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Flavonoids from *Isodon eriocalyx*

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Abstract – From the dried leaves of *Isodon eriocalyx* (Labiatae) six flavonoids were isolated and two of them were elucidated to be novel ones named 5,7,8,4'-tetrahydroxy-6-methoxyflavone (1), and isothymusin-8-O-β-D-glucoside (2).

Key words – *Isodon eriocalyx*, Labiatae, flavonoids.

Introduction

Isodon eriocalyx (Dunn) Hara, a perennial herb or shrub plant of Labiatae family, is widely distributed in Yunnan, Sichuan, Guizhou and Guangxi Province. It has long been used as folk medicine to treat sore throat, inflammation and interdigital disease (Kunming Institute of Botany *et al.*, 1977) as well as reducing blood pressure (Li *et al.*, 1988).

The *Isodon* genus is known to be rich in *ent*-kaurane diterpeonids. A series of new *ent*-kaurane diterpenoids have been isolated from the dried leaves of *I. eriocalyx* collected in different regions of Yunnan Province (Takeda *et al.*, 1995, Hassanm *et al.*, 1994).

During the course on a systematic investigation of the biologically active constituents from *Isodon* plants, we further examined the dried leaves of the same plant and isolated six flavonoids: 5,7,8,4'-tetrahydroxy-6-methoxyflavone (1) and isothymusin-8-O-β-D-glucoside (2), isothymusin (3) (Barbaran *et al.*, 1986), luteolin-7-methyl ether (4) (Wallace *et al.*, 1971), cirsimaritin (5) (Wang *et al.*, 1985) and genkwanin (6) (Wallace *et al.*, 1971). Compounds (1) and (2) were determined to be novel ones and the others were obtained from *I. eriocalyx* for the first time.

Experimental

Geneeral experimental procedures – All melting points were measured on a XRC-1 micro melting point apparatus produced by Sichuan University and uncorrected. Optical rotations were taken on a JASCO-20C digital polarimeter. IR spectra were recorded with a Perkin-Elmer 577 spectrometer. UV spectra were obtained on a UV 210A spectrometer. MS spectra were measured on a VG Auto Spec-3000 spectrometer. NMR spectra were run on a Bruker AM-400 spectrometer. The chemical shifts (δ) were expressed in ppm with reference to the solvent signals. Coupling constants (J) were given in Hz.

Plant Material – The dried leaves of *Isodon eriocalyx* (Dunn) Hara were collected in Jiangchuan County, Yunnan Province, China, in September 10, 1994 and identified by Prof. X.-W. Li. A voucher specimen is kept in the Herbarium of Kunming Institute of Botany.

Extraction and isolation – Dried powdered leaves of *I. eriocalyx* (Dunn) Hara (11.9 kg) were extracted with MeOH (4×40l) und-

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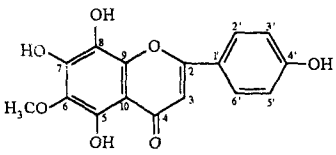

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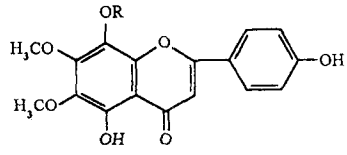
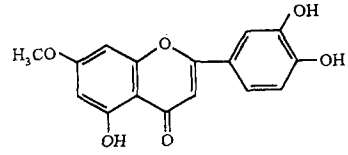


2 R=Glc
3 R=H



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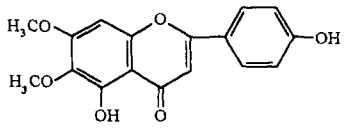

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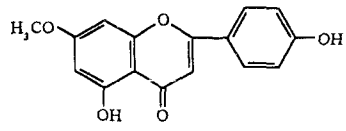

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er reflux and then concentrated in vacuum to give crude extract (978 g). The extract was dissolved in H_2O and successively extracted with petroleum ether (60-90℃) and ethyl acetate. The AcOEt extract (395 g) was chromatographed on a silica gel column (1.6 kg, 200-300 mesh) eluting with $CHCl_3$ by increasing amount of Me_2CO to yield seven fractions. Fraction I, II and III were submitted to MCI gel to decolor green pigments. Then fraction II-VII was subjected to silica gel column chromatography repeatedly and finally afforded six compounds: 1 (44 mg), 2 (60 mg), 3 (3.3 g), 4 (35 mg), 5 (45 mg) and 6 (53 mg).

