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NOTE

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Dihydroroseoside, a new cyclohexanone glucoside, from the leaves of shirakamba (*Betula platyphylla* Sukatchev var. *japonica* Hara)

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Abstract A new cyclohexanone glucoside (**II**) and a known cyclohexenone glucoside roseoside [**I**, (6*S*, 9*S*)-6-hydroxy-6-(9-β-D-glucopyranosyloxy-*trans*-7-butenyl)-1,5,5-trimethyl-1-cyclohexenone] were isolated from an ethanol extract of shirakamba (*Betula platyphylla* Sukatchev var. *japonica* Hara) leaves. The structure of **II** was determined to be (6*S*, 9*R*)-6-hydroxy-6-(9-O-β-D-glucopyranosyloxy-*trans*-7-butenyl)-1,5,5-trimethyl-1-cyclohexanone by ^{1}H-NMR and ^{13}C-NMR spectroscopic analyses. It was named dihydroroseoside.

Key words Roseoside · Dihydroroseoside · Vomifoliol · Dihydrovomifoliol · Leaves · Shirakamba · *Betula platyphylla* Sukatchev var. *japonica* Hara

Introduction

We studied extractives from the leaves of shirakamba (*Betula platyphylla* Sukatchev var. *japonica* Hara) to obtain basic information on their chemical components so we could utilize the leaves as a herbal tea.[1,2] We reported the isolation and structural determination of two lignan rhamnosides[1] and four *p*-hydroxyphenyl derivatives[2] from the leaves. In another study of the extractives of the leaves, two sesquiterpene glucosides were isolated. This paper deals with the structural determination of the two glucosides (Fig. 1).

Results and discussion

Compound **I** (M^+ 386) was isolated as a colorless oil and in a crystalline state after acetylation as a tetraacetate **IA** (M^+ 554, $C_{27}H_{38}O_{12}$). Compound **I** was identified as a known compound of 6-hydroxy-6-(9β-D-glucopyranosyloxy-*trans*-7-butenyl)-1,5,5-trimethyl-1-cyclohexenone (roseoside). The ^{1}H-NMR (nuclear magnetic resonance) spectral data of the tetraacetate **IA** is similar to that of the acetate of roseoside isolated from the needles of *Pinus sylvestris* (Pinaceae, A).[3] Two olefinic protons of the side chain of **IA** was confirmed by ^{1}H-NMR signals at C_8 [δ5.68 (1H, dd)] and at C_7 [δ5.75 (1H, d)] with J = 15.8 Hz indicating the *trans* relation, and an olefinic proton of the —CO—C*H*=C(CH_3)— group was attributed to a singlet at C_2 [δ5.93 (1H, s)]. Roseoside has been previously found also in the leaves and stems of *Vinca rosea* (Apocynaceae),[4] the dried seeds of *Astragulus complanatus* (Leguminosae, G),[5] and the leaves of *Alangium premnifolium* (Alangiaceae, B, C, and G).[6] The aglucone of the roseoside is vomifoliol, which has been found in the leaves of *Magnolia stellata* (Magnoliaceae),[7] *Betula alba* (Betulaceae),[8] and *Rauwolfia vomitoria* (Apocynaceae).[9] The assignment of the chemical shift of ^{13}C-NMR spectra data of roseoside (as acetate, **IA**) has not yet been reported (Table 1).

Compound **II** (M^+ 388) was isolated as colorless oil and in a crystalline state after acetylation as a tetraacetate **IIA** (M^+ 556, $C_{27}H_{40}O_{12}$). Acetate **IIA** was found to have a molecular formula with two hydrogen atoms less than that of acetate **IA**. The ^{1}H-NMR and ^{13}C-NMR spectral data of acetate **IIA** were similar to those of acetate **IA** except for the absence of the signal derived from an olefinic proton and carbon. Two olefinic protons in the side chain (C_7, C_8) was confirmed by ^{1}H-NMR signals at C_8 [δ5.83 (1H, dd)] and at C_7 [δ5.67 (1H, d)] with J = 16.0 Hz indicating the *trans* relation. Protons of methylene signals of C_2 [δ2.43 (1H, m), 2.21 (1H, m)] and a methine signal of C_1 [δ2.21 (1H, m)] are observed instead of the olefinic proton at C_2 of the partial structure of the —CO—C*H*=C(CH_3)— group of acetate **IA**, indicating that a double bond at C_2 of acetate **IA** was saturated. The structural relation between C_1 and C_2 of acetates **IA** and **IIA** was confirmed by ^{13}C-NMR spectral data (Table 1). Thus, the structure of compound **II** was clarified to be 6-hydroxy-6-(9-O-β-D-glucopyranosyloxy-*trans*-7-butenyl)-1,5,5-trimethyl-1-cyclohexanone, which to

