

狗脊化学成分研究

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摘要: 从狗脊(*Woodwardia japonica* (L. f.) Sm.)中分离得到五个化合物,经解析,分别鉴定为: 山柰素-3-O- α -L-(4-O-乙酰基)鼠李糖基-7-O- α -L-鼠李糖甙(kaempferol 3-O- α -L-(4-O-acetyl)rhamnopyranoside-7-O- α -L-rhamnopyranoside)(I)、山柰素-3-O- α -L-鼠李糖基-7-O- α -L-鼠李糖甙(kaempferol 3-O- α -L-rhamnopyranoside-7-O- α -L-rhamnopyranoside)(II)、狗脊蕨酸(woodwardinic acid)(III)、 β -谷甾醇(β -sitosterol)(IV)、胡萝卜甙(daucosterol)(V),这五个化合物均系首次从该植物中获得。

关键词: 狗脊; 化学成分; 山柰素; 狗脊蕨酸

中图分类号: Q946 **文献标识码:** A **文章编号:** 1005-3395(2002)04-0361-05

Studies on the Chemical Constituents of *Woodwardia japonica*

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Abstract: Five compounds have been firstly isolated from *Woodwardia japonica* (L. f.) Sm. On the basis of spectral analysis, the structures of these compounds were identified as kaempferol 3-O- α -L-(4-O-acetyl)rhamnopyranoside-7-O- α -L-rhamnopyranoside (I), kaempferol 3-O- α -L-rhamnopyranoside-7-O- α -L-rhamnopyranoside (II), woodwardinic acid (III), β -sitosterol (IV), daucosterol (V).

Key words: *Woodwardia japonica*; Chemical constituent; Kaempferitrin; Woodwardinic acid

Woodwardia japonica, a widespread fern in South China, is an indicator plant of acid soil^[1]. It is used as helminthic pesticide, detoxification of heat evil, and dissipater of ecchymosis^[2]. The chemical constituents of *W. japonica* were previously unknown.

1 Results and discussion

An EtOAc-soluble portion of the 95% EtOH extract of *W. japonica* was gradiently chromatographed on silica gel column using CHCl₃/MeOH system to give compound I and

Received: 2002-02-22 Accepted: 2002-04-16

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II. And in the same way, the CHCl_3 -soluble portion gave compound III, IV and V.

Compound I, was obtained as yellow acicular crystal. Its ^{13}C NMR and ^1H NMR spectra (Table 1 and 2) showed the characteristic signals of kaempferol, two rhamnosyl residues and an acetyl (Ac) group. From both the splitting characters of ^1H NMR spectrum and the COSY signals between the carbon and hydrogen atoms of the rhamnosyl in HMBC, the ^1H NMR signal of the 11 H-rhamnosyl were assigned to 0.78(3H, d, $J=6$ Hz, Me-6''), 1.21(3H, d, $J=6$ Hz, Me-6''), 2.08(3H, s, Ac), 3.27(1H, m, H-5''), 3.46(1H, t, $J=10$ Hz, H-4''), 3.60(1H, m, H-3''), 3.818(1H, t, $J=10$ Hz, H-5'''), 3.824(1H, t, $J=10$ Hz, H-3'''), 4.02(1H, br s; H-2'''), 4.21(1H, br s, H-2''), 5.51(1H, br s, H-1''), 5.55(1H, br s, H-1'''). The α -rhamnosyl can also be confirmed. The signal of the other H-4 of rhamnosyl was hidden in the signal of water according to a correlation of one C-rhamnosyl and the H- H_2O in the HMBC spectrum. The signals between H-1''' and C-7, H-1'' and C-3, and C-Ac and H-4'' were strong, and signals of the COSY between H-1'' and C-2'', 5'', C-1'' and H-6, H-8, H-1''' and C-3''', C-7 were weaker (Fig. 1). Therefore, the Ac was attached to the C-4'' which belonged to the rhamnosyl attaching to C-3, and the other rhamnosyl was attached to C-7. The attachment of Ac to C-4'' made the ^{13}C NMR and ^1H NMR signals of C-4'' downfield shift and those of neighbouring C-atoms upfield shift. By what has been discussed above, compound I was confirmed as kaempferol 3-O- α -L-(4-O-acetyl)rhamnopyranoside-7-O- α -L-rhamnopyranoside.

The data of I was conflict with a previous report^[3] of a compound that had had the same structure as I, and another previous research had reported a compound named kaempferol 3-O- α -L-(3-O-acetyl)rhamnopyranoside-7-O- α -L-rhamnopyranoside^[4], which had the same ^1H NMR and ^{13}C NMR data as compound I, but there were no explicit signal of the attachment of the Ac and the rhamnose in the NOSEY, by which all carbon atoms of that compound were assigned.

