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# GENKWANIN AND IRIDOID GLYCOSIDES FROM *Leonurus turkestanicus*

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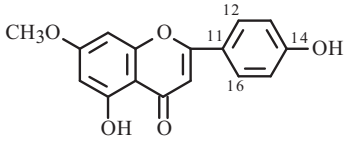
*Leonurus cardiaca* L. (Labiatae) and *L. quinquelobatus* Gilib. are used in galenics as sedative and hypotensive agents [1]. Two species of *Leonurus* are indigenous to Uzbekistan, *L. turkestanicus* V. Krecz. et Kupr. and *L. panceroides* M. Pop. [2], and are used as medicinal plants instead of the official species. However, the chemical compositions of these plants have not been reported. This prompted us to study *L. turkestanicus*.

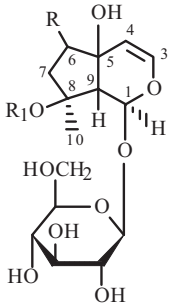
The air-dried aerial part of *L. turkestanicus* (2 kg) that was collected in Kara-Tepe, Tashkent Oblast on June 30, 2007, during flowering was extracted with MeOH (4 × 14 L). The MeOH extract was evaporated to a thick condensate and dissolved in MeOH (70%, 1 L). The solution was filtered. The filtrate was evaporated to remove completely MeOH. The solution volume was adjusted to 0.5 L with $H_2O$. The aqueous solution was extracted three times with *n*-BuOH. The *n*-BuOH extract was evaporated to dryness and dried. The yield of the *n*-BuOH extract was 63.42 g. The *n*-BuOH extract was chromatographed over a column of KSK silica gel with elution successively by $CHCl_3$, $CHCl_3$:MeOH (1, 100:1; 2, 50:1; 3, 10:1), and $CHCl_3$:MeOH:$H_2O$ (4, 70:12:1).

Fractions eluted by $CHCl_3$ and system 1 contained sterols.

System 2 eluted flavonoid **1** (40 mg).




**1**




**2 - 4**

**2:** R = H, $R_1$ = Ac
**3:** R = OH, $R_1$ = Ac
**4:** R = OH, $R_1$ = H

Further elution of the column by system 3 isolated a fraction containing **2**, rechromatography of which by the same system afforded **2** (6 mg).

Then, elution of the column by system 4 isolated **3** and **4**. A part of the fractions was rechromatographed over the column using the same system in order to purify the compounds from a yellow pigment. This afforded **3** (1.3 g) and **4** (0.756 g), which were the principal constituents of the extracted compounds. The aqueous phase remaining after work up with *n*-BuOH contained exclusively **3** and **4** in about a 1:1 ratio. Evaporation of the $H_2O$ afforded these two compounds (120 g). This led to the conclusion that the content of **3** and **4** in the air-dried plant was at least 6%.

**Genkwanin (1)**, $C_{16}H_{12}O_5$. Inspection of the PMR and $^{13}C$ NMR spectra of **1** (Table 1) showed that it was a 5,7,14-trisubstituted flavonoid, two substituents of which were hydroxyls. The third substituent was a methoxyl. The difference nuclear Overhauser effect (NOE) with saturation of the methoxyl exhibited couplings for the H-6 and H-8 resonances. This determined unambiguously the location of the methoxyl on C-7. Therefore, **1** had the structure 5,14-dihydroxy-7-methoxyflavonone. Genkwanin has the same structure [3, 4].

