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FLAVONOL GLYCOSIDES FROM *EPIMEDIUM SAGITTATUM*

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Key Word Index—*Epimedium sagittatum*; Berberidaceae; anhydroicaritin-3-*O*-α-rhamnoside; icaritin-3-*O*-α-rhamnoside; icariin; icarisid I.

Abstract—Two new flavonol glycosides were isolated from *Epimedium sagittatum* besides the known flavonol glycosides, icariin and icarisid I. On the basis of spectral analyses, the structures of the compounds were determined to be anhydroicaritin-3-*O*-α-rhamnoside and icaritin-3-*O*-α-rhamnoside.

INTRODUCTION

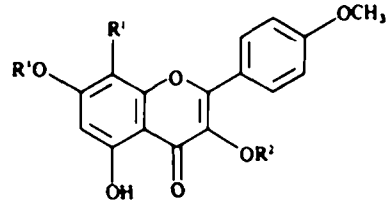
The aerial parts of *Epimedium* spp. ('Yin-Yang-Huo' in Chinese) have been used as crude drugs in China and Japan. The species are distributed in China (16 species) [1], Japan (nine species), Europe, the Middle East and the Himalayas. At present, *E. brevicornum*, *E. sagittatum*, *E. pubescens*, *E. Koreanum*, *E. grandiflorum* var. *thunbergianum* and *E. cremeum* are mainly used as 'Yin-Yang-Huo' in China and Japan. Constituents of *Epimedium* have been reported as flavonoids [2–13], alkaloids (magnoflorine) [14, 15] and lignans [16]. For the investigation of the chemotaxonomy of the genus *Epimedium*, we now report a preliminary study of the chemical constituents of *E. sagittatum* (Sieb. et Zucc.) Maxim. Two new flavonol glycosides (1 and 2) were isolated from the aerial parts, together with two other flavonol glycosides, icariin (3) and icarisid I (4).

RESULTS AND DISCUSSION

The EtOAc soluble portion of the 35% EtOH extract was chromatographed on silica gel to give four major compounds. These compounds responded to the Molisch and Shinoda (Mg-HCl) test.

Compound 1, mp 203–204° was obtained as yellow needles. Elemental analysis indicated the molecular formula as $C_{27}H_{30}O_{10}$. Its IR spectrum showed a strong absorption band at 1650 cm^{-1} for a chelated carbonyl group. The UV spectrum of 1 in MeOH showed absorption at 271 (band II), 310 and 350 sh (band I) nm, which indicated a sugar residue at C-3 in the flavonol skeleton [17]. The bathochromic shift of band I with $AlCl_3$/HCl (58 nm) is a characteristic feature of a 5-hydroxy-3-*O*-substituted flavonol. The bathochromic shift of band II (10 nm) with AcONa also indicated the presence of an unsubstituted hydroxy group at C-7 [17]. The bathochromic shift of band I (10 nm) with NaOMe suggested that no free 4'-hydroxy group existed in ring B [17]. The ^{1}H NMR spectrum showed five protons in the aromatic region; a singlet at 6.37 (1H) assignable to the A ring proton and a set of *ortho* coupled doublets at δ7.12 and 7.96 (each 2H, J = 9 Hz) to the 4'-substituted flavone. Furthermore, a signal of a methoxy group was observed at 3.89 ppm. In the EI mass spectrum, six major fragments were appeared at m/z 368, 353, 313, 300, 165 and 135. The fragment at m/z 368, which corresponded to the aglycone moiety of 1, suggested the presence of three hydroxyls, one methoxyl and one γ,γ-dimethylallyl group in the aglycone. Another fragment indicated that the methoxyl group was attached at C-4' (m/z; B_1') and that the γ,γ-dimethylallyl group was at C-6 or C-8 in ring A. The large bathochromic shift (58 nm) in the presence of $AlCl_3$/HCl showed that the γ,γ-dimethylallyl group was attached at C-8. On the basis of above data, the aglycone of compound 1 was confirmed to be 8-γ,γ-dimethylallyl-4'-methoxy-3,5,7-trihydroxyflavone (anhydroicaritin) [5]. In the ^{13}C NMR, a rhamnosyl moiety was confirmed in 1 (see Table 1). Consequently, the structure of 1 was elucidated to be anhydroicaritin-3-*O*-α-rhamnoside.

