# Constituents of the Fresh Leaves of *Aristolochia cucurbitifolia*

Tian-Shung Wu,* Yann-Lii Leu, and Yu-Yi Chan

*Department of Chemistry, National Cheng Kung University, Tainan, Taiwan, R.O.C.*
Received October 30, 1998; accepted December 14, 1998

**Continuing our investigation on the bioactive compounds from the plant of *Aristolochia genus* in Taiwan, we isolated one new aristolochic acid derivative, aristolochic acid-VII methyl ester; one new dihydrophenanthrenelactone, aristolide-C; four new benzenoids, 2′-hydroxyethyl 4-hydroxybenzoate, ariscucurbin-A, -B and -C, together with forty-six known compounds from the fresh *Aristolochia cucurbitifolia*.**

**Key words** *Aristolochia cucurbitifolia*; Aristolochiaceae; aristolide-C; ariscucurbin-A

*Aristolochia cucurbitifolia* Hayata (Aristolochiaceae) is a shrub.[1] The fruits and roots of this plant are used in traditional Chinese medicine as anodynes, antiphlogistics, antitussives, expectorants, and antiasthmatic agents and also for the treatment of snakebites and lung inflammation.[2] Aristolochic acids, aristolactams,[3] denitroaristolochic acids,[4] and sesquiterpenes[5] have been isolated from this plant. The present report describes the isolation and structure elucidation of six new compounds: aristolochic acid-VII methyl ester (**1**), aristolide-C (**2**), 2′-hydroxyethyl 4-hydroxybenzoate (**3**), ariscucurbin-A (**4**), -B (**5**) and -C (**6**) as well as forty-six known compounds from the fresh leaves of *A. cucurbitifolia*.

## Results and Discussion

Aristolochic acid-VII methyl ester (**1**) was obtained as yellow syrup and high resolution electron ionization mass spectrum (HR-EI-MS) (*m/z*: 385.0800 $[M]^+$) established the molecular formula as $C_{19}H_{15}NO_8$. The UV absorptions at 227, 273, 313 and 373 nm indicated the presence of a typical phenanthrene derivative.[6] In the aromatic region of $^1$H-NMR, a set of AB type signals at $\delta$ 8.85 and 7.46 (each 1H, d, $J$=9.6 Hz) were attributed to H-5 and H-6, respectively. The signal of H-5 appeared at lower field at $\delta$ 8.85, due to the deshielding effect of the A ring in aristolochic acid derivative. Two singlet signals appearing at $\delta$ 8.67 (1H, s) and 7.70 (1H, s) were assigned to H-9 and H-2, respectively. The methoxyl groups appeared at $\delta$ 4.06 (3H, s), 4.04 (3H, s) and 3.87 (3H, s). Consequently, the above spectral data support the structure of aristolochic acid-VII methyl ester as **1**, which was previously synthesized by Priestap.[7] This, however is the compound **1** isolated from a natural source.

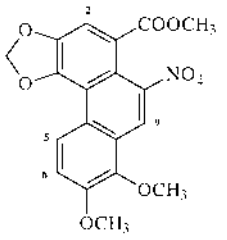
Aristolide-C (**2**) was isolated as an optically active colorless oil. Its molecular formula was determined to be $C_{18}H_{14}O_6$ (*m/z*: 326.0791 $[M]^+$) by HR-EI-MS. The UV spectrum of **2** with absorption maxima at 219 (sh), 270, 295 and 327 (sh) nm was similar to that of aristololide-A which is considered characteristic of a dihydrophenanthrenelactone nucleus.[8] The IR spectrum showed the presence of a lactonic carbonyl group at 1768 cm$^{-1}$. In the $^1$H-NMR spectrum, an AB-type system at $\delta$ 7.65 (1H, d, $J$=8.4 Hz) and 6.93 (1H, d, $J$=8.4 Hz) was attributed to H-5 and H-6, respectively. One singlet signal at $\delta$ 7.09 (1H, s) was assigned to H-2. Two methoxyl groups and a methylenedioxy signal appeared at $\delta$ 3.92, 3.83 (each 3H, s) and 6.25, 6.16 (each 1H, d, $J$=1.2 Hz), respectively. In addition, three signals clearly coupled at $\delta$ 5.33 (1H, dd, $J$=13.6, 6.4 Hz), 3.95 (1H, dd, $J$=13.6, 6.4 Hz) and 2.48 (1H, t, $J$=13.6 Hz) and were assigned to H-10ax., H-9eq. and H-9ax., respectively. The stereochemistry of C-10 was determined as *R*-configuration by the negative specific optical rotation.[8,9] Based on the above results, the structure of **2** was assigned as aristolide-C.

