Chemical Evaluation of *Betula* Species in Japan. VI.[1] Constituents of *Betula schmidtii*

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The constituents of *Betula schmidtii* REGAL were identified as follows. Fresh leaves: methyl (12*R*,20*S*)-20-hydroxy-12-β-D-xylopyranosyloxy-3,4-secodammara-4(28),24-dien-3-oate (betula-schmidtoside A)*, myricetin 3-*O*-α-L-arabinofuranoside, myricetin 3-*O*-α-L-rhamnopyranoside, myricetin 3*O*-β-D-galactopyranoside, (−)-rhododendrol 4′-*O*-β-D-glucopyranoside*. Outer bark: betulin, lupeol, oleanolic acid, betulone, betulonic acid. Inner bark: (+)-catechin, (3*R*)-3,5′-dihydroxy-4′-methoxy-3′,4″-oxo-1,7-diphenyl-1-heptene, (−)-lyoniresinol 3α-*O*-β-D-xylopyranoside, (+)-lyoniresinol 3α-*O*-β-D-glucopyranoside, monogynol A, ssioriside, (−)-rhododendrol 4′-*O*-β-D-glucopyranoside*, (−)-rhododendrol 4′-*O*-α-L-arabinofuranosyl-(1→6)-β-D-glucopyranoside*. Root bark: oleanolic acid 3-caffeate, 27-hydroxyoleanolic acid 27-caffeate. The three compounds with asterisks are new.

Key words *Betula schmidtii*; secodammarane; phenylbutanoid; lignan; diarylheptanoid; flavonoid

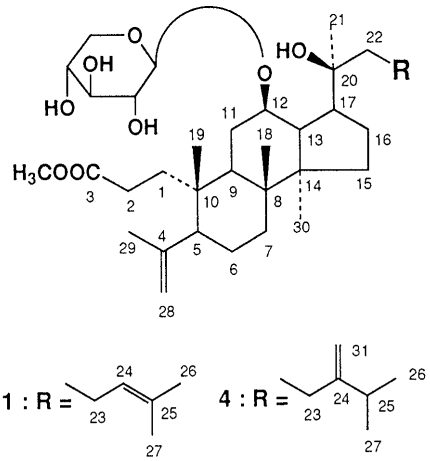
Among eleven species of *Betula* in Japan, *B. ovarifolia*,[1] *B. platyphylla* var. *japonica*,[2] *B. ermanii*,[3] *B. maximowicziana*[4] and *B. davurica*[5] have undergone investigation of their constituents. As the sixth subject of the series, we investigated *B. schmidtii* REGAL (Japanese name: Ono-ore-kanba), which is found in the eastern side of Honshu. The appearance of the tree is different from those of white birches such as *B. platyphylla* var. *japonica* and *B. ermanii*, having dark-brown outer bark which peels away in large thick sections. In this paper, we describe the chemical profile of *B. schmidtii* including the structural elucidation of three new compounds.

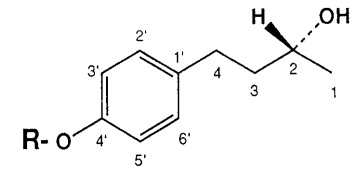
Constituents of Fresh Leaves From the fresh leaves collected in August, two new compounds, **1** and **2**, were isolated together with myricetin 3-*O*-α-L-arabinofuranoside, myricetin 3-*O*-α-L-rhamnopyranoside and myricetin 3-*O*-β-D-galactopyranoside.

1, named betula-schmidtoside A, was formulated as $C_{36}H_{60}O_8$ by high-resolution (HR) FAB-MS. The ^{13}C- and ^1H-NMR spectra suggested **1** to be a pentoside of a triterpene having a methyl ester, a secondary and a tertiary hydroxyl, a terminal methylene, tri-substituted olefine and seven methyls. On acid hydrolysis, **1** gave D-xylose, but no aglycone because this was acid-labile. Enzymatic hydrolysis of **1** with D-xylosidase, however, did not proceed probably due to steric hindrance. The structure of **1** was elucidated by two-dimensional shift correlation spectroscopy (2D-COSY). As shown in Fig. 1, the ^1H–^1H COSY and long-range ^{13}C–^1H COSY indicated a 3,4-secodammarane skeleton with a carbomethoxy, 12-β-D-xylopyranosyloxy, 20-hydroxy and 4(28), 24-diene. The structure resembled the methyl ester of alnustic acid 12-*O*-β-D-xylopyranoside (**4**) which had been isolated from the female flowers of *Alnus serrulatoides*.[6] The ^{13}C-NMR data of **1** were in good agreement with those of **4**, except for the signals C-23—C-27, Table 1. Confirming the stereochemistry by nuclear Overhauser effect correlation spectroscopy (NOESY), the structure of **1** was determined as methyl (12*R*,20*S*)-20-hydroxy-12-β-D-xylopyranosyloxy-3,4-secodammara-4(28),24-dien-3-oate. It may be an artifact derived from its acid form during extraction, although we could not detect the corresponding compound in the extract.