Compound 2, mp 238–239° was obtained as a yellowish brown powder. The IR, ^{1}H NMR, UV spectra were closely similar to those of 1. However, in the mass spectrum, the fragment corresponding to its aglycone appeared at m/z 386. The fragment ion at m/z 368 could be produced by loss of H_2O from the fragment at m/z 386. Consequently, it was considered that 2 was substituted with a $CH_2CH_2CH(OH)Me_2$ instead of a γ,γ-dimethylallyl group at C-8. The fact was further confirmed



1 R^1 = —$CH_2CH{=}C(CH_3)_2$, R^2 = rham; R^3 = H
2 R^1 = —$CH_2CH_2CHOH(CH_3)_2$, R^2 = rham; R^3 = H
3 R^1 = —$CH_2CH{=}C(CH_3)_2$, R^2 = rham; R^3 = Glc
4 R^1 = —$CH_2CH{=}C(CH_3)_2$, R^2 = H; R^3 = Glc

Table 1. ^{13}C NMR data of compounds 1–4*

Carbon no.	1	2	3	4
2	153.8	153.7	153.0	146.9
3	134.5	134.3	135.7	136.2
4	178.4	178.0	178.3	176.5
5	161.3	161.7	160.5	160.1
6	98.3	98.3	98.2	97.5
7	161.6	161.7	161.4	160.6
8	105.9	107.1	108.4	108.1
9	156.7	156.4	157.3	152.7
10	104.2	104.1	105.6	104.5
1′	122.4	122.3	122.2	123.4
2′	130.4	130.4	130.5	129.3
3′	114.0	113.9	114.1	114.1
4′	158.8	158.5	160.5	158.5
5′	114.0	113.9	114.1	114.1
6′	130.4	130.4	130.5	129.3
1″	21.1	42.5	21.1	21.5
2″	122.3	17.4	122.3	122.3
3″	131.0	68.8	131.1	131.1
4″	25.4	28.9	25.4	25.4
5″	17.7	29.1	17.5	17.9
1‴	101.9	101.9	102.0	100.5
2‴	70.4	70.4	70.4	73.4
3‴	70.6	70.6	70.6	76.7
4‴	71.2	71.1	69.7	69.7
5‴	70.1	70.0	70.1	77.2
6‴	17.5	17.4	17.9	60.7
1⁗			100.6	
2⁗			73.4	
3⁗			76.7	
4⁗			71.2	
5⁗			76.7	
6⁗			60.7	
-OMe	55.5	55.4	55.5	55.4

*Measured in DMSO-d_6.

by the signals in the ^{13}C NMR; 42.3 (*t*, C-1″), 17.4 (*t*, C-2″), 68.8 (*d*, C-3″) and 29.1 (*q*, C-4″,5″). On the basis of above data, the structure of **2** was determined to be icaritin-3-*O*-α-rhamnoside.

Compounds **3** (mp 223.3–225°) and **4** [248–249° (dec.)] were both obtained as yellow needles. The aglycone of both compounds was the same as that of **1**. The UV, IR, mass, ^1H NMR and ^{13}C NMR spectral data suggested that **3** and **4** were the known flavonols glycosides, icariin and icarisid [9], respectively.

It was previously supposed that icaritin was the aglycone of icariin. However, a recent study [9] has revealed the correct structure of the aglycone of icariin as anhydroicaritin. In this paper, we have also shown the presence icaritin glycoside in an *Epimedium* species.

EXPERIMENTAL

All mps are uncorr. MS were obtained at 70 eV. ^1H NMR spectra were recorded at 60 MHz; chemical shifts are given in δ values (ppm) with TMS as internal standard. ^{13}C NMR spectra were obtained with a spectral width of 3500 Hz. TLC was carried out on G-PF 254 (Merk) in $CHCl_3$–MeOH–H_2O (13:7:2; lower phase).

Extraction and isolation of flavonol glycosides. Commercial dried aerial parts of *E. sagittatum* (2 kg) were extracted with 35% EtOH (26 l.) at room temp. and the extract evapd *in vacuo* to give a greenish brown residue (300 g). The residue was suspended in H_2O and extracted with C_6H_6, EtOAc and *n*-BuOH, successively. The EtOAc fraction (41.8 g) was chromatographed on silica gel with a $CHCl_3$–MeOH gradient. The $CHCl_3$–MeOH (10:1) eluant gave a mixture of compounds **1**–**4**. Repeated CC, prep. TLC and recrystallization afforded compounds **1** (0.7 g), **2** (30 mg), **3** (2.2 g) and **4** (30 mg).

