monoterpene glucosides, schizonepetoside A and B [J]. Chem Pharm Bull, 1981, 29(6): 1636-1643.
[6] Munehito M, Michihide K, Yuji S, *et al*. Phenylpropenoic acid derivatives as antiinflammatory agent and its isolation from *Schizonepeta tenuifolia* [P]. Japan Kokai Tokkyo Koko JP 02, 306, 970 [90, 306, 9701(Cl. C 07D307/86) 20 Dec. 1990, Appl. 89/127, 488, 19 Mzy 1989, spp].

Chemical Constituents of *Pueraria peduncularis*

ZHANG Xiao-rong, WANG Ming-kui, PENG Shu-lin, LIU Fa-qiang, DING Li-sheng
(Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu Sichuan 610041, China)

Abstract **Object** To investigate the chemical constituents from the stem of *Pueraria peduncularis* (Grah. ex Benth.) Benth. **Methods** The constituents were isolated on silica gel column chromatography. Their structures were elucidated by chemical and spectroscopic evidence. **Results** Ten compounds were comfirmed as 3-*O*-[β-*D*-glucopyranosyl-(1→3)β-*D*-6-*O*-methyl-glucuronopyranosyl]-3β, 15α, 23-trihydroxy-olean-12-en-16-one (Ⅰ), daidzein (Ⅱ), genistein (Ⅲ), daidzin (Ⅳ), genistin (Ⅴ), 3β, 15α-dihydroxy-olean-12-en-16-one (Ⅵ), lupeol (Ⅶ), betulinic acid (Ⅷ), α-spinasterol-glucopyranoside (Ⅸ) and α-spinasterol (Ⅹ). **Conclusion** Compound Ⅰ exhibited antimicrobial activity against *Aspergillus niger*. Compounds Ⅱ ~ Ⅹ were first isolated from this plant.

Key words *Pueraria peduncularis* (Grah. ex Benth.) Benth; chemical constituent; *Aspergillus niger*

葛根的化学成分研究

张晓[illegible],王明奎,彭树林,刘发强, 丁立生*
(中国科学院成都生物研究所,四川 成都 610041)

摘 要: 目的 从豆科葛属植物云南葛藤 *Pueraria peduncularis* 中分离得到 10个化合物。方法 通过波谱和化学方法分别进行鉴定。结果 它们的化学结构为: 3-*O*-[β-*D*-吡喃葡萄糖(1→3)β-*D*-吡喃葡萄糖醛酸甲酯苷]-3β, 15α, 23-三羟基齐墩果-12-烯-16-酮(Ⅰ),大豆素(Ⅱ),染料木素(Ⅲ),大豆苷(Ⅳ),染料木苷(Ⅴ), 3β, 15α-二羟基齐墩果-12-烯-16-酮(Ⅵ),羽扇醇(Ⅶ),桦木酸(Ⅷ),α-菠甾醇葡萄糖苷(Ⅸ)和α-菠甾醇(Ⅹ)。结论 化合物Ⅰ对黑曲霉具有抑制活性。

关键词: 云南葛藤;化学成分;黑曲霉
中图分类号: R284.1 文献标识码: A 文章编号: 0253-2670(2002)01-0011-04

We have reported[1] two new triterpenoids and one known compound obtained from the stem of *Pueraria peduncularis* (Grah. ex Benth.) Benth. (Leguminosae) after acid hydrolysis. In the present study, ten compounds from the methanolic extract of the stem of this plant have been investigated, and compound Ⅰ showed antimicrobial activity against *Aspergillus niger*.

1 Results and discussion

The HRFABMS of Ⅰ gave a $[M-H]^-$ ion at *m/z* 823.452 9, in agreement with the molecular formula $C_{43}H_{68}O_{15}$ (calcd for $C_{43}H_{67}O_{15}$ *m/z* 823.448 0). The IR spectrum showed the absorption bands of hydroxyl and carbonyl groups at 3 419, 1 744 and 1 701 cm^{-1}. In the ^{1}HNMR and ^{13}CNMR spectra of Ⅰ, the presence of seven quaternary carbon atoms and the chemical shifts of C-12 at δ 125.8 and C-13 at δ 142.0 were characteristic of a $\triangle^{12}$-oleanene skeleton. Acid hydrolysis of Ⅰ gave aglycone Ⅰa, whose ^{1}HNMR and ^{13}CNMR signals were identical with those of 3β, 15α, 23-trihydroxy-olean-12-en-16-one[1]. Glucose and methyl glucuronate were identified by TLC. The EIMS of acetylated Ⅰ showed fragment ion at *m/z*

