HETEROCYCLES, Vol. 65, No. 3, 2005, pp. 661 - 666
Received, 21st December, 2004, Accepted, 28th January, 2005, Published online,1st February, 2005

A NEW FLAVONE FROM *LYCOPODIUM JAPONICUM*

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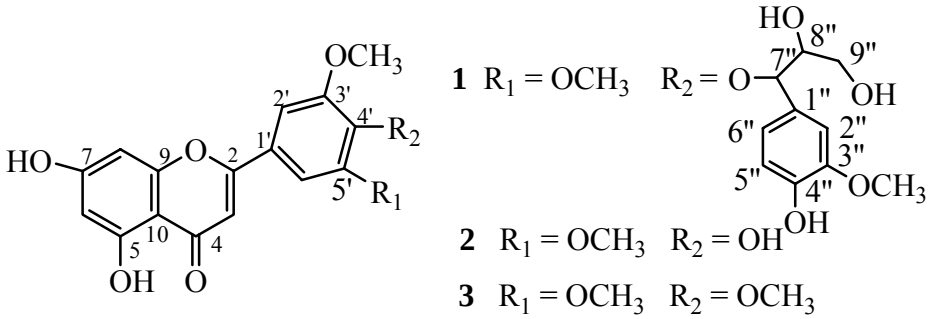
Abstract — A new flavone, lycopodone (**1**), together with other three known flavonoids tricin (**2**), tricetin 3`,4`,5`-OMe (**3**), 5,7,4`-trihydroxy-3`- methoxy flavone (**4**), were isolated from the fern *Lycopodium japonicum.* The structure of **1** was established on the basis of spectral measures. Compounds (**1**) and (**2**) showed moderate antitumor activity.

INTRODUCTION

Lycopodium japonicum is widely distributed in Guangdong, Guangxi, Yunnan, Guizhou Provinces.[1] This plant is one of the most commonly encountered traditional Chinese herbal medicines for treatments of arthritic pain, quadriplegia, dysmenorrhea, and contusion.[2-3] The chemical constituents of the genus *Lycopodium* have been investigated previously.[4-6] But flavones of the genus were hardly reported. Numerous biological activities of flavonoids have been reported. For example, tricin (**2**) has been evaluated with respect to antimicrobial[8] and cancer cell line activities.[9-10] In the course of our screening of biologically active constituents, four compounds (**1**), (**2**) [10-11], (**3**) [11], (**4**) [12] (see **Scheme 1**) were isolated from *L. japonicum.* In this paper, we would like to present the isolation and identification of the new compound, lycopodone.



1 $R_1 = OCH_3$ $R_2 =$ O

2 $R_1 = OCH_3$ $R_2 = OH$

3 $R_1 = OCH_3$ $R_2 = OCH_3$

4 $R_1 = H$ $R_2 = OH$

Scheme 1 the structures of compounds (**1-4)**

RESULTS AND DISCUSSION

Lycopodone (**1**) possessed the molecular formula $C_{27}H_{26}O_{11}$ by HRFAB-MS (neg.) (*m/z* 525.0867 $[M-H]^-$, calc. for $C_{27}H_{25}O_{11}$ 525.1396), which was consistent with its NMR data. The new compound exhibited UV maximum absorptions (273 and 331 nm) and IR absorptions spectral at 3417 and 1612 cm^{-1}, which were characteristic of a flavonoid. The ^{13}C NMR (DEPT) spectra presented nine carbon signals corresponding to a C_6-C_3 phenylpronane unit, and the frequencies were characteristic of a flavone skeleton. The ^1H NMR spectra showed signals (H-3, 6, 8, 2` and 6`) typical of a tricin-like flavone skeleton. Comparing those of **2,** the ^{13}C NMR spectra of **1** displayed an additional aromatic ring and an additional glycerol chain and a methoxy group. In the aromatic part of the ^1H NMR spectrum, the signals at δ 6.95 (1H, d, *J* = 1.8 Hz, H-2``), 6.76 (1H, d, *J* = 8.8 Hz, H-5``), and 6.70 (1H, dd, *J* = 1.8, 8.8 Hz, H-6``) defined a 1, 3, 4 tri-substituted aromatic ring. This was confirmed in the $^{13}C$ NMR spectrum by three methane peaks at $\delta_C$ 111.2 (d, C-2``), 119.4 (d, C-5``), 114.7 (d, C-6``). Due to deshielded effect, two signal peaks δ_H 4.36 (1H, H-7``); $\delta_C$ 86.3 (d, C-7``) and δ_H 4.80 (1H, H-8``); $\delta_C$ 72.2 (d, C-8``) and a methylene peaks δ_H 3.51 (2H, d, *J* = 9.3, H-9``); $\delta_C$ 60.1 (t, C-9``) were observed in the ^1H NMR and ^{13}C NMR spectrum, which suggested glycerol chain. Thus, **1** was primarily determined to be a flavonophenylpropanetriol. The HMQC and HMBC 2D NMR spectral analyses confirmed that the three phenol hydroxyl groups were situated at C-5, C-7 and C-4``, and three methoxy groups at C-3`, C-5` and C-3`` (see **Table 1**). From HMBC spectrum, the correlations H-7`` with C-1`` and C-4` were observed,

