TWO NEW ISOFLAVONOID MONOGALACTOSIDES FROM *Trifolium pratense* ROOTS

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*Formononetin and the new isoflavonoid glycosides formononetin-7-*O-β-*D-galactopyranoside and inermin-3-*O-β-*D-galactopyranoside were isolated from* Trifolium pratense *L. roots. The structures of the isolated compounds were proved using chemical transformations and UV, PMR, and* ^{13}*C NMR spectra.*

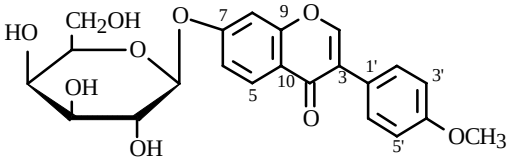
Key words: *Trifolium pratense* L., isoflavonoids, formononetin, formononetin-7-*O*-*β*-D-galactopyranoside, inermin-3-*O*-*β*-D-galactoppyranoside.

Numerous investigations of red clover (*Trifolium pratense* L., Fabaceae) indicate that it contains the isoflavonoids biochanin A, ononin, sissotrin, formononetin, diadzein, prunetin, genistein, pratensein, pseudobaptigenin, calycosin, and other compounds [1-3].

Total flavonoids of red clover are used in biologically active formulations for prophylaxis and auxiliary treatment of various diseases [4].

Herein we present results of a chemical investigation of isoflavonoids from the underground part of red clover collected in September 2005 in Surgut Region of Khanty-Mansiisk Autonomous District.

Column chromatography of the chloroform fraction of the ethanol extract affored isoflavonoid **1**; the ethylacetate fraction, isoflavonoids **2** and **3**.



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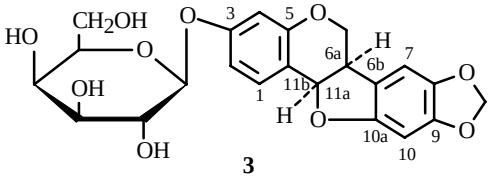

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Compound **1** was identified as formononetin (7-hydroxy-4′-methoxyisoflavone) by direct comparison of its UV and PMR spectra with those of an authentic sample [5]. The UV spectrum of **2** had absorption maxima characteristic of isoflavone derivatives [6].

PMR and ^{13}C NMR data (Table 1) showed that **2** was a monoglycoside. Acid hydrolysis of **2** produced formononetin and D-galactose. Galactose was identified by TLC and GC (as the trimethylsilyl ether) and comparison with an authentic sample.

The SSCC of the resonance for the anomeric proton (J = 6.4 Hz) in the PMR spectrum and the chemical shifts of C resonances for the D-galactose in the ^{13}C NMR spectrum of **2** indicated that the carbohydrate part had the *β*-D-galactopyranose structure [6, 7].

Therefore, **2** was formononetin-7-*O*-*β*-D-galactopyranoside.

Isoflavonoid **3** had a UV spectrum (λ_{max}, EtOH, nm: 278, 285, 310) typical of pterocarpanes [5].

Acid hydrolysis of **3** produced the aglycon and D-galactose (TLC, GC). The aglycon ($C_{16}H_{12}O_5$; λ_{max} 282, 287, 311 nm) was identified as 6a*R*,11a*R*-3-hydroxy-8,9-methylenedioxypterocarpane (inermin) based on spectral data and comparison with an authentic sample [5]. The resonance for the anomeric proton of D-galactose in the PMR spectrum of **3** appeared at 4.84 ppm as a doublet with SSCC 7.2 Hz; that of C-1, at δ 100.3 ppm in the ^{13}C NMR spectrum (Table 2).