* 收稿日期: 2000-10-12

331 [terminal glc(OAc)$_4$], suggested that glucose was terminal sugar. β-Configuraton at the anomeric positions was inferred from the coupling constants for both glucopyranose (J= 7.5 Hz) and methyl glucuronate (J= 7.5 Hz). In the ^{13}CNMR spectrum of Ⅰ, the signal of C-3 was shifted downfield to δ 82.2 as compared with analogous signal of Ⅰa, indicated that C-3 of Ⅰ was linked with the sugar chain. This conclusion was further supported by the HMBC spectrum of Ⅰ, in which the proton at H-1 (δ 5.17) of methyl glucuronate had cross-peak with C-3 (δ 82.2) of Ⅰ (Fig. 1). By analysis of HMQC and HMBC spectra of Ⅰ, the ^{1}HNMR and ^{13}CNMR signals could be assigned (see Table. 1). The HMBC correlation was observed between anomeric proton (δ 5.26) of glucose and C-3 (δ 87.3) of methyl glucuronate (Fig. 1). Therefore, the sugar chain was 6-*O*-Me-glu (3→1) glc. Except for C-6 of methyl glucuronate, the carbon signals due to these sugar moieties were in accord with the published data for similarly linked sugar moieties [-glu (3→1) glc][2].

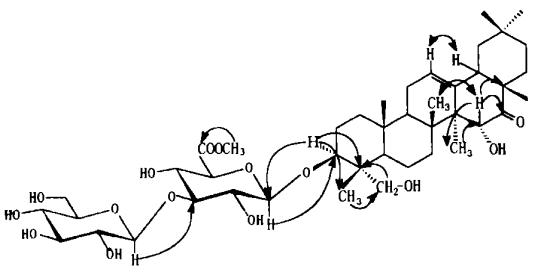

Fig. 1 Key HMBC (↷) and NOESY (↶) correlations of compound Ⅰ

The above evidence led to the elucidation of the structure of 3-*O*-[β-*D*-glucopyranosyl (1→3)β-*D*-6-*O*-methyl-glucuronopyranosyl]-3β, 15α, 23-trihydroxy-olean-12-en-16-one. According to the practice encountered among the plant glycosides, compoundⅠ may be an artifact during the extraction resulting from 3-*O*-[β-*D*-glucopyranosyl (1→3)β-*D*-glucuronopyranosyl]-3β, 15α, 23-trihydroxy-olean-12-en-16-one which was isolated from the same plant before[3].

In the light of direct comparison between published spectral data and that of the tested samples, the compounds Ⅱ ~ Ⅹ were identified as daidzein (Ⅱ)[4], genistein (Ⅲ)[5], daidzin (Ⅳ)[6,7], genistin (Ⅴ)[7], 3β, 15α-dihydroxy-olean-12-en-16-one (Ⅵ)[1], lupeol (Ⅶ)[8], betulinic acid (Ⅷ)[9], α-spinasterol-glucopyranoside (Ⅸ)[10] and α-spinasterol (Ⅹ)[10] respectively. ^{13}CNMR spectral data of compounds Ⅱ, Ⅲ, Ⅳ and Ⅴ, see Table 2. The nine compounds were first isolated from this plant.

Table 1 ^{1}HNMR and ^{13}CNMR spectral data of compounds Ⅰ (in C_5D_5N, 125 MHz) and Ⅰa (in $CDCl_3$, 75 MHz) (J Hz)

C, H atom	Ⅰ*	Ⅰa	Ⅰ	HMBC (H to C)
1	38.6 (t)	38.3		
2	26.2 (t)	26.7		
3	82.2 (d)	76.8	3.68, t, J= 8	C: 4, 24, glu-1
4	43.5 (s)	41.8		
5	47.2 (d)	49.6		
6	18.0 (t)	18.6		
7	35.6 (t)	35.0		
8	41.8 (s)	41.8		
9	47.2 (d)	46.7		
10	36.8 (s)	36.9		
11	24.1 (t)	23.7		
12	125.8 (d)	125.5	5.45, br.s	C: 11, 14, 18
13	142.0 (s)	141.5		
14	46.4 (s)	46.0		
15	72.8 (d)	72.4	4.77, s	C: 16, 17, 27
16	217.3 (s)	271.3		
17	54.2 (s)	54.1		
18	53.0 (d)	52.9	2.55, dd, J= 14.4	C13, 17, 19
19	47.9 (t)	47.7		
20	30.9 (s)	30.5		
21	35.9 (t)	35.3		
22	30.9 (t)	30.5		
23	63.8 (t)	72.1	4.30, 3.71, d, J= 10	C: 3, 4, 5, 24
24	13.6 (q)	11.4	0.943	C: 3, 4, 23
25	16.4 (q)	15.8	0.943	
26	17.8 (q)	17.6	1.18	
27	21.9 (q)	21.2	0.982	C: 13, 14, 15
28	28.1 (q)	28.1	1.24	C: 17, 18
29	33.1 (q)	32.9	0.794	
30	23.3 (q)	23.3	0.844	