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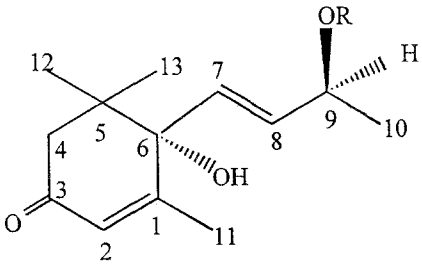
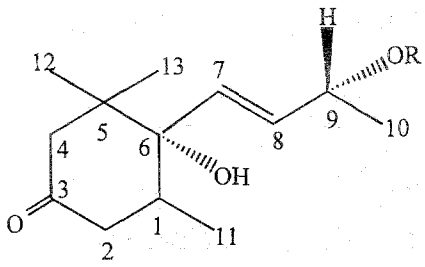
Fig. 1. Structures of cyclohexenone, cyclohexanone, and their glucosides isolated from the leaves of *Betula platyphylla* Sukatchev var. *japonica* Hara



(6*S*, 9*S*)

Ia: R = H, vomiforiol
I: R = β-D-glucopyranose, roseoside
IA: R = β-D-glucopyranose (Ac4)



(6*S*, 9*R*)

IIa: R = H, dihydrovomiforiol
II: R = β-D-glucopyranose, dihydroroseoside
IIA: R = β-D-glucopyranose (Ac4)

Table 1. ^{1}H- and ^{13}C-NMR spectral data of acetates **IA** and **IIA**

	IA		**IIA**	
	H	C	H	C
Aylycon moieties				
1	–	162.0	2.21 (1H, m)	36.3
2	5.93 (1H, s)	127.0	2.43 (1H, m); 2.21 (1H, m)	45.2
3	–	197.4	–	211.3
4	2.28 (1H, d, J = 17.2 Hz)	49.8	1.90 (1H, d, J = 13.4 Hz)	51.4
	2.44 (1H, d, J = 17.2 Hz)		2.85 (1H, d, J = 13.4 Hz)	–
5	–	41.1	–	43.0
6	–	79.1	–	76.9
7	5.75 (1H, d, J = 15.8 Hz)	132.3	5.67 (1H, d, J = 16.0 Hz)	132.3
8	5.68 (1H, dd, J = 6.7, 15.8 Hz)	131.8	5.83 (1H, dd, J = 6.2, 16.0 Hz)	132.4
9	4.36 (1H, t, J = 6.7 Hz)	74.8	4.30 (1H, t, J = 6.7 Hz)	77.2
10	1.31 (3H, d, J = 6.7 Hz)	22.0	1.28 (3H, d, J = 6.7 Hz)	21.3
11	1.93 (3H, d, J = 1.2 Hz)	18.1	0.86 (3H, d, J = 7.1 Hz)	15.9
12	1.01 (3H, s)	22.8	0.92 (3H, s)	19.5
13	1.10 (3H, s)	24.2	0.94 (3H, s)	24.5
Sugar moieties				
1′	4.50 (1H, d, J = 7.9 Hz)	98.3	4.58 (1H, d, J = 7.9 Hz)	99.8
2′	4.97 (1H, dd, J = 8.1, 9.4 Hz)	71.5	4.99 (1H, dd, J = 8.1, 9.6 Hz)	71.7
3′	5.15 (1H, t, J = 9.4 Hz)	73.0	5.20 (1H, t, J = 9.6 Hz)	72.9
4′	5.08 (1H, t, J = 9.4 Hz)	68.4	5.19 (1H, t, J = 9.6 Hz)	68.3
5′	3.62 (1H, m)	71.9	3.72 (1H, m)	71.9
6′	4.13 (1H, dd, J = 2.5, 12.3 Hz)	62.0	4.15 (1H, dd, J = 2.2, 12.3 Hz)	61.7
	4.25 (1H, dd, J = 4.9, 12.3 Hz)		4.21 (1H, dd, J = 4.2, 12.3 Hz)	
Alcoholic-OAc	2.01–2.05 (12H, m)		2.01–2.09 (12H, m)	

