Arch Pharm Res Vol 28, No 11, 1233-1235, 2005

Archives of Pharmacal Research
http://apr.psk.or.kr

3"-Hydroxyamentoflavone and Its 7-*O*-Methyl Ether, Two New Biflavonoids from *Aristolochia contorta*

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(Received February 28, 2005)

Two new biflavonoids, 3"-hydroxyamentoflavone-7-*O*-methyl ether (**1**) and 3"-hydroxyamentoflavone (**2**), were isolated from the fruits of *Aristolochia contorta* Bge. Their structures were elucidated by HR-ESI-MS, 1D-, and 2D-NMR spectroscopy.

Key words: *Aristolochia contorta*, Biflavonoids, 3"-Hydroxyamentoflavone-7-*O*-methyl ether, 3"-Hydroxyamentoflavone

INTRODUCTION

The genus *Aristolochia*, consisting of about 400 species in wide areas from the tropics to temperate zones, is an important genus in the family Aristolochiaceae. Some species of *Aristolochia* have been extensively used in traditional Chinese medicine. Literature survey showed that a great deal of interest in phytochemical studies on plants of the *Aristolochia* genus have yielded various type of compounds such as aristolochic acid and derivatives, aporphines, amides, benzylisoquinolines, isoquinolines, chlorophylls, terpenes, lignans, biphenyl ethers, flavonoids, tetralones, benzenoids, steroids, and miscellaneous with activities of antitumor, antiplatelet aggregation, immunomodulating and antifertility (Shi *et al.*, 2004; Priestap *et al.*, 2004; Damu *et al.*, 2003; Nascimento and Lopes, 2003). The aristolochic acids have been proved to be the most potent constituents of *Aristolochia* species and are known to be nephrotoxins and carcinogens (loset *et al.*, 2003).

The dried mature fruits of *A. contorta* Bge was used for the treatment of tuberculosis, cough, emptysis, and gall in traditional Chinese medicine (Jiangsu New Medical College, 1977). Previous investigation revealed the presence of aristolochic acids and derivatives (Tan and Liu, 1994; Lee and Han, 1992; Lou *et al.*, 1986). In the search for bioactive compounds from *Aristolochia* species, we investigated the plant fruits and isolated two biflavonoids, 3"-hydroxyamentoflavone-7-*O*-methyl ether (**1**) and 3"-hydroxyamentoflavone (**2**). We herein report the isolation and characterization of these two compounds.

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MATERIALS AND METHODS

Instruments and regents

MS were determined on an API Qstar Pulsa LC/TOF mass spectrometer. FAB-MS were analyzed on a VG Autospec-3000 mass spectrometer. NMR spectra were measured on a Bruker DRX-500 spectrometer with TMS as int. standard. Silica gel (200-300 mesh) was used for column chromatography and silica gel GF_{254} for TLC (Qingdao Marine Chemical Co., China). Solvents were of the industrial purity and distilled before using.

Plant materials

The fruits of *A. contorta* were purchased on July 2002 from Yunnan Provincinal Crude Drugs Company, Yunnan, China and identified by Prof. Xin-Rong Liao, Department of Traditional Chinese Medicine, Yunnan Institute of Traditional Chinese Medicine, China. The voucher specimen was deposited at the Department of Chemistry, Yunnan Normal University (No.20020725).

Extraction and Isolation

The dried and crushed fruits of *A. contorta* (1 kg) were extracted with 95% ethanol five times at room temperature. The extract (40 g) was concentrated and absorbed on silica gel, eluting with petroleum ether, chloroform, acetone and methanol successively. The acetone eluting fraction

Table I. ^{1}H and ^{13}C-NMR data for compounds **1** and **2** (CD_3COCD_3)

