Department of Chemistry[1], Yunnan Normal University, and Experimental Center[2], Yunnan University, Kunming, Yunnan, P.R. China

A new biflavonoid from *Aristolochia contorta*

LI-LI YU[1], RONG HUANG[2], YU-PING LV[1], YONG ZHAO[1], YEGAO CHEN[1]

Received December 8, 2004, accepted January 5, 2005

Dr. Ye-Gao Chen, Department of Chemistry, Yunnan Normal University, Street 121, 650092 Kunming, Yunnan, P. R. China
ygchen48@hotmail.com

Pharmazie 60: 789–791 (2005)

From the fruits of *Aristolochia contorta* Bge, beside the known aristolochic acids IVa and VII, aristolactam-*N*-β-D-glucopyanoside, aristoloctam Ia *N*-β-D-glucopyanoside, pinitol and daucosterol, a new biflavonoid was isolated. Its structure was determined as (±)-2″R,3″R-dihydro-3″-hydroxyamentoflavone (**1**) by means of spectral methods including 1D-, 2D-NMR and HR-ESIMS and the known compounds were identified on the basis of comparing their NMR data with those of the corresponding compounds in the literature.

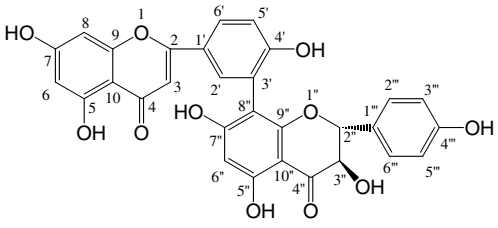
1. Introduction

The genus *Aristolochia* is extensively used in traditional Chinese medicine and has drawn much attention in phytochemical studies, which yielded various compounds such as aristolochic acids and derivatives, aporphines, amides, benzylisoquinolines, isoquinolones, chlorophylls, terpenes, lignans, biphenyl ethers, flavonoids, tetralones, benzenoids, steroids and miscellaneous with antitumor, antiplatelet aggregation, immunomodulating and antifertility activities (Damu et al. 2003; Nascimento and Lopes 2003; Priestap et al. 2003; Shi et al. 2004). The aristolochic acids proved to be the most potent constituents of *Aristolochia* species and are known to be nephrotoxins and carcinogens (Wu et al. 2003).

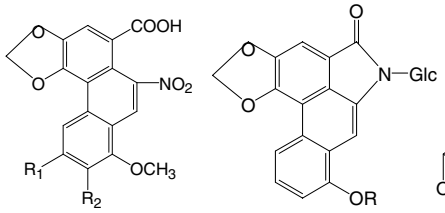
2. Investigations, results and discussion

In the search for bioactive compounds from *Aristolochia* species, we investigated the dried mature fruits of *A. contorta*, which was used for the treatment of tuberculosis, cough, emptysis and gall in traditional Chinese medicine (Jiangsu New Medical College 1977). Previous investigations revealed the presence of aristolochic acids and derivatives (Lee and Han 1992; Lou et al. 1986; Tan and Liu 1994). From the ethanol extract of the fruits of *A. contorta*, we isolated a new biflavonoid, along with the known aristolochic acids IVa (**2**) and VII (**3**), aristolactam-*N*-β-D-glucopyanoside (**4**), aristoloctam Ia *N*-β-D-glucopyanoside (**5**), pinitol (**6**) and daucosterol (**7**). The novel structure was determined as (±)-2″R,3″R-dihydro-3″-hydroxyamentoflavone (**1**) by means of spectral methods including 1D-, 2D-NMR and HR-ESIMS and the known compounds were identified on the basis of comparing their NMR data with those of the corresponding compounds in the literature.

(±)-2″R,3″R-dihydro-3″-hydroxyamentoflavone (**1**) was isolated as a primrose yellow amorphous powder. The molecular formula $C_{31}H_{20}O_{11}$ was deduced by HR-ESIMS

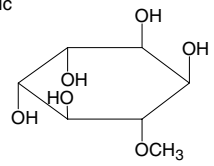


1



2 R_1= OH, R_2= H
3 R_1= H, R_2= OCH_3

4 R= OCH_3
5 R= OH



6

([M + H]$^+$ at m/z 557.1090, calcd. 557.1083) and its structure was determined by 1D- and 2D-NMR spectroscopy.

