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Note

Studies on the flavonoid compounds of *Origanum vulgare* L.

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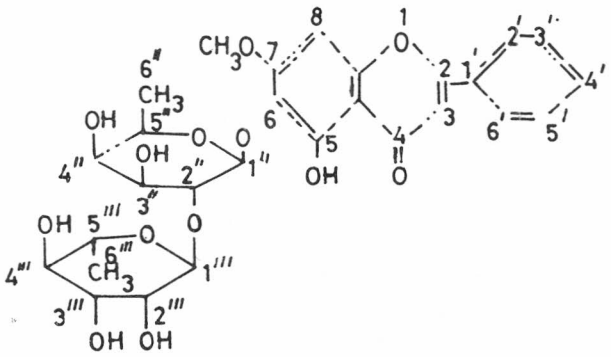
A novel flavone glycoside, 5-hydroxy-7-methoxy-6-*O*-[α-L-rhamnopyranosyl(1 → 2)-β-D-fucopyranosyl]flavone **2** has been isolated along with four known flavones and their structures have been deduced on the basis of their spectral data and chemical studies. This is the first report on the isolation of flavone-glycosides from *Origanum vulgare* L.

Origanum vulgare L. is an annual herbaceous lipped plant and is distributed mainly in Hunan, Gansu and Shanxi provinces of China. It is reported in traditional chinese folk medicine to have good antibiotic and anti-inflammatory activities. It also counteracts cancer cells of the liver properties[1]. However, very little is known about its chemical constituents. In continuation of our investigations[2-4] on the genus *Origanum*, we have examined the whole plants of *Origanum vulgare* L. and now report for the first time the isolation and identification of a new flavone glycoside **2** and four known flavonoids.

The known compounds were identified by comparing their spectral data with those of authentic samples or with those reported in literature as 5, 6-dihydroxy-7-methoxylflavone **1**[5], 5-hydroxyl-6,7-dimethoxylflavone **3**[2], isoskuranetin-7-*O*-β-neohesperidoside **4**[3] and 3′,4′,5′-trimethoxyfuranoflavone **5**[4].

Compound **2** was obtained as yellow crystals, m.p. 238-39.3°. The mass (FAB) spectrum of **2** gave the molecular ion peak at m/z 576[M^+]. This peak along with elemental analysis led to the molecular formula $C_{28}H_{32}O_{13}$. The usual colour tests indicated **2** to be a flavone-glycoside. The UV(MeOH) spectrum showed signals at 250, 279, 318 nm; (+ NaOMe) 278, 360; (+$AlCl_3$)258, 286; and (+HCl) 257, 286, 345, which are the characteristic absorptions of a flavone[6]. Acid hydrolysis of **2** resulted in the formation of aglycone **2a**. The monosaccharides were identified as L-rhamnose and D-fucose by paper co-chromatography. The purified aglycone **2a** was obtained as orange crystals, m.p. 223-24°. It gave the positive Molish test, and showed in its IR spectrum a strong absorption at 3446cm^{-1} indicating the presence of a hydroxyl group. In its ^{1}H NMR spectrum two single peaks appearing at δ 12.30 and 11, 24 supported the presence of two hydroxyl groups (C_5−OH, 1H, s; C_6−OH,1H,s, disappeared on the addition of D_2O). Besides, it showed three signals due to C_8-H, C_3-H and methoxy group protons at δ 6.90(1H,s), 6.92 (1H, d,J=5.0) and 3.92 (3H,brs), respectively. The MS(EI) of **2a** gave a molecular ion at m/z 284 and major fragments at m/z 283, 256, 182 and 152. Its melting point was consistent with that of 5,6-dihydroxy-7-methoxylflavone. Based on comparison of the spectral data of **2a** with those of **1**[5], the aglycone (**2a**) was characterized as 5,6-dihydroxy-7-methoxylflavone. In addition, the ^{1}H NMR spectrum of **2** exhibited signals ascribable to anomeric protons of two sugar moieties at 5.73 (1H,d, 7.5Hz), and 4.55(1H, t, 7.2Hz) (cf. Table I) indicating the presence of one β-linkage and one α-linkage. A detailed comparison of the ^{13}C NMR data of **2** with **2a** (Table II), showed downfield shift of 5.6 ppm for C-6 and an upfield shift of 5.9ppm for C-5 in the latter thereby indicating the glycosidic linkage between sugar moiety and the hydroxyl at C-6 of aglycone. The glycoside on partial hydrolysis liberated L-rhamnose and then D-fucose, thereby indicating that L-rhamnose was terminal sugar, and D-fucose was attached to the aglycone. The sugar carbon signals (Table II) were



(2)

Table I—^{1}H NMR chemical shifts of rhamnose and fucose in C_5D_5N (J values in Hz)

	Fucose		Rhamnose
C1′-H	4.55(t,J=7.2)	C1‴-H	5.73(d,J=7.5)
C2″-H	4.70(d,J=5.0)	C2‴-H	3.95(dd,J=1.5,2.5)
C3″-H	3.60(dd,J=5.0,8.0)	C3‴-H	3.79(dd,J=2.5,9.0)
C4″-H	3.42(d,J=6.0)	C4‴-H	3.40(dd,J=9.0,9.0)
C5″-H	4.50(dd,J=8.0,7.8)	C5‴-H	4.15(dd,J=9.0,6.5)
C6″-CH_3	1.40(d,J=6.8)	C6‴-CH_3	1.30(d,J=6.5)