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Table 1. Chemical Shifts of C and H Atoms in **1**

| C atom | DEPT | Data in $C_5D_5N$ | | Data in $(CD_3)_2CO$ | |
|---|---|---|---|---|---|
| | | $\delta_C$ | $\delta_H$ (J/Hz) | $\delta_C$ | $\delta_H$ (J/Hz) |
| 2 | C | 164.83 | – | 164.01 | – |
| 3 | CH | 103.95 | 6.82 s | 102.96 | 6.77 s |
| 4 | C | 182.80 | – | 181.82 | – |
| 5 | C | 158.07 | – | 157.16 | OH 12.90 s |
| 6 | CH | 98.57 | 6.50 d (2.4) | 97.86 | 6.31 d (2.2) |
| 7 | C | 165.80 | – | 165.05 | – |
| 8 | CH | 92.83 | 6.58 d (2.4) | 92.60 | 6.69 d (2.2) |
| 9 | C | 162.68* | – | 161.18 | – |
| 10 | C | 105.87 | – | 104.61 | – |
| 11 | C | 121.96 | – | 121.01 | – |
| 12 | CH | 128.98 | 7.83 d (8.9) | 128.45 | 7.89 d (8.8) |
| 13 | CH | 116.95 | 7.15 d (8.9) | 115.89 | 6.88 d (9) |
| 14 | C | 163.05* | – | 161.18 | – |
| 15 | CH | 116.95 | 7.15 d (8.9) | 115.89 | 6.88 d (9) |
| 16 | CH | 128.98 | 7.83 d (8.9) | 128.45 | 7.89 d (8.8) |
| $CH_3O$ | $CH_3$ | 55.90 | 3.65 s | 55.95 | 3.81 s |

*Assignment of resonances is arbitrary.

Table 2. Chemical Shifts of C and H Atoms in **2**-**4**

| C atom | DEPT | **2** ($C_5D_5N$) | | **3** ($C_5D_5N$) | | **3** ($D_2O$) | | **4** ($D_2O$) | |
|---|---|---|---|---|---|---|---|---|---|
| | | $\delta_C$ | $\delta_H$ (J/Hz) | $\delta_C$ | $\delta_H$ (J/Hz) | $\delta_C$ | $\delta_H$ (J/Hz) | $\delta_C$ | $\delta_H$ (J/Hz) |
| 1 | CH | 94.09 | 6.40 d (1.6) | 94.35 | 6.57 d (1.5) | 93.97 | 6.06 d (1.4) | 93.38 | 5.74 d (1.4) |
| 3 | CH | 141.07 | 6.39 d (6.4) | 141.81 | 6.39 d (6) | 142.81 | 6.46 d (6.4) | 141.83 | 6.39 d (6.4) |
| 4 | CH | 110.27 | 5.04 dd (6.3, 1.6) | 107.76 | 5.01 dd (6.4, 1.7) | 104.93 | 5.02 dd (6.5, 1.7) | 107.02 | 5.07 dd (6.4, 1.6) |
| 5 | C | 72.10 | – | 72.82 | – | 72.48 | – | 71.75 | – |
| 6 | $CH(CH_2)$ | 37.14 | 1.63 ddd (11.9, 6.5, 1.5), 2.21 m | 76.48 | 3.96 m | 76.42 | 3.84 dd (4.4, 1.5) | 76.92 | 3.83 t (4.7, 4.7) |
| 7 | $CH_2$ | 38.23 | 1.53 m, 1.96 ddt (13.6, 6.8, 1.3, 1.3) | 45.43 | 2.41 dt (15, 1.2, 1.2), 1.93 dd (14.9, 4.7) | 44.45 | 2.03 dd (15.6, 4.6), 2.18 dt (15.7, 1.2, 1.2) | 46.01 | 1.84 ddd (14.2, 4.2, 1), 2.02 dd (14.1, 5) |
| 8 | C | 88.27 | – | 86.68 | – | 88.06 | – | 77.65 | – |
| 9 | CH | 58.06 | 3.04 q (1.5) | 54.84 | 3.41 q (1.5) | 53.17 | 2.87 q (1.4) | 57.51 | 2.57 q (1.2) |
| 10 | $CH_3$ | 21.89 | 1.42 s | 22.26 | 1.46 s | 21.41 | 1.45 s | 24.59 | 1.27 s |
| Ac | $CH_3$ | 21.68 | 1.72 s | 21.70 | 1.71 s | 21.81 | 2.06 s | – | |
| | – | 170.88 | – | 170.53 | – | 174.30 | – | – | |
| | | | | | *β*-D-Glc*p* | | | | |
| 1 | CH | 98.64 | 5.13 d (8) | 98.75 | 5.18 d (8) | 98.82 | 4.74 d (8) | 98.92 | 4.74 d (8) |
| 2 | CH | 74.71 | 3.88 dd (9, 8) | 74.46 | 3.93 dd (9, 8) | 72.62 | 3.32 dd (9, 8) | 73.09 | 3.33 dd (9.4, 8) |
| 3 | CH | 78.45 | 4.11 m | 78.11 | 4.15 t (8.8) | 75.56 | 3.52 t (9) | 75.96 | 3.52 t (9) |
| 4 | CH | 71.59 | 4.11 m | 71.25 | 4.09 t (8.8) | 69.74 | 3.42 dd (9.7, 9) | 70.26 | 3.41 dd (9.8, 9) |
| 5 | CH | 78.68 | 3.86 m | 78.42 | 3.86 ddd (9, 5.4, 2.2) | 76.29 | 3.49 m | 76.86 | 3.51 m |
| 6 | $CH_2$ | 62.63 | 4.21 dd (11.9, 5.4), 4.37 dd (11.9, 2.5) | 62.36 | 4.18 dd (12, 5.6), 4.39 dd (12, 2.4) | 60.79 | 3.75 dd (12.5, 5.7), 3.94 dd (12.5, 2.2) | 61.29 | 3.74 dd (12.3, 5.8), 3.93 dd (12.4, 2.3) |

