SECONDARY METABOLITES FROM *Hedysarum setigerum*

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We previously reported the isolation of five flavonoids from the aerial part of *Hedysarum setigerum*. These included isorhamnetin, avicularin, roifolin, linarin, and diosmin [1]. In continuation of the study of secondary metabolites of *H. setigerum* to find antiviral agents, we isolated another six flavonoids and four sterols.

A precipitate containing two flavonoids formed upon standing in the aqueous methanol extract [1]. The precipitate was separated and chromatographed over silica gel ($CHCl_3$:CH_3OH:H_2O, 100:2:1) and polyamide (30% CH_3OH). Compounds **1** and **3** were isolated in this manner. The fraction obtained via chromatography of the butanol extracts over polyamide [1] (60-70% CH_3OH) was treated repeatedly with acetone. Chromatography over silca gel isolated **2** from the acetone-soluble fraction ($CHCl_3$:CH_3OH, 98:2) and **4** from the acetone-insoluble fraction ($CHCl_3$:CH_3OH:H_2O, 70:12:1). The fraction produced by elution of the butanol extracts over polyamide (15-35% CH_3OH) underwent flash chromatography over silica gel ($CHCl_3$:CH_3OH:H_2O, 70:23:4-63:23:3) and afforded two fractions enriched in flavonoids. Fraction 1 was chromatographed successively over silica gel ($CHCl_3$:CH_3OH:H_2O, 70:23:1), polyamide (33% CH_3OH), and Sephagel (DEAE in the OH^- form, 15% CH_3OH). This produced **6**. Fraction 2 was chromatographed repeatedly over silica gel ($CHCl_3$:CH_3OH:H_2O, 100:30:2) to isolate **5**.

Quercetin (1): mp 302°C (Et_2O). Mass spectrum (FAB^+, *m/z*; I_{rel}, %): 303 (46) $[M + H]^+$, 302 (13) $[M]^+$. Mass spectrum (HR-FAB): found $[M + H]^+$ 303.050; cald. for $C_{15}H_{11}O_7$, 303.0500.

3-O-Methylkaempferol (2): mp 270-272°C (EtOH). Mass spectrum (FAB^+, *m/z*; I_{rel}, %): 389 (5) $[M - 3H + 4Na]^+$, 323 (15) $[M + Na]^+$, 261 (100) $[M - 3H - 4Na - 2H_2O]^+$. Mass spectrum (HR-FAB): found $[M + Na]^+$ 323.053; cald. for $C_{16}H_{12}O_6Na$, 323.0528.

Quercitrin (3): mp 181-183°C (CH_3OH). Mass spectrum (FAB^+, *m/z*; I_{rel}, %): 449 (9) $[M + H]^+$, 303 (40) [M + H - Rha]$^+$, 263 (100) [M + H - $C_7H_4O_4$ - 2OH]$^+$. Mass spectrum (HR-FAB): found $[M + H]^+$ 449.108; cald. for $C_{21}H_{21}O_{11}$, 449.1080.

Kaempferol-3-O-α-L-arabinofuranoside (4): mp 226-227°C (CH_3OH). Mass spectrum (FAB^+, *m/z*; I_{rel}, %): 463 (8) $[M - H + 2Na]^+$, 441 (4) $[M + Na]^+$, 413 (100) [M + Na - CO]$^+$, 315 (8) [M - H + 2Na - OAra]$^+$. Mass spectrum (HR-FAB): found $[M + Na]^+$ 441.080; cald. for $C_{20}H_{18}O_{10}Na$, 441.0798.

Rutin (5): mp 190-192°C (EtOH). Mass spectrum (FAB^+, *m/z*; I_{rel}, %): 655 (13) $[M - H + 2Na]^+$, 633 (12) $[M + Na]^+$, 301 (77) [M - H - Glc - Rha]$^+$. Mass spectrum (HR-FAB): found $[M + Na]^+$ 633.143; cald. for $C_{27}H_{30}O_{16}Na$, 633.1428.

