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Four glucosides of *p*-hydroxyphenyl derivatives from birch leaves

Received: May 26, 1998 / Accepted: December 11, 1998

Abstract The extractive of shirakamba (*Betula platyphylla* Sukatchev var. *japonica* Hara) leaves was investigated. Four glucosides of *p*-hydroxyphenyl derivatives were isolated, and their structures were indentified as betuloside (I), 3,4'-dihydroxy-propiofenone-3- β -D-glucopyranoside (II), salidoside (III), and arbutin (IV). Arbutin was newly found in the leaves of shirakamba.

Key words Phenolic glucoside · Leaves · Birch · *Betula platyphylla* Sukatchev var. *japonica* Hara

Introduction

In a previous paper¹ we reported the isolation of two lignan rhamnosides in the extractives of the leaves of shirakamba (*Betula platyphylla* Sukatchev var. *japonica* Hara; Betulaceae) and described their structures. In a further study of the extractives of the leaves of the constituents, four glucosides of phenolic compounds were isolated. In a structural explanation of the four phenolic glucosides is provided in this paper (Fig. 1).

Results and discussion

Compound I was positive in diazotized sulfanilic acid (DSA), showing that it was a phenolic compound. The M^+ of compound I was observed at m/z 328, and the acetyl derivative of compound I (IA) gave M^+ at m/z 538. The mass difference between the two molecular ions indicate that compound I has five free hydroxyl groups. The proton nuclear magnetic resonance (¹H-NMR) spectrum of acetate

IA (Table 1) showed an AA'BB' type double doublet at δ 7.19 (2H, d, $J = 8.4$ Hz) and 6.96 (2H, d, $J = 8.4$ Hz) assignable to four aromatic protons of C_{2,6'} and C_{3,5'}, indicating the existence of a 1,4-disubstituted benzene ring in acetate IA. In the two-dimensional homonuclear chemical shift-correlated spectroscopy (¹H-¹H-COSY) of acetate IA, a correlation between two methylene protons at C₁ [δ 2.69 (2H, m)] and C₂ [δ 1.84 (1H, m), 1.72 (1H, m)] and a methine proton C₃ [δ 3.76 (1H, m)] and methyl protons at C₄ [δ 1.12 (3H, d, $J = 6.4$ Hz)] was observed. A singlet at δ 2.27 was assigned to the methyl protons of an acetoxy group on the aromatic ring. The alcoholic acetoxy groups gave four singlets at δ 2.01, 2.03, 2.04, and 2.05.

On the other hand, the presence of a glucopyranosyl moiety in compound I was suggested by a carbon 13 nuclear magnetic resonance (¹³C-NMR) analysis (Table 2), which showed six resonances at δ 103.7 (C-1"), 76.6 (C-2"), 79.6 (C-3"), 73.2 (C-4"), 79.2 (C-5"), and 64.3 (C-6"); and its acetyl derivative IA gave six signals at δ 99.2 (C-1"), 71.6 (C-2"), 73.0 (C-3"), 68.6 (C-4"), 71.7 (C-5"), and 62.1 (C-6").²⁻⁶ 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 4.50 (1H, d, $J = 7.9$ Hz) in the ¹H-NMR spectrum of acetate IA. The long-range connectivities observed in the heteronuclear multiple bond correlation (HMBC) spectrum of acetate IA revealed a connection between the glucosyl residue and the aglycone. The anomeric proton signal at δ 4.50 correlated with the signal of C-3. This result shows that the glucosyl residue is linked to C-3.

Thus, it is concluded that compound I is 3-(4'-hydroxyphenyl) butanol-3- β -D-glucopyranoside (betuloside). Compound I was identified as an R configuration by a direct comparison of the $[\alpha]_D$ value with published data for R-rhododendrin.⁴ Compound I had been obtained from the inner bark of *B. platyphylla*, *B. pendula*, and *B. pubescens* (Betulaceae).^{3,4,7}

Compound II was positive in DSA, indicating that it was a phenolic compound. The M^+ of compound II was observed at m/z 328, and the acetyl derivative of compound II (IIA) gave the M^+ at m/z 496. The mass difference between the two molecular ions indicates the existence of four hy-

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Fig. 1. Structures of the glucosides of *p*-hydroxyphenyl derivatives isolated from the leaves of *Betula platyphylla* Sukatchev var. *japonica* Hara