This is the second example of a secodammarane from *Betula*.[1] *Betula* and *Alnus* have been grouped as a subfamily Betuloideae, while other genera of Beturaceae are classed as Coryloideae.[7] The presence of secodammaranes in both genera supports their close relationship in Betulaceae.[6]

2 was formulated as $C_{16}H_{24}O_7$ by HR-FAB-MS. The ^1H- and ^{13}C-NMR spectra showed the presence of a *p*-substituted benzene, a methyl, two methylene, an oxymethine and a hexosyl group in the molecule. On acid hydrolysis, **2** gave (−)-rhododendrol (**2a**)[8] and D-glucose. On comparing the ^{13}C-NMR data of **2a** with those of **2**,





2 : R = β-D-Glc(*p*)
3 : R = α-L-Ara(*f*)-(1→6)-β-D-Glc(*p*)

Chart 1

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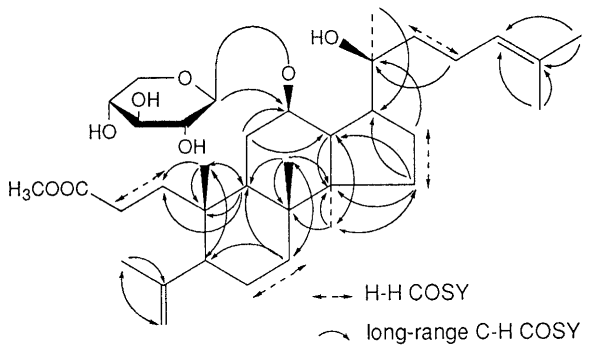

Fig. 1. ^{1}H–^{1}H COSY and Long-Range ^{13}C–^{1}H COSY Connections for **1**

Table 1. ^{13}C-NMR Data in C_5D_5N

C	1	4
1	24.7	24.7
2	28.6	28.7
3	174.3	174.1
4	147.5	147.3
5	40.5	40.5
6	28.7	28.7
7	33.5	33.5
8	39.7	39.6
9	50.3	50.4
10	39.7	39.6
11	28.2	28.2
12	76.5	76.4
13	46.8	46.8
14	52.8	52.8
15	31.5	31.6
16	27.2	27.2
17	53.9	53.5
18	15.5	15.4
19	20.2	20.2
20	72.7	72.6
21	27.0	27.2
22	34.4	34.3
23	23.0	35.1
24	126.6	157.3
25	130.5	34.3
26	17.7	22.0
27	25.8	22.0
28	114.0	114.0
29	23.3	23.3
30	17.3	17.4
31	—	106.2
MeO	51.5	51.4
Xyl-1	100.6	100.5
Xyl-2	75.0	74.7
Xyl-3	78.8	78.6
Xyl-4	70.7	70.5
Xyl-5	67.4	67.2

differences in the chemical shifts were observed around C-4′ (see Experimental), indicating the position of glucosylation to be C-4′. Thus, the structure of **2** was determined to be rhododendrol 4′-*O*-*β*-D-glucopyranoside.

Constituents of Outer Bark From the air-dried outer bark collected in August, five known triterpenes, betulin,[3] lupeol,[3] oleanolic acid,[3] betulone[3] and betulonic acid,[9] were isolated. Their structures were determined by direct comparison with authentic samples. The yield of betulin, which was over 10% for *B. platyphylla* var. *japonica* and about 5% for *B. ermanii*, was only 0.2%.

Constituents of Inner Bark From the air-dried inner bark collected in August, rhododendrol 4′-*O*-*β*-D-glucopyranoside (**2**) and another new compound, **3**, were isolated together with six known compounds, (+)-catechin,[3] (3*R*)-3,5′-dihydroxy-4′-methoxy-3′,4″-oxo-1,7-diphenyl-1-heptene,[2] (−)-lyoniresinol 3*α*-*O*-*β*-D-xylopyranoside,[3] (+)-lyoniresinol 3*α*-*O*-*β*-D-glucopyranoside,[10] monogynol A[3] and ssioriside.[11]

3 was formulated as $C_{21}H_{32}O_{11}$ by HR-FAB-MS. The ^{1}H- and ^{13}C-NMR data were similar to those of **2** except for the additional signals of an arabinofuranosyl and differences in the chemical shifts around C-6 of D-glucosyl which were due to glycosylation shifts.[12] On acid hydrolysis, **3** gave (−)-rhododendrol, D-glucose and L-arabinose. Thus, the structure of **3** was determined to be (−)-rhododendrol 4′-*O*-*α*-L-arabinofuranosyl-(1→6)-*β*-D-glucopyranoside.

