Chemical Evaluation of *Betula* Species in Japan. IV.[1] Constituents of *Betula davurica*

Hiroyuki FUCHINO, Tetsuya SATOH, Midori SHIMIZU, and Nobutoshi TANAKA*

Faculty of Pharmaceutical Sciences, Science University of Tokyo, Funakawara-machi, Ichigaya, Shinjuku-ku, Tokyo 162, Japan. Received August 1, 1997; accepted October 6, 1997

The constituents of *Betula davurica* PALL. were identified as follows: Fresh leaves: 12-*O*-acetylbetulafolienetetraol oxide I, 5,8-dihydroxy-6,7-dimethoxyflavone, rutin. Outer bark: betulin, betulin 3-*O*-caffeate, oleanolic acid, oleanolic acid 3-*O*-acetate, 3β-acetoxy-12α-hydroxyoleanan-28, 13β-olide, betulinic acid 3-*O*-caffeate, oleanolic acid 3-*O*-caffeate, betulonic acid. Inner bark: acerogenin E, (3*R*)-3,5′-dihydroxy-4′-methoxy-3′,4″-oxo-1,7-diphenyl-1-heptene, 17-*O*-methyl-7-oxoacerogenin E*, 15-methoxy-17-*O*-methyl-7-oxoacerogenin E*, (−)-lyoniresinol 3α-*O*-β-D-xylopyranoside (=nudiposide), (+)-catechin, (+)-catechin 7-*O*-β-D-xylopyranoside, 3,4,5-trimethoxyphenol β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside, monogynol A, roseoside. Root bark: betulin 3-*O*-caffeate, 3β,27-dihydroxyolean-12-en-28-oic acid 27-*O*-caffeate. The two compounds with an asterisk are new.

Key words *Betula davurica*; diarylheptanoid; dammarane; lupane; flavonoid; oleanane

Eleven species of the genus *Betula* are known in Japan. *B. ermanii* CHAM.,[2] *B. platyphylla* SUKAT. var. *japonica* HARA[3] and *B. maximowicziana* REGEL[1] have white bark and their constituents have been investigated in our earlier studies. *B. davurica* PALL., yaegawakanba in Japanese, has morphologically different bark from the former three, so a different chemical profile is anticipated. In this paper, we describe the constituents of *B. davurica*.

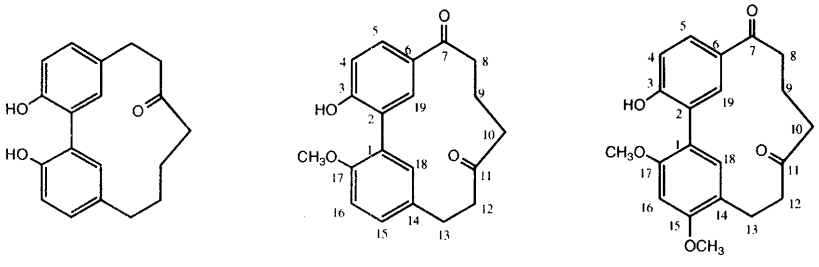
Constituents of Fresh Leaves The leaves of Siberian species had been reported[4] to have a dammarane-type triterpene, betulafolienetriol oxide. In this study, 12-*O*-acetyl betulafolienetetraol oxide I[1] was obtained. Other constituents were flavonoids, 5,8-dihydroxy-6,7-dimethoxyflavone[5] and rutin.[6] Their structures were determined by comparison of their physical properties and spectral data with those previously reported.

Constituents of Outer Bark From the air-dried outer bark collected in June, 8 known compounds: betulin,[2] betulin 3-*O*-caffeate,[2] betulinic acid 3-*O*-caffeate,[7] betulonic acid,[8] oleanolic acid,[7] oleanolic acid 3-*O*-acetate,[3] oleanolic acid 3-*O*-caffeate[9] and 3β-acetoxy-12α-hydroxyoleanan-28,13β-olide[10] were isolated. They are commonly obtained from other *Betula* species except for 3β-acetoxy-12α-hydroxyoleanan-28,13β-olide. Their structures were easily determined by comparison of their physical properties and spectral data with those previously reported. The yield of betulin, which was *ca.* 10% for *B. platyphylla* var. *japonica*[3] and *ca.* 5% for *B. ermanii*[2] and *B. maximowicziana*,[1] is less than 1%, and is proportional to whiteness of the bark.

Constituents of Inner Bark From the air-dried inner bark collected in June, acerogenin E (**1**),[11] (3*R*)-3,5′-dihydroxy-4′-methoxy-3′,4″-oxo-1,7-diphenyl-1-heptene,[3] (−)-lyoniresinol 3α-*O*-β-D-xylopyranoside (=nudiposide),[2] (+)-catechin 7-*O*-β-D-xylopyranoside,[2] (+)-catechin,[2] 3,4,5-trimethoxyphenol β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside,[2] monogynol A,[2] roseoside[12] and two new diarylheptanoids, **2** and **3**, were isolated (Chart 1).