Neobudofficide (6): mp 180-182°C (CH_3OH), $[\alpha]_D^{14}$ -57.06° (*c* 0.17, EtOH). Mass spectrum (FAB^+, *m/z*; I_{rel}, %): 761 (6) $[M + Na]^+$, 727 (5) [M + Na - 2OH]$^+$, 273 (22) [M + Na - 2OH - 2Rha - Glc]$^+$. Mass spectrum (HR-FAB): found $[M + Na]^+$ 761.228; found for $C_{34}H_{42}O_{18}Na$, 761.2268. For the PMR and ^{13}C NMR, see Table 1.

All physicochemical constants of **1** [2], **2** [3], **3-4** [4], and **6** [5] and spectral data (PMR and ^{13}C 2D) agreed with the literature data for these compounds [5, 7].

Compounds **2**, **4**, and **6** were obtained for the first time from plants of the *Hedysarum* genus. The isolation of neobudofficide, 5,7-dihydroxy-4′-methoxyflavone 7-O-α-L-rhamnopyranosyl-(1→6)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside, is the first observation of this compound in nature [5, 6].

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TABLE 1. PMR and ^{13}C NMR Data for Neobudofficide (**6**) (250 MHz, C_5D_5N, δ, ppm, J/Hz)

Atom	**6**		Atom		**6**	
	^{13}C	^{1}H			^{13}C	^{1}H
2	164.4		Glc	1″	99.8	5.7 (d, 7.5)
3	104.6	6.9 (s)		2″	77.5	4.5 (m)
4	182.6			3″	79.1	4.4 (m)
5	162.6			4″	72.6	4.5 (m)
6	100.6	6.3 (d, 1.8)		5″	77.3	4.1 (m)
7	163.7			6″	67.3	4.7, 4.0 (m)
8	95.1	6.7 (d, 1.8)	Rha (2)	1‴	102.3	6.4 (d, 1.2)
9	157.7			2‴	72.3*	4.8 (m)*
10	106.7			3‴	72.6*	4.5 (m)*
1′	124.1			4‴	73.9	4.3 (m)
2′	128.6	7.9 (dd, 8.8, 1.9)		5‴	69.8	4.8 (m)
3′	114.9	7.2 (dd, 8.8, 1.9)		6‴	19.2	1.9 (d, 6.2)
4′	162.9		Rha (6)	1⁗	102.4	5.5 (d, 1.2)
5′	114.9	7.2 (dd, 8.8, 1.9)		2⁗	71.9*	4.6 (m)*
6′	128.6	7.9 (dd, 8.8, 1.9)		3⁗	71.2*	4.0 (m)*
-OH (5)		13.4 (s)		4⁗	73.9	4.2 (m)
-OCH_3	55.4	3.7 (s)		5⁗	69.7	4.3 (m)
				6⁗	18.2	1.6 (d, 6.2)

*Alternate signal assignment.

Flash chromatography of the $CHCl_3$ fraction [1] over silica gel (C_6H_6) isolated the sterol fraction (0.09% of dry mass). GC-MS of the acetate derivatives identified campesterol (11.7%), stigmasterol (14.1%), β-sitosterol (69.2%), and stigmastanol (4.2% of the fraction mass).

REFERENCES

1. O. V. Neretina, A. S. Gromova, V. I. Lutskii, and A. A. Semenov, *Rastit. Resur.*, No. 1, 82 (2002).
2. V. A. Kurkin, V. B. Braslavskii, G. G. Zapesochnaya, and V. O. Tolkachev, *Khim. Prir. Soedin.*, 548 (1990).
3. E. Wollenweber and K. Egger, *Tetrahedron Lett.*, **19**, 1601 (1970).
4. W. Karrer, *Konstitution und vorkommen der organischen pflanzenstoffe*, Birkhauser Verlag Basel, Stuttgart (1958).
5. J. S. Li, Y. Y. Zhao, B. Wang, X. L. Li, and I. B. Ma, *Acta Pharm. Sin.*, **31**, No. 11, 849 (1996).
6. Y.-H. Liao, P. J. Houghton, and J. R. S. Hoult, *J. Nat. Prod.*, **62**, No. 9, 1241 (1999).
7. K. R. Markham, B. Ternai, R. Stanley, H. Geiger, and T. J. Mabry, *Tetrahedron*, **34**, 1389 (1978).