Table II—^{18}C NMR chemical shifts of compounds **2a** and **2** in C_5D_5N

Carbon	**2a**(δ_c)	**2**(δ_c)	Carbon Fucose (δ_c)		Carbon Rhamnose (δ_c)	
C-1	—	—	C-1″	101.8	C-1‴	101.5
C-2	163.06	162.99	C-2″	76.6	C-2‴	72.4
C-3	104.61	104.4[1]	C-3″	75.1	C-3‴	72.5
C-4	182.17	182.20	C-4″	72.9	C-4‴	74.2
C-5	146.07	140.93	C-5″	72.6	C-5‴	69.5
C-6	130.03	136.63	C-6″	16.9	C-6‴	19.1
C-7	154.53	154.23				
C-8	91.17	91.09				
C-9	149.72	149.58				
C-10	105.24	104.96				

easily assigned. The glycosidation shifts on C-1(+7ppm) and C-2(+4.6ppm) of fucose and on C-1(+6ppm) of rhamnose established the presence of a nodal rhamnopyranosyl residue glycosylated at C-2 of fucose, and C-1 connected with C-6 of aglycone. On the bases of these results compound **2** was identified as 5- hydroxy-7-methoxy-6-*O*-[α-L-rhamnopyranosyl(1→2)- β-D-fucopyranosyl]flavone.

Experimental Section

Melting points were determined on a Kofler hot plate and are uncorrected. Spectra were recorded using the following instruments: UV, Japan shimadzu UV-300 double beam spectrophotometer; IR, Perkin-Elmer 986 spectrometer (KBr disks); NMR, FT-80 Varian spectrometer (400MHz, for ^{1}H and 100MHz for ^{13}C), chemical shifts are given in δ ppm downfield from TMS internal standard; MS, MAT-44S and ZAB-HS mass spectrometer. Silica gel(200-300 mesh, 100-200 mesh) and polyamide(120-160 mesh) were for column chromatography.

Collection of plant material. The whole plants of *Origanum vulgare* L. were collected from Gansu Province of China, in June 1991; a voucher specimen identified by Prof. Y S Lian is preserved in the Herbarium of the Botany Department, Northwest Normal University, Lanzhou 730070, PR China.

Isolation and extraction. Dried plants (3kg) were pulverized and soaked in 95% EtOH(three times). The residue obtained by concentration of EtOH extract under reduced pressure was partitioned successively with pet. ether, Et_2O, EtOAc and *n*-BuOH in a soxhlet extractor.

The Et_2O solution portion after removal of the solvent under reduced pressure yielded a dark green gummy residue (120g), which was chromatographed over silica gel using chloroform, mixtures of chloroform-methanol and ethyl acetate-methanol in increasing polarity as eluants. Rechromatography of selected fractions and purifications using preparative TLC yielded four crystalline compounds **1**(110mg), **2**(130mg), **3**(50mg) and **4**(79mg). Rechromatography of selected fractions over polyamide column using MeOH-H_2O as eluants gave the crystals of **5**(38mg).

Flavone glycoside 2. Yellow crystals, m.p. 238-39.3°. UV and IR spectral data are given in the text; ^{1}H NMR(400MHz, DMSO-d_6): δ12.30 (1H,s,C_5 OH), 8.02 (2H,m,2′,6′-H), 7.05 (2H,m,3′,5′-H), 6.90(1H,s,C_8-H), 6.92 (1H,d,J=5.0Hz, C_8-H), 5.73(1H,d,J=7.5Hz, sugar anomeric proton), 4.55(1H,t, J=7.2Hz, sugar anomeric proton), 3.92(3H,s,OCH_8); sugar protons and carbon signals were unequivocally assigned(Tables I and II); MS(FAB): m/z 576[M^+], 430,284,283,264,238 (Found: C,58.28, H,5.64. $C_{28}H_{32}O_{12}$ requires C, 58.33; H, 5.56%).

Aglycone 2a. Orange crystals, m.p. 223-24°; ^{1}H NMR (400MHz, DMSO-d_6): δ3.92(3H,s,$-OCH_3$), 6.90 (1H,s,C_8-H), 6.92(1H,d,J=5.0, C_3-H), 7.05(2H,m,3′,5′-H), 8.02(2H,m,2′,6′-H), 12.30 (1H,s,C_5-OH), 11.24 (1H,s,C_6-OH); UV(MeOH):257, 278, 346nm; IR(KBr): 3446, 1615, 1510, 1370,1092, 1054 cm^{-1}; MS(EI): m/z 284 [M^+], 283, 256, 182, 154, 120, 77; ^{13}C NMR data are listed in Table II (Found: C, 67.55, H, 4.15. $C_{16}H_{12}O_5$ requires: C, 67.60, H, 4.22%).

Acknowledgement

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