2′-Hydroxyethyl 4-hydroxybenzoate (**3**) was obtained as colorless oil and showed a characteristic UV spectrum of benzenoid chromophore. The molecular formula, $C_9H_{10}O_4$, was deduced from the molecular ion at *m/z* 182.0579 in the HR-EI-MS. The IR spectrum of **3** revealed the presence of a hydroxyl and a carbonyl group at 3400 and 1710 cm$^{-1}$, respectively. In the $^1$H-NMR spectrum, the aromatic proton signals of *para*-substituted benzene ring appeared at $\delta$ 7.98 and 6.86 (each 2H, d, $J$=8.6 Hz). The presence of a partial structure–$OCH_2CH_2OH$ in this molecule was inferred by the signals at $\delta$ 5.60 (1H, br s, $D_2O$ exchangeable), 4.44 and 3.95 (each 2H, t, $J$=4.8 Hz). The glycol moiety should be attached to the carbonyl group and was confirmed by the fragment at *m/z* 93 ($M^+-COOCH_2CH_2OH$) in the mass spectrum. On the basis of these data, the structure of 2′-hydroxyethyl 4-hydroxybenzoate was established as **3**, which was previously synthesized by Heim.[10] This is also the first time this compound has been isolated from a natural source.

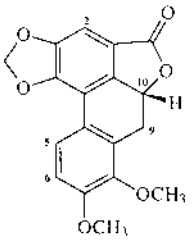
Ariscucurbin-A (**4**) was isolated as colorless oil. The molecular formula, $C_{12}H_{14}O_5$, was determined by the HR-EI-MS at *m/z* 238.0843. The IR spectrum showed intense absorption bands at 3300 cm$^{-1}$ for hydroxyl group and 1700 cm$^{-1}$ for $\alpha,\beta$-unsaturated carbonyl group. The UV absorptions at 216, 234, 297 and 326 nm suggested a benzenoid. In the $^1$H-NMR of **4**, a set of ABX type signals at $\delta$ 7.20 (1H, d, $J$=2.0 Hz), 7.08 (1H, dd, $J$=8.2, 2.0 Hz) and 6.82 (1H, d, $J$=8.2 Hz); the *trans*-olefinic protons of $\alpha,\beta$-unsaturated carbonyl group at $\delta$ 7.67 and 6.40 (each 1H, d, $J$=16.0 Hz); and one methoxyl group at $\delta$ 3.90 (3H, s) were inferred to be the feruloyl moiety. This was confirmed by negative Gibb's test and the fragment *m/z* 177 in the mass spectrum. In addition, ethylene protons appeared at $\delta$ 4.32 and 3.80 (each 2H, t, $J$=5.0 Hz). Based on the above data, ariscucurbin-A was assigned as **4**.

Ariscucurbin-B (**5**) and -C (**6**) showed the same molecular formula, $C_{11}H_{12}O_4$, by HR-EI-MS. Their IR spectra showed absorption bands at 3440, 3200 cm$^{-1}$ for hydroxyl groups and 1710 cm$^{-1}$ for $\alpha,\beta$-unsaturated carbonyl group, respectively. The UV spectra of **5** and **6** were consistent with typical absorption of the benzenoid derivatives. The mass spectra of **5** and **6** both showed the base peak at *m/z* 147 assignable to the cinnamoyl moiety. In the $^1$H-NMR spectra, **5** showed

* To whom correspondence should be addressed.



1



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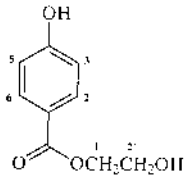

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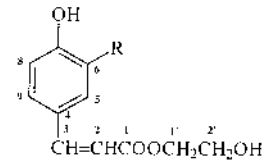

**4**: R=$OCH_3$, *trans*
**5**: R=H , *trans*
**6**: R=H , *cis*

the presence of *trans*-olefinic protons of $\alpha,\beta$-unsaturated carbonyl group at $\delta$ 7.69 and 6.36 (each 1H, d, $J$=16.0 Hz) and **6** *cis*-olefinic protons at $\delta$ 6.88 and 5.81 (each 1H, d, $J$=12.5 Hz). On the basis of these results, the structures of ariscucurbin-B and -C were established as **5** and **6**, respectively.

