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ISORHAMNETIN 3-(2,6-DIRHAMNOSYLGALACTOSIDE)-7-RHAMNOSIDE AND 3-(6-RHAMNOSYLGALACTOSIDE)-7-RHAMNOSIDE FROM *RHAZYA STRICTA*

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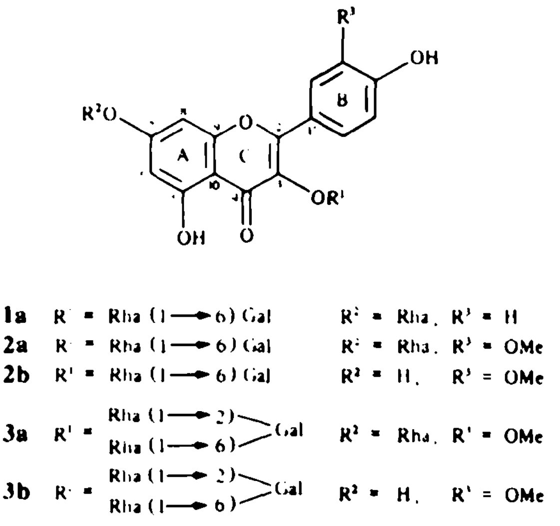
Key Word Index *Rhazya stricta*; Apocynaceae; *Primula veris*; *P. officinalis*; Primulaceae; isorhamnetin 3-(6-α-L-rhamnopyranosyl-β-D-galactopyranoside)-7-α-L-rhamnopyranoside; isorhamnetin 3-(2,6-di-α-L-rhamnopyranosyl-β-D-galactopyranoside)-7-α-L-rhamnopyranoside; robinin.

Abstract Robinin and two novel glycosides: isorhamnetin 3-(6-rhamnosylgalactoside)-7-rhamnoside and 3-(2,6-dirhamnosylgalactoside)-7-rhamnoside were isolated from a leaf extract of *Rhazya stricta*. Naringinase catalysed hydrolysis of both glycosides removed the rhamnose from the 7-hydroxyl. The product isorhamnetin 3-(2,6-dirhamnosylgalactoside) was shown by ^{1}H NMR to be identical with a glycoside previously isolated from *Primula veris*.

INTRODUCTION

The alkaloidal constituents of the Indian medicinal plant *Rhazya stricta* Decaisne (Apocynaceae) have been well investigated [1–4]. In contrast only one investigation concerning the polar constituents has been published [5]. Two glycosides were isolated (rhazianoside A and B) and both shown to contain one mole of isorhamnetin, one mole of rhamnose, and two moles of galactose [5]. The renewed interest in the biological effects of flavonoids [6], especially their activities as inhibitors of histamine release from mast cells [7–9], encouraged us to reinvestigate the polar constituents of *R. stricta*. This paper reports the isolation and structure elucidation of robinin (**1a**) and the two novel glycosides isorhamnetin 3-(6-rhamnosylgalactoside)-7-rhamnoside (**2a**) and isorhamnetin 3-(2,6-dirhamnosylgalactoside)-7-rhamnoside (**3a**).



1a R¹ = Rha (1→6) Gal R² = Rha, R³ = H
2a R¹ = Rha (1→6) Gal R² = Rha, R³ = OMe
2b R¹ = Rha (1→6) Gal R² = H, R³ = OMe
3a R¹ = Rha (1→2), Rha (1→6) Gal R² = Rha, R³ = OMe
3b R¹ = Rha (1→2), Rha (1→6) Gal R² = H, R³ = OMe

RESULTS AND DISCUSSION

After column chromatography and HPLC three glycosides **1a**, **2a**, and **3a** were obtained in yields of 0.04, 0.2 and 0.2% respectively. The sugar moieties of the three glycosides were shown to contain D-galactose and L-rhamnose units as shown by the method of Leontein *et al.* [10]. Thus, the reaction mixture obtained after acetylation of the non-volatile constituents of a heated acidic solution of **1a**, **2a** or **3a** in (*S*)-(+)-2-octanol was investigated by capillary GC. The obtained patterns were recognized in the chromatograms of the acetylated (*S*)-2-octyl glycosides of L-rhamnose and D-galactose. Interpretation of the ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) of the three glycosides revealed that all the rhamnosyl radicals were found in the α-L-rhamnopyranosyl form, and the galactosyl radicals were in the β-D-galactopyranosyl form (*cf.* [11]).