5,7,8,4'-Tetrahydroxy-6-methoxyflavone (1): $C_{16}H_{12}O_7$, red-orange crystals (from MeOH), mp: 162.5-164.5℃, $[\alpha]_D^{25}$ +5.4° (C_5H_5N, c 0.513); IR ν_{max}^{KBr} cm^{-1}: 3360, 3120, 1646, 1580, 1564, 1490, 1430, 1395, 1210, 1170, 1086, 1053, 1000, 955, 825 and 803; UV λ_{max}^{MeOH} nm (log ε): 333.5 (4.22), 277.0 (4.09); +NaOMe: 394.0, 275.5, 249.0; +$AlCl_3$: 362.0, 309.0, 283.5, 227.0 (sh); +$AlCl_3$+HCl: 384.0 (sh), 353.5, 309.5, 283.5, 228.5 (sh); +NaOAc: 392.5, 334.0, 292.0 (sh) and 277.0; +NaOAc+H_3BO_3: 339.0, 277.0; EIMS 70 ev m/z (rel. int. %): 316 $[M]^+$ (93), 301 $[M\text{-}CH_3]^+$ (100), 273 (10), 183 (58), 169 (9), 155 (33), 118 (31); HR EIMS m/z: 316.0548 (calc. 316.0583); ^1H NMR (DMSO)δ: 6.72 (1H, s, H-3), 8.00 (2H, d, J=8.8 Hz, H-2', 6'), 6.93 (2H, d, J=8.8 Hz, H-3', 5'), 12.54 (1H, s, OH-5), 3.77 (3H, s, OCH_3-6); ^{13}C NMR data see Table 1.

Isothymusin-8-O-β-D-glucoside (2), $C_{23}H_{24}O_{12}$, yellow crystals (from MeOH), mp: 310.5-312.5℃, $[\alpha]_D^{25}$-60.0° (C_5H_5N, c 0.300); IR ν_{max}^{KBr} cm^{-1}: 3450, 1644, 1600, 1560, 1370, 1300, 1220, 1070, 1040, and 830; UV λ_{max}^{MeOH} nm (log ε): 370.0 (4.17), 303.0 (4.39, sh), 286.0 (4.42), 223.5 (4.37); +Na-OMe: 378.0, 333.0 (sh), 279.0, 241.5; +$AlCl_3$: 414.0, 395.5 (sh), 354.5

Table 1. ^{13}C NMR data of compounds 1-6 in DMSO (100 M Hz, δ in ppm with reference to the signal of DMSO)

Carbon	1	2	3	4	5	6
2	163.6	164.5	164.1	164.2	164.0	164.0
3	102.1	102.3	102.4	103.0	102.6	102.9
4	182.5	182.6	182.7	181.8	182.1	181.8
5	145.2	148.9	144.7	161.2	152.5	161.1
6	131.5	136.1	136.1	97.9	131.7	97.9
7	147.2	153.1	148.0	165.1	158.5	165.1
8	125.5	129.0	130.5	92.5	91.4	92.6
9	141.6	145.3	141.3	157.2	152.0	157.2
10	102.9	106.2	106.3	104.6	105.0	104.6
1'	121.5	121.1	121.2	121.4	121.0	121.0
2'	128.7	129.1	128.7	113.5	128.4	128.4
3'	115.9	115.9	115.9	145.7	115.9	115.9
4'	161.2	161.4	161.3	149.8	161.2	161.2
5'	115.9	115.9	115.9	116.0	115.9	115.9
6'	128.7	129.1	128.7	119.0	128.4	128.4
OCH_3	60.1	61.6	61.0	56.0	59.9	55.9
		60.6	60.4		56.3	
1"		103.8				
2"		74.1				
3"		77.2				
4"		70.1				
5"		76.4				
6"		61.1				

