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# TRITERPENE GLYCOSIDES OF *Fatsia japonica*. IV. STRUCTURE OF GLYCOSIDES $D_1$ AND $D_2$ FROM SEEDS

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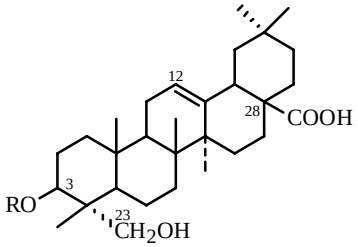
*Seeds of* Fatsia japonica *(Araliaceae) afforded the known hederagenin 3-O-β-D-glucopyranosyl-(1→2)-O-α-L-arabinopyranoside and the new triterpene glycoside $D_2$, for which the structure hederagenin 3-O-β-D-galactopyranosyl-(1→2)-O-α-L-arabinopyranoside was proposed based on chemical methods and NMR spectroscopy.*

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

We report results on isolation of glycosides $D_1$ and $D_2$ from fraction D obtained from seeds of *Fatsia japonica* [1] and determination of their structures. Fraction D was rechromatographed over highly effective microspherical silica gel Silpearl. Chromatographically pure glycosides $D_1$, $D_2$, and $D_3$ were obtained. According to preliminary $^1H$ and $^{13}C$ NMR spectra of signals for the anomeric C atoms and protons, glycosides $D_1$ and $D_2$ were pure compounds whereas $D_3$ was a chromatographically inseparable mixture of two triterpene glycosides.




**1:** R = β-D-Glc*p*″-(1→2)-O-α-L-Ara*p*′→
**2:** R = β-D-Gal*p*″-(1→2)-O-α-L-Ara*p*′→

Glycoside $D_1$ (**1**) was identified as hederagenin 3-O-β-D-glucopyranosyl-(1→2)-O-α-L-arabinopyranoside by comparing its chromatographic mobility with that of a known sample that we isolated previously from leaves of *F. japonica* [2]. Acid hydrolysis confirmed the composition of $D_1$. Alkaline hydrolysis and methylation with diazomethane revealed the site of attachment of the carbohydrate chain. The $^{13}C$ NMR spectrum of **1** is identical to that of glycoside F from leaves of *F. japonica* [2], which confirms the proposed structure. Additional confirmation of the structure, in particular the 1→2 bond between the monosaccharides, was obtained by complete assignment of PMR signals for the carbohydrate based on the two-dimensional (2D) COSY spectrum and unambiguous assignment of $^{13}C$ NMR signals for the carbohydrate based on the 2D HSQC spectrum (Table 1). A positive α-effect on C-2 of arabinose from the 1→2 bond and cross-peaks between glucose H-1 and arabinose H-2 and between arabinose H-1 and aglycone H-3 in the ROESY spectrum are observed, as expected. $^{13}C$ NMR signals for the aglycone of **1** were assigned by comparison with previous results [2] and other literature data for 3-substituted hederagenin [3] and are listed in Table 2.

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TABLE 1. Chemical Shifts of $^{13}C$ and $^{1}H$ in Carbohydrates of Glycosides $D_1$ (**1**) and $D_2$ (**2**) (δ, ppm, 0 = TMS, $C_5D_5N$)

| Atom | **1** | | Atom | **2** | |
|---|---|---|---|---|---|
| | $^{13}C$ | $^{1}H$ | | $^{13}C$ | $^{1}H$ |
| Ara′ | | | Ara′ | | |
| 1 | 103.9 | 5.17 | 1 | 103.8 | 5.14 |
| 2 | 80.6 | 4.57 | 2 | 81.0 | 4.55 |
| 3 | 73.5 | 4.28 | 3 | 73.5 | 4.25 |
| 4 | 68.3 | 4.32 | 4 | 68.3 | 4.30 |
| 5 | 65.0 | 4.25; 3.70 | 5 | 64.3 | 4.22; 3.64 |
| Glc′′ | | | Gal′′ | | |
| 1 | 105.4 | 5.18 | 1 | 106.2 | 5.05 |
| 2 | 76.0 | 4.05 | 2 | 73.6 | 4.50 |
| 3 | 78.0 | 4.25 | 3 | 75.0 | 4.09 |
| 4 | 71.4 | 4.15 | 4 | 69.7 | 4.55 |
| 5 | 78.2 | 3.80 | 5 | 76.8 | 3.95 |
| 6 | 62.6 | 4.45; 4.28 | 6 | 61.6 | 4.42; 4.34 |