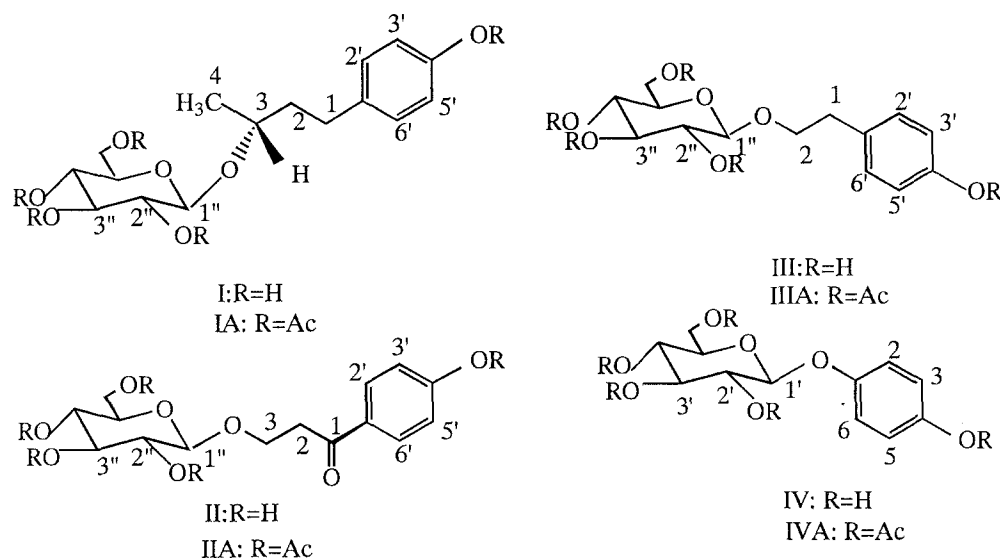


Table 1. $^1\text{H-NMR}$ spectral data of the acetates IA, IIA, IIIA, and IVA^a

Protons	IA	IIA	IIIA	IVA
Aglycone moieties				
Aromatic rings				
H-2', 6' (H-2,6)*	7.19 (2H, d, $J = 8.4$ Hz)	7.87 (2H, d, $J = 8.7$ Hz)	7.20 (2H, d, $J = 8.5$ Hz)	7.00 (4H, s, H-2, 6/3, 5)
H-3', 5' (H-3,5)*	6.96 (2H, d, $J = 8.4$ Hz)	6.86 (2H, d, $J = 8.7$ Hz)	6.98 (2H, d, $J = 8.5$ Hz)	
Phenolic-OAc	2.27 (3H, s)		2.28 (3H, s)	2.29 (3H, s)
Side chains				
H-1	2.69 (2H, m)		2.88 (2H, m)	
H-2	1.84 (1H, m)	3.29 (1H, m)	3.64 (1H, m)	
	1.72 (1H, m)	3.06 (1H, m)	4.14 (1H, m)	
H-3	3.76 (1H, m)	4.04 (1H, m)		
		4.22 (1H, m)		
H-4	1.12 (3H, d, $J = 6.4$ Hz)			
Sugar moieties				
H-1'' (H-1')*	4.50 (1H, d, $J = 7.9$ Hz)	4.58 (1H, d, $J = 8.0$ Hz)	4.47 (1H, m, $J = 8.1$ Hz)	5.03 (1H, d, $J = 7.6$ Hz)
H-2'' (H-2')*	5.00 (1H, dd, $J = 7.9, 9.8$ Hz)	4.95 (1H, dd, $J = 9.6, 8.0$ Hz)	4.99 (1H, dd, $J = 9.6, 8.1$ Hz)	5.27 (2H, m, H-2', 3')
H-3'' (H-3')*	5.21 (1H, t, $J = 9.8$ Hz)	5.20 (1H, t, $J = 9.6$ Hz)	5.18 (1H, t, $J = 9.6$ Hz)	
H-4'' (H-4')*	5.11 (1H, t, $J = 9.8$ Hz)	5.07 (1H, t, $J = 9.6$ Hz)	5.08 (1H, t, $J = 9.6$ Hz)	5.16 (1H, m)
H-5'' (H-5')*	3.66 (1H, m)	3.70 (1H, m)	3.68 (1H, m)	3.83 (1H, m)
H-6'' (H-6')*	4.14 (1H, dd, $J = 2.5, 12.3$ Hz)	4.12 (1H, m)	4.11 (1H, m)	4.16 (1H, dd, $J = 2.9, 12.8$ Hz)
	4.24 (1H, dd, $J = 4.4, 12.3$ Hz)	4.26 (1H, m)	4.25 (1H, dd, $J = 4.9, 12.3$ Hz)	4.28 (1H, dd, $J = 5.4, 12.1$ Hz)
Alcoholic-OAc	2.01–2.05 (12H, m)	1.90–2.07 (12H, m)	1.91–2.09 (12H, m)	2.03–2.08 (12H, m)

s, singlet; d, doublet; dd, doublet doublet; m, multiplet; J , coupling constant; *, acetate IVA