No	1		2	
	^{1}H	^{13}C	^{1}H	^{13}C
2		164.4		164.2
3	6.80 (s)	103.6	6.76 (*s*)	103.5
4		182.0		182.2
5		160.9		162.5
6	6.33 (d, 2.2)	98.0	6.26 (*d*, 2.0)	98.9
7		165.9		164.0
8	6.69 (d, 2.2)	94.2	6.54 (*d*, 2.0)	94.0
9		157.9		158.0
10		105.2		104.6
1'		122.6		122.6
2'	8.17 (d, 2.4)	131.9	8.16 (*d*, 2.4)	131.8
3'		119.9		119.9
4'		159.6		159.5
5'	7.28 (d, 8.7)	116.8	7.27 (*d*, 8.6)	116.7
6'	8.08 (dd, 8.7, 2.4)	128.1	8.06 (*dd*, 8.6, 2.4)	128.0
2"		146.3		146.3
3"		135.8		135.8
4"		176.0		176.0
5"		161.0		160.9
6"	6.49 (s)	98.4	6.49 (*s*)	98.4
7"		162.2		161.7
8"		103.3		103.4
9"		154.3		154.3
10"		103.7		103.6
1"'		122.6		122.6
2"'	7.91 (d, 9.0)	129.5	7.92 (*d*, 9.0)	129.6
3"'	6.83 (d, 9.0)	115.4	6.83 (*d*, 9.0)	115.4
4"'		159.2		159.2
5"'	6.83 (d, 9.0)	115.4	6.83 (*d*, 9.0)	115.4
6"'	7.91 (d, 9.0)	129.5	7.92 (*d*, 9.0)	129.6
CH_3O-7	3.90 (s)	55.5		
HO-	12.36 (s)		12.36 (*s*)	
	13.04 (s)		13.00 (*s*)	

(10 g) was subjected to column chromatography on silica gel eluting with a gradient of acetone in petroleum ether to yield fractions 1-15 monitored by TLC tests. Fr. 11 (2 g) was further subjected to cc on silica gel eluting with petroleum ether-EtOAc (2:1, v/v) and then further purified by a Sephadex LH-20 (20~80 um) column chromatography eluting with MeOH to yield pure compounds **1** (6 mg) and **2** (10 mg).

Compound 1

EIMS *m/z* (rel. int., %): 279 (5), 223 (3), 205 (12), 167 (14), 149 (91), 121 (10), 101 (71), 98 (35), 83 (43), 59 (100); FAB-MS $[M+H]^+$ *m/z* 569; HR-ESI-MS $[M+H]^+$ *m/z* 569.1096, calcd for $C_{31}H_{21}O_{11}$, 569.1083; ^{1}H- (CD_3COCD_3, 500 MHz) and ^{13}C-NMR (CD_3COCD_3, 125 MHz): Table I.

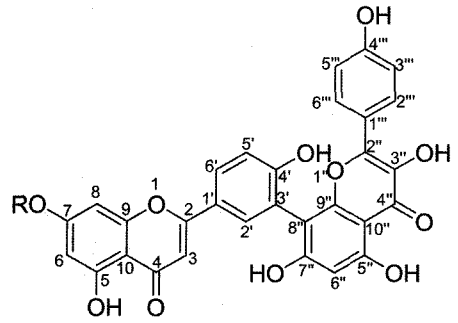
Compound 2

EIMS *m/z* (rel. int., %): 279 (3), 223 (5), 205 (6), 167 (12), 149 (100), 121 (4), 104 (5), 98 (8), 83 (15); FAB-MS $[M+H]^+$ *m/z* 555; HR-ESI-MS $[M+H]^+$ *m/z* 555.0924, calcd for $C_{30}H_{19}O_{11}$, 555.0927; ^{1}H- (CD_3COCD_3, 500 MHz) and ^{13}C-NMR (CD_3COCD_3, 125 MHz): Table I.

RESULTS AND DISCUSSION

The chromatographic separation of the acetone fraction from the fruits of *A. contorta* led to the isolation of 3"-hydroxyamentoflavone-7-*O*-methyl ether (**1**) and 3"-hydroxyamentoflavone (**2**) (Fig. 1).