Conditions: in $CDCl_3$, TMS as an internal standard, δ, ppm

our knowledge has not been reported so far; we named it dihydroroseoside. The aglucone of the new glucoside **II** is therefore named dihydrovomifoliol (**IIa**). The ^{13}C-NMR data of dihydroroseoside acetate **IIA** is shown in Table 1. The isolation of the glucosides of cyclohexanone and cyclohexenone from shirakamba leaves has not yet been reported. The aglucons of the two isolated compounds (**I** and **II**) belonged to sesquiterpenes related to abscisic acid (C15), although the carbon number is C13. Biogenesis of the aglucons might be β-oxidation of the side chain of abscisic acid.

The absolute configurations at C-9 of β-D-glucopyranosides of 3-oxo-α-ionol moiety (E and F in Table 2) were established by Pabst et al. using the Heluchen method, which was developed to determine the absolute configuration of chiral secondary alcohols.[10] The ^{13}C-NMR chemical shifts of the C-9 of β-D-glucopyranoside of (9*S*)-3-oxo-α-ionol and (9*R*)-3-oxo-α-ionol are δ74.7 and 77.0, respectively (Table 2). The difference of the chemical shifts reflects the stereochemistry of C_9, precisely. The relation of the ^{13}C-NMR chemical shifts and absolute configurations of the secondary alcohols (E and F) was used by Otsuka et al.[6] to determine the absolute configuration of the stereoisomers of roseoside (A, B, C, G) as shown in Table 2 (Fig. 2). Applying the relation of the absolute configurations (*R* or *S*) and ^{13}C-NMR data (Table 2), the absolute configurations of the C-9 positions of acetates **IA** (δ74.8) and **IIA** (δ77.2) were assigned as 9*S* and 9*R*, respectively.

The positive values in $[\alpha]_D$ of acetates **IA** and **IIA** indicate that the absolute configurations at C-6 of acetates **IA**

Table 2. Relation between ^{13}C-NMR chemical shifts and absolute configuration of C-9

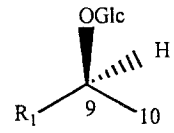
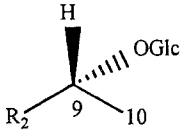
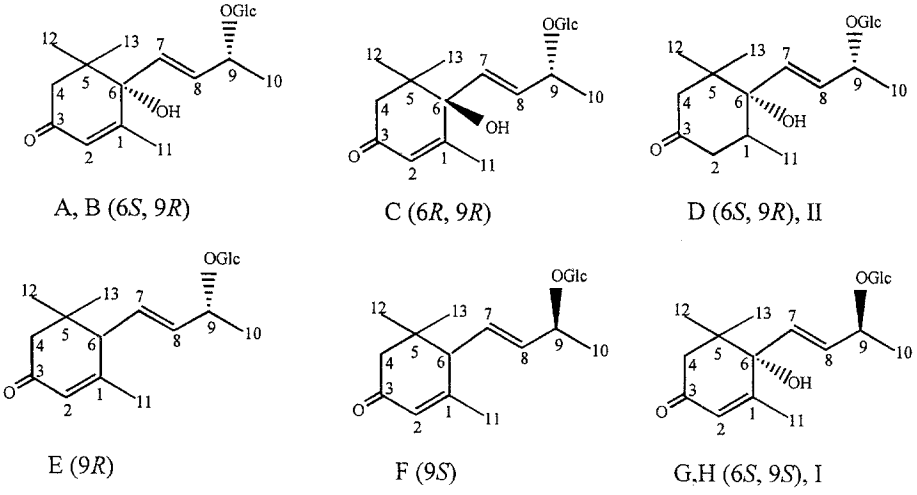
Compound	Chemical shift of C-9		Reference
			
	9*S*	9*R*	
A: Roseoside (6*S*, 9*R*)	–	77.3	3
B: Roseoside (6*S*, 9*R*)	–	77.0	6
C: Roseoside (6*R*, 9*R*)	–	77.3	6
D: Dihydroroseoside (6*S*, 9*R*), **IIA**	–	77.2	This study
E: 3-Oxo-α-ionol-β-D-glucopyranoside (9*R*)	–	77.0	10
F: 3-Oxo-α-ionol-β-D-glucopyranoside (9*S*)	74.7	–	5, 10
G: Roseoside (6*S*, 9*S*)	73.2	–	5, 6
H: Roseoside (6*S*, 9*S*), **IA**	74.8	–	This study

