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C_{31}-SECODAMMARANE-TYPE TRITERPENOID SAPONINS FROM THE MALE FLOWERS OF *ALNUS PENDULA*

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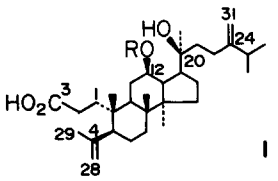
Key Word Index—*Alnus pendula*, Betulaceae, male flowers, alnustic acid, alnustic acid saponins

Abstract—Six C_{31}-secodammarane-type triterpenoid saponins, in addition to alnustic acid, were isolated from the male flowers of *Alnus pendula*. Two of these saponins were new and were shown to be the 12-*O*-(2′-*O*-acetyl)-β-D-xylopyranoside and the 12-*O*-(2′-*O*-acetyl)-β-D-glucopyranoside of alnustic acid, respectively, on the basis of their physico-chemical data.

INTRODUCTION

Previously, we investigated the chemical constituents of the male flowers of *Alnus pendula* Matsum. (Japanese name: Hime-yashabushi), and elucidated the structure of a new ketone, alnustone, identified fifteen aromatic compounds and fatty acids, and provided evidence for the presence of a triterpenic acid [1]. The same triterpenic acid has been isolated recently from the male flowers of *A. sieboldiana* and named alnustic acid, and its structure has been elucidated [2]. The further investigation on the chemical constituents of the male flowers of *A. pendula* resulted in the isolation of six triterpenoid saponins (**2**–**7**) in addition to alnustic acid (**1**). In this paper, we report the evidence which led to the establishment of the structures of the two new saponins, **3** and **4**, and the identification of saponins **2** and **5**–**7**.



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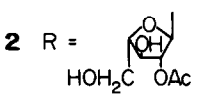

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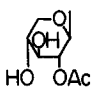

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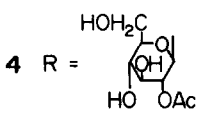

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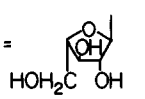

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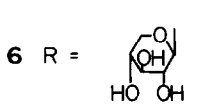

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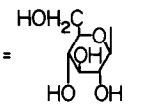

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RESULTS AND DISCUSSION

Six saponins **2**–**7** were isolated, in addition to alnustic acid **1**, by a combination of centrifugal and thin-layer chromatographic separations of an acetone extract of the male flowers. The saponins **2** and **5**–**7** were identified as the 12-*O*-(2′-*O*-acetyl)-α-L-arabinofuranoside (**2**), the 12-*O*-α-L-arabinofuranoside (**5**), the 12-*O*-β-D-xylopyranoside (**6**) and the 12-*O*-β-D-glucopyranoside (**7**) of alnustic acid, respectively; all of which have been isolated from the female flowers of *A. serrulatoides* [3]. The saponins are numbered in the order of increasing polarity on TLC of their methyl esters (**9**–**14**).

Saponin 3

The FDMS of the methyl ester (**11**) of saponin **3** exhibited a peak due to the $[M+H]^+$ ion at *m/z* 677, and this indicated that the MW of the methyl ester (**11**) was larger than that of the methyl ester (**12**) of saponin **6** by 42 amu. This difference was due to an acetyl group, which was confirmed by the ^1H and ^{13}C NMR signals at δ_H 2.06 (3H, *s*, –O–CO–Me) and δ_C 169.3 (*s*, –O–CO–Me) and 21.0 (*q*, –O–CO–Me), respectively. The methyl ester (**11**), on saponification followed by methylation with diazomethane, gave a deacetyl methyl ester (**15**) whose spectral data were identical with those of **12**. From these observations, **11** was presumed to be a monoacetate of **12**. This acetoxyl group was shown to be located at C-2′ of the xylopyranosyl moiety on the basis of the appearance of an ion peak at *m/z* 175 due to the [MeCO·xylopyranosyl]$^+$ ion in the FDMS of **11** and application of the acetylation shift rule [4–7] to the displacement of the ^{13}C NMR chemical shifts of C-1′ and C-3′ between **11** and **15**, as shown in Table 1. The β-glycosidic linkage of this sugar was indicated on the basis

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Table 1 ^{13}C NMR chemical shifts for compounds **11**, **13**, **15** and **16** (δ_C, in C_5D_5N)

