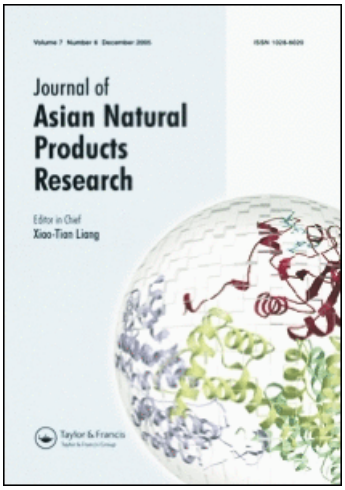


Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Note: Two new bicoumarins from *Trifolium repens*L.

Qing-Feng Zhan; Zeng-Hua Xia; Jun-Lin Wang; Ai-Na Lao

Online publication date: 12 May 2010

To cite this Article Zhan, Qing-Feng , Xia, Zeng-Hua , Wang, Jun-Lin and Lao, Ai-Na(2003) 'Note: Two new bicoumarins from *Trifolium repens*L.', Journal of Asian Natural Products Research, 5: 4, 303 — 306
To link to this Article: DOI: 10.1080/1028602031000111978
URL: http://dx.doi.org/10.1080/1028602031000111978

Journal of Asian Natural Products Research, 2003 VOL. 5 (4), pp. 303–306

Note

TWO NEW BICOUMARINS FROM *TRIFOLIUM REPENS* L.

QING-FENG ZHAN, ZENG-HUA XIA, JUN-LIN WANG and AI-NA LAO*

Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China

(Received 20 December 2002; Revised 26 February 2003; In final form 5 March 2003)

Two new bicoumarins, named repensin A (**1**) and B (**2**), have been isolated from *Trifolium repens*. On the basis of spectral data, the structures of **1** and **2** have been established as 7-methoxy-7′,8′-dihydroxy-8,6′-bicoumarinyl and 7,5′-dihydroxy-3,6′-bicoumarinyl, respectively.

Keywords: *Trifolium repens*; Leguminosae; Bicoumarin; Repensin A; Repensin B

INTRODUCTION

Trifolium repens L. (Leguminosae), which is distributed world-wide, is used as an important health food for human. It has estrogenic, antispasmodic and expectorant properties [1]. Previous studies on *Trifolium repens* have led to the isolation of flavonoids [2,3], isoflavonoids [2,3], bicoumarins [4] and triterpenoid saponins [5]. This paper deals with the isolation and structural elucidation of two new bicoumarins, named repensin A (**1**) and B (**2**), respectively.

RESULTS AND DISCUSSION

The EtOAc fraction from the ethanol extract of the *Trifolium repens* was repeatedly chromatographed on silica gel to afford repensin A (**1**) and B (**2**).

Repensin A (**1**), an amorphous solid, has a molecular formula $C_{19}H_{12}O_7$, $\Omega = 14$, determined by HREI-MS (*m/z*: 352.0580 $[M]^+$, calcd. for $C_{19}H_{12}O_7$ 352.0583) as well as ^{13}C and DEPT NMR data. The IR spectrum of **1** showed absorption bands due to hydroxyl (3369.1 cm^{-1}), lactone group (1708.6 cm^{-1}) and aromatic bands (1602.6, 1417.3,

*Corresponding author. Tel.: +86-021-64311833-316. Fax: +86-021-64370269.
E-mail: anlao@mail.shcnc.ac.cn

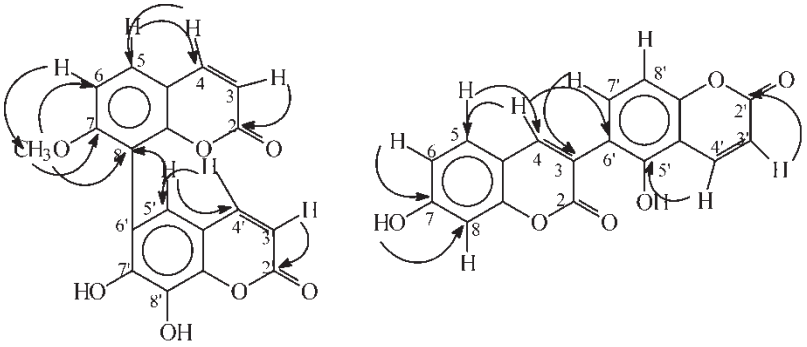
ISSN 1028-6020 print/ISSN 1477-2213 online
DOI: 10.1080/1028602031000111978