*: Sugar moiety of Ⅰ: 6-OMe-glu: 106.1 (d), 74.2 (d), 87.3 (d), 71.6 (d), 76.7 (d), 170.3 (s), 52.2 (q); glc: 105.9 (d), 75.7 (d), 78.3 (d), 71.5 (d), 78.8 (d), 62.5 (t).

2 Experimental

2.1 General experimental procedures: Optical rotation was detected with a PE-241 polarimeter. IR spectrum was detected on a Nicolet MX-1 spectrometer as a pressed KBr disk. NMR spectra were recorded on Bruker AP-300 and DRX-500 MHz spectrums with TMS as the internal standard. MS spectra were detected on a VG AutoSpec-3000

mass spectrometer. 200~ 300 mesh silica gel and Lichroprep RP-8 were used for column chromatography.

2.2 Plant material: The whole plant of *P. pedunculoris* was collected in Liangshan, Sichuan Province, China and identified by professor Yang Guanghui, from Sichuan Agricultural College.

2.3 Extraction and isolation: The dried stems (0.5 kg) were extracted with MeOH for seven days at room temperature. This operation was repeated three times. After removal of the solvent, the residue was suspended in H_2O and successively extracted with petroleum ether (bp 60℃~ 90℃), EtOAc and *n*-BuOH respectively. The EtOAc extracts (15 g) were repeatedly chromatographed on silica gel and eluted with petroleum ether-acetone (25∶ 1) to obtain compounds Ⅱ (43 mg), Ⅲ (40 mg), Ⅳ (16 mg), Ⅴ (131 mg), Ⅸ (12 mg) and Ⅹ (55 mg). The *n*-BuOH extracts (22 g) were chromatographed on silica gel column and eluted with $CHCl_3$-MeOH (35∶ 1) to obtain compound Ⅷ (19 mg) and three fractions. Fraction 1 was chromatographed on silica gel column and eluted with $CHCl_3$-MeOH (15∶ 1) to afford compounds Ⅵ (65 mg) and Ⅶ (13 mg). Fraction 2 was repeatedly chromatographed on silica gel and RP-8 column and eluted with $CHCl_3$-MeOH (10∶ 1) and MeOH-H_2O (80∶ 20, 500 mL; 90∶ 10, 500 mL) separately to obtain compound Ⅰ (53 mg).

2.4 Identification: Compound Ⅰ: White powder. mp: 207℃~ 209℃. $[\alpha]_D^{20}$: - 9.5° (c 0.084, MeOH). IR ν_{max}^{KBr} (cm^{-1}): 3 420, 2 950, 1 744, 1 702, 1 458, 1 386, 1 079, 1 033. HR-FABMS $[M-H]^-$ m/z: 823.452 9 ($C_{43}H_{67}O_{15}$, calcd. 823.448 0). FABMS (-) m/z: 823 $[M-H]^-$, 471 $[M-\{6\text{-}O\text{-}Me\text{-}glu\text{-}(3\rightarrow 1)\text{-}glc\}]^-$.

Hydrolysis of Ⅰ: Compound Ⅰ (18 mg) was hydrolyzed with 4N HCl (5 mL) and benzene (5 mL) under reflux for 6 h. The organic phase was separated and the water phase was extracted with benzene three times. After removal of benzene under reduced pressure the residue was purified by CC on silica gel and eluted with petroleum ether-acetone (3∶ 1) to obtain Ⅰa (4 mg). ^{1}HNMR δ (300 MHz, $CDCl_3$) of Ⅰa: 5.51 (1H, br.s, H-12), 4.57 (1H, s, H-15), 3.74 (1H, d, J= 10 Hz, H-23), 3.66 (1H, t, J= 8 Hz, H-3), 3.44 (1H, d, J= 10 Hz, H-23), 2.54 (1H, dd, J= 14, 4 Hz, H-18), 1.25, 1.22, 1.19, 1.03, 0.95, 0.87, 0.85 (each 3 H, s, 7× CH_3).

Acetylation of Ⅰ: Compound Ⅰ (5 mg) was acetylated with Ac_2O-pyridine (1∶ 1, 2 mL) at room temperatur for 60 h to yield acetate of Ⅰ. EIMS (m/z): 331, 290, 248.