Compound **2**, a colorless amorphous powder, was formulated as $C_{20}H_{20}O_4$ by high-resolution electron impact mass spectrum (HR-EI-MS). The ^{13}C-NMR data showed the presence of two carbonyl groups (δ 199.7, 211.9), an aromatic methoxyl group (δ 56.7), five methylenic (δ 21.9, 28.1, 39.5, 41.6, 44.8) and twelve aromatic carbons (δ 111.6, 118.3, 125.2, 126.7, 127.7, 129.1, 129.4, 133.9, 133.9, 139.1, 153.3, 158.5) and the UV spectrum of **2** was similar to that of **1**.[11] These results suggested that **2** was a biphenyl-type diarylheptanoid. The structure of **2** was determined as 17-*O*-methyl-7-oxoacerogenin E by measurement of the ^{1}H–^{1}H, long-range ^{13}C–^{1}H correlation spectroscopy (COSY) and nuclear Overhauser effect correlation spectroscopy (NOESY) (Fig. 1).

Compound **3**, colorless needles, mp 223—224 °C, was formulated as $C_{21}H_{22}O_5$ by HR-EI-MS. The UV, IR spectral data were similar to those of **2** but ^{1}H- and ^{13}C-NMR spectra showed the presence of an additional aromatic methoxyl group compared to those of **2**. The coupling patterns of aromatic protons in ^{1}H-NMR spectra



1 **2** **3**

Chart 1. Chemical Structures of **1**, **2** and **3**

* To whom correspondence should be addressed.

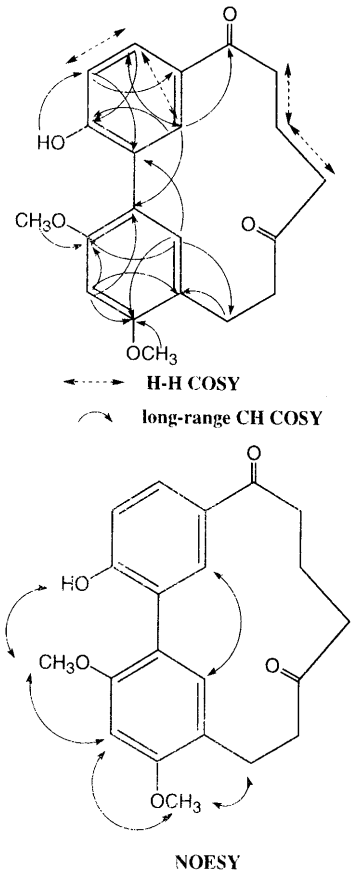


Fig. 1. ^{1}H–^{1}H COSY, Long-Range ^{13}C–^{1}H COSY and NOESY Connections for **2**

(δ 6.51 (s), 6.73 (s)) suggested that the additional methoxyl group was situated at C-5 or C-15. As the NOESY correlation between one methoxyl proton (δ 3.92) and a benzylic methylene proton (δ 2.96, H-22) was observed, the structure of **3** was determined to be 15-methoxy-17-*O*-methyl-7-oxoacerogenin E. The 2D-NMR (^{1}H–^{1}H COSY, long-range ^{13}C–^{1}H COSY) spectra supported its structure (Fig. 2).

Constituents of Root Bark From the air-dried root bark collected in June, betulin 3-*O*-caffeate and 3β,27-dihydroxyolean-12-en-28-oic acid 27-*O*-caffeate, which had been isolated from *Melianthus comosus* by Anderson and his colleagues[13] were obtained.

In this study, 22 compounds including two new ones, **2** and **3**, were isolated and the following remarkable features were revealed. 1: The dammarane-type triterpenes of leaves of *B. davurica* have 3α,17α,20-hydroxyl groups, while those of *B. ermanii* have 3β,11α,20-hydroxyl groups and those of *B. maximowicziana* have 3α,12β,20-hydroxyl groups. 2: It is noteworthy that (+)-catechin and its xyloside are included in large amounts, 0.4% and 0.9%, respectively. 3: The content of betulin is below 1%, which is the least amount in all species we have examined to date.