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TABLE 1. PMR and ^{13}C NMR Spectra of **2** (DMSO-d_6)

C atom	Compound **2**	
	δ_C, ppm	δ_H (J/Hz)
Aglycon		
2	153.7	8.42 s
3	124.0	
4	174.8	
5	127.0	8.05 d (8.4)
6	115.7	7.15 dd (8.4, 1.8)
7	161.5	
8	103.5	7.23 br. d
9	157.1	
10	118.5	
1′	123.4	
2′/6′	130.1	7.52 d (8.7)
3′/5′	113.7	6.99 d (8.7)
4′	159.1	
OCH_3	55.2	3.78 s
Gal*p*		
1″	100.1	5.11 d (6.4)
2″	73.2	3.28 - 3.77 m
3″	76.5	3.28 - 3.77 m
4″	69.7	3.28 - 3.77 m
5″	77.2	3.28 - 3.77 m
6″	60.3	3.28 - 3.77 m

TABLE 2. PMR and ^{13}C NMR Spectra of **3** (DMSO-d_6)

C atom	Compound **3**	
	δ_C, ppm	δ_H (J/Hz)
Aglycon		
1	131.9	7.36 d (8/7)
2	110.4	6.71 dd (8.7, 1.8)
3	158.5	
4	104.0	6.55 d (1.8)
5	156.2	
6	65.9	4.27 dd (3.6, 10.2)
6a	39.5	3.59 - 3.70 m
6b	118.3	
7	105.4	6.98 s
8/9	147.5	
10	93.3	6.52 s
10a	153.7	
11a	77.7	5.57 d (6.9)
11b	114.2	
OCH_2O	101.2	5.92 d (11.2)
Gal*p*		
1′	100.3	4.84 d (7.2)
2′	73.2	3.12 - 3.36 m
3′	76.5	3.12 - 3.36 m
4′	69.7	3.12 - 3.36 m
5′	77.1	3.12 - 3.36 m
6′	60.7	3.12 - 3.36 m

Circular dichroism spectra (see Experimental) confirmed the 6a*R*,11a*R*-configuration of the chiral centers in the aglycon of **3** [8]. The structure of **3** was established as inermin-3-*O*-*β*-D-galactopyranoside based on these data.

Formononetin-7-*O*-*β*-D-galactopyranoside and inermin-3-*O*-*β*-D-galactopyranoside have not been reported and are new natural compounds [9].

EXPERIMENTAL

We used Sorbfil PTLC-P-A-UV plates for TLC and the following solvent systems: $CHCl_3$:EtOAc (9:1, 1), $CHCl_3$:C_2H_5OH (9:1, 2); $CHCl_3$:EtOAc:C_2H_5OH (6:1:3, 3), *n*-BuOH:EtOH:H_2O (5:3:2, 4).

Spots of flavonoids on plates were examined in UV light in a UFS-254/365 chromatogram illuminator and developed by vanillin in alcohol (3%) mixed with conc. HCl in a 4:1 ratio. Monosaccharides were detected by spraying with H_2SO_4 followed by heating at 100-110°C. Column chromatography was performed over KSKG silica gel (100/160 μm particle size). PMR and ^{13}C NMR spectra were recorded on a Bruker AVACE AV300 instrument at operating frequency 300 (PMR) and 75 MHz (^{13}C NMR). UV spectra were recorded on a SF-2000 spectrophotometer. Circular dichroism spectra were recorded on a Jasco J-20 spectropolarimeter. Melting points were measured by a capillary method in H_2SO_4.

Extraction and Isolation of Flavonoids. An air-dried portion of the ground underground part (0.5 kg) was extracted five times with ethanol (85%) at room temperature. The resulting extract was dried by evaporation in vacuo and treated successively with hexane, $CHCl_3$, EtOAc, and *n*-BuOH. The resulting fractions were evaporated to dryness in vacuo to afford $CHCl_3$ (1.0 g), EtOAc (5.8), and BuOH (9.6) fractions.

The $CHCl_3$ fraction was chromatographed (1.0 g) over a column (1.5 × 60 cm) of silica gel (25.0 g) using hexane:$CHCl_3$ (increasing $CHCl_3$ gradient from 70 to 100%) and then EtOH in $CHCl_3$ (from 0 to 6%). Fractions of 50 mL were collected. Elution by $CHCl_3$:EtOH (98:2) afforded formononetin (88.3 mg).

The EtOAc fraction (5.8 g) was chromatographed over a column (3.5 × 90 cm) of silica gel (175 g). Compounds were eluted by $CHCl_3$:EtOH (increasing EtOH gradient from 0 to 20%). Fractions of 100 mL were collected. Elution by $CHCl_3$:EtOH (94:6) afforded inermin-3-*O*-*β*-D-galactopyranoside (500 mg) and formononetin-7-*O*-*β*-D-galactopyranoside (250 mg).