^a At 500 Hz, in CD_3OD , TMS as an internal standard

droxyl groups in compound II. An $^1\text{H-NMR}$ spectrum of the acetate IIA (Table 1) showed four singlets at δ 2.07, 2.02, 1.98, and 1.90, which were assigned to the methyl protons of four alcoholic acetoxy groups. However, no phenolic acetoxy group was observed. The conflicting findings that compound II was positive in DSA and no phenolic acetoxy group was observed in the $^1\text{H-NMR}$ spectrum of acetate IIA suggest that the phenolic hydroxyl group of compound II is resistant to the acetylation procedure. The AA'BB' type double doublet at δ 7.87 (2H, d, $J = 8.7$ Hz) and δ 6.86 (2H, d, $J = 8.7$ Hz) assignable to four aromatic protons of $\text{C}_{2',6'}$, $\text{C}_{3',5'}$ in the $^1\text{H-NMR}$ spectrum indicates the existence of a 1, 4-disubstituted benzene ring. The $^{13}\text{C-NMR}$ spectrum of acetate IIA revealed a signal from a carbonyl group at δ

196.3. The proton signals of $\text{C}_{2',6'}$ of acetate IIA (δ 7.87) in the $^1\text{H-NMR}$ spectrum, compared with those of acetate IA δ 7.19, shifted obviously downfield. The downfield shift was explained by the deshielding effect of the protons of $\text{C}_{2',6'}$ by the carbonyl group located at C_1 . This partial structure was supported further by the occurrence of a fragment ion at m/z 121 due to an $(\text{HO})\text{ArC} \equiv \text{O}^+$ moiety, also showing that a phenolic hydroxyl group is attached to C_4 . In the $^1\text{H-}^1\text{HCOSEY}$ of acetate IIA, a correlation between two methylene protons C_2 [δ 3.06 (1H, m), 3.29 (1H, m)] and C_3 [δ 4.04 (1H, m), 4.22 (1H, m)] was observed.

The presence of a glucopyranosyl moiety in acetate IIA was clarified by comparing the $^{13}\text{C-NMR}$ spectrum (Table 2) with that of acetate IA. The mode of the glucosidic

Table 2. ^{13}C -NMR spectral data of the acetates IA, IIA, IIIA, IVA and compound I^a

C	IA ^b	I ^c	IIA ^b	IIIA ^b	IVA ^b
Aglycone moieties					
Side chains					
C-1	30.7	33.3	196.3	35.3	—
C-2	38.5	42.0	38.1	70.5	—
C-3	74.7	76.6	65.9	—	—
C-4	19.9	19.9	—	—	—
Aromatic rings					
C-1' (C-1)*	139.7	136.1	130.1	136.1	154.5
C-2', 6' (C-2, 6)*	129.5	131.9	130.9	130.0	122.6
C-3', 5' (C-3, 5)*	121.3	117.5	115.4	121.1	118.0
C-4' (C-4)*	148.8	157.7	160.4	149.2	146.3
Sugar moieties					
C-1'' (C-1'')*	99.2	103.7	101.4	100.8	99.6
C-2'' (C-2'')*	71.6	76.6	71.3	71.1	71.2
C-3'' (C-3'')*	73.0	79.9	72.8	72.8	72.7
C-4'' (C-4'')*	68.6	73.2	68.4	68.4	68.3
C-5'' (C-5'')*	71.7	79.2	71.8	71.9	72.1
C-6'' (C-6'')*	62.1	64.3	61.9	61.9	62.0

^a At 125MHz, TMS as internal standard^b Measured in CDCl_3 ^c Measured in CD_3OD

*, Acetate IVA

linkage was determined to be β -form based on the coupling constant of the anomeric proton signal at δ 4.58 (1H, d, $J = 8.0\text{Hz}$) in the ^1H -NMR spectrum of acetate IIA. The long-range connectivities observed in the HMBC spectrum of acetate IIA indicate that the anomeric proton signal at δ 4.58 correlates with the signal of C-3, showing that the location of the glucosyl residue is at the C-3 position. All these results support the idea that compound II is 3,4'-dihydroxypropiofenone-3- β -D-glucopyranoside, which had been obtained from the leaves of *B. alba*⁸ and *B. platyphylla* var. *japonica*.⁵

