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Phenolic Glucosides from Inner Bark of Shirakamba Birch, *Betula platyphylla* Sukatchev var. *japonica* Hara

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

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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, platyphyllenone, 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, Jappan 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 temperatures 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, 40:10:1, 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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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 (0.68g) 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 (0.48g) 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 mainly compound 1 and F3-2 containing compound 6 was carried out until each compound became pure on TLC (CMW). Purification of F3-1 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).

**Instrumental Analyses**

Spectra were obtained with the following instruments: NMR: a Bruker AM-500FT-NMR 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 Polarmeter DIP-360.

**Physico-chemical properties of the isolated compounds**

Structures of the compounds 1-6 are shown in Fig.1.

**Compound 1**

(1')-3,3',4',5,7-pentahydroxyflavan (1'-catechin) was positive to DSA. TLC (AEAW): RF 0.68. (Yield: 91.6mg). FD-MS m/z : 290 [M⁺] (100). El-HR-MS m/z : 290.0789 [M⁺] (100). El-MS m/z (rel. int.): 290 (43.21), 139 (100), 123 (35.55), 107 (6.53); 1'H-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-8'), 158.1 (C-8a'), 132.6 (C-15'), 115.6 (C-15'''), 146.1 (C-15''''), 146.0 (C-14''''), 96.6 (C-5''), 116.2 (C-6'').

**Compound 2**

(1',7-di-(4-hydroxyphenyl)-5-hepten-3-one (platyphylloene) was positive to DSA, obtained as colorless oil (Yield: 5.5mg). 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⁺] (100); El-MS m/z (rel. int.): 296 (15.93), 176 (14.49), 107 (100), 75 (6.07); 1'H-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-1'''), 133.4 (C-1''', or C-1'''), 129.5 (C-2'', 2'', 6'', 6''), 115.4 (C-3'', 3'', 5'', 5'').

**Compound 3**

(4'-hydroxy-3'-methoxyphenol-β-D-6-O-(4''-hydroxy-3''-5''-dimeth-oxybenzoate)-glucopyranoside) was positive to DSA. (Yield: 6.0mg). TLC (CMW, 40:10:1, v/v): RF 0.43. FD-MS m/z (rel. int.): 482 [M⁺] (100). El-HR-MS m/z : 482.1345 [M⁺] (100). El-MS m/z (rel. int.): 429 (6.12), 282 (9.20), 207 (6.35), 75 (5.10), 71 (1.06).

**Compound 4**

((1,7-di-(4-hydroxyphenyl)-heptan-3-one-5-01-β-D-glucopyranose (platyphylloside)) was positive to DSA, obtained as colorless powder (Yield: 22.8mg). TLC (CMW, 40:10:1, v/v): RF 0.35. Mp 189.5-190°C. 1'H-NMR (ppm, CD₃OD): δ 7.27 (2H, s), 6.94 (1H, d, J = 2.0 Hz), 6.92 (1H, d, J = 2.0 Hz), 6.10 (1H, dd, J = 3.0, 2.0 Hz), 6.42 (1H, s), 3.80 (6H, s, OMe x 2), 3.71 (3H, s, OMe). 13C-NMR (ppm, CD₃COCD₃): δ 104.7(C-1), 75.3 (C-2), 78.2 (C-3), 72.1 (C-4), 75.8 (C-5), 65.4 (C-6), 155.2 (C'''), 107.6 (C-2'''), 152.3 (C-3'''), 141.4 (C-4'''), 130.4 (C-5'''), 121.0 (C-6'''), 142.4 (C-1''), 108.7 (C-2''), 148.9 (C-3''), 102.3 (C-4''), 167.0 (C-α').

**Compound 5**

(3,4,5-trimethylhydroxybenzal-β-D-glucopyranoside) was negative to DSA, obtained as white needles from F3 (Yield: 8.0mg). TLC (CMW, 40:10:1, v/v): RF 0.37. mp 201-203. FD-MS m/z (rel. int.): 346 [M⁺] (100). El-HR-MS m/z : 346.1229 [M⁺] (100). El-MS m/z (rel. int.): 346 (43.77), 211 (1.54), 184 (100), 169 (64.29), 141 (11.26); 1'H-NMR (ppm, CD₃OD): δ 6.48 (2H, s), 4.82 (1H, d, J = 7.6 Hz), 3.92 (1H, dd, J = 12.3, 5.3 Hz), 3.80 (6H, s, OMe x 2), 3.69 (3H, s, OMe), 3.66 (1H, dd, J = 12.3, 2.6 Hz), 3.32-3.47 (4H, overlapping). 13C-NMR (ppm, CD₃OD): δ 103.5 (C-1), 75.3 (C-2), 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'), 78.4 (C-5), 63.0 (C-6), 156.3 (C'-1), 96.5

Command 6
(4'-hydroxyphenylbutane -2-ol-ß-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]25° D -45.3° ± 0.1° (C 2.30, MeOH); FD-MS m/z (rel. int.): 328 [M]+ (100); El-HR-MS m/z: 328.1497 [M]+ (calcd. for C16H24O7: 328.1522); EI-MS m/z: 328 (4.53), 296 (1.61), 207 (6.90), 177 (15.35), 148 (32.32), 133 (14.14), 107 (100); 13C-NMR (ppm, CD3OD): δ 7.03 (2H, J = 8.0 Hz), 6.67 (2H, d, J = 8.0 Hz), 3.30 (1H, m), 2.60 (2H, m), 1.84 (1H, m), 1.68 (1H, m), 1.20 (3H, d, J = 6.4 Hz).