Constituents of Root Bark From the air-dried root bark collected in August, oleanolic acid 3-caffeate[3] and 27-hydroxyoleanolic acid 27-caffeate[5] were isolated.

Experimental

The instruments, materials and experimental conditions were the same as described in Part I of this series.[3]

Isolation *B. schmidtii* samples were collected at Kawaba-mura, Gunma Prefecture, in August.

Fresh Leaves: Fresh leaves (2 kg) were extracted with MeOH (20 l) at room temperature for 2 weeks. The extract and 10 l MeOH were passed over activated charcoal (130 g) to give frac. M. The column was further eluted with 30% $CHCl_3$/MeOH (10 l) to give frac. C—M. Each fraction was concentrated to a syrup under reduced pressure. The syrup of frac. M was partitioned with $CHCl_3$–MeOH–H_2O (4:4:3) and the upper layer was evaporated. The residue was chromatographed on Sephadex LH-20 by using 90% MeOH. The fractions containing flavonoids were collected and rechromatographed on Chromatorex ODS by using 60% MeOH, and then refined by HPLC (Shim-pack ODS, 80% MeOH) to obtain myricetin 3-*O*-*α*-L-arabinofuranoside (610 mg) and myricetin 3-*O*-*α*-L-rhamnopyranoside (18 mg). The fractions containing **1** and **2** were collected and chromatographed on silica gel by using $CHCl_3$–MeOH and on Sephadex LH-20 by using MeOH to obtain **1** (62 mg) and **2** (570 mg). The syrup of frac. C—M was partitioned with $CHCl_3$–MeOH–H_2O (4:4:3) and the upper layer was evaporated. The residue was chromatographed on Sephadex LH-20 by using MeOH and on Chromatorex ODS by using MeOH to obtain myricetin 3-*O*-*β*-D-galactopyranoside (112 mg).

Outer Bark[13]: Air-dried outer bark (380 g) was extracted with $CHCl_3$ (2 l) under reflux for 4 h. The extract was concentrated to a syrup under reduced pressure. The syrup was chromatographed on silica gel by using $CHCl_3$–MeOH and *n*-hexane–EtOAc to obtain betulin (510 mg), lupeol (483 mg), oleanolic acid (19 mg), betulone (9 mg) and betulonic acid (51 mg).

Inner Bark[13]: Air-dried inner bark (1.1 kg) was extracted with MeOH (4 l) under reflux for 4 h. The extract was concentrated to a syrup under reduced pressure. The syrup was chromatographed on silica gel by using $CHCl_3$–MeOH. The fractions containing triterpenes were collected and subjected to rotation locular counter-current chromatography (RLCC) using *n*-hexane–MeOH–H_2O (10:9:1) to obtain monogynol A (80 mg) and (3*R*)-3,5′-dihydroxy-4′-methoxy-3′,4″-oxo-1,7-diphenyl-1-heptene (55 mg). The fractions containing phenolic glycosides were collected and chromatographed on Sephadex LH-20 by using 80% MeOH and on Chromatorex ODS by using 90% MeOH to obtain (+)-catechin (1.8 g), (−)-lyoniresinol 3*α*-*O*-*β*-D-xylopyranoside (210 mg), (+)-lyoniresinol 3*α*-*O*-*β*-D-glucopyranoside (206 mg), ssioriside (173 mg), **2** (1.1 g) and **3** (188 mg).

Root Bark: Air-dried root bark (250 g) was extracted with MeOH (2 l) under reflux for 4 h. The extract was concentrated and partitioned with $CHCl_3$–MeOH–H_2O (4:4:3). The lower layer was evaporated and chromatographed on silica gel by using $CHCl_3$–EtOAc to obtain

oleanolic acid caffeate (280 mg) and 27-hydroxyoleanolic acid 27-caffeate (142 mg).

Methyl (12*R*,20*S*)-20-Hydroxy-12-β-D-xylopyranosyloxy-3,4-seco-dammara-4(28),24-dien-3-oate (Betula-schmidtoside A, 2) A colorless amorphous powder, $[\alpha]_D$ +1.2° (c=0.5, MeOH). ^{1}H-NMR (C_5D_5N) δ: 0.74, 0.87, 0.94, 1.33, 1.71 (each 3H s), 1.64 (6H, s), 3.78 (1H, t, J=11.0 Hz), 4.30 (1H, dd, J=11.0, 4.9 Hz), 4.76 (1H, s), 4.92 (1H, s), 5.18 (1H, d, J=7.6 Hz), 5.33 (1H, t, J=7.0 Hz). HR-FAB-MS (negative mode) m/z : 619.420 $[M-H]^-$. Calcd for $C_{36}H_{59}O_8$: 619.421.

