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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*-acetylbetulafolienetriol, (3*R*)-3,5′-dihydroxy-4′-methoxy-3′,4″-oxo-1,7-diphenyl-1-heptene and rutin. Betulin, betulin 3-caffeate, rhododendrin, (+)-catechin, (+)-catechin 7-*O*-*β*-D-xylopyranoside 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.[2] 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*-acetylbetulafolienetriol oxide I,[3] (3*R*)-3,5′-dihydroxy-4′-methoxy-3′,4″-oxo-1,7-diphenyl-1-heptene[3] 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-en-20(*S*)-ol, was deduced by fitting the ^{1}H- and ^{13}C-NMR data to those of dammar-24-ene-3*β*,11*α*,20(*S*)-triol (**4b**).[4] 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 serrulatoides*.[5] The NMR data of **1** 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_3-29). 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 **1**.

Ovalifoliolide B (**2**) was formulated as $C_{30}H_{48}O_4$ by HR-EI-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 3*β*,11*α*-diacetoxy-20(*S*),24(*R*)-epoxy-25-hydroxydammarane (**4a**),[4] (Table 1). After confirmation of the structure by 2D-COSY, **2** was determined to

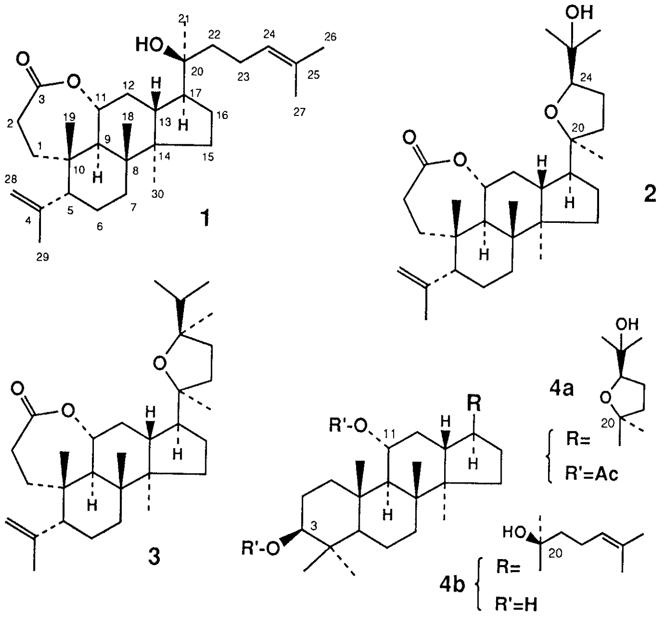

Chart 1

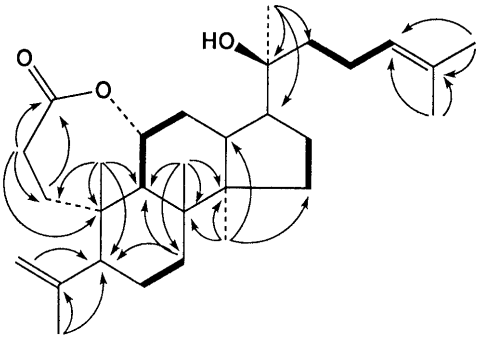

Fig. 1. Diagnostic Correlations Observed in the Long-Range C–H COSY (⌒) and H-H COSY (▬) for **1**

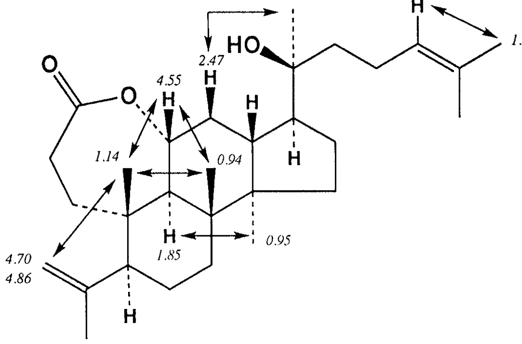

Fig. 2. Chemical Shifts of ^{1}H Signals and Diagnostic Correlations Observed in the NOESY for **1**