Compound 6

The displacement of double bond by a glucopyranosyl moiety. The molecular formula of compound 6 was deduced from the FD-MS spectrum of compound 4. It showed a molecular ion peak at m/z 482 in the FD-MS. The spectrum of 13C-NMR of compound 6 showed the presence of two aromatic rings. The 1H-NMR spectrum (Table 1) revealesd that C-2' and C-6' were adjacent to carbonyl group, because the chemical shift of these two protons were in downfield. A singlet at δ 3.80 (6H) indicated the presence of two symmetric methoxyl groups, suggesting the presence of syringyl nucleus. A singlet at δ 3.71 (3H) was due to another methoxyl group of the another benzene ring. The presence of a glucopyranosyl moiety in compound 6 was confirmed by 13C-NMR spectrum (Table 1), which showed six signals at δ 104.7(C-1), 75.3(C-2), 78.2(C-3), 72.1(C-4), 75.8(C-5) and 65.4(C-6). The mode of the glucosidic linkage was determined to be β-form based on the coupling constant of the anomeric proton signal at δ 4.66 (1H, d, J = 7.6 Hz) in the 1H-NMR spectrum. Thus, compound 4 was supposed to be a 4-hydroxy-3.5-dimethoxy benzonic acid ester of 4-hydroxy-3-methoxy-ß-D-glucopyranoside as 4'-hydroxy-ß-D-glucopyranoside. To our knowledge, the compound has not been reported so far.

Compound 4
1,7-di-(4'-hydroxyphenyl)-5-hepten-3-one (platyphyllonol)
The FD-MS of compound 4 showed a fragment ion at m/z 476, resulting from the loss of a glucosyl moiety. The molecular formula of compound 4 was deduced from the FD-MS spectrum of m/z 476 (M+, C16H12O6). Compound 4 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-from based on the
large coupling constant of the anomic proton signal at \( \delta 4.82 \) (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-B-D-gluco-pyranoside, which was isolated firstly by Terazawa et al. (1984) and named as platyphyilloside. The absolute configuration of C-5 was identified as an S by a direct comparison of the \( [\alpha]D \) value with published data for platyphyilloside, 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-trimethoxyphenol-8-D-glucopyranoside

Compound 5 showed a molecular ion peak at \( m/z \) 346 (M+) in the FD-MS. By the data of \(^1\)H- and \(^13\)C-NMR spectra, the existence of three aromatic methoxyl groups, an aromatic ring and a glucopyranosyl moiety was indicated. A singlet at \( \delta 6.48 \) in the \(^1\)H-NMR spectrum indicated the presence of two aromatic protons. The data of \(^13\)C-NMR spectrum of compound 5 suggested the existence of a 3, 4, 5-trimethoxy benzene ring. A singal at \( \delta 156.3 \) in \(^13\)C-NMR spectrum was assigned to the aromatic carbon C-1' which connected to the glucosyl residue. The presence of a glucopyranosyl moiety in compound 5 was confirmed by \(^13\)C-NMR spectrum, which showed six signals at \( \delta 103.5 \) (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 a-configuration based on the large coupling constant of the anomeric proton signal at \( \delta 4.82 \) (1H, \( d, J = 7.6 \) Hz) in the \(^1\)H-NMR spectrum. Thus, the structure of compound 5 was determined to be 3, 4, 5-trimethoxyphenol-8-D-glucopyranoside. (Hayashi et al. 1990; Shimomura et al. 1988).

**Compound 6**

4-hydroxyphenylbutane-2-ol-8-D-glucopyranoside (betuloside)

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 \(^1\)H-NMR showed a doublet of AA'BB' type at \( \delta 7.03 \) (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 \( \delta 1.20 \) (3H, \( m \)), two methylenes at \( \delta 2.60 \) (2H, \( m \)) and \([1.84 \) (1H, \( m \), 1.68 (1H, \( m \))] and a methine at \( \delta 3.30 \) (1H, \( m \)) were observed. Furthermore, the presence of a glucopyranosyl moiety was suggested by \(^13\)C-NMR spectrum, which showed six signals at \( \delta 103.1 \) (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 glucoside linkage was determined to be \( \beta \)-form based on the large coupling constant of the anomeric proton signal at \( \delta 4.33 \) (1H, \( d, J = 7.9 \) Hz) in the \(^1\)H-NMR spectrum. The HMBC spectrum revealed a connection between the glucosyl residue and the aglycone: the anomeric proton signal of glucosyl moiety at \( \delta 4.33 \) 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) butan-2-8-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 \([\alpha]D \) value with published data for \( R \)-rhododendrin (Fuchino et al. 1996).

**Acknowledgments**

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**References**


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