which suggested that C-7`` and C-4` were linked its *O* bond. The $^{1}$H-$^{1}$H COSY spectra revealed the coupling patterns of the oxygenated methyne and methane protons of H-7``, H-8``, and H-9``. The relatively small constant between H-8`` and H-7`` (J = 5.0 Hz) suggested an erythro-configuration in the glycerol chain.[11-12] Thus, lycopodone (**1)** was formulated as *rel*-(7``*R,* 8``*S*) - tricin-4`-*O*-(4``-hydroxy-3``-methoxyphenyl propanetriol).

EXPERIMENTAL

General Experimental Procedures. Column chromatography (CC): Silica gel (200-300 mesh, Qingdao Marine Chemical, China); and Lichroprep RP-18 (40-63um, Merck, Darmstadt, German). All melting points: YANACO-MP-52 apparatus, uncorrected. Optical rotations: Horiba SEAP-300 spectropolarimeter. IR spectra: Shimadzu IR-450 instrument; in cm^{-1}; KBr pellets. UV spectral data: UV 210A spectrometer. FAB-MS and HRFAB-MS: VG-AUTOSPEC-3000 spectrometer; in *m/z* (rel. int. in % of the base peak). NMR specta: Bruker AV-400, or DRX-500 instruments; Chemical shifts (δ) in ppm; TMS as the internal standard. Fractions were monitored by TLC, and spots were visualized by heating TLC sprayed with 10% H_2SO_4.

Plant Material. The whole body of *L. japonicum* Thunb was obtained from the Chinese herbal market. It was identified by Prof. S. G. Wu. A voucher specimen (KUN No. 001143) was deposited at the Laboratory of Phytochemistry, Kunming Institute of Botany.

Extraction and Isolation. The dried, milled whole body of *L. japonicum* (19.0 kg) was exhaustively extracted with 90% MeOH (3×10 L) under reflux for 4, 4, 3 h. The MeOH extract was evaporated under reduce pressure to yield syrup (910 g), which was suspended in water: MeOH (9:1, 1500 mL) and extracted successively with EtOAc (3×2000 mL) and n-BuOH part (3×2000 mL) to give EtOAc-soluble (410 g) and n-BuOH-soluble fractions (101 g). The AcOEt extract was absorbed on 600 g silica gel and was fractionated by CC [silica gel (1.5 kg), $CHCl_3$: Me_2CO 10:0, 9:1, 8:2, 7:3, 6:4 0:10, *v*:*v*] to afford six fractions (*Fr.*): 1 (oil), 2 (104 g), 3 (119 g), 4 (64 g), 5 (49 g), 6 (22 g).

Fr. 2 (104 g) was subjected to CC (silica gel, $CHCl_3$: MeOH 40:1, 30:1, 20:1, *v*:*v*) to get four

subfractions. *Fr. 2.1* was further purified by repeated CC (silica gel, $CHCl_3$: MeOH 60:1, 50:1, *v*:*v*) to yield **3** (30 mg). *Fr. 2.2* was dissolved in $CHCl_3$ and yielded yellow powder of compound (**2)** (10 mg). *Fr.2.4* was further purified by CC (silica gel, $CHCl_3$: MeOH 35:1, *v*:*v*) to give **4** (40 mg).