Formononetin (1) (7-hydroxy-4′-methoxyisoflavone), $C_{16}H_{12}O_4$, $[M]^+$ 268, mp 261-263°C, UV spectrum (EtOH, λ_{max}, nm): 250, 263, 295.

PMR spectrum (300 MHz, C_5D_5N, δ, ppm, J/Hz): 3.57 (3H, s, OCH_3), 6.99 (2H, d, J = 9.0, H-3′, H-5′), 7.07 (1H, br.s, H-8), 7.14 (1H, dd, J = 9.0, 2.0, H-6), 7.72 (2H, d, J = 9.0, H-2′, H-6′), 8.10 (1H, s, H-2), 8.40 (1H, d, J = 9.0, H-5).

Formononetin-7-*O*-*β*-D-galactopyranoside (2), $C_{16}H_{12}O_4$, mp 213°C, UV spectrum (EtOH, λ_{max}, nm): 261, 263, 303.

Table 1 gives the PMR and ^{13}C NMR spectra.

Acid Hydrolysis of 2. A solution of **2** (25 mg) in a mixture of HCl (5%) and EtOH (1:1, 20 mL) was boiled for 2 h. The resulting precipitate of the aglycon was filtered off and recrystallized from benzene to afford formononetin (8 mg), mp 261-263°C. TLC (system 4) and GC (trimethylsilyl ether) identified in the hydrolysate D-galactose. The trimethylsilyl ether was prepared as follows. Carbohydrate was dissolved in pyridine, silylated by BSTFA for 20 min, and evaporated to dryness. The dry solid was dissolved in hexane.

Inermin-3-*O*-*β*-D-galactopyranoside (3), $C_{16}H_{12}O_4$, mp 193°C, UV spectrum (EtOH, λ_{max}, nm): 278, 285, 310. Circular dichroism spectrum [*c* 0.1, EtOH, Δε = –12.3 (233 nm), Δε = –1.2 (278 nm), Δε = +3.6 (310 nm)].

Table 2 gives the PMR and ^{13}C NMR spectra.

Acid Hydrolysis of 3. The hydrolysis was performed as described above (30 mg). The aglycon was extracted from the hydrolysate by $CHCl_3$, evaporated to dryness, and recrystallized from benzene to afford inermin (10 mg), mp 180-181°C, $[\alpha]_D$ -211.4° (EtOH). TLC and GC of the hydrolysate identified D-galactose.

REFERENCES

1. *Plant Resources of the USSR: Flowering Plants, Their Chemical Composition and Use. Hydrangeaceae-Haloragaceae Families* [in Russian], Leningrad (1987), pp. 137-142.

2. S. M. Bouea, T. E. Wiese, S. Nehls, M. E. Burow, S. Elliott, C. H. Carter-Wientjes, B. Y. Shih, J. A. Mclachlan, and T. E. Cleveland, *J. Agric. Food Chem.*, **51**, 2193 (2003).
3. S.-M. Heinonen, K. Wahala, and H. Adlercreutz, *J. Agric. Food Chem.*, **52**, 6802 (2004).
4. G. P. Yakovlev and K. F. Blinova, eds., *Medicinal Plant Raw Material. Pharmacognosy* [in Russian], SpetsLit., St. Petersburg (2004), p. 728.
5. S. S. Yusupova, E. Kh. Batirov, F. Kiyamitdinova, and V. M. Malikov, *Khim. Prir. Soedin.*, 639 (1986).
6. T. J. Mabry, K. R. Markham, and M. B. Thomas, *The Systematic Identification of Flavonoids* Springer-Verlag, New York (1970).
7. P. K. Agrawal and R. P. Rastogi, *Heterocycles*, **16**, 2181 (1981).
8. J. B. Harborne, T. J. Mabry, and H. Mabry, eds., *The Flavonoids*, Chapman and Hall, London (1975), p. 774.
9. O. M. Andersen and K. R. Markham, eds., *Flavonoids. Chemistry, Biochemistry and Applications*, CRC, Taylor and Francis, Boca Raton, FL (2006).