Chemical Evaluation of *Betula* Species in Japan. V. 1) Constituents of *Betula ovalifolia*

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Two new seco-dammarane-type triterpenes, called ovalifoliolides A and B, were isolated from the methanol extract of fresh leaves of *Betula ovalifolia* together with 12-O-acetylbutyrolifolenetriol, (3R)-3',5'-dihydroxy-4'-methoxy-3',4'-oxo-1,7-diphenyl-1-heptene and rutin. Betulin, betulin 3-caffeate, rhododendrin, (+)-catechin, (+)-catechin 7-O-[α]-xylropyranoside and procyanidin B-3 were also isolated from the bark. Their structures were determined by spectral data and chemical evidence.

Key words *Betula ovalifolia*; seco-dammarane; ovalifoliolide; betulin; catechin; flavonoid

*Betula ovalifolia* RUPR. is a small shrub found in wet habitats. It is rare and local in Hokkaido and listed in the red data book in Japan. A small amount of fresh leaves and bark of twigs was collected from a cultivated shrub and their constituents were investigated.

From methanol extract of the fresh leaves, two new seco-dammarane-type triterpenes termed ovalifoliolides A and B were isolated together with 12-O-acetylbutyrolifolenetriol oxide 1, (3R)-3',5'-dihydroxy-4'-methoxy-3',4'-oxo-1,7-diphenyl-1-heptene and rutin.

Ovalifoliolide A (1) was formulated as C$_{30}$H$_{48}$O$_3$ by high resolution (HR) EI-MS. The presence of a typical side chain for dammaranes, that is, 24-ene-20(S)-ol, was deduced by fitting the $^1$H- and $^{13}$C-NMR data to those of dammar-24-ene-3β,11β,20(S)-triol (4b). The remaining data contained those for an isopropenyl, δ$_{H}$: 1.74 (3H, s), 4.70 (1H, br s), 4.86 (1H, br s), δ$_{C}$: 23.7 (CH$_3$), 114.1 (CH$_2$), 146.6 (C), and an ester, δ$_{H}$: 4.54 (1H, m), δ$_{C}$: 76.5 (CH), 176.3 (C), suggesting that 1 has a seco-dammarane skeleton like alnuselide (3) which has been isolated from the Betulaceous tree, *Alnus serrulata* (3). The NMR data of I were in good agreement with those of 3 except for the side chain, δ$_{C}$: 176.3 (C-3), 146.7 (C-4), 76.5 (C-11), 17.0 (C-18), 18.8 (C-19), 114.1 (C-28), 23.7 (C-29), 15.6 (C-30); δ$_{H}$: 4.73 (H-28), 4.88 (H-28), 1.79 (H$_2$-29). Final confirmation of the structure was accomplished by two-dimensional shift correlation spectroscopy (2D-COSY). As shown in Figs. 1 and 2, the long-range $^{13}$C-$^1$H COSY and nuclear Overhauser effect correlation spectroscopy (NOESY) confirmed the plane structure and the stereochemistry of I.

Ovalifoliolide B (2) was formulated as C$_{30}$H$_{48}$O$_4$ by HR-El-MS. The $^1$H- and $^{13}$C-NMR data were the same as those of 1 except that the signals for side chain agreed more with those of 3β,11α,12α-diaceoxy-20(S),24(R)-epoxy-25-hydroxydammarane (4a), (4) (Table 1). After confirmation of the structure by 2D-COSY, 2 was determined to

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Fig. 1. Diagnostic Correlations Observed in the Long-Range C–H COSY (---) and H–H COSY (——) for 1

Fig. 2. Chemical Shifts of $^1$H Signals and Diagnostic Correlations Observed in the NOESY for 1

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be a 20(S),24(R)-epoxy-25-hydroxy-derivative of 1.

From the methanol extract of the dried bark, betulin,\(^3\) betulin 3-O-cafeate,\(^3\) rhododendrin (= betuloside),\(^3\) (+)-catechin,\(^4\) (+)-catechin 7-O-β-D-xylopyranoside\(^4\) and procyanidin B-3\(^6\) were isolated.