Table 1 ^{13}C and ^{1}H NMR spectra data of compound of **1**

No	^{13}C	^{1}H	HMBC	COSY
2	162.9 s	/	H-3, H-2`, H-6`	
3	104.7 d	7.00 (s)		
4	181.7 s	/	H-3, H-6	
5	161.3 s	/	H-6, 5-OH	
6	98.8 d	6.70 (d, 2.0)	H-8	
7	164.2 s	/	H-6, H-8	
8	94.2 d	6.22 (d, 2.0)	H-6	
9	157.3 s	/	H-8	
10	103.8 s	/	H-6, H-8, H-3, 5-OH	
1`	125.2 s	/	H-3, H-2`, H-6`	
2`	104.4 d	7.30 (s)	H-6`	
3`	152.9 s	/	3`-OMe, H-2`	
4`	139.5 s	/	H-2`, H-6`, H-7``	
5`	152.9 s	/	5`-OMe, H-6`	
6`	104.4 d	7.30 (s)	H-2`	
3`,5`-OMe	56.3 q	3.76 (s, 6H)		
1``	133.1 s	/	H-2``, H-6``	
2``	111.2 d	6.95 (d, 1.8)	H-6``, H-8``	
3``	146.9 s	/	3``-OMe, H-2``, H-5``	
4``	145.4 s	/	4``-OH, H-5``	
5``	119.4 d	6.76 (d, 8.8)	H-6``, H-8``, 4``-OH	
6``	114.7 d	6.70 (dd, 1.8, 8.8)	H-2``, H-5``, 4``-OH	
7``	86.3 d	4.36 (br s)	H-8``, H-9``	H-8``
8``	72.2 d	4.80 (br s)	H-7``, H-9``	H-7``, H-9``

9``	60.1 t	3.51 (d, 9.3)	H-8``, H-7``	H-8``
3``-OMe	55.6 q	3.65 (s, 3H)		
5-OH		12.83 (br s, 1H)		
4``-OH		8.62 (br s, 1H)		
8``-OH		5.07 (br s, 1H)		
9``-OH		4.06 (br s, 1H)		

Recorded at 500 MHz in DMSO-d_6, at room temperature.

Fr. 3(119 g) was purified by repeated CC (silica gel, $CHCl_3$: MeOH 25:1, 15:1, 10:1, *v*:*v*) to afford five fractions. *Fr.3.2* (200.0 mg) was a mixture of compound (**1)** and other compounds. And the mixture was repeatedly subjected to HPLC (MeOH : H_2O 70:30, *v*:*v*) to yield compound (**1)** (50.0 mg).

Compound (**1),** yellow powder, mp>300^oC; $[\alpha]^{23}_D$ – 11.76 ° (*c* 1.7, MeOH); UV λ_{max} ^{MeOH} nm(log ε): 204(4.16), 273(3.55), 306(3.53), 331(3.55); ^{13}C and ^1H NMR spectrum, see **Table 1**; IR (KBr) ν_{max} 3417 (br.s), 2941, 2842, 1656, 1612, 1591, 1556, 1455, 1356, 1250, 1170, 1048, 1031, 989, 918, 838, 786 cm^{-1}; FAB$^-$MS (m/z, %): 525([M-1]$^-$, 55), 329([M-$C_{10}H_{13}O_4$], 100); HRFAB-MS: 525.0867 (calcd for $C_{27}H_{25}O_{11}$ 525.1396)

Antitumor Activity: Human tumor A549, K562 cells line assay were performed at Kunming Medical College prof. Q. Chen and her coworkers and previously described bioassay methods were adopted. [15-17] The IC_{50} values for compounds (**1)** and (**2)** (10-100 μg/mL, 11.68 μg/mL, respectively, against human tumor K562 cells) indicated moderate antitumor activity.

ACKNOWLEDGEMENTS

This work was supported by NKIP Foundation of CAS (KSCZX-SW-301-08) and Fundation of Key State Lab of Phytochemistry.

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