945.0 cm^{-1}). In the ^1H NMR spectrum of **1** (Table I), the characteristic AB quartet at δ 6.22 (2H, d, $J = 9.4$ Hz, H-3, H-3′) and 8.02 (2H, d, $J = 9.4$ Hz, H-4, H-4′) indicated that **1** was a bicoumarin [6]. In addition, the signals of four protons at δ 6.22 (1H, d, $J = 9.4$ Hz, H-3), 6.98 (1H, d, $J = 8.6$ Hz, H-6), 7.60 (1H, d, $J = 8.6$ Hz, H-5) and 8.02 (1H, d, $J = 9.4$ Hz, H-4) revealed the presence of 7,8-di-substituted coumarin unit in **1**. Moreover, the signals of three protons at δ 6.22 (1H, d, $J = 9.4$ Hz, H-3′), 7.37 (1H, s, H-5′) and 8.02 (1H, d, $J = 9.4$ Hz, H-4′) were also observed, suggesting **1** to have the other 6,7,8-tri-substituted coumarin unit; The ^1H NMR spectrum of **1** also showed the signals of two hydroxyl groups at δ 10.25 (2H, br s), and one methoxy group at δ 3.98 (3H, s). The ^{13}C NMR spectrum of **1** showed 18 carbon signals at lowfield. In the light of assigned ^1H NMR spectrum, four carbon signals at δ 111.0 (C-3′), 111.5 (C-3) and 144.8 (C-4, C-4′) were assigned to olefinic carbons based on HMQC spectrum. The two coumarin units in **1** are linked by C-8 with C-6′, determined by the observed cross peak (H-5′/C-8) in the HMBC spectrum of **1** (Fig. 1). In the HMBC spectrum of **1** the cross peaks (H-6/—OCH_3 and —OCH_3/C-8) indicated that —OCH_3 was located at C-7. Based on the spectroscopic data, the structure of **1** was established as 7-methoxy-7′,8′-dihydroxy-8,6′-bicoumarinyl. The assignments of the protons and carbons of **1** were based on the results of its ^1H NMR, ^{13}C NMR, HMQC and HMBC.

Repensin B (**2**), an amorphous solid, has a molecular formula $C_{18}H_{10}O_6$, $\Omega = 14$, determined by HR-EIMS (*m/z*: 322.0481 $[M]^+$, calcd. mass for $C_{18}H_{10}O_6$ 322.0478) and ^{13}C and DEPT NMR data. The ^1H and ^{13}C NMR spectrum of **2** (Table I) indicated that **2** is a bicoumarin, too. In the ^1H NMR spectrum of **2**, the signals of eight protons at δ 6.22 (1H, d, $J = 9.6$ Hz, H-3′), 6.80 (1H, s, H-8), 6.85 (1H, d, $J = 8.4$ Hz, H-6), 6.98 (1H, d, $J = 8.8$ Hz, H-8′), 7.59 (1H, d, $J = 8.8$ Hz, H-7′), 7.60 (1H, d, $J = 8.4$ Hz, H-5), 8.00 (1H, s, H-4) and 8.01 (1H, d, $J = 9.6$ Hz, H-4′) suggested the presence of 3,7-di-substituted coumarin unit, and the other 5′,6′-di-substituted coumarin unit, in **2**. The ^1H NMR spectrum of **2** also showed the signals of two hydroxyl groups at δ 10.72 (2H, br s). The two coumarin units were linked by C-3 with C-6′, confirmed by the observed cross peaks (H-4/C-6′ and H-7′/C-3) in the HMBC spectrum of **2** (Fig. 1). Consequently, the structure of **2** was established as 7,5′-dihydroxy-3,6′-bicoumarinyl.

TABLE I ^1H and ^{13}C NMR spectral data of repensin A (**1**) and B (**2**) (DMSO)

	Repensin A (**1**)		*Repensin B* (**2**)	
1a		153.3		158.8
2		160.5		161.1
3	6.22 (1H, d, $J = 9.4$ Hz)	111.5		115.0
4	8.02 (1H, d, $J = 9.4$ Hz)	144.8	8.00 (1H, s)	144.5
4a		106.9		111.0
5	7.60 (1H, d, $J = 8.6$ Hz)	129.2	7.60 (1H,d, $J = 8.4$ Hz)	129.5
6	6.98 (1H, d, $J = 8.6$ Hz)	112.8	6.85 (1H, d, $J = 8.4$ Hz)	113.3
7		159.4		154.9
8		111.3	6.80 (1H, s)	102.0
1a′		147.8		153.0
2′		160.7		160.0
3′	6.22 (1H, d, $J = 9.4$ Hz)	111.0	6.22 (1H, d, $J = 9.6$ Hz)	111.0
4′	8.02 (1H, d, $J = 9.4$ Hz)	144.8	8.01 (1H, d, $J = 9.6$ Hz)	144.5
4a′		107.6		111.3
5′	7.37 (1H, s)	108.6		158.9
6′		110.3		110.5
7′		149.1	7.59 (1H, d, $J = 8.8$ Hz)	129.0
8′		149.1	6.98 (1H, d, $J = 8.8$ Hz)	113.0
—OCH_3	3.98 (3H, s)	56.1		
—OH	10.25 (2H, br s)		10.72 (2H, br s)	



1 **2**

FIGURE 1 Selected HMBC cross peaks of **1** and **2**.