Carbon	11	13	15	16
1	24.7	24.7	24.7	24.7
2	28.7	28.7	28.7	28.5
3	174.3	174.4	174.3	174.1
4	147.4	147.5	147.5	147.3
5	40 4	40.3	40.6	40.4
6	28 7	28.7	28.7	28.5
7	33 5	33.6	33 6	33.5
8	39 7	39 7	39.7	39.5
9	50.4	50.5	50 5	50.3
10	39 7	39.7	39 7	39.5
11	28.7	28.7	28.7	28.5
12	76.3	75.9	76 7	76.8
13	46.8	46.8	46.8	46.7
14	52 9	52.8	52.9	52 5
15	31.4	31.3	31.7	31.4
16	27 1	27.0	27 3	26.7
17	53 9	53.9	53.8	53.7
18	15.6	15.6	15.3	15.4
19	20 3	20.2	20.1	20.0
20	72.7	73.0	72 7	72.9
21	27.1	27.0	27.3	26.7
22	34.4*	34.3*	34.4*	34.1*
23	35.8*	35.7*	35.1*	35.0*
24	157.6	157.4	157.5	157 1
25	34.4	34.3	34.4	34 1
26	22.2	22.2	22.1	22.0
27	22.2	22 2	22.1	22 0
28	114.0	114.0	114.0	114.0
29	23.2	23.3	23.3	23.3
30	17.2	17.0	17.3	17 1
31	106.2	106.4	106.1	106 3
–OMe	51.4	51 9	51.4	51.5
–COMe	169.3	169.4		
–COMe	21.0	21.1		
Sugar moiety				
1′	98.4	97.8	100.9	99.9
2′	74.7	74.9	74.9	74.7
3′	77.3	77.7	78.7	78.0
4′	70.9	71.3	70.7	70.7
5′	67.3	78 7	67 4	78 0
6′		62 3		62 2

*Values in any vertical column may be interchanged although those given here are preferred.

of the coupling constant ($J = 7$ Hz) for the anomeric proton and the application of Klyne's rule [8] to the $[M]_D$ value calculated for the sugar moiety of **15** (Table 2) by use of the $[M]_D$ values of the compounds listed in Table 3. Saponin **3** was consequently defined as (12*R*, 20*S*)-12-*O*-(2′-*O*-acetyl-β-D-xylopyranosyl)-20-hydroxy-24-methylene-3,4-secodammar-4(28)-en-3-oic acid.

Saponin **4**

The IR and ^1H NMR spectra of the methyl ester (**13**) of saponin **4** were similar to those of **11**. The FDMS of **13** exhibited peaks at *m/z* 707 and 205, which were assigned to an $[M+H]^+$ ion and a fragment ion originating from a monoacetylated hexose moiety, respectively. The similar treatment of **13** as **11** gave a deacetyl methyl ester (**16**), whose physical data and spectra were identical with those of the methyl ester (**14**) of saponin **7**. By considering the ^{13}C NMR chemical shifts of **13** and **16** (Table 1) and the molecular rotation evaluated for the sugar moiety of **16** (Table 2), it was apparent that the methyl ester **13** was the 12-*O*-β-D-(2′-*O*-acetyl)-glucopyranoside of methyl alnustate **8**. Thus, the structure of saponin **4** was established as (12*R*, 20*S*)-12-*O*-(2′-*O*-acetyl-β-D-glucopyranosyl)-20-hydroxy-24-methylene-3,4-secodammar-4(28)-en-3-oic acid.

EXPERIMENTAL

NMR: 90 MHz for ^1H and 22.6 MHz for ^{13}C with TMS as int. standard, EIMS: direct inlet, 70 eV; FDMS of the methyl esters **11** and **13**: JEOL JMS-D 300 mass spectrometer equipped with a carbon emitter; the emitter current was 10–24 mA, Analytical TLC (0.25 mm) and prep TLC (0.75 mm) silica gel (Merck 60 GF_{254}) Compounds were visualized as coloured spots by spraying with vanillin–H_2SO_4 (1 134 w/w) and then by heating

Table 3 $[M]_D$ of methyl alnustate, xyloside and glucoside used for the calculations

Compound	$[M]_D$	References
Methyl alnustate	+214.4°	[2]
Methyl β-D-xylopyranoside	−107°	[9]
Methyl α-D-xylopyranoside	+249°	[9]
Methyl β-D-glucopyranoside	−62°	[10, 11]
Methyl α-D-glucopyranoside	+305°	[10, 11]

Table 2. $[M]_D$ of the deacetyl methyl esters **15** and **16** and determination of the C-1 configuration of the sugars

Compound	Obs. $[M]_D$	Calc. $[M]_D$	$[M]_D$ evald. for the sugars	Configuration at C-1 of the sugars
15	+33 9°	+107.4°		
Xylose in **15**			−180 5°	β
16	−25.7°	+152.4°		
Glucose in **16**			−240.1°	β

on a hot plate or by spraying with 0.3% *p*-bromocresol green soln in H_2O–MeOH (1:4) adjusted to pH 8.0.