Compounds **1** was obtained as yellow amorphous powders. HR-ESI-MS exhibited $[M+H]^+$ of **1** at *m/z* 569.1096 (calcd for $C_{31}H_{21}O_{11}$, 569.1083). The ^{1}H- NMR, ^{13}C-NMR (Table I), and DEPT spectra of **1** revealed the presence of thirty-one carbon signals among which eleven were methines and nineteen quaternary carbons including two carbonyls (δ_C 182.0 and 176.0). The ^{1}H-NMR spectrum displayed signals for one methoxy and eleven aromatic protons. From the analysis of the ^{1}H-^{1}H COSY and HMBC spectra of **1**, it was deduced that the aromatic protons were distributed into one para-disubstituted [δ_H 7.91 (2H, d, *J*= 9.0 Hz, H-2"', 6"'), 6.83 (2H, d, *J*= 9.0 Hz, H- 3"', 5"')], one 1, 3, 4-trisubstituted [δ_H 8.08 (1H, dd, *J*= 8.7, 2.4 Hz, H- 6'), 7.28 (1H, d, *J*= 8.7 Hz, H- 5'), 8.17 (1H, d, *J*= 2.4 Hz, H- 2')], one 1, 2, 3, 5-tetrasubstituted [δ_H 6.33 (1H, d, *J*= 2.2 Hz, H-6), 6.69 (1H, d, *J*= 2.2 Hz, H-8)] and one pentasubstituted aromatic ring [δ_H 6.49 (1H, s, H-6")], indicating that **1** was a biflavonoid. Detailed analysis of ^{1}H-NMR data allowed to the deduction that **1** was an amentoflavone series of biflavonoid linked between C-3' and C-8" (Lu *et al.*, 2004), which was conformed by ^{13}C-



1 R=CH_3
2 R=H

Fig. 1. Structure of biflavones isolated from *Aristolochia contorta*

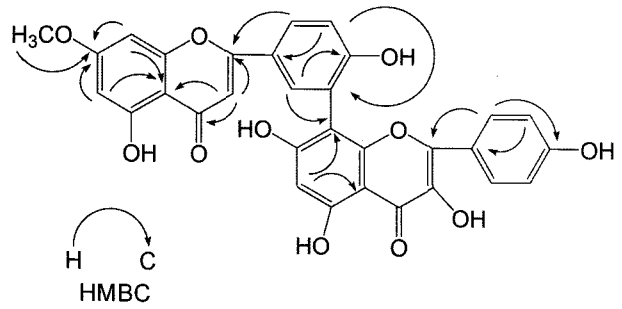


Fig. 2. HMBC correlation of compound **1**

NMR and the HMBC experiment (Fig. 2) by showing long-range correlations between H-5' and C-3' (δ_C 119.9), H-2', H-6" and C-8" (δ_C 103.3). An additional hydroxyl group was assigned to linked at C-3" as correlations between signals at δ_H 6.80 (H-3) and δ_C 164.4 (C-2) and 182.0 (C-4) were observed, but there was no any cross peaks between δ_C 176.0 (C-4") and 135.8 (C-3") and protons. The attachment of methoxy was also determined by cross peaks between H-8, H-6 and methoxy protons and C-7 (δ_C 165.9) in HMBC spectrum, which was verified by correlations between methoxy protons and H-8 and H-6 in 2D NOESY plot. Thus, all the substitution positions were established and **1** was characterized as 3"-hydroxy-amentoflavone-7-O-methyl ether.

Compound **2** was obtained also as a yellow amorphous powder. The molecular formula $C_{30}H_{18}O_{11}$ was inferred by HR-ESI-MS ($[M+H]^+$ at *m/z* 555.0924, calcd for 555.0927). The ^{1}H- NMR, ^{13}C-NMR (Table I), and DEPT spectra of **2** was pretty similar with that of **1**. The only structural difference between the two compounds laid in the substitution at C-7. The studies on its ^{1}H-NMR and ^{13}C-NMR spectra suggested a hydroxy group at C-7 in **2**, instead of a methoxy at C-7 in **1**. The analysis of its 1D- and 2D-NMR spectra led to the unambiguous assignment of its structure as 3"-hydroxyamentoflavone. It is the second time biflavonoids were found in plants of the genus *Aristolochia* (Fernando *et al.*, 2000).

ACKNOWLEDGEMENTS

This investigation was supported by a grant (No. 30160093) from National Science Foundation of China, the Excellent Young Teachers Program (No. 2003 192) of MOE, China and a grant (No. 2000 C007) for international collaborative research by Yunnan Provincial Committee of Science and Technology, China.

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