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NEW FLAVANONE AND OTHER CONSTITUENTS OF *Helichrysum arenarium* INDIGENOUS TO CHINA

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*A new flavonoid for which the structure (2*S*)-5,8,4′-trihydroxy-6,7-vinylenedioxyflavanone was established and the known compounds stigmasterol, stigmasterol* β-*D-glucopyranoside, caffeic acid ethyl ester, and 3,4-methylenedioxycinnamic acid were isolated from* Helichrysum arenarium.

Keywords: *Helichrysum arenarium*, triterpenoids, phenylcarboxylic acid derivatives, flavonoids, PMR and ^{13}C NMR spectra.

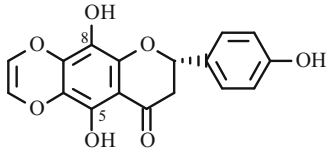
Helichrysum arenarium L. or everlasting is a perennial medicinal plant that is included in the pharmacopoeias of several European countries and has a long history of use in folk and practical medicine for treating various diseases [1, 2]. Recent research found strong antibacterial and anti-oxidant properties for various extracts of this plant [3–6].

This species of everlasting has a very broad natural distribution because it grows in all regions of Europe, Asia Minor, Central Asia and in the Altai, Mongolia, and China [1].

Populations of this plant indigenous to Europe are well studied with respect to their chemistry. It was found that the characteristic secondary metabolites of everlasting are isoprenoids (mono-, sesqui-, di-, and triterpenoids) and phenolic compounds (aromatic phenols and acids, coumarins, phthalides, flavonoids, etc.) [6–20].

It is well known that the qualitative and quantitative compositions of plant secondary metabolites can change depending on the habitat and vegetation period. Studies of the chemistry of the *H. arenarium* population indigenous to China were begun because of this and due to its broad use in traditional Chinese medicine [21–23].

In continuation of the study of the chemical composition of everlasting indigenous to Xinjiang Autonomous Province of the PRC, the known compounds *β*-sitosterol (**1**), stigmasterol (**2**), *β*-sitosterol *β*-D-glucopyranoside (**3**), stigmasterol *β*-D-glucopyranoside (**4**), caffeic acid ethyl ester (**5**), 3,4-methylenedioxycinnamic acid (**6**), 3,5-dihydroxy-6,7,8-trimethoxyflavonol (**7**), and the new flavanone **8** were isolated by separation of the total EtOAc extract obtained by work up of the alcohol extract.



8

Flavanone **8** is a yellow crystalline compound with formula $C_{17}H_{12}O_7$, mp 224–225°C, $[\alpha]_D$ –77.5°. The IR spectrum of **8** shows absorption bands characteristic of hydroxyls (3298 cm^{-1}), carbonyl (1630), and an aromatic ring (1602 and 1498).

The structure of **8** was elucidated using the PMR and ^{13}C NMR spectral data presented in Table 1.

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TABLE 1. PMR and ^{13}C NMR Spectra of Compound **8** (DMSO-d_6, HMDSO 0.0 ppm, δ, J/Hz)

C atom	δ_C	δ_H	C atom	δ_C	δ_H
2	78.52	5.43 (dd, J = 12.6, 3.0)	1′	128.94	
3	42.04	2.68 (dd, J = 17.2, 3.0)	2′,6′	128.44	7.32 (d, J = 8.6)
		3.26 (dd, J = 17.2, 12.6)	3′,5′	115.26	6.79 (d, J = 8.6)
4	196.49		4′	166.72	
5–8	147.06, 157.79,		5-OH		12.14 s
	163.02, 163.56		8-OH		10.87 s
9	172.09		4′-OH		9.65 s
10	101.85		-OCH=CHO-	95.06, 95.88	5.88 s

An ABX system was observed in the PMR spectrum as a doublet of doublets with chemical shifts δ 5.43, 3.26, and 2.68 ppm with SSCC characteristic of the H-2*β*, H-3_{ax}, and H-3_{eq} protons of flavanone ring C [24].

Next two 2H resonances of AA′BB′ character were observed from the symmetric system (2′,6′ and 3′,5′) of *p*-substituted ring B.

The 1H singlets at 9.65, 10.87, and 12.14 ppm belonged to aromatic hydroxyls. The chemical shift of the resonance at weakest field indicated the presence of a strong intramolecular H-bond of the hydroxyl and the C-4 carbonyl. The presence of this bond was confirmed by recording the PMR spectrum at elevated temperature (100°C). The chemical shift of the hydroxyl proton did not change at elevated temperature. Therefore, this hydroxyl was located on C-5. According to the symmetry of the *p*-substituted aromatic system, the SSCC of the protons of this AA′BB′ system, and the chemical shifts of the hydroxyl-proton resonances, the second hydroxyl was situated on C-4′ of ring B.