The ^{1}H-, ^{13}C NMR (Table) and DEPT spectra of **1** revealed the presence of thirty carbon signals due to two sp^3 oxygenated methines (δ_C 83.2 and 72.2) and twenty-eight sp^2 carbons among which eleven were sp^2 methines and seventeen quaternary carbons including two carbonyls (δ_C 182.2 and 197.8). The ^{1}H NMR spectrum displayed signals for 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 7.41 (2 H, d, J = 8.6, H-2‴, 6‴), 6.76 (2 H, d, J = 8.6, H-3‴, 5‴)], one 1,3,4-trisubstituted [δ_H 7.83 (1 H, dd, J = 8.7, 2.5, H-6′), 7.04 (1 H, d, J = 8.7, H-5′), 8.02 (1 H, d, J = 2.5, H-2′)], one 1,2,3,5-tetrasubstituted [δ_H 6.28 (1 H, d, J = 2.0, H-6), 6.60 (1 H, d,

Table: ^{1}H and ^{13}C NMR spectra data (CD_3COCD_3) for (±)-2″R,3″R-dihydro-3″-hydroxyamemtoflavone (1)*

Position	δ_H	δ_C
2		164.8
3	6.66 (s)	103.3
4		182.2
5		162.6
6	6.28 (d, 2.0)	98.8
7		163.5
8	6.60 (d, 2.0)	93.8
9		157.7
10		104.5
1′		122.1
2′	8.02 (d, 2.5)	131.7
3′		120.3
4′		159.1
5′	7.04 (d, 8.7)	116.5
6′	7.83 (dd, 8.7, 2.5)	127.4
2″	5.20 (d, 11.5)	83.2
3″	4.68 (d, 11.5)	72.2
4″		197.8
5″		160.8
6″	6.21 (s)	96.4
7″		163.9
8″		104.8
9″		163.4
10″		100.9
1‴		122.1
2‴	7.41 (d, 8.6)	129.1
3‴	6.76 (d, 8.6)	114.9
4‴		158.0
5‴	6.76 (d, 8.6)	114.9
6‴	7.41 (d, 8.6)	129.1
HO−	11.88 (s)	
	13.08 (s)	

* Coupling constants in Hz are in parentheses.

J = 2.0, H-8)] and one pentasubstituted aromatic ring [δ_H 6.21 (1 H, s, H-6″)], indicating that **1** was a biflavonoid. The HMBC experiment (Fig.) showed long-range correlations between H-5′ and C-3′ (δ_C 120.3), H-2′, H-6″ and C-8″ (δ_C 104.8), suggesting the biflavone was linked between C-3′ and C-8″, like the known biflavone amentoflavone previously isolated from *Selaginella doederleinii* (Lu YP et al. 2004). The two sp^3 oxygenated methines [δ_H 5.20 and 4.68 (each 1 H, d, J = 11.5)] were assigned to be C-2″ and C-3″ respectively, as shown by the coupling action and cross peak between the two protons in the ^{1}H–^{1}H COSY spectrum. Correlations between signals at H-2″ and C-4″ (δ_C 197.8), C-2‴ and C-6‴ (δ_C 129.1), H-3″ and C-10″ (δ_C 100.9) and C-1‴ (δ_C 122.1), H-6″ and C-10″ further confirmed the deduction. The coupling constant between H-2″ and H-3″ displayed the two protons in trans-orientation, which was validated by no cross peak between H-2″ and H-3″ in the 2D NOESY spectrum. The compound showed no optical activity, leading to the conclusion that it is a racemate of **1** (2″R,3″R-dihydro-3″-hydroxyamentoflavone) and its enantiomorph 2″S,3″S-dihydro-3″-hydroxyamentoflavone. On the basis of the results mentioned above, the novel biflavonoid was thus determined as dl-form of **1**.

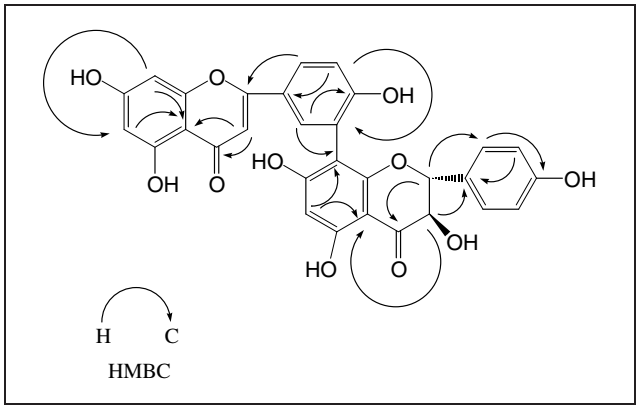


Fig.: HMBC correlation of (±)-2″R,3″R-dihydro-3″-hydroxyamentoflavone (1)

The known compounds aristolochic acids IVa (**2**) and VII (**3**), aristolactam-*N*-β-D-glucopyanoside (**4**), aristoloctam Ia *N*-β-D-glucopyanoside (**5**), pinitol (**6**) and daucosterol (**7**) were also isolated and characterized by comparison of their spectroscopic data (NMR and MS) with literature values (Lee and Han 1992; Leu et al. 1998; Lou et al. 1986; Tan and Liu 1994; Wu et al. 1999).