Compound III was positive in DSA, indicating that it is a phenolic compound. The M^+ of compound III was observed at m/z 300, and the acetyl derivative of compound III (IIIA) gave an M^+ at m/z 510. The mass difference of the two molecular ions indicate that compound III had five free hydroxyl groups. An ^1H -NMR spectrum of the acetate IIIA (Table 1) showed the AA'BB' type double doublet at δ 7.20 (2H, d, $J = 8.5\text{Hz}$) and 6.98 (2H, d, $J = 8.5\text{Hz}$) assignable to four aromatic protons of $\text{C}_{2',6'}$ and $\text{C}_{3',5'}$, indicating the existence of a 1,4-disubstituted benzene ring. In the ^1H - ^1H COSY of acetate IIIA, a correlation between two methylene protons C_2 [δ 3.64 (1H, m), 4.14 (1H, m)] and C_1 [δ 2.88 (2H, m)] was observed. A singlet at δ 2.28 was assigned to the methyl protons of a phenolic acetoxy group. The alcoholic acetoxy groups gave four singlets at δ 2.09, 2.02, 1.96, and 1.91.

The presence of a glucopyranosyl moiety in acetate IIIA was suggested by ^{13}C -NMR analysis (Table 2). The spectrum showed six resonances at δ 100.8 (C-1''), 72.8 (C-3''), 71.9 (C-5''), 71.1 (C-2''), 68.4 (C-4''), and 61.9 (C-6''), which were similar to those of acetate IA. The mode of the glucosidic linkage was determined to be β -form based on the coupling constant of the anomeric proton signal at δ 4.47 (1H, d, $J = 8.1\text{Hz}$) in the ^1H -NMR spectrum of acetate

IIIA. The long-range connectivities observed in the HMBC spectrum revealed the connection between the glucosyl residue and aglycone. The anomeric proton signal at δ 4.47 was correlated with the signal of C-2, which shows that the glucosyl residue was linked to C-2.

Thus, it is concluded that compound III is 2-(4'-hydroxyphenyl) ethanol-2- β -D-glucopyranoside (sali-droside), which had been obtained from the inner bark of *B. platyphylla* Sukatchev var. *japonica* Hara.⁹

Compound IV was obtained as an acetyl derivative IVA. The molecular ion peak (M^+) of the acetate IVA was observed at m/z 482. An ^1H -NMR spectrum of acetate IVA (Table 1) showed a singlet at δ 7.00 (4H) assignable to four aromatic protons that have the same environmental condition. A singlet at δ 2.29 was derived from the methyl protons of an acetoxy group on the aromatic ring.

On the other hand, the existence of a glucopyranosyl moiety was suggested by ^{13}C -NMR analysis of acetate IVA (Table 2). The spectrum six resonances at δ 99.6 (C-1'), 71.2 (C-2'), 72.7 (C-3'), 68.3 (C-4'), 72.1 (C-5'), and 62.0 (C-6') were assigned to those of a glucosyl residue by a comparison of the ^{13}C -NMR spectrum with those of acetate IA. The mode of the glucosidic linkage was determined to be β -form based on the coupling constant of the anomeric proton signal at δ 5.03 (1H, d, $J = 7.6\text{Hz}$) in the ^1H -NMR spectrum of acetate IVA. Placement of the glucosyl residue on the aglycone was verified by use of the HMBC spectrum of acetate IVA. The anomeric proton signal at δ 5.03 correlated with the signal of the aromatic carbon of C-1. This result proves that the glucosyl residue was linked to C-1.

Thus, it is concluded that compound IV is 4-hydroxyphenyl- β -D-glucopyranoside (arbutin), which had been isolated from *Vaccinium vitis-idaea* L (Ericaceae)¹⁰ and *Magnolia stellata* (Magnoliaceae).¹¹ The isolation of arbutin from shirakamba has not been reported so far.

