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—Six phenolic glucosides containing a new glucoside 4'-hydroxy-3'-methoxyphenol-β-D-[6-O-(4''-hydroxy-3'',5''-dimethoxybenzoate)]-glucopyranoside—

JIANG Hongzhou, SHEN Yanbo, YASUDA Eri, CHIBA Motoi and TERAZAWA Minoru*

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Abstract

Alcoholic extracts obtained from the inner bark of shirakamba birch, Betula platyphylla Sukatchev var. japonica Hara were further investigated. A new phenolic glucoside 4'-hydroxy-3'-methoxyphenol-β-D-[6-O-(4''-hydroxy-3'',5''-dimethoxybenzoate)]-glucopyranoside along with five known phenolic compounds was isolated. The structures of the isolated compounds were determined on the basis of spectroscopic studies.

Key Words: Betula, birch, 4'-hydroxy-3'-methoxyphenol-β-D-[6-O-(4''-hydroxy-3'',5''-dimethoxybenzoate)]-glucopyranoside, 3,4,5-trimethoxyphenyl-β-D-glucopyranoside, platyphylloside, betuloside, catechin

Introduction

Shirakamba birch (Betula platyphylla Sukatchev var. japonica Hara) is a tall deciduous tree that grows on Hokkaido and Honshu islands in Japan (Shibuya and Igarashi 1995). It has not been widely used in Japan except as pulp chip and chopstick production from its wood. However, in Europe, birch flowers and leaves have been utilized as herbal tea. The production of herbal tea has started in Bifuka, Hokkaido, Japan since 1995 (Terazawa 1995). Traditionally, extracts from the leaves have been expected to be a source of compounds that increase the activity of the anti-oxidative defense of human blood plasma (Drozdova et al. 1995). Chemical constituents of the birch leaves have been investigated to be isolated as rhamnosides of lignans (Shen et al. 1999-a), glucosides of p-hydroquinone derivatives (Shen et al. 1999-b), glucosides of sesquiterpenoids (Shen et al. 2001) and glycosides of flavonoids (Shen et al. 2000; Fuchino et al. 1995). The inner bark and leaves of shirakamba birch contains a large amount of phenolic glucosides, including salidroside, betuloside and platyphylloside (Terazawa et al. 1984). It was found later that platyphylloside has an inhibitory effect on ruminant digestibility in vitro (Sunnerheim et al. 1988).

In this paper, we report the isolation of six phenolic glucosides including a new phenolic glucoside 4'-hydroxy-3'-methoxyphenol-β-D-[6-O-(4''-hydroxy-3'',5''-dimethoxybenzoate)]-glucopyranoside from the inner bark of shirakamba birch.

Material and Methods

Material

Fresh inner bark of shirakamba birch (Betula platyphylla Sukatchev var. japonica Hara) was collected in July 1998 at the Nakagawa Experiment Station of Hokkaido University Forestry.

Extraction and Isolation

Extraction: The fresh inner bark (2.51 kg) of shirakamba birch (Betula platyphylla Sukatchev var. japonica Hara) was cut into small sections and extracted with 95% ethanol (EtOH) at room temperature for 2 weeks. The EtOH extracts were concentrated under reduced pressure to give syrup (170g). The syrup (26g) was mixed with silica-gel (1:2) and dried under reduced pressure to remove the solvent and water.

Chromatography: The dried cake was impregnated sufficiently with the developing solvent (ethyl acetate (EtOAc) saturated with water) before charging it on a silica-gel (Wakogel C-200) column. Each fraction was collected in 300 ml portion. The eluents were monitored by TLC (CMW: CHCl₃/MeOH/H₂O, 80:10:1, v/v), and the fractions containing the same compounds were combined. Three fractions of F1, F2 and F3 were obtained. Chromatographic purification of the each fraction was carried out until each compound became pure on TLC (CMW).

