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TRITERPENOIDS FROM THE FEMALE AND MALE FLOWERS OF *ALNUS SIEBOLDIANA*

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Key Word Index—*Alnus sieboldiana*; Betulaceae; female flowers; male flowers; C_{31}-secodammarane-type triterpenoids.

Abstract—The major triterpenoids in both the female and male flowers of *Alnus sieboldiana* are a C_{31}-secodammarane-type, alnustic acid, and its 12-*O*-α-L-arbinofuranoside and 12-*O*-β-D-xylopyranoside.

Previous studies on the chemical constituents of *Alnus sieboldiana* Matsum. (Japanese name: Ohba-yashabushi) have shown the presence of flavonoids in the pollen [1, 2] and hydrocarbons [3], flavonoids and stilbenes [4], diarylheptanoids [5, 6] and a C_{31}-secodammarane-type triterpenoid, such as alnustic acid [7], in the male flowers. Alnustic acid and its monoglycosides have been also isolated from the female flowers of *A. serrulatoides* Call. [8] and the male flowers of *A. pendula* Matsum. [9].

We examined the flowers of *A. sieboldiana*, and found the presence of alnuserrudiolone (**1**) and alnustic acid (**2**) and its 12-*O*-α-L-arabinofuranoside (**3**), 12-*O*-β-D-xylopyranoside (**4**) and 12-*O*-β-D-glucopyranoside (**5**) in the female flowers and the presence of the glycosides **3** and **4**, in addition to alnustic acid (**2**), in the male flowers. Table 1 shows the relative quantities, the triterpenoids being numbered in the order of increasing polarity on TLC.

Alnustic acid (**2**) and its 12-*O*-α-L-arabinofuranoside (**3**) and 12-*O*-β-D-xylopyranoside (**4**) predominate in the female and male flowers (Table 1). Alnuserrudiolone (**1**) [10], a C_{31}-dammarane-type triterpenoid, and alnustic acid 12-*O*-β-D-glucopyranoside (**5**) were present in only the female but not the male flowers. Compounds **2**–**5** have been also found to be present in the female flowers of *A. serrulatoides* [8] and the male flowers of *A. pendula* [9]. Although various triterpenoids had been isolated from the leaves, barks, cortices and twigs of many species of Betulaceae [11–14], no C_{31}-secodammarane-type has yet been isolated. Thus, the present findings and our previous results [8, 9] indicate that the C_{31}-secodammarane-type triterpenoid is a characteristic compound in the flowers of this genus. Investigations on triterpenoids in the flowers of other *Alnus* species are now in progress.

EXPERIMENTAL

All the equipment used has been described previously [9]. Analytical TLC was performed on silica gel (Merck No. 5715).

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Table 1. Triterpenoids in the female and male flowers of *A. sieboldiana*

Compounds*	Relative amount†		R_f‡		
	Female	Male	(a)	(b)	(c)
Alnuserrudiolone (**1**)	+	—	0.19	0.81	
Alnustic acid (**2**)	+++	+++	0.03	0.59	0.91
Al 12-*O*-α-L-arabinofuranoside (**3**)	++++	++++		0.29	0.74
Al 12-*O*-β-D-xylopyranoside (**4**)	+++	+++		0.19	0.68
Al 12-*O*-β-D-glucopyranoside (**5**)	++	—			0.59

*Al = Alnustic acid.

†Estimated by visual examination on TLC plates.

‡Solvent systems: (a) EtOAc–hexane (2:3); (b) $CHCl_3$–MeOH (9:1) and (c) $CHCl_3$–MeOH–H_2O (40:10:1).

Compounds were visualized as coloured spots by spraying with vanillin–H_2SO_4 (1:134, w/w) and then by heating on a hot-plate and/or by spraying with 0.3% *p*-Bromocresol Green soln in H_2O–MeOH (1:4) adjusted to pH 8.0.

Extraction and isolation. The female flowers (483 g) of *A. sieboldiana*, growing wild on a mountain in the suburbs of Hiroshima City, were collected in the middle of March (*ca* 1 week after the flowering of the male flowers). The female flowers, after they had been minced mechanically, were immersed in Me_2CO at room temp for 10 months. Removal of the solvent from the Me_2CO soln gave a brown, viscous oily substance (20.0 g). This oily substance was subjected to centrifugal chromatography on silica gel [Merck 60; 5 mm × 30 cm (diam)] by means of gradient elution with $CHCl_3$–MeOH as eluant (MeOH increasing from 0 to 100%), followed by purification with prep. TLC to give compounds **1**–**5**. The male flowers (5.0 kg) were also collected just after flowering from the same trees and extracted as described for the case of the female flowers to give an Me_2CO extract (130 g), which showed the presence of three triterpenoids **2**–**4** on TLC.

Identification of compounds. The triterpenoids isolated from the female flowers were alnuserrudiolone (**1**, 5 mg, an amorphous solid) and alnustic acid (**2**, 96 mg, mp 154–156°) and its 12-*O*-α-L-arabinofuranoside (**3**, 144 mg, an amorphous solid), 12-*O*-β-D-xylopyranoside (**4**, 101 mg, an amorphous solid) and 12-*O*-β-D-glucopyranoside (**5**, 45 mg, an amorphous solid). All these compounds showed identical behaviour on TLC, physicochemical data and spectra as those of known samples. Compounds **2**–**4** in the male flowers were identified by co-TLC with those isolated from the female flowers.

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