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TAMARIXETIN GLYCOSIDES FROM THE FLOWERS OF *VERBASCUM PHLOMOIDES*

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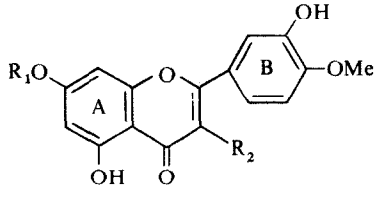
Key Word Index—*Verbascum phlomoides*; Scrophulariaceae; flavonoid glycosides; tamarixetin 7-glucoside; tamarixetin 7-rutinoside.

Flowers of *Verbascum phlomoides* L. contain a mixture of flavonoids which are insoluble in water and in methanol. Diosmin (**1**), hesperidin (**2**), tamarixetin 7-*O*-glucoside (**3**) (previously only known synthetically [1]) and tamarixetin 7-*O*-rutinoside (**4**) were isolated after acetylation followed by column chromatography. According to Hein [2], the flowers of *Verbascum phlomoides* L. contain a glycoside of the hesperidin type and the tamarixetin glucorhamnoside, verbascoside. This latter name has, however, already been used for a derivative of caffeic acid [3].



Diosmin (**1**) R_1 = rutinose, R_2 = H
3 R_1 = Glc, R_2 = OH
4 R_1 = rutinose, R_2 = OH
Tamarixetin (**5**) R_1 = H, R_2 = OH

Diosmin (**1**) has been described before in *Verbascum* [4]. Up to now, only the 3-*O*-glucoside [5, 7] and 3-*O*-SO_3-K [6, 7] derivatives of tamarixetin have been reported. Tamarixetin 7-*O*-rutinoside, as well as tamarixetin 7-*O*-neohesperioside, were prepared synthetically, for ^{1}H NMR studies.

The flavonoid mixture of *Verbascum phlomoides* L. was peracetylated and four of the six compounds were isolated by column and thick layer chromatography. The main substance was the tamarixetin 7-*O*-rutinoside whose structure has been confirmed by ^{1}H NMR spectroscopy of the acetate in a double resonance experiment (irradiation of the OMe-proton) [8]. A nuclear-Overhauser-effect was observed for the proton with *ortho*-coupling (ring B of the flavonoid skeleton). This corresponds to a substitution pattern of a OMe-group at C-4'. Similar results were obtained from the spectrum of diosmin acetate. The measured ^{1}H NMR spectrum of the 7-*O*-rutinoside of tamarixetin (**4**) agreed exactly with literature values [1, 9]. In the glycosides **3** and **4** of the tamarixetin, the position of the sugar moiety is determined by ^{1}H NMR spectroscopy [9]. In both cases, the carbohydrate part is attached to C-7 of the ring A nucleus; mass spectroscopic studies show a molecular ion being also the base peak: $M^+ = m/e$ 316 ($C_{16}H_{12}O_7$; MW calc. for 316.055; found 316.058). Another fragment was found at m/e 301 (57.7%) for M − Me [10]. The sugars were identified as L-rhamnose and D-glucose by PC and TLC comparison with authentic samples [11, 12]. Silylation and gas chromatography of the 1-*O*-methyl-pertrimethylsilyl sugar mixture gave a ratio of glucose–rhamnose as 1:0.98 [13]. Hydrolysis of **3** produced the aglycone, **5** and glucose.