The absence of other aromatic protons indicated that flavanone ring A had four substituents, one of which should be assigned as a third hydroxyl. Its presence on C-6 was excluded because a positive qualitative reaction with $CrCl_2$ that is characteristic of the *ortho*-dihydroxy group in ring A was not observed [25]. Flavanone **8** gave a positive gossypetin test that is characteristic of a *para*-dihydroxy group. This indicated that the third hydroxyl was positioned on C-8 [25]. The remaining 2H singlet at 5.88 ppm could be assigned to either protons of a dioxymethylene group, which is often encountered in flavonoids, or protons of a dioxyvinylene group. In the second instance, the resonances of two protons should have the same chemical shifts. Trifluoroacetic acid was added to the DMSO-d_6 solution of the compound in order to answer this question using PMR spectroscopy. With this, the 2H singlet at 5.88 split into two 1H doublets with SSCC 2.2 Hz, indicating the presence of a vinylenedioxy moiety. According to the allowed positions for hydroxyls in ring A, the dioxyvinylene group was located on C-6 and C-7.

This structure was confirmed using ^{13}C NMR spectral data. Analysis of DEPT spectra showed the following qualitative hydrocarbon composition: nine quaternary C atoms, seven tertiary, and one secondary belonging to the C-3 methylene. The H-containing C atoms were assigned based on heteronuclear correlations. As it turned out, the 2H singlet at 5.88 ppm in the PMR spectrum corresponded to two tertiary C resonances in the ^{13}C NMR spectrum at 95.06 and 96.88 ppm. These indicated the presence in the structure of a vinylenedioxy group. Resonances of quaternary C atoms were assigned using literature data for flavonoids [22].

Flavanones contain one asymmetric C atom at C-2. The *S*-isomers usually dominate in plants [26]. These are found as the levo-rotary forms [27–29], like in flavanone **8** isolated by us.

According to the results (Table 1) and additional 2D NMR experimens performed by us, which agreed with results from an analogous analysis of a similar structure [30], the chemical shifts and SSCC for the protons (characteristic [24] of H-2*β*, H-3_{ax}, and H-3_{eq}) of the ABX system of flavanone **8** ring C belonged to the 2*S*-isomer.

According to the comprehensive analysis, the physicochemical properties, and the literature, the isolated flavanone **8** had the structure (2*S*)-5,8,4′-trihydroxy-6,7-vinylenedioxyflavanone.

According to the literature, flavonoids containing a vinylenedioxy group have not previously been isolated from natural sources.

Thus, eight compounds, of which **2**, **4**, **5**, **6**, and **8** were observed for the first time from this plant species, were isolated and characterized from *H. arenarium* indigenous to China. The structure of the new flavonoid **8** was elucidated.

EXPERIMENTAL

General Comments. IR spectra were recorded on a Perkin–Elmer model 2000 (KBr) Fourier spectrometer and a Nicolet Magna 4500 (KBr) IR spectrometer. PMR spectra were taken on a Unity-400+ spectrometer at operating frequency 400 MHz. Samples were prepared in DMSO-d_6 and DMSO-d_6 + Py-d_5 with HMDS internal standard (0 ppm). Spectra were recorded at room temperature on the δ-scale. Mass spectra were recorded on an Agilent Technologies 5973 Inert GC–MS (HP ChemStation data processing system). Constituents of the mixture were separated on a quartz capillary column (30 m × 0.25 mm) with a bonded stationary phase (5%) of phenylmethyltrisiloxane. Constituents were identified from mass spectra and retention times using the Wiley GC–MS library. TLC analysis used Silufol UV 254 chromatographic plates with detection by iodine vapor, ammonia vapor, a UV lamp at 254 and 365 nm, and 1% vanillin in conc. H_2SO_4.

Extraction and Isolation of *H. arenarium* Secondary Metabolites. Air-dried ground plant raw material (10 kg) that was collected in Xinjiang Autonomous Province of the PRC during full flowering was extracted with EtOH (70%, 3 × 40 L) at room temperature for 8 h. The combined extracts were filtered from the precipitate. The filtrate was vacuum distilled. The condensed extract (1120.3 g) was diluted with water and extracted successively with petroleum ether (10 × 2 L), EtOAc (10 × 2 L), and *n*-BuOH (10 × 2 L). The yields of the extracts were petroleum-ether (110.4 g), EtOAc (168 g), *n*-BuOH (270 g), and aqueous residue (310 g).