**Experimental**

The instruments, materials and experimental conditions were the same as described in Part 1 of this series.\(^3\)

**Isolation. Leaves** Fresh leaves (450 g) collected in August at Kushiro, Hokkaido Prefecture, were extracted with MeOH (7 l) at room temperature for 2 weeks. The extract and then MeOH (8 l) were passed over activated charcoal (50 g) packed in a column of 5 cm diameter to obtain fraction M. The column was further eluted with a mixture of CHCl\(_3\) and MeOH (3:7) to obtain fraction C-M. Fraction M was concentrated to a syrup under reduced pressure. The syrup was chromatographed on silica gel using CHCl\(_3\) and MeOH. The fractions containing tripterenes were collected and recrystallized on silica gel using n-hexane and EtOAc to obtain 1 (128 mg), 2 (88 mg) and 12-O-acetyltetrahydrofuranoteriolo (11 mg), while fractions containing (3R,\(\_\)S,3\(-\))-dihydroxy-4-methoxy-3,4\(-\)-di-n-propyl-1,7-dihydropyrene-1-carboxylic acid were collected and recrystallized on Sephadex LH-20 using 90% MeOH for this purpose. Fraction C-M was concentrated and partitioned with CHCl\(_3\)-MeOH-H\(_2\)O (4:4:3). The upper layer was concentrated and chromatographed on Sephadex LH-20 to obtain rutin (218 mg).

**Bark** Air-dried bark (107 g) was extracted twice with MeOH (1 l) under reflux for 6 h. The extracts and 3 l of MeOH were passed over activated charcoal (15 g) packed in a column of 3 cm diameter. The resulting solution was concentrated to a syrup under reduced pressure: this was chromatographed on silica gel using CHCl\(_3\) and MeOH. The fractions containing tripterenes were recrystallized on silica gel using n-hexane and EtOAc to obtain betulin (81 mg) and betulin 3-O-cafeate (34 mg). Those containing phenolics were recrystallized on Sephadex LH-20 using 90% MeOH to obtain rhododendrin (174 mg), (-)-catechin (214 mg), (+)-catechin 7-O-β-D-xylopyranoside (90 mg). The fractions containing proanthocyanidins were recrystallized on Sephadex LH-20 using 85% EtOH to obtain procyanidin B-3 (87 mg).

**Ovalifoliolide A (1)** A colorless amorphous powder. \([\alpha]\) \(_D\) +90° (c=1.0, CHCl\(_3\)). \(^1\)H-NMR (CDCl\(_3\), \(δ\)): 0.94 (3H, s, H\(_2\)-18), 0.95 (3H, s, H\(_2\)-20), 1.14 (3H, s, H\(_2\)-19), 1.15 (3H, s, H\(_2\)-21), 1.63 (3H, s, H\(_2\)-27), 1.69 (3H, s, H\(_2\)-26), 1.74 (3H, s, H\(_2\)-29), 4.54 (1H, m, H-1), 4.70 (1H, brs, H-28), 4.86 (1H, brs, H-29), 5.11 (1H, t, J=7.7 Hz, H-24). EI-MS \(m/z\): 456, 438, 109. HR-ESI-MS \(m/z\): 456.360 [M\(^+\)]. Calcd for C\(_{20}\)H\(_{24}\)O\(_3\): 456.360.

**Ovalifoliolide B (2)** A colorless amorphous powder. \([\alpha]\) \(_D\) +73° (c=2.3, CHCl\(_3\)). \(^1\)H-NMR (CDCl\(_3\), \(δ\)): 0.93 (3H, s, H\(_2\)-18), 0.95 (3H, s, H\(_2\)-20), 1.12 (3H, s), 1.13 (3H, s), 1.21 (3H, s, H\(_2\)-29), 3.72 (1H, t, J=7.6 Hz, H-24), 4.52 (1H, m, H-1), 4.70 (1H, brs, H-28), 4.86 (1H, brs, H-29). EI-MS \(m/z\): 472, 457, 143. HR-ESI-MS \(m/z\): 472.355 [M\(^+\)]. Calcd for C\(_{20}\)H\(_{24}\)O\(_3\): 472.355.

**References**

2) Red data book lists plant communities which need urgent protection and conservation.