The ^{1}H NMR spectrum of **1a** showed the aglycone to be kaempferol and the sugar moiety to consist of two moles of rhamnose and one mole of galactose. Thus, robinin and **1a** are formed from the same units. Comparison of the ^{1}H and ^{13}C NMR spectra of **1a** and authentic robinin showed them to be identical.

The aglycone of **2a** was shown by ^{1}H NMR spectroscopy to be isorhamnetin. UV-Absorption shifts [12] indicated that the 3- and 7-hydroxy groups were substituted. Comparison of the ^{13}C NMR spectra of **1a** and **2a** showed that the signals originating in analogous carbons of the sugar moieties were found at the same chemical shift values. Thus **2a** must have the same sugar moieties as **1a**, and consequently we suggest the structure isorhamnetin 3-(6-rhamnosylgalactoside)-7-rhamnoside.

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Table 1. ^{1}H NMR data for **2a**, **2b**, **3a** and **3b**. Chemical shifts (δ) of aglycone, anomeric protons and methyl groups

	2a	**2b**	**3a**	**3b**
Aglycone				
H-6	6.47 *d* (2.1)	6.23 *br*	6.46 *d* (2.0)	6.23 *d* (2.0)
H-8	6.83 *d* (2.1)	6.46 *br*	6.83 *d* (2.0)	6.46 *d* (2.0)
H-2′	8.03 *d* (1.8)	8.02 *d* (2.0)	8.05 *d* (2.0)	8.04 *d* (2.0)
H-5′	6.93 *d* (8.3)	6.92 *d* (8.5)	6.93 *d* (8.5)	6.93 *d* (8.5)
H-6′	7.59 *dd* (1.8, 8.3)	7.53 *dd* (2.0, 8.5)	7.60 *dd* (2.0, 8.5)	7.52 *dd* (2.0, 8.5)
CH_3O-	3.88 *s*	3.87 *s*	3.91 *s*	3.91 *s*
Sugars				
Gal-H-1	5.49 *d* (7.6)	5.47 *d* (7.6)	5.71 *d* (7.6)	5.70 *d* (7.9)
7-Rha-H-1	5.58 *br*	-	5.57 *br*	-
$-CH_3$	1.14 *d* (6.1)		1.14 *d* (6.1)	
6^{Gal}-Rha-H-1	4.45 *br*	4.45 *br*	4.43 *br*	4.43 *br*
CH_3	1.07 *d* (6.1)	1.07 *d* (5.8)	1.07 *d* (6.4)	1.08 *d* (6.1)
2^{Gal}-Rha-H-1			5.02 *br*	5.02 *br*
CH_3	–	–	0.73 *d* (6.1)	0.73 *d* (6.1)

The spectra were recorded at 270 MHz in DMSO-d_6 solutions with 5% CF_3COOH using TMS (δ = 0) as internal standard. Coupling constants in Hz are given in parentheses. The remaining sugar protons were found in the region δ3.0-4.0.

Table 2. ^{13}C NMR data for **2a** and **3a**

Carbons	**2a**	**3a**
Aglycone 2	156.62*	156.61*
3	133.51	132.85
4	177.34	177.31
10	105.58	105.53
5	160.80	160.81
6	98.62†	98.91†
7	161.64	161.52
8	94.55	94.64
9	155.87*	155.86*
1′	120.85	120.80
2′	113.89	113.35
3′	149.63	149.49
4′	146.96	146.92
5′	115.11	115.05
6′	122.26	121.95
CH_3O-	55.99	55.82
Gal 1	101.83	100.76
2	71.21	75.11
3	73.78‡	73.35‡
4	68.00	68.09
5	73.08‡	73.53‡
6	65.41	64.93
Rha 1	99.20†, 99.99†	98.28†, 99.95†, 99.24†
2	69.78, 70.18	69.98, 70.19, 70.32
3	70.60, 70.37	70.54, 70.32, 70.48
4	71.70, 71.92	71.68, 71.54, 71.79
5	69.63, 68.00	69.73, 68.17, 68.38
6	17.46, 17.46	16.95, 17.79, 18.43

The spectra were recorded at 67.9 MHz in DMSO-d_6 solution at 368 K using TMS (δ = 0) as internal standard. In order to avoid a negative NOE the protons were only decoupled during the aquisition time.

*,†,‡ in the same vertical column: assignments may be interchanged.

Further evidence for this structure was obtained by naringinase catalysed hydrolysis of **2a**. As shown by UV and ^{1}H NMR spectroscopy only the phenolic acetal bond was cleaved leaving the glycoside **2b**.