Compound **1** (*anhydroicaritin-3-O-α-rhamnoside*). Yellow needles, mp 203–204°. $C_{27}H_{30}O_{10}$ (calcd. C 63.04, H 5.84; found C 62.89, H 5.85). UV λ_{max}^{MeOH} nm: 271, 310, 350 sh, + NaOMe: 282, 380, + $AlCl_3$: 281, 305 sh, 342, 410, + $AlCl_3$/HCl: 281, 307, 345, 408, + AcONa: 281, 360, + AcONa/H_3BO_3: 271, 310 sh, 350. IR ν_{max}^{KBr} cm^{-1}: 3200 (OH), 1650 (chelated C=O), 1600. EIMS (*m/z*): 368, 353, 313, 300, 165, 135. ^1H NMR (DMSO-d_6): δ0.83 (3H, rhamnosyl Me), 1.70 (6H, *br s*, C-4″,5″, Me), 3.89–5.38 (*m*, sugar protons), 3.89 (3H, *s*, OMe), 6.37 (1H, *s*, H-6), 7.12 (2H, *d*, *J* = 9 Hz, H-3′,5′), 7.96 (2H, *d*, *J* = 9 Hz, H-2′,6′), 10.63 (1H, *s*, C-7, OH), 12.85 (1H, *s*, C-5, OH).

Compound **2** (*icaritin-3-O-α-rhamnoside*). Yellowish brown powder, mp 238–239° (C_6H_6–Me_2CO). $C_{27}H_{32}O_{11}$. UV λ_{max}^{MeOH} nm: 272, 313, 350 sh, + NaOMe: 281, 385 sh, + $AlCl_3$: 279, 308, 353, 400 sh, + $AlCl_3$/HCl: 281, 306, 350, 400 sh, + AcONa: 276, 350, + AcONa/H_3BO_3: 271, 315, 355 sh. IR ν_{max}^{KBr} cm^{-1}: 3400, 1650. EIMS (*m/z*): 386, 368, 353, 313, 300, 165, 135. ^1H NMR (DMSO-d_6): δ0.85 (3H, rhamnosyl Me), 1.09, 1.20 (each 3H, *s*, C-4″,5″, Me), 1.27 (2H, H-2″), 2.56 (2H, H-1″), 3.88 (3H, *s*, OMe), 4.20–5.38 (*m*, sugar protons), 6.31 (1H, *s*, H-6), 7.07 (2H, *d*, *J* = 9 Hz, H-3′,5′), 7.95 (2H, *d*, *J* = 9 Hz, H-2′,6′).

Compound **3** (*icariin*). Yellow needles (MeOH), mp 223–225°. $C_{33}H_{40}O_{15}$. UV λ_{max}^{MeOH} nm: 270, 316, 350 sh, + NaOMe: 273, 370, + $AlCl_3$: 280, 305, 345, 410, + $AlCl_3$/HCl: 280, 305, 339, 410, + AcONa: 270, 315, 350 sh, + AcONa/H_3BO_3: 270, 315, 350 sh. IR ν_{max}^{KBr} cm^{-1}: 3300, 1650, 1600. EIMS (*m/z*): 368, 353, 313, 300, 165, 135. ^1H NMR (DMSO-d_6): δ0.80 (3H, rhamnosyl Me), 3.89 (3H, *s*, OMe), 4.00–5.40 (*m*, sugar protons), 1.65, 1.72 (each 3H, C-4″,5″, Me), 6.63 (1H, *s*, H-6), 7.14 (2H, *d*, *J* = 9 Hz, H-3′,5′), 7.94 (2H, *d*, *J* = 9 Hz, H-2′,6′).

Compound **4** (*icarisid I*). Yellow needles (MeOH), mp 248–248.8° (dec.). $C_{27}H_{30}O_{11}$. UV λ_{max}^{MeOH} nm: 272, 328, 375, + NaOMe: 260, 422, + $AlCl_3$: 269, 356, 430, + $AlCl_3$/HCl: 269, 353, 430, + AcONa: 265, 370, 418, + AcONa/H_3BO_3: 272, 325, 373. IR ν_{max}^{KBr} cm^{-1}: 3400, 1650, 1600. EIMS (*m/z*): 368, 353, 313, 300, 165, 135. ^1H NMR (DMSO-d_6): δ 1.70, 1.83 (each 3H, *s*, C-4″,5″, Me), 2.90 (2H, H-1″), 3.87 (3H, *s*, OMe), 3.83–5.40 (*m*, sugar protons), 6.64 (1H, *s*, H-6), 7.16 (2H, *d*, *J* = 9 Hz, H-3′,5′), 8.23 (2H, *d*, *J* = 9 Hz, H-2′,6′).