EXPERIMENTAL

General Experimental Procedures

^{1}H, ^{13}C, DEPT and all 2D NMR spectra were run on an INVOA-600 Spectrometer in DMSO, operating at 100 MHz for ^{13}C NMR spectra, 400 MHz for ^{1}H NMR spectra, and 600 MHz for 2D NMR spectra. EIMS and HR-EIMS were recorded on a MAT-95 Mass Spectrometer. IR spectra were recorded on a DTGS KBr Spectrum Detector. Optical rotation was obtained on a WZZ-1S polarimeter. The UV spectra were recorded on a Shimadzu UV-260. Silica gel H (Qingdao Haiyang Chemical Group Co. Ltd. in China) was used for column chromatography and TLC was performed on silica gel $HSGF_{254}$ (Yantai Zhifu Huangwu Co. Ltd). Spots were visualized under UV and by spraying with 10% H_2SO_4 in 95% EtOH followed by heating.

Plant Material

The herb of *Trifolium repens* was collected from Shanghai (China) in June 2001. The botanical identification was made by Professor Xuesheng Bao (Shanghai Institute of Drug Control). A voucher specimen (No. 0107) is deposited at the Herbarium of the Department of Phytochemistry, Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Extraction and Isolation

The powdered whole plant of *Trifolium repens* (20.5 kg) was extracted three times with 95% EtOH. After evaporation of ethanol *in vacuo*, the residue (2.5 kg) was suspended in water and then extracted successively with light petroleum, EtOAc and BuOH. The EtOAc fraction was 420 g, of which 140 g was chromatographed on a silica gel column, eluting with a $CHCl_3$–MeOH (100:0–0:100) gradient. The fraction (10 g) eluted with $CHCl_3$–MeOH (10:1) was chromatographed on silica gel using $CHCl_3$–MeOH (30:1) as eluent to afford repensin A (**1**) (18 mg). The fraction (20 g) eluted with $CHCl_3$–MeOH (5:1) was chromatographed on silica gel using $CHCl_3$–MeOH (10:1) as eluent to afford repensin B (**2**) (140 mg).

Repensin A (1)

This was obtained as white amorphous powder. $[\alpha]_D^{24}$ 13.6 (*c* 0.10, MeOH); **UV** (DMSO) λ_{max} (nm) ($\log \epsilon$) 220.4 (3.95), 256.8 (4.02), 326.6 (4.44); IR ν_{max}^{KBr} (cm^{-1}): 3369.1, 2917.8,

1708.6, 1602.6, 1417.4, 1294.0, 1119.5, 1029.8, 945.0; ^{1}H, ^{13}C NMR see Table I; EIMS *m/z*: 352 $[M]^+$ (100), 335 $[M + H - H_2O]^+$ (42), 290 $[M - CO_2 - H_2O]^+$ (20); HR-EIMS (*m/z*: 352.0580 $[M]^+$, calcd. mass for $C_{19}H_{12}O_7$ 352.0583).

Repensin B (2)

This was obtained as white amorphous powder. $[\alpha]_D^{24}$ 13.3 (*c* 0.11, MeOH); UV (DMSO) λ_{max} (nm) (log ϵ): 210.4 (4.04), 256.4 (4.11), 327.8 (4.50); IR ν_{max}^{KBr} (cm^{-1}): 3214.8, 2919.7, 1710.6, 1610.3, 1465.7, 1234.2, 1124.3, 840.8; ^{1}H, ^{13}C NMR see Table I; EIMS *m/z*: 322 $[M]^+$ (100), 294 $[M - CO]^+$ (44), 262 $[M - CO - CO]^+$ (60); HR-EIMS (*m/z*: 322.0481 $[M]^+$, calcd. mass for $C_{18}H_{10}O_6$ 322.0478).

References

[1] Leung, A.Y. and Foster, S. (1996), *Encyclopedia of Common Natural Ingredient used in Food, Drugs, Cosmetics*, (Wiley), pp. 177–178.
[2] Foo, L.Y., Lu, Y., Molan, A.L. and McNabb, W.C. (2000), *Phytochemistry* **54**, 539–548.
[3] Saxena, V.K. and Jain, A.K. (1986), *Phytochemistry* **25**, 2687–2688.
[4] Spencer, R.R., Witt, S.C., Lundin, R.E. and Bickoff, E.M. (1967), *J. Agric. Food Chem.* **15**, 536–538.
[5] Sakamoto, S., Kofuji, S., Kuroyanagi, M. and Sekita, S. (1992), *Phytochemistry* **35**, 1773–1777.
[6] Murray, R.D., Mendez, J. and Brown, S.A. (1982), *The Natural Coumarins: Occurrence, Chemistry and Biochemistry* (John Wiley and Sons), pp. 272–273.