Experimental

The instruments, materials and experimental conditions were the same as described in Part 1 of this series.[2]

Isolation. Leaves Fresh leaves (2 kg) collected in June at Yachi-ho Highland in Nagano Prefecture, were extracted with MeOH (20 l) at room temperature for 2 weeks. The extract and 10 l of MeOH were passed over activated charcoal (130 g) to give fr. M. The column was further eluted with 30% $CHCl_3$/MeOH to give fr. C–M. Each fraction

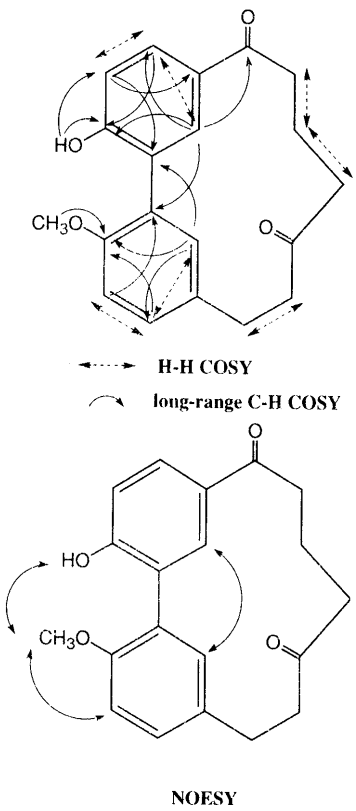


Fig. 2. ^{1}H–^{1}H COSY, Long-Range ^{13}C–^{1}H COSY and NOESY Connections for **3**

was concentrated to a syrup under reduced pressure. The syrup from fr. M was subjected to column chromatography on silica gel using $CHCl_3$–MeOH to give 20 fractions. Fractions 15—16 were crystallized from MeOH to afford rutin (148 mg). Fraction 8 was rechromatographed on Sephadex LH-20 using MeOH, on silica gel using $CHCl_3$–MeOH–H_2O (100:15:1) and on Chromatorex ODS (Fuji Silysia Chemical, Ltd.) using 40% H_2O/MeOH to give 12-*O*-acetyl betulafolienetetraol oxide I (12 mg). The syrup from fr. C–M was rechromatographed on Sephadex LH-20 using MeOH to give 6-methoxygalangin 3-methylether (30 mg).

Outer Bark Air-dried outer bark (447 g) was extracted with 4 l of $CHCl_3$ under reflux for 5 h. The extract was concentrated to a syrup and chromatographed on silica gel using $CHCl_3$–EtOAc to obtain 20 fractions. Oleanolic acid 3-*O*-caffeate (91 mg) was obtained from fr. 12—15. Fractions 2—6 were rechromatographed on silica gel using *n*-hexane–AcOEt, $CHCl_3$–AcOEt and on silica gel impregnated with 20% $AgNO_3$ using *n*-hexane–AcOEt to obtain betulin (442 mg), oleanolic acid 3-*O*-acetate (1055 mg), 3β-acetoxy-12α-hydroxyoleanan-28,13β-olide (122 mg) and betulonic acid (10 mg). Fractions 7—11 were rechromatographed on silica gel using *n*-hexane–AcOEt, $CHCl_3$–AcOEt and on Sephadex LH-20 using MeOH to gain oleanolic acid (256 mg). Fractions containing betulin 3-*O*-caffeate and betulinic acid 3-*O*-caffeate were subjected to acetylation with acetic anhydride in pyridine, and then rechromatographed on silica gel using *n*-hexane–AcOEt to obtain 28-*O*-acetylbetulin 3-*O*-(3,4-di-*O*-acetyl)caffeate (3.3 mg) and betulinic acid 3-*O*-(3,4-di-*O*-acetyl)caffeate (49 mg).

Inner Bark Air-dried inner bark (937 g) was extracted with 3 l of MeOH under reflux for 6 h. The extract was concentrated and partitioned with $CHCl_3$–MeOH–H_2O (4:4:3). Then the upper layer was concentrated and chromatographed on silica gel using $CHCl_3$ and MeOH to obtain 20 fractions. Fractions were rechromatographed on Sephadex LH-20 using MeOH–H_2O or EtOH–H_2O, on Chromatorex ODS using MeOH–H_2O, then on silica gel using $CHCl_3$–MeOH–H_2O (100:15:1) and subjected to HPLC (Capcellpak C-18 SG with 30% CH_3CN/H_2O and Carbon 500 with 80% MeCN/H_2O) to obtain nudiposide (187 mg), (+)-catechin 7-*O*-β-D-xylopyranoside (8850 mg), (+)-catechin (3630 mg), 3,4,5-trimethoxyphenol β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (670 mg) and roseoside (30 mg). The lower layer was concentrated and chromatographed on silica gel using $CHCl_3$ and MeOH to obtain 20 fractions. Fractions 11—12 were rechromatographed on silica gel

using *n*-hexane–AcOEt and subjected to HPLC (Carbon 500 with CH_3CN) to gain **1** (14 mg), **2** (17 mg) and **3** (22 mg). Fractions 13—16 were purified by Sephadex LH-20 column chromatography using MeOH and preparative thin-layer chromatography (TLC) using *n*-hexane–AcOEt to gain (3*R*)-3,5′-dihydroxy-4′-methoxy-3′,4″-oxo-1,7-diphenyl-1-heptene (30 mg) and monogynol A (150 mg).