EXPERIMENTAL

GLC was carried out on a 2 m column (OV-17) with a temp. program (160°, 2°/min) with N_2-flowing rate of 10 ml/min. Chromatographic separations were carried out with Si PF (254) (TLC) and PF 254 + 366 (DC) Merck, unsieved Si of Hermann, Cologne (SC). The following solvent systems were used for chromatography: (A) petrol–Me_2CO (5:3), (B) cyclohexane–Me_2CO (3:1), (C) C_6H_6–Me_2CO (7:1), (D) C_6H_6–Me_2CO (5:1), (E) $CHCl_3$–Me_2CO (5:1), (F) $CHCl_3$–MeOH (50:1), (G) $CHCl_3$–MeOH–H_2O (65:30:6), (H) EtOAc Py H_2O (3.6:1:1.5), (I) *n*-BuOH–EtOH–H_2O (4:1:5). 2 kg of a viscous MeOH extract obtained from the flowers of *Verbascum phlomoides* L. (commercial sample) were dissolved in 4 l. H_2O and then extracted with 2 l. *n*-BuOH. In this mixture of *n*-BuOH–H_2O, a dark yellow residue separated. It was washed successively with 250 ml EtOH. Finally ca 2 g light yellow powder with mp 310–317° were obtained; it was soluble only in Py and DMSO. Acetylation with Ac_2O/Py at room temp. gave 2.32 g acetylated product. The IR spectrum did not show any OH absorption. 2 g peracetate mixture were subjected to column chromatography on 200 g Si gel (changing the ratio of the soln successively from 30:1 to 5:1). Six different fractions were collected.

Tamarixetin 7-O-glucoside peracetate. Fraction I was purified several times by PLC with solvents A–C. Finally it was possible to obtain a pure peracetate of tamarixetin 7-*O*-glucoside, mp 156–157°. ^{1}H NMR (90 MHz, PFT, $CDCl_3$) [ppm]: δ 7.69 (*dd*, J = 8.2 and 2 Hz, 1H at C-6'); 7.53 (*d*, J = 2 Hz, 1H at C-2'); 7.08 (*d*, J = 8.2 Hz, 1H at C-5'); 7.00 (*d*, J = 2.1 Hz, 1H at C-8); 6.70 (*d*, J = 2.1 Hz, 1H at C-6); 5.36 (*m*, 5H); 4.21 (2H); 3.91 (*s*, 3H of OMe); 2.42; 2.36; 2.31; 2.09; 2.08; 2.06; 2.05 (*s*, 21H of 7Me-acetate). (MW calc. for $C_{36}H_{36}O_{19}$ (MS): 772.1851. Found: 772.1847. $[\alpha]_D^{20}$ −6.9° (*c* = 1, $CHCl_3$).

Tamarixetin 7-O-rutinoside peracetate. Fraction III was purified several times on PLC with solvents D–F and was finally separated from diosmin acetate in pure form as tamarixetin 7-*O*-rutinoside, mp 128–130°. (MW calc. for $C_{46}H_{50}O_{25}$ (MS): 1002.2642. Found: 1002.2596). $[\alpha]_D^{20}$ −30.0°, (*c* = 1, $CHCl_3$). UV λ_{max}^{MeOH} nm (log ε): 228 (4.208), 255 (4.044), and 315 (4.22). ^{1}H NMR (90 MHz, PFT, $CDCl_3$) [ppm]: δ 7.69 (*dd*, J = 8 and 2 Hz, 1H at C-6'); 7.53 (*d*, J = 2 Hz, 1H at C-2'); 7.06 (*d*, J = 8 Hz, 1H at C-5'); 6.93 (*d*, J = 2.1 Hz, 1H at C-8); 6.64 (*d*, J = 2.1 Hz, 1H at C-6); 5.4–5.0 (7H of sugar); 4.69 (*d*, J = 0.6 Hz, 1H at C-1 of rhamnose); 3.90 (*s*, 3H of OMe-group); 4.02–3.7 (*m*, 4H of sugar); 2.40; 2.33; 2.29; 2.05; 2.04; 2.03; 2.02; 2.0 and 1.91 (*s*, 27H of 9 Me-acetate); 1.12 (*d*, J = 6 Hz, 3H of Me-rhamnose).