Purification of F1: The fraction F1 (0.33g) containing compound 1 was subjected to a silica-gel column and eluted with a developing solvent (CHCl₃/MeOH/H₂O 80:10:1, v/v). Each 15 ml eluent

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Drozdova, et al. 1995. Chemical constituents of the birch leaves have been investigated to be isolated as rhamnosides of lignans (Shen et al. 1999-a), glucosides of p-hydroquinone derivatives (Shen et al. 1999-b), glucosides of sesquiterpenoids (Shen et al. 2001) and glycosides of flavonoids (Shen et al. 2000; Fuchino et al. 1995). The inner bark and leaves of shirakamba birch contains a large amount of phenolic glucosides, including salidroside, betuloside and platyphylloside (Terazawa et al. 1984). It was found later that platyphylloside has an inhibitory effect on ruminant digestibility in vitro (Sunnerheim et al. 1988).
was collected as a fraction and the fractions containing the same compounds were combined. Purification of F1-1 containing mainly compound 1 was carried out until compound 1 became pure on TLC (CMW).

**Purification of F2**: The fraction F2 (0.68 g) was subjected to a silica-gel column and eluted with a developing solvent (CHCl₃/MeOH/H₂O, 60:10:1, v/v). Each 15 ml eluent was collected as a fraction and the fractions containing the same compounds were combined. Purification of F2-1 containing compound 2, F2-2 containing compound 3 and F2-3 containing compound 4, was carried out until each compound became pure on TLC (CMW).

**Purification of F3**: Fraction F3 (0.48 g) was subjected to a silica-gel column and eluted with a developing solvent (CHCl₃/MeOH/H₂O, 50:10:1, v/v). Each 15 ml eluent was collected as a fraction and the fractions containing the same compounds were combined. Purification of F3-1 containing compound 5 and F3-2 containing compound 6 was carried out until each compound became pure on TLC (CMW).

**Instrumental Analyses**
Spectra were obtained with the following instruments: NMR: a Bruker AM-500FT-NMR spectrometer and a JEOL JNM-EX 270 FT-NMR system; FD-MS and EI-MS: a JEOL JMS01SG-2 mass spectrometer and a JMS-DX-300 mass spectrometer, respectively. Melting points were measured with a Yanagimoto Micro Melting Apparatus. Optical rotatory was measured with a JASCO Digital Polarimeter DIP-360.

**Physico-chemical properties of the isolated compounds**
Structures of the compounds 1-6 are shown in Fig. 1.

**Compound 1**
((+)-3,3',4',5,7-pentahydroxyflavan ((+)- catechin)) was positive to DSA, TLC (AEAW): RF 0.68. (Yield: 91.6 mg). FD-MS m/z : 290 [M⁺] (100). El-HR-MS m/z : 290.0879 [M⁺] (calcd. for C₁₅H₁₄O₆: 290.0879); EI-MS m/z (rel. int.): 290 (43.21), 139 (100), 123 (35.55), 107 (6.53). 1H-NMR (ppm, CD₃OD): δ 6.85 (1H, d, J = 2.0 Hz), 6.74 (1H, s), 6.73 (1H, dd, J = 2.4, 3.0 Hz), 5.98 (1H, d, J = 2.4 Hz), 5.84 (1H, s), 4.53 (1H, d, J = 7.8 Hz), 3.97 (1H, m), 2.88 (1H, dd, J = 5.4 Hz), 2.52 (1H, dd, J = 8.3 Hz). 13C-NMR (ppm, CD₃COCD₃): δ 80.1 (C-2'), 68.8 (C-3'), 30.5 (C-4'), 101.1 (C-4a), 157.2 (C-5'), 96.0 (C-6'), 157.6 (C-7'), 20.5 (C-8a), 158.1 (C-8'), 132.6 (C-1'), 115.6 (C-2'''), 146.1 (C-3'''), 146.0 (C-4''), 96.6 (C-5''), 116.2 (C-6'').