Constituents of the total EtOAc extract (168 g) were separated by chromatography over a column (10 × 120 cm) of silica gel (1700 g) that was eluted successively with petroleum ether and then solvent systems (with an increasing gradient of the last) petroleum ether:EtOAc (100:1→1:1) and $CHCl_3$:MeOH (100:1→1:1).

***β*-Sitosterol (1) and Stigmasterol (2).** Identical fractions obtained by elution with petroleum ether:EtOAc (10:1) were combined and recrystallized from MeOH. The resulting crystals represented a mixture of the two related sterols **1** and **2** according to PMR spectral data (one spot in different systems by TLC). According to the integrated intensity of the resonance for the gem-hydroxyl protons and olefinic protons H-22 and H-23 of stigmasterol, the ratio of **1** and **2** in the mixture was about 2:1. Compounds **1** and **2** were identified by comparing their spectral properties with the published data [31].

***β*-Sitosterol *β*-D-glucopyranoside (3) and stigmasterol *β*-D-glucopyranoside (4)** were isolated from effluents of the $CHCl_3$:MeOH (30:1) system also as a mixture according to PMR spectral data. They were identified by comparing their spectral properties with the published data [32].

Caffeic acid ethyl ester (5) was isolated from effluents of the $CHCl_3$:MeOH (20:1) system as a crystalline compound, mp 144–145°C.

PMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 1.349 (3H, t, J = 7.2, –O–CH_2–C$\underline{H}_3$), 4.253 (2H, q, J = 7.2, –O–C$\underline{H}_2$–CH_3), 6.289 (1H, d, J = 16.2, *α*–CH=CH–), 6.814 (1H, d, J = 8.2, H-5), 6.978 (1H, dd, J = 8.2, 2.3, H-6), 7.073 (1H, d, J = 2.3, H-2), 7.572 (1H, d, J = 16.2, *β*–CH=CH–), 9.097 (1H, s, OH), 9.530 (1H, s, OH).

The compound was identified by comparing physical and spectral constants with the published data [33, 34].

3,4-Methylenedioxycinnamic Acid (6). Evaporated effluents remaining after isolation of **5** were dissolved in the minimum amount of MeOH and analyzed by GC–MS. A compound of formula $C_{10}H_8O_4$ was identified by comparing its retention time and mass spectrum, *m*/*z* (%): 192 (100) $[M]^+$, 175 (21), 163 (2.8), 145 (20), 117 (8.6), 89 (21), 63 (15.7), 39 (5.7) with those of the library spectrum of 3,4-methylenedioxycinnamic acid.

3,5-Dihydroxy-6,7,8-trimethoxyflavonol (7). Elution of the column with petroleum ether:EtOAc (10:1) isolated a flavonoid with mp 153–154°C. A comparison of PMR and ^{13}C NMR spectral data with those in the literature for this flavonoid identified it as 3,5-dihydroxy-6,7,8-trimethoxyflavonol, which was isolated earlier from this plant [22].

(2*S*)-5,8,4′-Trihydroxy-6,7-vinylenedioxyflavanone (8) was isolated from effluents of the $CHCl_3$:MeOH (20:1) system as yellow crystals, mp 224–225°C. IR spectrum (KBr, ν, cm^{-1}): 3298, 3127, 3034, 2921, 2852, 2359, 2342, 1630, 1602, 1520, 1498, 1463, 1420, 1387, 1341, 1312, 1250, 1182, 1157, 1084, 1064, 1014, 970, 890, 832, 760, 731, 667, 650, 554, 531, 492. Table 1 lists the PMR and ^{13}C NMR spectral data.

REFERENCES

1. *Plant Resources of the USSR: Flowering Plants, Their Chemical Composition and Use. Family Asteraceae (Compositae)* [in Russian], Vol. **7**, Nauka, St. Petersburg, 1993, p. 120.