Diosmin-peracetate. Mp 129–130°, lit. 129–130° [1]; (MW calc. for $C_{44}H_{48}O_{23}$ (MS): 944.2687. Found: 944.2577). ^1H NMR (90 MHz, PFT, $CDCl_3$) [ppm]: δ 7.74 (*dd*, $J = 9$ and 2.2 Hz, 1H at C-6'); 7.56 (*d*, $J = 2.2$ Hz, 1H at C-2'); 7.09 (*d*, $J = 9$ Hz, 1H at C-5'); 6.73 (*d*, $J = 2.1$ Hz, 1H at C-8); 6.64 (*d*, $J = 2.1$ Hz, 1H at C-6); 6.51 (*s*, 1H at C-3); 5.33–4.87 (*m*, 7H of sugar); 4.71 (*d*, $J = 0.6$ Hz, 1H of C-1 of rhamnose); 3.91 (*s*, 3H of OMe group); 4.36–3.60 (*m*, 4H of sugar); 2.43, 2.37, 2.10, 2.08, 2.07, 2.06, 2.05, 2.04, 1.92 (*s*, 27H of 9 Me-acetate), 1.14 (*d*, $J = 6$ Hz, 3H of Me-rhamnose).

Tamarixetin 7-O-rutinoside **4**. 120 mg peracetylated tamarixetin 7-*O*-rutinoside were dissolved in 25 ml dry MeOH and 0.5 ml 5% NaOMe were added. The soln was heated at 65° for 10 min and evapd to dryness. It was then dissolved in 50 ml H_2O and extracted with 25 ml *n*-BuOH (×3). The *n*-BuOH was removed *in vacuo* and the substance obtained was subjected to column chromatography on a 2 g Si gel column for purification: crystals, mp 293–295°. IR (KBr) cm^{-1}; 3600–3200, 2945, 1650, 1620, 1580, 1480, 1320, 1175, 1145, 1125, 1090, 1060, 1040.

Hydrolysis of tamarixetin 7-O-rutinoside. 50 mg **4** were dissolved in 20 ml 2 N H_2SO_4 and the soln was refluxed for 6 hr at 165°. After cooling, the reaction mixture was diluted with 100 ml H_2O and extracted with 25 ml (×4) of *n*-BuOH. (a) The *n*-BuOH layer was washed with H_2O and evapd to dryness: mp 255–258° (lit. [5] 259–260°). (MW calc. for $C_{16}H_{12}O_7$ (MS): 316.058. Found: 316.055). MS *m/e*: 316 (M^+, 100%); 301 (M − Me, 56.7); 287 (M − CHO, 5); 273 M − C_2H_3O, 15.7); 151 (M − $C_8H_5O_4$, 21); 149 (M − $C_8H_7O_4$, 17); 83; 71; 69. The H_2O layer was neutralized with $BaCO_3$ and evapd. In this way 15 mg mixture of sugars were obtained. These were identified by means of PC and TLC as L-rhamnose and D-glucose. Quantitative sugar analyses were also carried out [13].

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A NEW FLAVONE FROM *GOMPHRENA MARTIANA*

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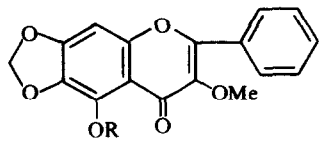
Key Word Index—*Gomphrena martiana*; Amarantaceae; flavonoid; 3,5-dimethoxy-6,7-methylenedioxyflavone.

Little is known of the chemical constituents of the genus *Gomphrena*. Isolation of betacyanins from *G. globosa* L. is the only previous work reported in the literature [1]. Present examination of *G. martiana* led to the isolation of a flavone that was characterized as 3,5-dimethoxy-6,7-methylenedioxy flavone (**1**) by means of spectroscopic and chemical procedures. This compound has been previously synthesized [2] but there are no reports on its isolation from natural sources.



1 R = Me
2 R = H

RESULTS AND DISCUSSION

From the petrol extract of whole plants a precipitate separated on concentration. On repeated crystallizations from EtOH it yielded white needles, mp 198–200°. The compound was not phenolic, as shown by a negative $FeCl_3$ reaction, but it gave positive Labat reaction indicating the presence of a methylenedioxy group [3]. The UV and a positive Shinoda test [4] were consistent with a flavone structure. The ^1H NMR signals of the aromatic protons at δ 8 (2H, *m*, H-2' and H-6') and 7.43 (3H, *m*,