3. Experimental

3.1. General

MS were determined on an API Qstar Pulsa LC/TOF mass spectrometer. NMR spectra were measured on a Bruker DR-500 spectrometer with TMS as int. standard. Optical rotation was run on a Horiba-SEPA-300 polarimeter. 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.

3.2. Plant materials

The fruits of *Aristolochia contorta* Bge were purchased on July, 2002 from Yunnan Provincinal Crude Drugs Company, Yunnan, P. R. 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).

3.3. Extraction and isolation

The dried and crushed fruits (1 kg) were extracted for five times with 95% EtOH at room temperature. The extract was concentrated and absorbed on silica gel, eluted with petroleum ether, chloroform, acetone and methanol successively. The acetone fraction (10 g) was subjected to CC on silica gel eluted with a gradient of acetone in petroleum ether to yield fractions 1–15 monitored by TLC tests. Fr. 11 was further subjected to CC over silica gel, eluting with petroleum ether/EtOAc 3 : 1 and rechromatographied on Sephadex LH-20 (MeOH/$CHCl_3$ 95 : 5) to yield compounds **1** (8 mg), **2** (28 mg) and **3** (14 mg). Using the same procedure, Fr. 12 yielded **4** (20 mg), **5** (2 mg), **6** (3 mg) and **7** (2 mg).

(±)-2″R,3″R-Dihydro-3″-hydroxyamentoflavone (**1**), Yellow amorphous powder; $[\alpha]_D^{25}$ 0° (Me_2CO; c 0.82); HR-ESIMS m/z: 557.1090 $[M+H]^+$ (calcd. for $C_{30}H_{20}O_{11}$, 557.1083); ^{1}H- and ^{13}C NMR: See Table.

Aristolochic acid IVa (**2**), Salmon pink amorphous powder; EIMS m/z (%): 357 (9), 323 (11), 313 (22), 312 (100), 311 (46), 310 (49), 309 (61), 297 (25), 296 (21), 295 (41), 294 (47), 280 (23), 266 (28), 225 (6), 196 (4), 139 (13); ^{1}H NMR (CD_3COCD_3, 500 MHz): δ 8.62 (1 H, s), 8.20 (1 H, s), 7.79 (1 H, s), 6.88 (1 H, d, J = 2), 6.48 (2 H, s), 4.06 (3 H, s, $-OCH_3$); ^{13}C NMR (CD_3COCD_3, 125 MHz): δ 167.1, 161.0, 158.6, 146.7, 145.8, 132.0, 132.0, 124.8, 120.6, 119.6, 118.7, 113.4, 112.3, 111.3, 102.8, 99.3, 55.8.

Aristolochic acid VII (**3**), Salmon pink amorphous powder; EIMS m/z (%): 371 (6), 341 (30), 325 (14), 295 (100), 310 (4), 297 (24), 266 (14), 237 (10), 195 (6), 168 (11), 139 (17); ^{1}H NMR (CD_3COCD_3, 500 MHz): δ 8.81 (1 H, dd, J = 9.0, 0.6), 8.53 (1 H, d, J = 0.6), 7.77 (1 H, s), 7.52 (1 H, d, J = 9.0), 6.49 (2 H, s), 4.08 (6 H, s).

Aristolactam-*N*-β-D-glucopyanoside (**4**), Yellow amorphous powder; EIMS m/z (%): 455 (15), 322 (9), 294 (24), 293 (100), 278 (46), 263 (12), 250 (11), 137 (8); ^{1}H NMR (C_5D_5N, 500 MHz): δ 8.20 (1 H, d, J = 8.1), 7.77 (1 H, s), 7.65 (1 H, s), 7.57 (1 H, t, J = 8.1), 7.26 (1 H, d, J = 8.1), 6.50 (2 H, d, J = 6.9), 5.34 (1 H, d, J = 9.3), 5.27 (1 H, d, J = 5.4), 5.23 (1 H, d, J = 4.6), 5.13 (1 H, d, J = 5.4), 4.62 (1 H, t, J = 5.5); ^{13}C NMR (C_5D_5N, 125 MHz) δ 167.4, 156.1, 148.6, 147.9, 134.4, 126.2, 126.2, 125.9, 125.4, 124.8, 119.5, 118.9, 112.2, 108.1, 106.0, 103.4, 101.3, 83.7, 81.7, 79.5, 71.8, 71.7, 62.8, 55.5.