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REFERENCES

1. Xu, S. M., and Zhu, C. D. (1985) *Zhongcaoyao* **16**, 33.
2. Akai, S. (1935) *Yakugaku Zasshi* **55**, 537.
3. Akai, S. and Matsukawa, T. (1935) *Yakugaku Zasshi* **55**, 705.
4. Akai, S. and Nakazawa, K. (1935) *Yakugaku Zasshi* **55**, 719.
5. Akai, S, Imaida, M. and Matsukawa, T. (1935) *Yakugaku Zasshi* **55**, 1139.
6. Takemoto, T., Daigo, K. and Tokuoka, Y. (1975) *Yakugaku Zasshi* **95**, 312.

7. Tokuoka, Y., Daigo, K. and Takemoto, T. (1975) *Yakugaku Zasshi* **95**, 321.
8. Tokuoka, Y., Daigo, K. and Takemoto, T. (1975) *Yakugaku Zasshi* **95**, 698.
9. Tokuoka, Y., Daigo, K. and Takemoto, T. (1975) *Yakugaku Zasshi* **95**, 825.
10. Xu, S. X., Wan, Z. X., Wu, L. J., Wan, N. L. and Chen, Y. J. (1982) *Zhongcaoyao* **13**, 9.
11. Liu, B. Q., Ma, H. S. and Mou, P. (1980) *Zhongcaoyao* **11**, 201.
12. Yang, C. X., Liu, X. K. and Wu, W. L. (1981) *Zhongcaoyao* **11**, 444.
13. Xu, S. X., Wan, Z. X. Wu, L. J., Wan, N. L. and Chen, Y. J. (1981) *J. Shenyang Coll. Pharm.* **14**, 234.
14. Tomita, M. and Ishii, H. (1957) *Yakugaku Zasshi* **77**, 114.
15. Tomita, Y. and Ishii, H. (1957) *Yakugaku Zasshi* **77**, 212.
16. Tokuoka, Y., Daigo, K. and Takemoto, T. (1975) *Yakugaku Zasshi* **95**, 557.
17. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*. Springer, Berlin.

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PYRROLE-3-CARBAMIDINE: A LETHAL PRINCIPLE FROM *NIEREMBERGIA HIPPOMANICA*

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Key Word Index—*Nierembergia hippomanica*; Nicotianeae; Solanaceae; lethal principle; structure elucidation; pyrrole-3-carbamidine.

Abstract—Pyrrole-3-carbamidine has been isolated and identified as the lethal constituent of *Nierembergia hippomanica*.

INTRODUCTION

Nierembergia hippomanica Miers. is an Argentinian plant toxic to livestock. Since the last century there are records of the plant being poisonous to cattle, sheep, goats, horses and rabbits in Argentina. Attempts to identify the toxin and pharmacological tests with guinea-pigs, dogs, toads, pigeons and gasteropods have been reviewed [1]. However no success was achieved in the isolation and identification of the toxic constituent(s).

Death may occur some hours after eating the plant and is preceded by symptoms of diarrhoea, midriasis, locomotor ataxia, excitement, weakened heart action, dyspnoea, and strong convulsions. On autopsy, acute cases showed evidence of gastro-intestinal irritation and hyperaemia of brain and meninges. Some of these symptoms may be explained by the identification of sympathomimetic β-phenethylamines [2], pentacyclic triterpenes [2] and a parasympatholytic tropane alkaloid [3] which we have previously reported. But none of these and other compounds we isolated [4–7] accounted for the lethality observed by ingestion of the plant.

In the present paper the novel pyrrole-3-carbamidine **1** is reported as the lethal principle of this plant. The structure was elucidated by chemical and spectroscopic methods. Toxicity was monitored by i.p. injection in mice.

RESULTS AND DISCUSSION

The methanolic extract of whole plants of *N. hippomanica* was toxic to mice when injected i.p. Therefore, this extract was successively percolated on polyamide with chloroform, water and methanol. Only the aqueous percolate was lethal to mice. Fractionation based on toxicity led to an Ehrlich positive fraction that was further chromatographed on a Bio-Gel P-2 column [8]. Further purification led to compound **1**. Upon alkaline hydrolysis of **1**, pyrrole-3-carboxylic acid and ammonia were obtained.

The ^{1}H NMR spectrum of **1** showed an ABX system of the pyrrolic protons (J_{AX} = 1.4, J_{BX} = 1.4 and J_{AB} = 2.8 Hz). ^{13}C NMR spectral data were in complete agreement with the structure **1**, on the basis of published chemical shifts of related pyrrolic compounds [9]. Both spectra suggested a 3-substituted pyrrole and the latter indicated the presence of an amidine carbon (160.3 ppm). Moreover, the mass spectrum of **1** showed the molecular ion at *m/z* 109 and main fragments at *m/z* 93, 66 and 43 (Scheme 1) indicative of an amidine group. This fact was

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