2. M. D. Mashkovskii, *Drugs* [in Russian], Vol. **1**, Meditsina, Moscow, 1984, p. 513.
3. A. Rancic, M. Sokovic, J. Vukojevic, A. Simic, P. Marin, S. Duletic-Lausevic, and D. Djokovic, *J. Essent. Oil Res.*, **17**, No. 3, 341 (2005).
4. U. Oezgen, A. Mavi, Z. Terzi, M. Coskun, and A. Yildirim, *Turkish J. Pharmaceut. Sci.*, **1**, No. 3, 203 (2004).
5. B. Tepe, M. Sokmen, A. H. Askin, and A. Sokmen, *Food Chem.*, **90**, No. 4, 685 (2004).
6. E. Czinner, K. Hagymasi, A. Blazovics, A. Kery, E. Szoke, and E. Lemberkovics, *J. Ethnopharmacol.*, **73**, No. 3, 437 (2000).
7. J. Jelink, Z. Jiricka, I. Janku, and M. Hava, *Cesk. Fysiol.*, **9**, 289 (1960).
8. O. Y. Rashba and G. A. Mostovova, *Mikrobiol. Zh., Akad. NU*, **24**, No. 2, 48 (1962).
9. O. Y. Rashba, *Mikrobiol. Zh., Akad. NU*, **26**, No. 2, 26 (1964).
10. E. Czinner, E. Lemberkovics, K. E. Bihatsi, G. Vitanyi, and L. Lelik, *J. Essent. Oil Res.*, **12**, No. 6, 728 (2000).
11. E. Dombrowicz, L. Swiatek, and W. Kopycki, *Pharmazie*, **47**, No. 6, 469 (1992).
12. A. H. Mericli, B. Damadyan, and B. Cubukcu, *Sci. Pharm.*, **54**, No. 4, 363 (1986).
13. A. I. Derkach, N. F. Komissarenko, and V. T. Chernobai, *Khim. Prir. Soedin.*, 777 (1986).
14. A. P. Prokopenko, V. N. Spiridonov, V. I. Litvinenko, and V. T. Chernobai, *Khim. Prir. Soedin.*, 649 (1972).
15. J. Vrkoc, L. Dolejs, P. Sedmera, S. Vasickova, and F. Sorm, *Tetrahedron Lett.*, No. 3, 247 (1971).
16. E. Lemberkovics, E. Czinner, A. Balazs, K. E. Bihatsi, G. Vitanyi, L. Lelik, J. Bernath, and E. Dzoke, *Acta Pharm. Hung.*, **71**, No. 2, 187 (2001).
17. E. Czinner, L. Kursiinszki, D. Baumann, M. Hamburger, A. Kery, and E. Lemberkovics, in: *Proceedings of the Phytochem. Society of Europe*, 47 (Natural Products in the New Millennium: Prospects and Industrial Application), 99 (2002).
18. J. Vrkoc, K. Ubik, and P. Sedmera, *Phytochemistry*, **12**, No. 8, 2062 (1973).
19. J. Vrkoc, M. Budesinsky, L. Dolejs, and S. Vasickova, *Phytochemistry*, **14**, No. 8, 1845 (1975).
20. I. B. Chinou, V. Ronsiss, and A. Loukis, *Planta Med.*, **62**, 377 (1996).
21. *Chinese Medicinal Materials Company, "Chinese Medicine Resources Status"*, Beijing. The Press of Chinese Science, 1302 (1994).
22. Lv Hui, Li Quan, Zhong Jie, Liao Li-Xin, and Haji Akber Aisa, *Chin. Pharm. J.*, **43**, No. 1, 11 (2008).
23. K. A. Eshbakova and H. A. Aisa, *Khim. Prir. Soedin.*, 774 (2009).
24. P. Bagri, M. Ali, S. Sultana, and V. Aeri, *Khim. Prir. Soedin.*, 169 (2010).
25. K. R. Markham, *Isolation Techniques for Flavonoids*, in: *The Flavonoids*, J. B. Harborne (ed.), Chapmann and Hall, London, 1975, p. 244.
26. D. Yu. Korul′kin, Zh. A. Abilov, R. A. Muzychkina, and G. A. Tolstikov, *Natural Flavonoids* [in Russian], Geo, Novosibirsk, 2007, p. 17.
27. M. Shoja, *Z. Kristallogr.*, **189**, 89 (1989).
28. N. T. Dat, K. Lee, Y.-S. Hong, Y. H. Kim, C. V. Minh, and J. J. Lee, *Planta Med.*, **75**, 803 (2009).
29. P. Basnet, S. Kadota, M. Shimizu, H.-X. Xu, and T. Namba, *Chem. Pharm. Bull.*, **41**, No. 10, 1790 (1993).
30. E. A. Khamidullina, M. Purevdash, I. V. Ushakov, O. V. Neretina, and S. A. Medvedeva, *Khim. Prir. Soedin.*, 81 (2005).
31. N. V. Kovganko, Zh. N. Kashkan, and E. V. Borisov, *Khim. Prir. Soedin.*, 751 (1999).
32. I. M. Isaev, R. P. Mamedova, M. A. Agzamova, and M. I. Isaev, *Khim. Prir. Soedin.*, 296 (2007).
33. H. Wagner, Y. H. Heur, A. Obermeier, G. N. Tittel, and S. Bladt, *Planta Med.*, **44**, 193 (1982).
34. N. I. Kulesh, N. P. Krasovskaya, and O. B. Maksimov, *Khim. Prir. Soedin.*, 506 (1986).