The ^{1}H NMR spectrum of the third glycoside **3a** revealed that this glycoside was also an isorhamnetin glycoside but with one galactose and three rhamnose radicals. UV spectroscopy showed that the aglycone was substituted in the same positions as in **2a**. Naringinase catalysed hydrolysis only cleaved the phenolic acetal bond leaving a glycoside consisting of isorhamnetin glycosylated at the 3-hydroxyl group, one galactose and two rhamnose radicals. The structure of the trisaccharide was established by investigation of the two-dimensional homonuclear ^{1}H-^{1}H chemical shift correlation diagram (COSY) of the peracetate of **3a** [13]. Comparison of this spectrum with the spectrum of **3a** revealed downfield shifts of 1-2 ppm for all the protons attached to the carbohydrate moieties except for the anomeric protons, the methyl protons, the four H-5, and the H-6 and H-2 of galactose. Since these shifts are caused by acetylation of a hydroxy group geminal to the proton, the considerably smaller shifts of the H-6 and H-2 of galactose must mean that the two rhamnosyl moieties are bound to the 2- and 6-hydroxyl groups. Careful interpretation of the COSY spectrum enabled assignments of all the proton signals except a differentiation between the signals of the different acetyl groups (Table 3). Based on the information from the COSY spectrum the structure of the acetate was elucidated as **3c**, and consequently **3a** must be isorhamnetin 3-(2,6-dirhamnosylgalactoside)-7-rhamnoside and the naringinase hydrolysed derivative must possess the structure **3b**. The branched trisaccharide moiety in **3a** has previously been found in rhamnustrioside [14]. The differences in the NMR data of the trisaccharide in **3a** and in rhamnustrioside can be explained by the different aglycones and the different locations of the sugar moiety. The two glycosides **2a** and **3a** are both built from the same units as are the previously

Table 3. ^{1}H NMR data for **3c**

Aglycone	H-6	H-8	H-2′	H-5′	H-6′	CH_3O
	6.78 *d* (2.5)	7. 13 *d* (2.5)	7.83 *d* (2.0)	7.11 *d* (8.5)	7.62 *dd* (2.0, 8.5)	3.92 *s*
Sugars	**H-1**	**H-2**	**H-3**	**H-4**	**H-5**	**H-6**
Gal	5.52 *d* (7.5)	3.94 *dd* (7.5, 10.0)	5.11 *dd* (10.0, 3.5)	5.38 *dd* (3.5, 1.0)	3.74 *ddd* (1.0, 6.0, 5.5)	3.20 *dd* (6.0, 10.5) 3.43 *dd* (5.5, 10.5)
7-Rha	5.58 *d* (2.0)	5.44 *dd* (2.0, 4.0)	5.48 *dd* (4.0, 10.0)	5.07 *dd* (10.0, 10.0)	4.51 *dq* (10.0, 6.0)	1.22 *d* (6.0)
6^{Gal}-Rha	4.46 *d* (1)	5.07 *dd* (1, 4.0)	5.48 *dd* (4.0, 10.0)	5.19 *dd* (10.0, 10.0)	3.91 *dq* (10.0, 6.0)	1.06 *d* (6.0)
2^{Gal}-Rha	4.98 *d* (2.0)	5.16 *dd* (2.0, 3.5)	5.07 *dd* (3.5, 9.5)	4.95 *dd* (9.5, 9.5)	3.59 *dq* (9.5, 6.0)	0.93 *d* (6.0)

The spectrum was recorded at 500 MHz in a $CDCl_3$ solution using TMS ($\delta = 0$) as internal standard.
Coupling constants in Hz are given in parentheses.
The 13 singlets from the acetate methyl groups were found in the region δ 1.9-2.7.

reported rhazianoside A and B [5]. The stoichiometry, however, is different. Isorhamnetin glycosides possessing the same monosaccharides with the same stoichiometry have been isolated from *Primula veris*[*] [15] and *Convalaria majalis* [16], but their structures were not fully elucidated. Comparison of the ^{1}H NMR spectra of **3b** and the *P. veris* glycoside disclosed the two compounds to posses the same structure.

Investigations of the biological properties of the flavonoids, especially their abilities to stabilize peritoneal rat mast cells, are in progress.

EXPERIMENTAL

A Waters pump 6000 A, a Waters injection valve U6K, and a Perkin-Elmer LC-55 spectrophotometer were used for HPLC. CC was performed over acid washed silica gel (70-230 mesh, Merck 7734) standardized to contain 10% of water. TLC was performed on Kiselgel 60 F 254 (DC-Alufolien Merck 5549). The NMR spectra were obtained on a Bruker HX-270S spectrometer in the FT mode. The COSY spectrum was recorded on a Bruker AM 500 spectrometer. GC was performed on a Finnigan 9500 gas chromatograph connected to a Finnigan 3500 D MS; column BP 5, 25 m × 0.2 mm (SGE).

