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TRITERPENE GLYCOSIDES OF *Fatsia japonica*. VI. STRUCTURES OF GLYCOSIDES D_{3a} AND D_{3b}

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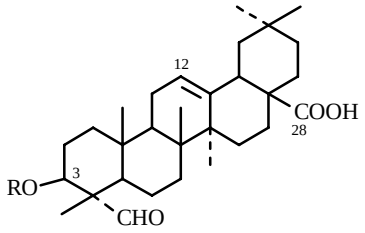
Seeds of Fatsia japonica *(Araliaceae) yielded the new glycosides of gypsogenin: 3-O-β-D-glucopyranosyl-(1→2)-O-β-D-glucopyranoside and 3-O-β-D-galactopyranosyl-(1→2)-O-β-D-glucopyranoside. The structures of these compounds were established by chemical methods and NMR spectroscopy.*

Key words: *Fatsia japonica*, Araliaceae, triterpene glycosides, gypsogenin glycosides.

We have previously described the isolation of glycoside D_3 (**1**) from glycoside fraction D of *Fatsia japonica* seeds [1]. TLC analysis of **1** in various solvent systems showed a chromatographically pure glycoside. Total acid hydrolysis of **1** detected the aglycone gypsogenin, which was identified by chromatographic mobility compared with an authentic sample, and a saccharide containing glucose and galactose in an approximately 7:1 ratio. Comparison of the relative chromatographic mobilities of **1** and several other known glycosides indicate that it is a gypsogenin bioside. Alkaline hydrolysis of **1** does not cause any changes in its chromatographic mobility. Furthermore, **1** is methylated by diazomethane in ether. Therefore, it is a monodesmoside glycoside of gypsogenin with the carbohydrate chain on C-3 of the aglycone.



1a, b

1a: R = *β*-D-Glc*p*-(1→2)-O-*β*-D-Glc*p*→
1b: R = *β*-D-Gal*p*-(1→2)-O-*β*-D-Glc*p*→

The ^{13}C NMR spectrum of **1** in the range 95-110 ppm revealed four signals for anomeric C atoms. Of these, signals at δ 105.2 and 102.7 ppm were approximately three times as strong as those at δ 105.9 and 102.8 ppm. The results lead to the conclusion that glycoside D_3 is a chromatographically inseparable mixture of two gypsogenin glycosides denoted D_{3a} (**1a**) and D_{3b} (**1b**) in approximately a 3:1 ratio. Then it is obvious that total acid hydrolysis is consistent with the predominant glycoside having two glucose units; the minor one, glucose and galactose. Additional chemical confirmation of the nature of the aglycone in glycosides **1a** and **1b** was provided by borohydride reduction of the assumed aldehyde to a primary alcohol. As a result, a chromatographically inseparable mixture of glycosides of lower chromatographic mobility was produced. Only hederagenin was found in the acid hydrolysate of the reduction products. The chromatographic mobilities of these compounds agree with those of previously described glycoside F from *F. japonica* seeds [2], which is hederagenin 3-O-*β*-sophoroside.

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TABLE 1. ^{13}C and ^{1}H Chemical Shifts of Aglycones and Carbohydrates of Glycosides D_{3a} (**1**) and D_{3b} (**2**) (δ, ppm, 0 = TMS, C_5D_5N)

Atom	**1**		**2**	
	^{13}C	^{1}H	^{13}C	^{1}H
		Aglycone		
1	38.0	1.60; 1.05	38.0	1.60; 1.05
2	24.6	2.36; 2.01	24.7	2.36; 2.01
3	83.0	4.20	83.1	4.26
4	55.1	-	55.0	-
5	47.8	1.48	47.8	1.48
6	20.2	1.56; 1.13	20.3	1.56; 1.13
7	33.1	1.55; 1.27	33.1	1.55; 1.27
8	39.9	-	39.9	-
9	47.9	1.83	47.9	1.83
10	36.2	-	36.2	-
11	23.7	2.03; 2.05	23.7	2.03; 2.05
12	122.1	5.64	122.1	5.64
13	144.9	-	144.9	-
14	42.1	-	42.1	-
15	28.2	2.25; 1.28	28.2	2.25; 1.28
16	23.6	2.25; 2.10	23.6	2.25; 2.10
17	46.7	-	46.7	-
18	42.0	3.43	42.0	3.43
19	46.5	1.96; 1.45	46.5	1.96; 1.45
20	30.9	-	30.9	-
21	34.2	1.60; 1.36	34.2	1.60; 1.36
22	32.4	2.17; 1.96	32.4	2.17; 1.96
23	208.8	10.01	209.0	10.09
24	10.6	1.55	10.8	1.57
25	15.6	0.96	15.6	0.95
26	17.3	1.09	17.3	1.09
27	26.1	1.45	26.1	1.45
28	180.8	-	180.8	-
29	33.2	1.15	33.2	1.15
30	23.8	1.19	23.8	1.19
		Carbohydrate		
1	102.7	4.79	102.8	4.76
2	82.5	4.06	82.5	4.03
3	78.3	4.25	78.2	4.19
4	71.1	4.06	71.1	4.01
5	78.1	3.86	78.0	3.83
6	62.5	4.47; 4.26	62.5	4.46; 4.25
	Glc″		Gal′	
1	105.2	5.26	105.9	5.15
2	76.6	4.09	74.2	4.48
3	77.9	4.21	74.8	4.10
4	71.2	4.25	70.0	4.53
5	78.3	3.96	78.2	3.79
6	62.6	4.55; 4.41	62.0	4.49; 4.26