Compound II, was obtained as yellow acicular crystal. Its ^{13}C NMR and ^1H NMR spectra (Table 2) showed the characteristic signals of kaempferol and two rhamnosyl residues. Two carbon signals assigned to the C-7 and C-3 of the aglycone were shifted to the C-7 and C-3 of kaempferol themselves^[5], and were accordant with those of I. These findings supported the fact that the two rhamnoses were attached to C-3 and C-7, respectively. Accordingly, compound II was considered to be kaempferol 3-O- α -L-rhamnopyranoside-7-O- α -L-rhamnopyranoside.

Compound III, obtained as white powder, had a molecular weight 456 (ESI). The ^{13}C NMR and DEPT spectra showed that there were six methyl groups, eleven methene groups, six methyne groups, four quaternary carbon atoms, a carboxyl, and an ethenyl unit in all of the thirty carbons. The carbon signal at $\delta 66.7$ was attached to hydroxy. The molecular formula was $\text{C}_{30}\text{H}_{48}\text{O}_3$. The six single peaks of methyne in the ^1H NMR showed that each methyne group was attached to a quaternary carbon atom. According to the degree of unsaturation, the other nineteen carbon atoms were assigned to a six-ring-terpene unit. The EI spectrum showed

the connection of the six methyne groups, the carboxyl, the ethenyl unit and the terpene unit^[6]. From what had been discussed above, compound III was identified as woodwardinic acid.

Table 1 ¹³C NMR Data of compound I

Carbon	ppm	Carbon	ppm
C-2	159.9	C-2''	71.7
C-3	135.9	C-3''	70.1
C-4	179.6	C-4''	75.0
C-5	163.0	C-5''	69.6
C-6	100.6	C-6''	17.6
C-7	163.5	C-1'''	99.9
C-8	95.7	C-2'''	71.7
C-9	158.0	C-3'''	71.3
C-10	102.6	C-4'''	73.6
C-1'	122.3	C-5'''	72.1
C-2', -6'	132.0	C-6'''	18.1
C-3', -5'	116.6	COCH ₃	21.0
C-4'	161.8	COCH ₃	172.5
C-1''	107.6		

Assigned by HMBC experiments.

Run at 500 MHz in MeOH-*d*₄.

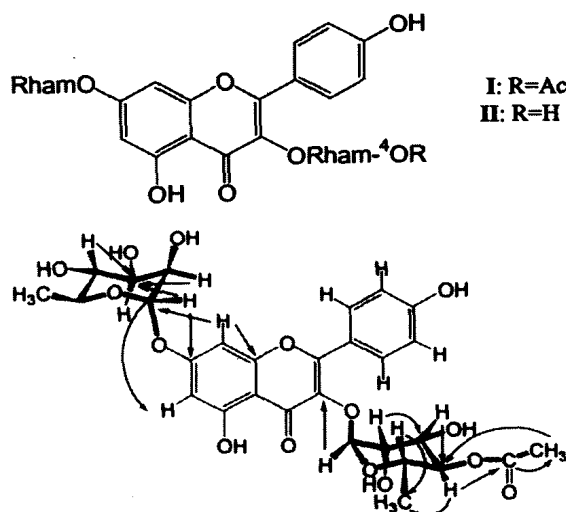


Fig. 1 HMBC correlations of compound I

Table 2 ¹H NMR spectrum data of compound I and II

H	I	II	H	I	II
6	6.44 d (2)	6.44 d (2)	6''	0.78 d (6)	0.92 d (6)
8	6.68 d (2)	6.69 d (2)	1'''	5.55 br s	5.55 br s
2',6'	7.72 d (9)	7.75 dt (9,3)	2'''	4.02 br s	4.01 br s
3',5'	6.93 d (9)	6.91 dt (9,3)	3'''	3.60 m	3.81 dd (10, 3.5)
1''	5.51 br s	5.39 br s	4'''	3.46 t (10)	3.46 t (10)
2''	4.21 br s	4.21 br s	5'''	3.818 t (10)	3.59 m
3''	3.824 t (10)	3.70 dd (10, 3.5)	6'''	1.21 d (6)	1.25 d (6)
4''	4.55 t (10)	3.32 t (10)	Ac	2.08 s	
5''	3.27 m	3.29 m			

Assignments of I based on HMBC, II based on ¹H-¹H COSY; Run at 500 MHz in MeOH-*d*₄.

Compound IV, obtained as white powder, having melting point at 134–135°C, was identified as β-sitosterol compared with authorized compound in TLC using both CHCl₃/MeOH and PE/EtOAc systems.

Compound V, obtained as white powder, had melting point at 271–273°C. The EI spectrum showed the characteristic signals of sitosterol^[7]. Compound V was considered to be daucosterol compared with authorized compound in TLC using CHCl₃/MeOH system.