**Compound 2**
(1,7-di-(4-hydroxyphenyl)-5-heptanone (platyphylloside)) was positive to DSA, obtained as colorless oil (Yield: 5.5 mg). TLC (CMW, 40:10:1, v/v): RF 0.66. FD-MS m/z : 296 [M⁺] (100); El-HR-MS m/z : 296.1374 [M⁺] (calcd. for C₁₅H₁₄O₆: 296.1413); EI-MS m/z (rel. int.): 296 (15.93), 176 (14.49), 107 (100), 75 (6.07); 1H-NMR (ppm, CDCl₃): δ 6.98 (4H, dd, J = 8.0 Hz), 6.74 (4H, dd, J = 8.0 Hz), 6.84 (1H, m), 6.08 (1H, brd), 2.80 (4H, m), 2.65 (2H, m), 2.47 (2H, q, J = 7.1 Hz). 13C-NMR (ppm, CDCl₃): δ 34.4 (C-1'), 33.6 (C-2'), 146.5 (C-3'), 130.8 (C-4'), 199.8 (C-5'), 42.0 (C-6'), 29.7 (C-7'), 132.9 (C-9', or C-1''), 133.4 (C-1''), 129.5 (C-2'', 2'', 6'', 6''), 151.5 (C-3''', 5''), 154.0 (C-4''), 75.3 (C-5''), 78.7 (C-3), 72.0.
(C-4), 78.4 (C-5), 63.0 (C-6), 156.3 (C'-1), 96.5 (C-2', 6'), 155.1 (C-3', 5'), 134.8 (C-4').

Compound 6
(4-hydroxyphenylbutane -2-ol-β-D- glucopyranoside (betuloside)) was positive to DSA., obtained as colorless crystals, mp 188-189 °C, (Yield:4.8mg).

TLC (CMW, 40:10:1, v/v): Rf 0.33. [α] D 18.4°. 13C-NMR (ppm, CD3OD): δ 7.03 (2H, d, J = 8.0 Hz), 6.67 (2H, d, J = 8.0 Hz), 3.30 (1H, m), 2.60 (2H, m), 1.84 (1H, m), 1.87 (1H, m), 1.30 (2H, d, J = 6.4 Hz).

1H-NMR (ppm, CD3OD): δ 7.03 (2H, d, J = 8.0 Hz), 6.67 (2H, d, J = 8.0 Hz), 3.30 (1H, m), 2.60 (2H, m), 1.84 (1H, m), 1.87 (1H, m), 1.30 (2H, d, J = 6.4 Hz).

The results and discussion

Compound 1
(+)-3',3',4',5,7-pentahydroxyflavan ([+)-catechin]

Compound 1 was positive to DSA, showing that it was a phenolic compound. The molecular weight of compound 1 was 290 in the FD-MS. The spectrum of 13C-NMR of compound 1 showed the presence of two aromatic rings, two methine carbons, and one methylene carbons. The 1H-NMR spectrum revealed the aromatic proton signals at 8.0 Hz assigned to eight aromatic protons of C-2', 6' and C-3', 5'.

Results and Discussion

Compound 1
(+)-3',3',4',5,7-pentahydroxyflavan ([+)-catechin]

Compound 1 was positive to DSA, showing that it was a phenolic compound. The molecular weight of compound 1 was 290 in the FD-MS. The spectrum of 13C-NMR of compound 1 showed the presence of two aromatic rings, two methine carbons, and one methylene carbon. The 1H-NMR spectrum revealed the aromatic proton signals at 8.0 Hz assigned to eight aromatic protons of C-2', 6' and C-3', 5'.

Compound 2
1,7-di-(4-hydroxyphenyl)- 5-hepten-3-one (platyphyllosone)

Compound 2 showed a molecular ion peak at m/z 482 in the FD-MS. The spectrum of 13C-NMR of compound 2 showed the presence of two aromatic rings. The nuclear magnetic resonance (NMR) spectrum (Table 1) revealed the aromatic proton signals at 8.0 Hz due to two symmetric benzene protons was assigned to the protons of C-2' and C-6'.