Table 2 ^{13}**CNMR spectral data of compounds** Ⅱ, Ⅲ, Ⅳ **and** Ⅴ **(in DMSO-d_6, 75 MHz)**

C	Ⅱ	Ⅲ	Ⅳ	Ⅴ
2	152.7	153.8	153.2	153.2
3	122.4	122.2	123.6	122.5
4	174.6	180.1	174.6	180.4
5	127.2	161.9	126.8	161.6
6	115.0	98.8	115.5	99.8
7	162.4	164.1	161.3	163.0
8	102.2	93.5	99.9	94.5
9	157.3	157.3	157.1	157.3
10	116.5	104.4	118.4	106.0
1′	123.4	121.1	122.2	120.9
2′	130.0	130.0	130.0	130.1
3′	114.8	114.9	114.8	114.9
4′	157.0	157.5	156.9	157.0
5′	114.8	114.9	114.8	114.9
6′	130.0	130.0	130.0	130.1
glc-1			103.0	99.8
glc-2			73.0	73.0
glc-3			76.3	76.3
glc-4			69.5	69.5
glc-5			77.1	77.1
glc-6			60.5	60.5

Compound Ⅱ: White powder. ^{1}HNMR δ (300 MHz, DMSO-d_6): 10.82 (1H, s, 7-OH), 9.57 (1H, s, 4′-OH), 8.30 (1H, s, H-2), 7.96 (1H, d, J= 9 Hz, H-5), 7.38 (2H, d, J= 9 Hz, H-2′, 6′), 6.95 (1H, d, J= 2 Hz, H-8), 6.87 (1H, dd, J= 9, 2 Hz, H-6), 6.81 (2H, d, J= 9 Hz, H-3′, 5′).

Compound Ⅲ: White powder. ^{1}HNMR δ (300 MHz, DMSO-d_6): 12.97 (1H, s, 5-OH), 10.89 (1H, s, 7-OH), 9.63 (1H, s, 4′-OH), 8.32 (1H, s, H-2), 7.37 (2H, d, J= 9 Hz, H-2′, 6′), 6.82 (2H, d, J= 9 Hz, H-3′, 5′), 6.38 (1H, d, J= 2 Hz, H-8), 6.22 (1H, d, J= 2 Hz, H-6).

Compound Ⅳ: White powder. ^{1}HNMR δ (300 MHz, DMSO-d_6): 9.55 (1H, s, 4′-OH), 8.38 (1H, s, H-2), 8.05 (1H, d, J= 9 Hz, H-5), 7.40

(2H, d, J= 9 Hz, H-2′, 6′), 7.23 (1H, d, J= 2 Hz, H-8), 7.14 (1H, dd, J= 9, 2 Hz, H-6), 6.82 (2H, d, J= 9 Hz, H-3′, 5′), 5.09 (1H, d, J= 7 Hz, H-1glc). (See Table 3)

Compound Ⅴ: White powder. ^{1}HNMR δ (300 MHz, DMSO-d$_6$): 12.94 (1H, s, 5-OH), 9.61 (1H, s, 4′-OH), 8.42 (1H, s, H-2), 7.40 (2H, d, J= 9 Hz, H-2′, 6′), 6.83 (2H, d, J= 9 Hz, H-3′, 5′), 6.72 (1H, br. s, H-8), 6.48 (1H, br. s, H-6), 5.06 (1H, d, J= 7 Hz, H-1″).

Compound Ⅵ: White needles. mp 194 ℃~195 ℃. ^{1}HNMR δ (300 MHz, CDCl$_3$): 5.50 (1H, br. s, H-12), 4.57 (1H, s, H-15), 1.22, 1.17, 1.02, 1.02, 0.975, 0.863, 0.850, 0.819 (each 3H, s, 8× CH$_3$).

Table 3 ^{13}C NMR spectral data of compounds Ⅵ, Ⅶ, Ⅷ, Ⅸ and Ⅹ (in CDCl$_3$, 75 MHz)