The structures of **1a** and **1b** were established using a combination of one-dimensional ^{1}H- and ^{13}C-NMR spectra and two-dimensional COSY, TOCSY, HSQC, and ROESY spectra. First, HSQC spectra showed doublets for anomeric protons

corresponding to those found in the ^{13}C-NMR spectra for the anomeric C atoms. TOCSY, COSY, and HSQC spectra enabled signals of the remaining skeletal protons and C atoms of the four monosaccharide residues to be assigned taking into account the relative intensity of the C signals for **1a** and **1b** and the areas of the cross-peaks. Considering the nature of the splitting and the magnitude of the spin—spin coupling constants (SSCC), these are three β-glucopyranose and one β-galactopyranose units. Then it is obvious that the carbohydrate part of **1a** is β-Glc*p*-β-Glc*p*; of **1b**, β-Gal*p*-β-Glc*p*. A comparison of the chemical shifts of the signals for C atoms in the ^{13}C-NMR spectrum of **1** and values found in the literature [2, 3] shows that one of the glucose units (in **1a**) and the galactose (in **1b**) are terminal (unsubstituted) whereas the other two glucose units (in **1a** and **1b**) are substituted (glycosylated) at C-2 because these atoms show positive α-effects (~6 ppm) compared with the unsubstituted glucose.

Thus, the disaccharides of **1a** and **1b** are β-D-glucopyranosyl-(1→2)-O-β-D-glucopyranosyl and β-D-galactopyranosyl-(1→2)-O-β-D-glucopyranosyl fragments.

The sequence of monosacchride bonding, the type of glycoside bond between them, and the site of attachment of the carbohydrate chain to the aglycone were confirmed as follows. The two-dimensional ROESY spectrum of **1** contains cross-peaks between the anomeric protons of terminal glucose (for **1a**) and galactose (in **1b**) and H-2 protons of the inner glucoses and between the anomeric protons of the inner glucoses (in **1a** and **1b**) and H-3 of the aglycone. The HMBC spectrum contains cross-peaks between anomeric protons of terminal glucose or galactose and C-2 of the inner glucoses and between anomeric protons of the inner glucoses and C-3 of the aglycone. Chemical shifts of ^{1}H and ^{13}C atoms of the carbohydrate parts of **1a** and **1b** are listed in Table 1.

Final confirmation of the nature of the aglycone in **1a** and **1b** was obtained by analyzing ^{13}C-NMR and two-dimensional HSQC and COSY spectra of **1**. Thus, a signal for a C atom at 208.8 ppm and a proton singlet at 10.01 ppm unambiguously confirm that the aglycone contains an aldehyde; signals at 122.1 and 144.9 ppm, a triply substituted double bond in an oleanolic acid derivative [4]. Remaining signals for C atoms of the aglycone were unambiguously assigned by combining COSY and HSQC analyses. This is based on the relatively low-field position of reference protons H-12, H-3, and H-18 and the isolated spin systems H-1—H-3, H-5—H-7, H-9—H-12, H-15—H-16, H-18—H-19, and H-21—H-22 in the aglycone. Signal assignments (Table 1) agree with literature data for chemical shifts of ^{13}C atoms of the aglycone for gypsogenin glycosides [5]. It should be noted that signals for C atoms of the aglycone (C-2, C-3, C-6, and C-24) in the immediate vicinity of the carbohydrate are doubled in an approximately 3:1 ratio. Therefore, the signals of these atoms are unambiguously assigned to glycosides **1a** or **1b**. Chemical shifts of the remaining C atoms of the aglycones of both glycosides are identical.

Thus, glycosides D_{3a} and D_{3b} are gypsogenin 3-O-β-D-glycopyranosyl-(1→2)-O-β-D-glucopyranoside and 3-O-β-D-galactopyranosyl-(1→2)-O-β-D-glucopyranoside.

EXPERIMENTAL

General comments and the hydrolysis method have been published [2]. The isolation method of glycoside D_3 from fraction D has been described [1].

Borohydride reduction was performed by dissolving glycoside (10 mg) in anhydrous methanol (2 mL), adding $NaBH_4$ (100 mg), and holding the mixture at room temperature for 10 h with constant stirring and TLC monitoring. After the reduction was complete (disappearance of starting material and formation of a product of lower chromatographic mobility), the solvent was removed in vacuo. The solid was dissolved in water-saturated butanol and washed three times with water. The butanol layer was evaporated to dryness and analyzed by TLC with an authentic sample of hederagenin 3-O-β-sophoroside [2].

The total acid hydrolysate of **1** contained glucose and galactose in an approximately 7:1 ratio and gypsogenin. The total acid hydrolysate of the borohydride-reduction product of **1** contained the same sugars and hederagenin.

Chemical shifts for ^{1}H and ^{13}C atoms of the carbohydrate and aglycone parts of **1a** and **1b** are listed in Table 1.

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