Compound 3
4'-hydroxy- 3'-methoxyphenol-β-D-[6-0-(4'-hydroxy-3',5'-dimethoxybenzoate)]-gluco- pyranoside, a new compound

Compound 3 was positive to DSA, showing that it was a phenolic compound. It showed a molecular ion peak at m/z 482 in the FD-MS. Compound 3 was proved to be closely related to compound 2, the largest difference being the displacement of double bond by a glucopyranosyl moiety (13C-NMR data). The mode of glucosidic linkage was determined to be β-form based on the
large coupling constant of the anomic proton signal at δ 4.82 (1H, d, J = 7.6 Hz). Based on the spectral aspects, compound 4 was concluded to be 1,7-di-(4-hydroxyphenyl)-heptane-3-one-5-ol-β-D-glucopyranoside, which was isolated firstly byTerazawa et al. (1984) and named as platyphylloside. The absolute configuration of C-5 was identified as an S by a direct comparison of the [α]D value with published data for platyphylloside, which is known as a compound showing an inhibitory effect on ruminant digestibility in vitro (Sunnerheim, K. et al. 1988).

**Compound 5**

3,4,5-trimethoxophenol-β-D-glucopyranoside

Compound 5 showed a molecular ion peak at m/z 346 (M⁺) in the FD-MS. By the data of 1H- and 13C-NMR spectra, the existence of three aromatic methoxyl groups, an aromatic ring and a glucopyranosyl was indicated. A singlet at δ 6.48 in the 1H-NMR spectrum indicated the presence of two aromatic protons. The data of the 13C-NMR spectrum of compound 5 suggested the existence of a 3, 4, 5-trimethoxyl benzene ring. A singal at δ 156.3 in 13C-NMR spectrum was assigned to the aromatic carbon C-1' which connected to the glucosyl residue. The presence of a glucopyranosylo moiety in compound 5 was confirmed by 13C-NMR spectrum, which showed six signals at δ 103.5 (C-1), 75.3 (C-2), 78.7 (C-3), 72.0 (C-4), 78.4 (C-5), and 63.0 (C-6). The mode of the glucosidic linkage was determined to be β-form based on the large coupling constant of the anomeric proton signal at δ 4.82 (1H, d, J = 7.6 Hz) in the 1H-NMR spectrum. Thus, the structure of compound 5 was determined to be 3, 4, 5-trimethoxophenol-β-D-glucopyranoside. (Hayashi et al. 1990; Shimomura et al. 1988).

**Compound 6**

4-hydroxyphenylbutane-2-ol-β-D-glucopyranoside

Compound 6 was positive to DSA, showing that it was a phenolic compound. The molecular weight was observed at m/z 328 in the FD-MS. The 1H-NMR showed a double doublet of AA′BB′ type at δ 7.03 (2H, d, J = 8.0 Hz) and 6.67 (2H, d, J = 8.0 Hz) assignable to four aromatic protons of C2′, 6′ and C3′, 5′, indicating the existence of a 1, 4-disubstituted benzene ring in compound 5. One methyl at δ 1.20 (3H, m), two methylenes at δ 2.60 (2H, m) and [1.84 (1H, m), 1.68 (1H, m)] and a methine at δ 3.30 (1H, m) were observed. Furthermore, the presence of a glucopyranosyl moiety was suggested by 13C-NMR spectrum, which showed six signals at δ 103.1 (C-1′), 75.9 (C-2′), 79.0 (C-3′), 75.9 (C-4′), 78.6 (C-5′) and 63.7 (C-6′). The mode of the glucosidic linkage was determined to be β-form based on the large coupling constant of the anomic proton signal at δ 4.33 (1H, d, J = 7.9 Hz) in the 1H-NMR spectrum. The HMBC spectrum revealed a connection between the glucosyl residue and the aglycone: the anomic proton signal of glucosyl moiety at δ 4.33 correlated with the signal of C-2. This result showed that the glucosyl residue is linked to C-2. Thus, compound 6 is concluded to be 4-(4′-hydroxyphenyl) butanol-2-β-D-glucopyranoside, which was known as betuloside. The absolute configuration of C-2 in compound 6 was identified as R by a direct comparison of the [α]D value with published data for R-rhododendrin (Fuchino et al. 1996).

**Acknowledgments**

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Table 1. NMR spectral data of compound 3
(In CD$_3$COCD$_3$, 270 MHz)

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Fig. Compounds isolated from inner bark of shirakamba

*Betula platyphylla* Sukatchev var. *japonica* Hara