*Assignment of resonances is arbitrary. H-9 has one constant $^3J$ and two $^4J$ of similar value. Because of this the H-9 resonance is observed as a 1:3:3:1 quartet.

**8-Acetylharpagide (3)**, $C_{17}H_{26}O_{11}$, and **harpagide (4)**, $C_{15}H_{24}O_{10}$. Interpretation of PMR and $^{13}C$ NMR spectra of **3** (Table 2) and DEPT, COSY, Hetcor, and NOESY spectra were consistent with a structure identical with that of 8-acetylharpagide [5]. Analogously, **4** was identified as harpagide [6]. As expected, alkaline hydrolysis of 8-acetylharpagide gave a product that was identified as **4**.

**6-Deoxy-8-acetylharpagide (2)**, $C_{17}H_{26}O_{10}$. PMR and $^{13}C$ NMR spectra of **2** showed that the compound differed from 8-acetylharpagide by the lack of a secondary hydroxyl on C-6, i.e., the compound was 6-deoxy-8-acetylharpagide. The iridoid glycoside was identical to that reported [7].

Thus, genkwanin (**1**), 6-deoxy-8-acetylharpagide (**2**), 8-acetylharpagide (**3**), and harpagide (**4**) were isolated and identified from the aerial part of *L. turkestanicus*. It is quite certain that the sedative and hypotensive properties of *L. turkestanicus* are due to iridoid glycosides.

PMR spectra were recorded from $C_5D_5N$ and DMSO-$d_6$ (δ, ppm, 0 = HMDS) and $D_2O$ solutions on a UNITYplus 400 (Varian) spectrometer. The internal standard for the last solution was acetone, the resonance of which had chemical shift δ 2.22 or *tert*-butyl alcohol, the resonance of which was found at δ 1.24. $^{13}C$ NMR spectra were also recorded on the UNITYplus 400 spectrometer (Varian) with full suppression of C–H coupling and under DEPT conditions. 2D NMR spectra ($^1H$–$^1H$ COSY, Hetcor, NOESY) were obtained using standard Varian programs. Chemical shifts of C atoms are given relative to the $\beta$-carbon atoms of $C_5D_5N$ (δ 123.493 vs. TMS). Spectra taken from $D_2O$ solutions have chemical shifts vs. that of $CD_3OD$ (δ 49.00).


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