(sh), 322.5, 232.0; +$AlCl_3$+HCl: 407.0, 390.0 (sh), 315.5, 246.0 (sh), 231.0; +NaOAc: 374.5, 336.0, 281.5; +NaOAc+H_3BO_3: 412.5 (sh), 387.5, 307.0 (sh), 283.0; EIMS 70 ev m/z (rel. int. %): 330 [aglycone]$^+$ (100), 315 [aglycone-CH_3]$^+$ (84), 297 (25), 212, 197 (41), 169 (27), 118 (38) and 69 (55); FABMS (negative ion mode) m/z:491 [M-H]$^+$, 329 [aglycone-H]$^+$; HR FABMS (negative ion mode) m/z: 492.1246 (calc. 492.1268); ^{1}H NMR (DMSO)δ:6.88 (1H, s, H-3), 8.15 (2H, d, J=8.8 Hz, H-2', 6'), 6.89 (2H, d, J=8.8 Hz, H-3', 5'), 12.90 (1H, s, OH-5), 10.42 (1H, s, OH-4'), 3.81 (3H, s, OCH_3-6), 4.01 (3H, s, OCH_3-7) and 4.82 (1H, d, J=8.0 Hz, H-1"); ^{13}C NMR data see Table 1.

Enzymatic hydrolysis of 2 A solution of glycoside 2 (50 mg) and β-glucosidase (50 mg) in an acetate buffer (PH 5.0, 10 ml) was incubated at 37℃ for 24 h. The resulting precipitate was collected by filtration, then crystallized from MeOH and finally yielded 3.

Results and Discussion

Compound 1 was obtained as red-orange crystals (form MeOH). The analysis of ^{1}H and ^{13}C NMR spectra indicated 1 was a flavone with tetra-substituted ring A and 4'-substituted ring B. The EI mass spectra exhibited a molecuar ion peak at m/z 316 ($C_{16}H_{12}O_7$) in accordance with flavone containing one methoxyl and four hydroxyl groups. The UV spectra recorded after the addition of shift reagents (sodium methylate and aluminum chloride with hydrochloric acid) showed the presence of free hydroxyl groups at C-4' and C-5 positions.

All these facts mentioned above further revealed 1 was tetrahydroxymonomethoxy-flavone with a free 5-hydroxyl group in the fully oxygenated ring A and a ring B with 4'-hydroxyl group.

The location of methoxyl was determined by the long-range heteronuclear correlation NMR experiment (COLOC). The OH group (δ_H 12.54 ppm) at C-5 (δ_c 145.2 ppm) showed correlations with C-6 (δ_c 131.5 ppm) and C-10 (δ_c 102.9 ppm). COLOC correlation was also observed between OMe and C-6. Therefore, the methoxyl was determined to locate at C-6 and the structure of compound 1 was 5,7,8, 4'-tetrahydroxy-6-methoxyflavone.

Compound 2 was established to have a molecular formula of $C_{23}H_{24}O_{12}$ by the analysis of negative FAB mass spectra (ion at m/z 491 [M-H]$^-$) and ^{13}C NMR data including DEPT technique.

The ^{1}H and ^{13}C NMR spectra suggested 2 was a flavone glucoside with a tetrasubstitued ring A and 4'-substituted ring B. The coupling constant of the doublet for H-1" in the ^{1}H NMR spectrum (J=8.0 Hz) indicated β-D-glucose. The UV spectral data showed the existence of two free hydroxyl groups at C-5 and C-4' positions, respectively (Beijing College of Traditional Chinese Medicine *et al.*, 1986).

The aglycone was yielded by enzyme hydrolysis in buffer solution. The isolation of isothymusin (5,8,4'-trihydroxy-6,7-dimethoxyflavone) (3) in this plant reminded us maybe isothymusin was the aglycone of 2. The comparison of EI mass and UV spectra together with TLC detection confirmed this assumption. Thus, 2 was identified as isothymusin-8-O-β-D-glucoside.

The chemical shifts of the carbons in ring A agreed well with glycosylation effect (Yao *et al.*, 1995). In general terms the carbon at the site of glycosylation is shifted to a higher field following glycosylation, whereas the ortho- and para- related carbons shifted downfield. The chemical shifts of 2 were only consistent with glycosylation at C-8: upfield shift for C-8 (-1.5 ppm), and downfield shifts for C-7 (+5.1 ppm), C-9 (+4.0 ppm), and C-5 (+4.2 ppm) compared with 3, respectively. The assignments of carbon signals were achieved by long-range heteronuclear correlation NMR experiment (COLOC).

Finally, compound 2 was identified as isothymusin-8-O-β-D-glucoside.

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