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Table 1. ^{13}C-NMR Data in $CDCl_3$

C	**1**	**2**	**4a**	**4b**
1	39.1	39.2	40.9	39.8
2	29.7	29.8	27.5	23.8
3	176.3	176.3	78.4	80.3
4	146.6	146.7	39.0	38.2
5	57.3	57.3	55.7	55.7
6	25.0	25.1	18.1	18.0
7	33.5	33.6	36.2	35.5
8	40.5	40.5	40.7	40.8
9	52.1	52.2	56.0	52.6
10	39.4	39.5	39.4	38.6
11	76.5	76.5	71.1	72.5
12	35.8	35.5	39.7	34.7
13	38.8	39.6	41.3	39.8
14	49.8	49.6	49.8	50.0
15	30.6	30.9	31.0	30.6
16	24.9	25.6	25.6	24.9
17	51.0	50.7	49.3	49.8
18	17.0	16.9	16.7	16.9
19	18.8	18.9	16.6	16.0
20	74.8	85.5	85.9	75.0
21	25.3	23.5	23.2	25.6
22	40.9	36.5	36.0	40.2
23	22.6	26.3	26.2	22.6
24	124.4	83.4	83.3	124.5
25	131.8	71.4	71.4	131.7
26	25.7	24.3	24.2	25.7
27	17.7	27.4	27.4	17.7
28	114.1	114.1	28.2	28.1
29	23.7	23.7	15.4	16.4
30	15.6	15.6	16.3	16.8

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*-caffeate,[3] rhododendrin(=betuloside),[3] (+)-catechin,[4] (+)-catechin 7-*O*-*β*-D-xyropyranoside[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 triterpenes were collected and rechromatographed on silica gel using *n*-hexane and EtOAc to obtain **1** (128 mg), **2** (88 mg) and 12-*O*-acetylbetulafolienetriol (11 mg), while fractions containing (3*R*)-3,5′-dihydroxy-4′-methoxy-3′,4″-oxo-1,7-diphenyl-1-heptene were collected and rechromatographed on Sephadex LH-20 using 90%MeOH for this purpose. Fraction C-M was concentrated and partitioned with $CHCl_3$–MeOH–H_2O (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 resultant solution was concentrated to a syrup under reduced pressure; this was chromatographed on silica gel using $CHCl_3$ and MeOH. The fractions containing triterpenes were rechromatographed on silica gel using *n*-hexane and EtOAc to obtain betulin (81 mg) and betulin 3-*O*-caffeate (34 mg). Those containing phenolics were rechromatographed on Sephadex LH-20 using 90%MeOH to obtain rhododendrin (174 mg), (+)-catechin (214 mg), (+)-catechin 7-*O*-*β*-D-xyropyranoside (390 mg). The fractions containing proanthocyanidins were rechromatographed 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_3-18), 0.95 (3H, s, H_3-30), 1.14 (3H, s, H_3-19), 1.15 (3H, s, H_3-21), 1.63 (3H, s, H_3-27), 1.69 (3H, s, H_3-26), 1.74 (3H, s, H_3-29), 4.54 (1H, m, H-11), 4.70 (1H, br s, H-28), 4.86 (1H, br s, H-28), 5.11 (1H, t, J=7.3Hz, H-24). EI-MS *m/z*: 456, 438, 109. HR-EI-MS *m/z*: 456.361 [M^+], Calcd for $C_{30}H_{48}O_3$: 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_3-18), 0.95 (3H, s, H_3-30), 1.12 (3H, s), 1.13 (3H, s), 1.21 (3H, s), 1.73(3H, s, H_3-29), 3.72 (1H, t, J=7.6 Hz, H-24), 4.52 (1H, m, H-11), 4.70 (1H, br s, H-28), 4.86 (1H, br s, H-28). EI-MS *m/z*: 472, 457, 143. HR-EI-MS *m/z*: 472.355 [M^+], Calcd for $C_{30}H_{48}O_4$: 472.355.

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