We have isolated five glucosides of *p*-hydroquinone derivatives: 4-hydroxy-2-methoxyphenol-1-O- β -D-glucopyranoside (V), 4-hydroxy-3-methoxyphenol-1-O- β -D-glucopyranoside (VI), and 4-hydroxy-3-methoxyphenol-1-O- β -D-(6'-O-syringoyl) glucopyranoside (VII) from the inner bark of *Ostrya japonica* Sarg.; and 3,4-dimethoxy-5-hydroxyphenol-1-O- β -D-glucopyranoside (VIII) and 3,4,5-trimethoxyphenol-1-O- β -D-glucopyranoside (IX) from the sapwood of birch.^{12,13} Confirmation of arbutin in birch leaves allowed us to report that all four types of the different *p*-hydroquinone glucosides correspond to the *p*-hydroxyphenyl type (P), guaiacyl type (G), G-5-OH, and syringyl type (S) pendant groups of phenylpropanoids in the wood of Betulaceae (Fig. 2).

Four glucosides of *p*-hydroxyphenyl derivatives have been found in many genera, and their formation may be a common phenomenon in plants (Table 3). However, the co-occurrence of $\text{C}_6\text{-C}_4$ (I), $\text{C}_6\text{-C}_3$ (II), $\text{C}_6\text{-C}_2$ (III), and $\text{C}_6\text{-C}_0$ (IV) structural compounds in shirakamba leaves is interesting from the viewpoint of their biogenetic relationship. These compounds may be precursors of arbutin, as all of them contained a *p*-hydroxyphenyl group, as discussed by Hayashi et al.¹⁷

Compound II

Compound II was positive in DSA. Mp 158°–163°C, TLC (CMW, 40:10:1, v/v): Rf 0.2. FD-MS m/z: 328. Acetylation of the compound II gave tetraacetate IIA. TLC (HEA, 1:1, v/v): Rf 0.24. FD-MS m/z: 496 (M⁺). $[\alpha]_D^{26} -19.9$ (c = 0.41 in CDCl₃). UV $\lambda_{\max}^{\text{CHCl}_3}$ nm (log ϵ): 272.5 (4.10). IR ν_{\max}^{KBr} cm⁻¹ (%): 2959 (24), 1751 (100), 1604 (50), 1374 (45), 1225 (59), 1041 (73), and 757 (37). EI-MS m/z: 331, 148, 121, 93, and 43. ¹H-NMR(CDCl₃): see Table 1. ¹³C-NMR(CDCl₃): see Table 2.

Compound III

Compound III was positive in DSA. TLC (CMW, 40:10:1, v/v): Rf 0.25. FD-MS m/z: 300 (M⁺). Acetylation of compound III gave pentaacetate IIIA. TLC (HEA, 1:1, v/v): Rf 0.56. FD-MS m/z: 510 (M⁺). $[\alpha]_D^{25} -9.24$ (c = 0.24 in CDCl₃). UV $\lambda_{\max}^{\text{EtOH}}$ nm (log ϵ): 240 (3.89) and 270 (3.62). IR ν_{\max}^{KBr} cm⁻¹ (%): 2925 (74), 1751 (100), 1509 (30), 1370 (45), 1221 (57), 1041 (63), and 909 (26). ¹H-NMR (CDCl₃): see Table 1. ¹³C-NMR(CDCl₃): see Table 2.

Compound IV

Compound IV was positive in DSA. TLC (CMW, 40:10:1, v/v): Rf 0.12. Acetylation of compound IV gave pentaacetate IVA. TLC (HEA, 1:1, v/v): Rf 0.45. FD-MS m/z: 482 (M⁺). $[\alpha]_D^{25} -7.99$ (c = 0.24 in CDCl₃). UV $\lambda_{\max}^{\text{CHCl}_3}$ nm (log ϵ): 240 (3.22) and 282 (2.93). IR ν_{\max}^{KBr} cm⁻¹ (%): 2924 (69), 1756 (100), 1504 (52), 1371 (45), 1225 (74), 1046 (59), and 909 (27). EI-MS m/z: 331, 271, 229, 211, 169, 152, 127, 109, and 43. ¹H-NMR(CDCl₃): see Table 1. ¹³C-NMR(CDCl₃): see Table 2.

Acknowledgments The authors are grateful to Dr. E. Fukushi and Mr. K. Watanabe, GC-MS and NMR Laboratory, Faculty of Agriculture, Hokkaido University for their helpful advice on the NMR and mass spectral measurements; and to Dr. Y. Uraki and Dr. Y. Ozawa, Laboratory of Wood Chemistry, Faculty of Agriculture, Hokkaido University for their help with the infrared and specific rotation measurements. The study was supported by a Grant-in-Aid from the Ministry of Education of Japan (0556026).

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