Aristoloctam Ia *N*-β-D-glucopyanoside (**5**), Yellow amorphous powder; ^{1}H NMR (DMSO, 500 MHz): δ 8.10 (1 H, d, J = 8.1Hz), 7.74 (1 H, *s*), 7.64 (1 H, s), 7.44 (1 H, t, J = 7.8), 7.12 (1 H, d, J = 7.5), 6.50 (2 H, d, J = 6.9), 5.36 (1 H, d, J = 9.3), 5.31 (1 H, d, J = 5.5), 5.24 (1 H, d, J = 5.0), 5.19 (1 H, d, J = 5.5), 4.68 (1 H, t, J = 5.6), 4.02 (1 H, m), 3.78

(1 H, m), 3.29 (1 H, m); ^{13}C NMR (DMSO, 125 MHz): δ 166.9, 154.5, 149.3, 148.1, 133.3, 127.0, 125.8, 125.7, 123.3, 118.1, 118.0, 113.0, 111.8, 106.1, 103.8, 101.7, 82.4, 80.6, 78.2, 70.9, 70.4, 61.8.

Pinitol (**6**), white amorphous powder; EIMS m/z (%): 194 (5), 176 (3), 158 (2), 144 (4), 116 (10), 102 (11), 87 (100), 85 (34), 73 (77), 60 (17), 57 (14); ^{1}H NMR (C_5D_5N, 500 MHz): δ 3.93 (3 H, s, $-OCH_3$), 4.17 (1 H, t, J = 9.3), 4.65 (1 H, t, J = 9.3), 4.75 (1 H, q, J = 9.2, 3.6), 4.77 (1 H, m), 4.80 (1 H, m), 4.82 (1 H, m); ^{13}C NMR (C_5D_5N, 125 MHz): δ 85.6, 74.4, 73.9, 73.5, 72.8, 72.0, 60.4.

Daucosterol (**7**), white amorphous powder; FAB-MS: 577$[M + H]^+$; identified by mixed melting point, co-TLC and comparison of IR spectrum with that of authentic samples.

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.

References

Damu AG, Kuo PC, Leu YL et al. (2003) Chemical constituents and pharmacology of the Formosan *Aristolochia* species. Chin Pharm J 55: 1–33.

Jiangsu New Medical College (1977) Dictionary of Traditional Chinese Medicine. Shanghai People's Press, Shanghai, p. 294.

Lee HS, Han DS (1992) A new acylated N-glycosyl lactam from *Aristolochia contorta*. J Nat Prod 55: 1165–1169.

Leu YL, Chan YY, Hsu MY et al. (1998) The constituents of the stem and roots of *Aristolochia foveolata*. J Chin Chem Soc 45: 539–541.

Lou FC, Ding LS, Wu MY et al. (1986) Chemical constituents form *Aristolochia contorta*. Acta Pharm Sin 21: 702–705.

Lu YP, Chen YG, Wen J (2004) A new biflavone from *Selaginella doederleinii*. Acta Bot Yunnan 26: 226–228.

Nascimento IR, Lopes LMX (2003) Diterpene esters of aristolochic acids from *Aristolochia pubescens*. Phytochemistry 63: 953–957.

Priestap HA, Baren CM, Lira PDL et al. (2003) Volatile constituents of *Aristolochia argentina*. Phytochemistry 63: 221–225.

Shi LS, Kuo PC, Tsai YL et al. (2004) The alkaloids and other constituents from the root and stem of Aristolochia elegans. Bioorg Med Chem 12: 439–446.

Tan HG, Liu YQ (1994) Chemical constituents form the roots of *Aristolochia contorta*. Zhongguo Zhong Yao Za Zhi 19: 677–678.

Wu PL, Su GC, Wu TS (2003) Constituents from the Stems of *Aristolochia manshuriensis*. J Nat Prod 66: 996–998.

Wu TS, Leu YL, Chan YY (1999) Constituents of the fresh leaves of *Aristolochia cucurbitifolia*. Chem Pharm Bull 47: 571–573.