Acid Hydrolysis of 1 A mixture of **1** (15 mg) and 3% HCl (5 ml) was refluxed for 1 h. The mixture was extracted with EtOAc and the water layer was evaporated. The residue was chromatographed on silica gel by using 10% MeOH in $CHCl_3$ to obtain D-xylose (3.1 mg), $[\alpha]_D$ +16° (c=0.3, H_2O). Its trimethylsilyl ether was identified by comparison with an authentic sample on GLC. Decomposition of the aglycone was checked by TLC of the EtOAc extract.

(−)-Rhododendrol 4′-*O*-β-D-Glucopyranoside (2) A colorless amorphous powder, $[\alpha]_D$ −52° (c=1.0, MeOH). UV (MeOH) λ_{max} nm (log ε): 220 (4.39), 275 (3.62). ^{1}H-NMR (CD_3OD) δ: 1.34 (3H, d, J=5.9 Hz), 1.70—2.00 (2H), 2.65—3.00 (2H), 3.84—4.10 (2H), 5.55 (1H, d, J=7.3 Hz), 7.19 (2H, d, J=8.6 Hz), 7.30 (2H, d, J=8.6 Hz). ^{13}C-NMR (CD_3OD) δ: 23.5 (C-1), 67.8 (C-2), 42.2 (C-3), 32.2 (C-4), 137.6 (C-1′), 130.2 (C-2′, 6′), 117.8 (C-3′, 5′), 157.2 (C-4′), 102.5 (Glc-1), 74.9 (Glc-2), 78.0 (Glc-3), 71.4 (Glc-4), 77.9 (Glc-5), 62.5 (Glc-6). HR-FAB-MS (negative mode) m/z: 327.145 $[M-H]^-$. Calcd for $C_{16}H_{23}O_7$: 327.145.

Acid Hydrolysis of 2 A mixture of **2** (40 mg) and 3% HCl (7 ml) was refluxed for 30 min and extracted with EtOAc. The extract was washed with water, dried over Na_2SO_4 and evaporated. The residue was chromatographed on silica gel by using $CHCl_3$–MeOH to obtain (−)-rhododendrol (13 mg), $[\alpha]_D$ −11° (c=1.0, MeOH). ^{13}C-NMR (CD_3OD) δ: 23.5 (C-1), 67.9 (C-2), 42.4 (C-3), 32.2 (C-4), 134.4 (C-1′), 130.2 (C-2′, 6′), 116.1 (C-3′, 5′), 156.3 (C-4′). Its spectral data were identical with those of an authentic sample. The water layer was evaporated and the residue was chromatographed on silica gel by using 10% MeOH in $CHCl_3$ to obtain D-glucose (7 mg), $[\alpha]_D$ +53° (c=0.7, H_2O). Its trimethylsilyl ether was identified by comparison with an authentic sample on GLC.

(−)-Rhododendrol 4′-*O*-α-L-Arabinofuranosyl-(1→6)-β-D-glucopyranoside (3) A colorless amorphous powder, $[\alpha]_D$ −83° (c=1.0, MeOH). UV (MeOH) λ_{max} nm (log ε): 220 (4.30), 273 (3.28). ^{1}H-NMR (C_5D_5N) δ: 1.31 (3H, d, J=6.6 Hz), 1.65—2.00 (2H), 2.60—2.90 (2H), 4.60—4.90 (4H), 5.41 (1H, d, J=6.6 Hz), 5.61 (1H, s), 7.22 (2H, d, J=8.6 Hz), 7.34 (2H, d, J=8.6 Hz). ^{13}C-NMR (CD_3OD) δ: 23.5 (C-1), 67.8 (C-2), 42.1 (C-3), 32.2 (C-4), 137.5 (C1′), 130.2 (C-2′, 6′), 117.7 (C-3′, 5′), 157.0 (C-4′), 102.4 (Glc-1), 74.8 (Glc-2), 77.8 (Glc-3), 71.7 (Glc-4), 76.7 (Glc-5), 68.0 (Glc-6), 109.9 (Ara-1), 83.1 (Ara-2), 78.8 (Ara-3), 85.8 (Ara-4), 62.9 (Ara-5). HR-FAB-MS (negative mode) m/z: 459.186 $[M-H]^-$. Calcd for $C_{21}H_{31}O_{11}$: 459.187.

Acid Hydrolysis of 3 **3** (50 mg) was hydrolyzed with 3% HCl (10 ml) in the same manner as **2** to obtain (−)-rhododendrol (11 mg), D-glucose (4 mg) and L-arabinose (6 mg), $[\alpha]_D$ +106° (c=0.6, H_2O). The sugars were identified as their trimethylsilyl ethers by comparison with authentic samples on GLC.

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13) All tissues outside the innermost cork cambium comprise the outer bark. All the secondary phloem between the vascular cambium and the innermost cork cambium is the inner bark. In the case of Betulaceous trees, the outer bark is in the form of large sheets and is easy to separate from the inner bark.