TRITERPENOIDS FROM THE FEMALE AND MALE FLOWERS OF ALNUS SIEBOLDIANA

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Key Word Index—Alnus sieboldiana; Betulaceae; female flowers; male flowers; C₃₁-secodammarane-type triterpenoids.

Abstract—The major triterpenoids in both the female and male flowers of Alnus sieboldiana are a C₃₁-secodammarane-type, alnustic acid, and its 12-0-L-arabinofuranoside and 12-0-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 C₃₆-secodammarane-type triterpenoid, such as alnustic acid [7], in the male flowers. Alnustic acid and its monoglycosides have also been isolated from the female flowers of A. serrulataoides Call. [8] and the male flowers of A. pendula Matsum. [9].

We examined the flowers of A. sieboldiana, and found the presence of alnusserrudiolone (1) and alnustic acid (2) and its 12-0-L-arabinofuranoside (3), 12-0-D-xylopyranoside (4) and 12-0-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 12-0-L-arabinofuranoside (3) and 12-0-D-xylopyranoside (4) predominate in the female and male flowers (Table 1). Alnusserrudiolone (1) [10], a C₃₆-dammarane-type triterpenoid, and alnustic acid 12-0-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. serrulataoides [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 C₃₁-secodammarane-type has yet been isolated. Thus, the present findings and our previous results [8, 9] indicate that the C₃₁-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).

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

<table>
<thead>
<tr>
<th>Compounds*</th>
<th>Relative amount†</th>
<th>Rf‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Alnusserrudiolone (1)</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>Alnustic acid (2)</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>Al 12-0-L-arabinofuranoside (3)</td>
<td>+ + + +</td>
<td>+ + + +</td>
</tr>
<tr>
<td>Al 12-0-D-xylopyranoside (4)</td>
<td>+ + + +</td>
<td>+ + + +</td>
</tr>
<tr>
<td>Al 12-0-D-glucopyranoside (5)</td>
<td>+ +</td>
<td>—</td>
</tr>
</tbody>
</table>

*Al = Alnustic acid.
†Estimated by visual examination on TLC plates.
‡Solvent systems: (a) EtOAc–hexane (2:3); (b) CHCl₃–MeOH (9:1) and (c) CHCl₃–MeOH–H₂O (40:10:1).

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Compounds were visualized as coloured spots by spraying with vanillin-H₂SO₄ (1:134, w/w) and then by heating on a hot-plate and/or by spraying with 0.3% p-Bromocresol Green soln in H₂O-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 Me₂CO at room temp for 10 months. Removal of the solvent from the Me₂CO 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 x 30 cm (diam)] by means of gradient elution with CHCl₃-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 Me₂CO 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 alnusradiolone (1, 1.5 mg, an amorphous solid) and alnustic acid (2, 96 mg, up 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.

**REFERENCES**