Fig. 2. Structures of the compounds A–H



and **IIA** were both *S*, because roseoside with the *R* configuration showed a strong negative value in $[\alpha]_D$.[6,11]

The aglucone of compound **I**, (+)vomifoliol, has been reported to exhibit properties similar to those of (±)abscisic acid on the stomatal aperture in epidermal strips from *Eichhornia crassipes* and is supposed to play an important role as an endogenous regulator of the stomatal aperture.[12] The physiological role of roseoside (**I**) and dihyroroseoside (**II**) in the leaves of shirakamba is not known and is an interesting problem to be solved in the future.

Experiment

All spectroscopic and chromatographic methods in this work are the same as those described previously.[1]

Isolation of compounds

Shirakamba leaves (2.72kg) were collected in September 1995 at the Sapporo nursery garden of Hokkaido University Experimental Forestry. The leaves were extracted 3 times with 95% ethanol (EtOH) at room temperature for 24h each time. The EtOH solutions were combined and concentrated to a syrup (279.2g) under reduced pressure. Then a part of the syrup (66.33g) was extracted successively with ethyl acetate (EtOAc), EtOAc saturated with water, and EtOH. The yields of the EtOAc-soluble, EtOAc saturated with water-soluble, and EtOH-soluble fractions were 15.7, 8.1, and 25.4g, respectively.

The fraction soluble in EtOAc saturated with water (8.1g) was chromatographed on a silica gel (Wakogel C-200) column using an eluent solution (EtOAc saturated with water). Each fraction was collected in 500-ml portions, and 55 fractions (f1–f55) were obtained. The fractions were combined to form six fractions [F1 (f1–f9, 2.53g), F2 (f10–f15, 0.75g), F3 (f16–f20, 0.68g), F4 (f21–f30, 0.78g), F5 (f31–f43, 0.48g), F6 (f44–f55, 0.23g)] by monitoring with thin-layer chromatography (TLC) using CMW [chloroform ($CHCl_3$)/methanol (MeOH)/H_2O, 40:10:1 v/v] as a developing solvent.

Fraction F5 (f31–f43, 0.48g) was rechromatographed on a silica gel column using CMW (60:10:1 v/v) as an eluting solvent. Each fraction was collected in 30-ml portions; com-

pounds **I** (5.1 mg) and **II** (4.3 mg) were obtained from f82–85 and f107–113, respectively.

Acetylations of the compounds were conducted with acetic anhydride and pyridine at 55°C for 24 h. The acetylation products were purified by a preparative TLC with HEA (*n*-hexane/EtOAc, 1:1 v/v), and the purified acetates **I**A (3.7 mg) and **II**A (2.0 mg) were obtained.

Compound **I**

Compound **I**: TLC (CMW 40:10:1 v/v): Rf 0.30, M^+ 386. Tetraacetate **I**A: TLC (HEA 1:1 v/v): Rf 0.36, M^+ 554. EI-HR-MS: 554.2325 (calculated for $C_{27}H_{38}O_{12}$: 554.5900). $[\alpha]_D^{25}$ + 48.0° (c = 0.13 in $CHCl_3$). UV: λ_{max}^{EtOH} 232 nm. IR: ν_{max}^{KBr} 3449 (OH), 1755 (OAc), 1655 (enone) cm^{-1}, ^{1}H-NMR and ^{13}C-NMR ($CDCl_3$): see Table 1.

Compound **II**

Compound **II**: TLC (CMW 40:10:1 v/v): Rf 0.35, M^+ 388. Tetraacetate **II**A: TLC (HEA 1:1 v/v): Rf 0.44, M^+ 556. EI-HR-MS: 556.2496 (calculated for $C_{27}H_{40}O_{12}$: 556.6058). $[\alpha]_D^{25}$ + 4.4° (c = 0.32 in $CHCl_3$). UV: λ_{max}^{EtOH} 217 nm. IR: ν_{max}^{KBr} 3449 (OH), 1755 (OAc) cm^{-1}. ^{1}H-NMR and ^{13}C-NMR ($CDCl_3$): see Table 1.

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