Plant material. Leaves of *Rhazya stricta* Decaisne were collected in February 1984 in the Quasium province, Saudi Arabia. Their identity was confirmed by the Botany Dept. Faculty of Science, King Saud University and a voucher specimen (no. 9) is on deposit at the Faculty of Agriculture, King Saud University.

Isolation of glycosides. The leaves were dried, powdered and percolated with 75% EtOH at room temp. The plant material was dried and extracted with hot distilled H_2O. The aq. extract was lyophilized, and the remaining powder extracted with hot MeOH. After conc. of the methanolic extract a residue was obtained, which was fractionated over silica gel using $CHCl_3$ MeOH H_2O (75:23:2) and $CHCl_3$-MeOH H_2O (25:23:2) as eluents. The fractions containing constituents with R_f values between 0.6 and 0.4 [$CHCl_3$-MeOH-H_2O (25:23:2)], and the fractions containing more polar constituents were concentrated. The residue containing the non-polar constituents was fractionated by HPLC (LiChrosorb RP 18, Knauer prepacked column, 16 × 250 mm) using MeOH-H_2O-AcOH (40:60:1) as eluent, flow 9 ml/min, to give **1a** (R_t 18 min) and **2a** (R_t 22 min) as amorphous yellow powders. The residue containing the polar constituents was fractionated by HPLC using the same system to give **3a** (R_t 10 min) as an amorphous yellow powder. The homogeneities of the isolated glycosides were verified by HPLC.

Sugar identification. A solution of the glycoside (1 mg) was dissolved in (S)-(+)-2-octanol (0.5 ml) added CF_3COOH (20 μl) and left at 130° for 12 hr in a sealed vessel. The reaction mixture was freeze dried, the residue dissolved in 1 ml of pyridine-Ac_2O (1:1) and left for 30 min at 100°. The mixture was conc. and the residue dissolved in MeOH and investigated by GC (oven temp. 150-230° with a rate of 4°/min).

Cleavage of the phenolic acetal bond. To an aq. soln of the glycoside (1 mg/ml) was added 0.1 unit of naringinase (Sigma N 1385) per mg of glycoside, incubated at 37° for 30 min and then left at room temp. until no starting material could be detected by TLC. The product was isolated by HPLC.

Acetylation. **3a** (4.5 mg) was dissolved in 600 μl of pyridine Ac_2O (1:1) and left for 12 hr at room temp. The residue after freeze drying was purified by CC using toluene EtOAc (1:1) as eluent. ^{13}C NMR data for **3c** [125.7 MHz, $CDCl_3$, TMS (δ = 0)] 171.70, 170.61, 170.16, 170.07, 170.01, 169.94, 169,89, 169,71, 169.66, 168.43, 159.29, 157.60, 151.00, 150.58, 141.70, 136.18, 129.29, 122.61, 121.36, 113.93, 112.87, 109.28, 102.04, 99.78, 98.71, 97.78, 95.78, 75.55, 72.80, 71.64, 71.06, 70.77, 70.54, 69.38, 69.23, 68.94, 68.89, 68.55, 68.08, 68.00, 66.75, 66.54, 66.14, 56.09, 31.95, 29.72, 29.38, 22.71, 21.03, 20.94, 20.83, 20.75, 20.71, 20.65, 20.44, 17.42, 17.16, 16.77, 14.12.

UV data: λ_{max} nm (log ε) in MeOH: **2a**: 253 (4.2), 265 (sh), 355 (4.0); + NaOMe: 270, 415; + NaOAc: 253, 265 (sh), 355, 420 (sh); + $AlCl_3$: 269, 300 (sh), 370 (sh), 405. **2b**: 253 (4.0), 265 (sh), 355 (3.9); + NaOMe: 272, 325, 415; + NaOAc: 253 (sh), 274, 315, 370. **3a**: 253 (4.2), 265 (sh), 355 (4.1), 420 (sh); + NaOMe: 269, 405; + NaOAc: 253, 265 (sh), 355, 420 (sh); + $AlCl_3$: 270, 300 (sh), 370 (sh), 403. **3b**: 253 (4.2), 265 (sh), 355 (4.0), 420 (sh); + NaOMe: 270,

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[*] In ref. [15] the obsolescent synonym *P. officinalis* is used.

325, 405; + NaOAc: 253 (sh), 270, 315, 360; + $AlCl_3$: 270, 300 (sh), 370 (sh), 405.

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