2 Experiments

Plant material *Woodwardia japonica* was collected at Baoshan District, Yunnan Province, China, and identified by Prof. Zhang Xian-chun, Institute of Botany, the Chinese

Academy of Sciences.

Extraction and isolation Dried plant (1.4 kg) was extracted three times with 95% EtOH. After concentration, the combined extract (200 g) was suspended in H₂O, and extracted with CHCl₃ and EtOAc successively. The CHCl₃ layer (27.3 g) was subjected repeatedly to CC on silica gel with CHCl₃ to give **IV** (588 mg), CHCl₃-MeOH (100:1), to give **III** (214 mg), CHCl₃-MeOH (100:3) to give **V** (29 mg). The EtOAc layer (13.9 g) was subjected to CC on silica gel with CHCl₃-MeOH (100:10) to give **I** (42 mg) and **II** (50 mg).

Compound I Yellow acicular crystal (MeOH): UV λ (max)(MeOH) 265, 315 (sh), 345; IR $\nu^{\text{KBr}}\text{cm}^{-1}$: 3422, 2922, 2852, 1723, 1656, 1603, 1492, 1376, 1351, 1257, 1209, 1178, 1114, 1058, 1024, 973; EI m/z 286(100)[kaempferol]⁺, 285(25)[kaempferol-1]⁺, 258(10)[kaempferol-18]⁺, 257(10)[kaempferol-29]⁺, 153(4)[C₇H₄O₄+1]⁺, 124(12)[C₇H₄O₄-28]⁺, 134(4)[C₈H₆O]⁺, 121(25)[C₇H₅O₂]⁺, 93(8)[C₇H₅O₂-28]⁺, 129(11); ¹³C NMR and ¹H NMR see Table 1 and 2.

Compound II Yellow acicular crystal (MeOH): IR $\nu^{\text{KBr}}\text{cm}^{-1}$: 3330, 2976, 2926, 2854, 1656, 1604, 1515, 1491, 1478, 1351, 1260, 1208, 1179, 1137, 1117, 1097, 1060, 1024, 994, 973; EI m/z 286(100)[kaempferol]⁺, 285(25)[kaempferol-1]⁺, 258(10)[kaempferol-18]⁺, 257(12)[kaempferol-29]⁺, 153(6)[C₇H₄O₄+1]⁺, 124(12)[C₇H₄O₄-28]⁺, 134(5)[C₈H₆O]⁺, 121(20)[C₇H₅O₂]⁺, 93(8)[C₇H₅O₂-28]⁺, 129(11); ¹H NMR see Table 2; ¹³C NMR (500 MHz, MeOH-*d*₄) δ 17.7 (C-6''), 18.1 (C-6'''), 71.3, 71.7, 71.9, 72.1, 72.1, 72.2, 73.3, 73.7 (Rham-2''-5'', 2'''-5'''), 95.7 (C-8), 99.9 (C-1'''), 100.6 (C-6), 103.6 (C-10), 107.6 (C-1''), 116.6 (C-3', 5'), 122.5 (C-1'), 132.0 (C-2', 6'), 136.5 (C-3), 158.1 (C-9), 159.8 (C-2), 161.7 (C-4'), 163.0 (C-5), 163.6 (C-7), 179.8 (C-4).

Compound III White powder; IR $\nu^{\text{KBr}}\text{cm}^{-1}$: 3410, 2942, 2610, 1691, 1442, 1382, 1351, 1332, 1292, 1254, 1171, 1128, 1092, 1076, 1022, 994, 884, 837, 735, 663; ESI m/z 456 [M]⁺, 438 [M-H₂O]⁺, 411 [M-COOH]⁺; EI m/z 456 [M]⁺, 189(100), 237(40), 219(10), 175(30), 218(15); ¹H NMR (500 MHz, Pyridine) δ 0.73(3H, s, Me), 0.92(3H, s, Me), 1.02(3H, s, Me), 1.45(3H, s, Me), 1.71(3H, s, Me), 1.85(3H, s, Me), 4.89(2H, s, H-30); ¹³C NMR (500 MHz, Pyridine, assigned by DEPT) δ 16.4 (C-Me), 16.8 (C-Me), 16.9 (C-Me), 19.2 (C-Me), 20.1 (C-Me), 25.1 (Me-24), 66.7 (C-3), 110.6 (C-22), 148.7 (C-30), 181.7 (C-23).

Acknowledgements We are indebted to Dr. Tu Guang-zhong, Analytical Center, Beijing Institute of Microchemistry for NMR measurements, He Wei, Institute of Botany, the Chinese Academy of Sciences for MS measurements, and Prof. Zhang Xian-chun, Institute of Botany, the Chinese Academy of Sciences for identification of the plant.

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