TABLE 2. Chemical Shifts of $^{13}C$ in Aglycones of Glycosides $D_1$ (**1**) and $D_2$ (**2**) (δ, ppm, 0 = TMS, $C_5D_5N$)

| C-atom | Compound | | C-atom | Compound | |
|---|---|---|---|---|---|
| | **1** | **2** | | **1** | **2** |
| **1** | 38.7 | 38.7 | **16** | 23.7 | 23.7 |
| **2** | 25.9 | 25.8 | **17** | 46.7 | 46.7 |
| **3** | 82.2 | 81.9 | **18** | 42.0 | 42.0 |
| **4** | 43.5 | 43.4 | **19** | 46.5 | 46.5 |
| **5** | 47.5 | 47.5 | **20** | 30.9 | 30.9 |
| **6** | 18.1 | 18.1 | **21** | 34.2 | 34.2 |
| **7** | 32.8 | 32.8 | **22** | 33.2 | 33.2 |
| **8** | 39.7 | 39.7 | **23** | 64.9 | 64.6 |
| **9** | 48.1 | 48.1 | **24** | 13.4 | 13.5 |
| **10** | 36.9 | 36.9 | **25** | 16.0 | 16.0 |
| **11** | 23.8 | 23.8 | **26** | 17.5 | 17.5 |
| **12** | 122.5 | 122.5 | **27** | 26.2 | 26.2 |
| **13** | 144.9 | 144.9 | **28** | 180.5 | 180.5 |
| **14** | 42.2 | 42.2 | **29** | 33.3 | 33.3 |
| **15** | 28.3 | 28.3 | **30** | 23.8 | 23.8 |

The chromatographic mobility of glycoside $D_2$ (**2**) did not identify it as any of the glycosides from leaves or fruit pericarp of *F. japonica*. Total acid hydrolysis of **2** produced galactose, arabinose, and hederagenin. Alkaline hydrolysis and treatment with diazomethane in ether indicated that **2** is a monodesmoside glycoside with the carbohydrate chain on C-3 of the aglycone. Partial acid hydrolysis of **2** has produced galactose and hederagenin 3-O-*α*-L-arabinopyranoside, which defines the bonding sequence of the monosaccharides. The structure of **2** was further established using various NMR spectroscopy methods.

Two signals of anomeric C atoms were easily found in the $^{13}C$ NMR of **2**; two doublets of anomeric protons in the PMR spectrum. This confirms that **2** is a bioside. Signals of the remaining skeletal protons of the monosaccharides were assigned based on TOCSY and COSY spectra. The splitting patterns and spin—spin coupling constants indicated that they were *β*-galactopyranose and α-arabinopyranose. Signals for C atoms of the carbohydrates were completely assigned based on the two-dimensional HSQC spectrum (Table 1). It was confirmed that the galactose is terminal (unsubstituted). Chemical shifts of its

C atoms agree well with literature data [4]. The arabinose is substituted on C-2 because this atom exhibits a positive $\alpha$-effect compared with unsubstituted arabinose [2, 3]. The type of bond between monosaccharides was independently confirmed by analysis of ROESY and HMBC spectra. The cross-peaks between galactose H-1 and arabinose H-2 and between galactose H-1 and arabinose C-2 were unambiguously identified. Thus, the carbohydrate of **2** is a 3-O-$\beta$-D-galactopyranosyl-(1→2)-O-$\alpha$-L-arabinopyranosyl fragment. Chemical shifts of C atoms in the aglycone of **2** were assigned by analogy with **1** and are identical to 3-substituted hederagenin [2, 3]. Attachment of the carbohydrate chain at the C-3 hydroxyl also follows from the ROESY and HMBC spectra in which cross-peaks between arabinose H-1 and hederagenin H-3 and between arabinose H-1 and hederagenin C-3 were found. Therefore, **2** is hederagenin 3-O-$\beta$-D-galactopyranosyl-(1→2)-O-$\alpha$-L-arabinopyranoside and is a new triterpene glycoside.

It is interesting that seeds of *Hedera helix* and *H. taurica* contain exclusively glycosides with glucose bound directly to aglycone C-3 [5-8] whereas seeds of *F. japonica* contain also glycosides in which the aglycone is bound to arabinose, like in fruit pericarp [8].

## EXPERIMENTAL

General comments on the hydrolysis methods and preparation of fraction D have been published [1].

Fraction D (160 mg) was separated over a Silpearl (200 g, Chemapol, Czech Rep.) silica-gel column with elution by water-saturated $CHCl_3$—$C_2H_5OH$ (7:3) to give glucosides $D_1$ (**1**, 40 mg), $D_2$ (**2**, 70 mg), and $D_3$ (50 mg). Acid hydrolysis of **1** gave glucose, arabinose, and hederagenin; of **2**, galactose, arabinose, and hederagenin. Alkaline hydrolysis conditions have no effect on **1** and **2** (TLC monitoring using $CHCl_3$—$CH_3OH$—$H_2O$, 100:30:5). Treatment of **1** and **2** with diazomethane in ether converted them to the methyl esters with greater chromatographic mobility (TLC monitoring). Tables 1 and 2 list chemical shifts in $^1H$ and $^{13}C$ NMR spectra of **1** and **2**.

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