, mp 262–264°, $[\alpha]_D^{23}$ + 42 (*c* 0.15); HR-EI-MS: *m/z* 472.7500 (Calc. for $C_{31}H_{52}O_3$: 472.7500); IR ν_{max} cm^{-1}: 3412 br (OH), 1645, 1459, 1106, 1055, 862 and 796; ^{1}H and ^{13}C NMR: see Tables 1 and 2; EI-MS *m/z* (rel. int.): 472 $[M]^+$ (1), 454 (74), 421 (96), 356 (32), 323 (14), 234 (24), 221 (61), 203 (54), 189 (73), 135 (97) and 95 (100). Acetylation of **7** (10 mg) with Ac_2O–pyridine (1:1, 4 ml) afforded a residue, which was recrystallized to give diacetate (**7a**), 11 mg, mp 256–259.5° (MeOH–$CHCl_3$), $[\alpha]_D^{23}$ + 6 (*c* 0.31) (lit. [11] mp 260–260.5°, $[\alpha]_D^{24}$ + 6.5); IR ν_{max} cm^{-1}: 1730, 1645, 1253, 1242, 858 and 790; ^{1}H and ^{13}C NMR: see Tables 1 and 2; EI-MS *m/z* 556 (1) $[M]^+$. Physical and spectral data of **7a** were almost identical to those already published [11].

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