Extraction and isolation Male flowers (8.3 kg) of *A. pendula*, naturally grown on a hill in the suburbs of Hiroshima City, were collected just before flowering in March. The flowers, after they had been minced mechanically, were extracted with Me_2CO at room temp for 2 months. The Me_2CO soln, after concn *in vacuo*, was extracted with Et_2O. Removal of the solvent from the extract gave a brown, viscous oil (185 g). A portion (21.0 g) of this oil was subjected to centrifugal chromatography on silica gel [Merck 60; 5 mm × 30 cm (diam)] with gradient elution using C_6H_6–MeOH as eluant(MeOH increasing from 0 to 10%) and separated into four fractions: (i) alnustic acid (**1**, 0.7 g), (ii) saponin **2** (1.0 g), (iii) a mixture of saponins **3** and **4** (1.2 g) and (iv) a mixture of saponins **5**–**7** (0.7 g). Each of these fractions gave a spot with R_f 0.28, 0.20, 0.12 and 0.07, respectively, on analytical TLC with C_6H_6–MeOH (9:1)

Fraction (iii) was methylated with CH_2N_2 and subjected to prep. TLC (Me_2CO–hexane, 2:3) to give the methyl ester (**11**) (0.044 g, R_f 0.36, an amorphous solid) of saponin **3** and the methyl ester (**13**) (0.063 g, R_f 0.16, an amorphous solid) of saponin **4**. **11** exhibited the following data: $[\alpha]_D^{25}$ +9.1° ± 1.0° ($CHCl_3$, *c* 0.42), IR ν_{max}^{Nujol} cm^{-1}: 3380 (OH), 3075, 1635 and 888 (C=CH_2) and 1738 (C=O); ^1H NMR (C_5D_5N): δ0.86–1.30 (18H, Me × 6), 1.72 (3H, *s*, >C=C(Me)–), 2.06 (3H, *s*, –O–CO–Me), 3.63 (3H, *s*, –CO–O–Me), 4.74–5.01 (4H, *br*, >C=CH_2 × 2) and 5.32 (1H, *d*, *J* = 7 Hz, anomeric H), ^{13}C NMR (C_5D_5N): see Table 1; EIMS *m/z* (rel. int.): 658 $[M-H_2O]^+$ (1), 405 (20), 387 (25), 175 (100) and 43 (98); FDMS *m/z* (rel. int.): 677 $[M+H]^+$ (100), 580 (81) and 175 (95). Compound **13** exhibited the following data. $[\alpha]_D^{25}$ +1.4° ± 0.9° ($CHCl_3$, *c* 0.43); IR ν_{max}^{Nujol} cm^{-1}: 3380 (OH), 3075, 1636 and 888 (C=CH_2) and 1734 (C=O); ^1H NMR (C_5D_5N): δ0.83–1.31 (18H, Me × 6), 1.73 (3H, *s*, >C=C(Me)–), 2.09 (3H, *s*, –O–CO–Me), 3.71 (3H, *s*, –CO–O–Me), 4.78–5.03 (4H, *br*, >C=CH_2 × 2) and 5.40 (1H, *d*, *J* = 7 Hz, anomeric H); ^{13}C NMR (C_5D_5N): see Table 1; EIMS *m/z* (rel. int.): 688 $[M-H_2O]^+$ (3), 405 (5), 387 (9) and 43 (100); FDMS *m/z* (rel. int.): 707 $[M+H]^+$ (100), 610 (59) and 205 (44).

Fraction (iv), after methylation with CH_2N_2, was separated on prep. TLC (Me_2CO–hexane, 2:3) to give the methyl ester (**10**) (0.227 g) of saponin **5**, the methyl ester (**12**) (0.061 g) of saponin **6** and the methyl ester (**14**) (0.163 g) of saponin **7**.

Saponification of the methyl esters **11** *and* **13**. The methyl esters **11** and **13** were saponified with 5% NaOH–MeOH to give deacetyl derivatives, which were then converted to their methyl esters **15** and **16**, respectively. The physical and spectral data of **15** and **16** were identical with those of the methyl esters of saponin **6** and **7** [3], respectively.

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