Root Bark Air-dried root bark (519 g) was extracted with 2 l of MeOH under reflux for 5 h. The extract was concentrated and partitioned with $CHCl_3$–MeOH–H_2O (4 : 4 : 3). The lower layer was chromatographed on silica gel using $CHCl_3$–AcOEt to obtain 20 fractions. Fractions 7—10 were rechromatographed on Chromatorex ODS using MeOH to obtain betulin-3-*O*-caffeate (444 mg). Fractions 14—20 were purified by silica gel column chromatography using $CHCl_3$–MeOH–H_2O–AcOH (460 : 30 : 2 : 1) and preparative TLC using $CHCl_3$–MeOH–H_2O–AcOH (480 : 30 : 2 : 1) to gain 3β,27-dihydroxyolean-12-en-28-oic acid 27-*O*-caffeate (17 mg).

17-*O*-Methyl-7-oxoacerogenin E (2) A colorless amorphous powder, $[\alpha]_D$ 0° ($c=0.5$, $CHCl_3$). UV λ_{max} (MeOH) nm (log ε): 283 (4.18), 250 (4.32). EI-MS *m/z*: 324 (M^+), 296, 253, 240, 212, 120. HR-EI-MS *m/z*: 324.1371 (M^+); Calcd for $C_{12}H_{20}O_4$, 324.1361. IR (KBr) cm^{-1}: 3300, 2910, 1700, 1670, 1562, 1510, 1235. ^{1}H-NMR ($CDCl_3$) δ: 2.18 (2H, quint, $J=6.9$ Hz), 2.80—2.91 (4H, m), 2.96 (2H, t, $J=6.9$ Hz), 3.05—3.14 (2H, m), 4.01 (3H, s), 6.82 (1H, d, $J=2.3$ Hz), 6.96 (1H, d, $J=8.6$ Hz), 7.02 (1H, d, $J=8.6$ Hz), 7.23 (1H, dd, $J=8.6$, 2.3 Hz), 7.64 (1H, d, $J=2.3$ Hz), 7.88 (1H, dd, $J=8.6$, 2.3 Hz). ^{13}C-NMR ($CDCl_3$) δ: 125.2 (C-1), 126.7 (C-2), 153.3 (C-3), 111.6 (C-4), 129.4 (C-5), 133.9 (C-6), 28.1 (C-7), 41.6 (C-8), 211.9 (C-9), 44.8 (C-10), 21.9 (C-11), 39.5 (C-12), 199.7 (C-13), 127.7 (C-14), 129.1 (C-15), 118.3 (C-16), 158.5 (C-17), 139.1 (C-18), 133.9 (C-19), 56.7 (CH_3O).

5-Methoxy-3-*O*-methyl-7-oxoacerogenin E (3) Colorless needles from acetonitrile, mp 223—224 °C, $[\alpha]_D$ 0° ($c=0.5$, $CHCl_3$). UV λ_{max} (MeOH) nm (log ε): 288 (4.15), 261 (4.42). EI-MS *m/z*: 354 (M^+), 326, 283, 269, 242, 135. HR-EI-MS *m/z*: 354.1469 (M^+); Calcd for $C_{21}H_{22}O_5$, 354.1467. IR (KBr) cm^{-1}: 3380, 2920, 1600, 1658, 1620, 1570, 1510. ^{1}H-NMR ($CDCl_3$) δ: 2.08—2.20 (2H, m), 2.68—2.76 (2H, m), 2.96 (4H, s), 3.06 (2H, br t, $J=7.6$ Hz), 3.92 (3H, s), 4.01 (3H, s), 6.51 (1H, s), 6.73 (1H, s), 7.00 (1H, d, $J=8.6$ Hz), 7.65 (1H, d, $J=2.5$ Hz), 7.83 (1H, dd, $J=8.6$, 2.5 Hz), 8.00 (1H, s). ^{13}C-NMR ($CDCl_3$) δ: 125.1 (C-1), 117.7 (C-2), 154.0 (C-3), 95.1 (C-4), 158.9 (C-5), 121.6 (C-6), 21.5 (C-7), 40.2 (C-8), 211.1 (C-9), 42.2 (C-10), 22.7 (C-11), 38.8 (C-12), 199.9 (C-13), 128.1 (C-14), 128.3 (C-15), 117.9 (C-16), 158.2 (C-17), 139.2 (C-18), 134.6 (C-19), 55.7, 57.0 (CH_3O).

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