C	Ⅵ	Ⅶ	Ⅷ	Ⅸ*	Ⅹ
1	39.0	38.7	38.8	37.3	37.2
2	27.2	27.4	27.2	30.0	31.4
3	78.9	79.0	79.1	77.0	71.1
4	38.7	38.9	38.9	34.7	37.9
5	54.9	55.3	55.4	40.1	40.3
6	18.4	18.3	18.3	30.0	29.8
7	35.3	34.3	34.4	117.9	117.5
8	41.3	40.9	40.7	139.5	139.6
9	46.7	50.5	50.6	49.6	49.5
10	36.6	37.2	37.2	34.5	34.0
11	23.7	20.9	20.9	21.7	21.6
12	125.6	25.2	25.6	39.6	39.5
13	141.5	38.1	38.3	43.5	43.3
14	46.0	42.8	42.5	55.3	55.1
15	72.7	27.5	30.6	23.3	23.0
16	217.4	35.6	32.3	28.9	28.5
17	54.0	43.0	56.3	56.0	55.9
18	52.9	48.3	47.0	12.6	12.1
19	47.7	48.0	49.3	13.1	13.1
20	30.8	151.0	150.7	41.1	40.8
21	35.2	29.9	29.7	21.3	21.1
22	30.5	40.0	37.2	138.7	138.2
23	28.0	28.0	28.0	129.6	129.5
24	15.6	15.4	15.4	51.4	51.3
25	16.6	16.1	16.0	32.2	31.9
26	17.6	16.0	16.1	21.6	21.4
27	21.3	14.6	14.7	19.2	19.0
28	28.0	18.0	179.4	25.7	25.4
29	32.9	109.3	109.6	12.2	12.3
30	23.3	19.3	19.4		

* in C_5D_5N; glc: 102.2, 75.3, 78.6, 71.7, 78.5, 62.9

References

[1] 丁立生, 窦永泽, 张晓 , 等. 苦葛蔓中的新三萜成分 [J]. 药学学报, 1999, 34(2): 125-127.

[2] Sahu N P, Mahato S B, Sarkar S K, *et al*. Triterpenoid saponins from *Gymnema sylvestre* [J]. Phytochemistry, 1996, 41(4): 1181-1185.

[3] 李 娜, 杨若林, 闵知大, 等. 云南葛的化学成分研究 [J]. 中国药科大学学报 [J], 1999, 30(3): 166-170.

[4] Jha H C, Zilliken F, Breitmaier E. Carbon-13 Chemical shifts assignments of chromones and isoflavones [J]. Can J Chem, 1980, 58: 1211-1219.

[5] Lane G A, Newman R H. Isoflavones from *Lupinus angustifolius* root [J]. Phytochemistry, 1987, 26(1): 295-300.

[6] Ohshima Y, Okuyama T, Takahashi K, *et al*. Isolation and high performance liquid chromatography (HPLC) of isoflavonoids from the Pueraria root [J]. Planta Medica, 1988: 250-254.

[7] Kinjo J E, Takeshita T, Abe Y, *et al*. Studies on the constituents of *Pueraria lobata*. Ⅳ. Chemical constituents in the flowers and the leaves [J]. Chem Pharm Bull, 1988, 36(3): 1174-1179.

[8] Reynolds W F, Mclean S, Poplawski J. Total assignment of ^{13}C and ^{1}H spectra of three isomeric triterpenol derivatives by 2D NMR: an investigation of the potential utility of ^{1}H chemical shifts in structural investigations of complex natural products [J]. Tetrahedron, 1986, 42(13): 3419-3428.

[9] Siddiqui S, Hafeez F, Begum S, *et al*. Oleanderol, a new pentacyclic triterpene from the leaves of *Nerium oleander* [J]. J Nat Prod, 1988, 51(2): 229-233.

[10] Kojima H, Sato N, Hatano A, *et al*. Sterol glucosides from *Prunella vulgaris* [J]. Phytochemistry, 1990, 29(7): 2351-2355.

栝楼化学成分的研究及其 α-菠菜甾醇的含量测定 (Ⅰ)

时岩鹏, 姚庆强*, 刘拥军, 韦兴光, 王保国**, 栾 杨*

(山东省医学科学院药物研究所, 山东 济南 250062)

摘 要: 目的 研究栝楼 *Trichosanthes kirilowii* Maxim. 的化学成分并建立栝楼中 α-菠菜甾醇的含量测定方法。方法 采用反复硅胶柱层析分离纯化, 通过理化常数测定和光谱分析鉴定其化学结构。采用双波长薄层扫描法研究

* 收稿日期: 2001-04-20

基金项目: 山东省自然科学基金研究课题资助 (No. Q99C08)

作者简介: 时岩鹏 (1973-), 男, 河北邢台人, 助理研究员, 1995年毕业于河北医科大学获学士学位, 1998年毕业于山东省医学科学院获硕士学位, 1998年至今在山东省医学科学院药物研究所工作, 主要研究方向为心血管药物和抗肿瘤药物的研究与开发。Tel: 2919970 E-mail: ypsht@yeah.net

* 课题负责人

* * 山东医科大学 99级本科实习生