The known compounds aristolochic acid-I (**7**),[11] -IV (**8**),[11] -IVa (**9**),[11] -VIIa (**10**),[7] sodium aristolochate-I (**11**),[12] -VIIa (**12**),[12] aristolochic acid-IV methyl ester (**13**),[11] aristolic acid (**14**),[4] aristolic acid methyl ester (**15**),[4] aristofolin-A (**16**),[4] sodium aristofolin-A (**17**),[4] aristofolin-B (**18**),[4] -C (**19**),[4] -D (**20**),[4] aristolactam-I (**21**),[12] -II (**22**),[11] 9-methoxyaristolactam-I (**23**),[11] aristolactam-C *N*-$\beta$-D-glucoside (**24**),[11] aristolactam-AII (**25**),[11] -BII (**26**),[11] ceparadione-A (**27**),[11] 4,5-dioxodehydroasimilobine (**28**),[3] aristolide-A (**29**),[8] quercetin 3-*O*-glucoside (**30**),[13] kaempferol 7-*O*-glucoside (**31**),[12] kaempferol 3-*O*-glucoside (**32**),[14] quercetin 3-*O*-rutinoside (**33**),[14] apigenin (**34**),[14] kaempferol 3,7-*O*-diglucoside (**35**),[14] kaemfperol 3-*O*-rutinoside (**36**),[14] quercetin 7-*O*-glucoside (**37**),[14] methylparaben (**38**),[11] vanillic acid (**39**),[15] *p*-hydroxybenzoic acid (**40**),[15] *p*-hydroxybenzaldehyde (**41**),[16] *p*-hydroxycinnamic acid (**42**),[11] methyl *p*-hydroxycinnamate (**43**),[11] *p*-coumaroyl-D-glucoside (**44**),[17] sodium (2*R*)-(*p*-hydroxyphenyl)lactate (**45**),[15] indole-3-carboxylic acid methyl ester (**46**),[18] *N*-acetyltyramine (**47**),[19] glyceryl-1-stearate (**48**),[20] $\beta$-sitoserol (**49**),[16] $\beta$-sitosteryl 3-*O*-glucoside (**50**),[16] 6$\beta$-hydroxystigmast-4-en-3-one (**51**)[16] and methyl-21-hydroxy-(21*R*)-pheophorbide-a (**52**)[21] were also isolated from the fresh leaves of *A*. *cucurbitifolia*. Their structures were characterized by comparison of their spectroscopic data (UV, IR, NMR and mass spectrometry) in the literature.

## Experimental

UV spectra were recorded in MeOH, and IR spectra were determined as KBr discs. $^1$H-NMR spectra were obtained on a Bruker NMR spectrometer (200 MHz), with tetramethylsilane (TMS) as internal standard. EI-MS was measured with a 70 eV direct inlet system on a VG70-250AS spectrometer. Melting points were uncorrected. Optical rotations were recorded on a Jasco DIP-370 digital polarimeter.

**Plant Material** *Aristolochia cucurbitifolia* HAYATA was collected at Yen Chao, Kaoshiung Hsien, Taiwan, in April 1992 and verified by Prof. C. S. Kuoh. A voucher specimen is deposited in the Herbarium of Cheng Kung University, Taiwan.

**Extraction and Separation** Fresh leaves (1.44 kg) were extracted with MeOH (×6) at room temperature and concentrated to give a deep brown syrup. The MeOH extract was then partitioned successively between $H_2O$ and $CHCl_3$, and then *n*-BuOH. The $CHCl_3$ layer was chromatographed directly on silica gel and eluted with a gradient of $CHCl_3$ and MeOH to give 4 fractions. Fraction 1 was rechromatographed on silica gel and eluted with *n*-hexane–EtOAc (40 : 1) to give **13** (1 mg), **1** (0.5 mg), **15** (3 mg), **19** (1.4 mg), **29** (6 mg), **2** (0.5 mg), **46** (4 mg) and **51** (3 mg), respectively. Fraction 2 was treated as fraction 1 to gain **21** (0.6 mg), **22** (0.6 mg), **23** (1 mg), **25** (1 mg), **26** (3 mg), **27** (1 mg), **38** (3 mg), **41** (7 mg), **43** (4 mg), **47** (0.5 mg) and **48** (3.5 mg). Fraction 3 was also rechromatographed on silica gel and eluted with $C_6H_6$–$(CH_3)_2CO$ (9 : 1) to gain **14** (2 mg), **28** (1 mg), **3** (2 mg), **49** (2 mg) and **52** (10 mg), successively. Fraction 4 was chromatographed on silica gel and eluted with $CHCl_3$–$(CH_3)_2CO$ (9 : 1) to obtain **7** (22 mg), **8** (0.7 mg), **11** (592 mg), **12** (24 mg), **39** (2 mg), **40** (3 mg), **42** (2 mg) and **50** (20 mg). The *n*-BuOH layer was chromatographed directly on Sephadex LH-20 and eluted with a gradient of $H_2O$ and MeOH to afford 18 fractions. Fraction 4 was chromatographed on Diaion HP-20 and eluted with a gradient of $H_2O$ and MeOH to give **45** (2.5 mg). Fraction 6 was rechromatographed on silica gel and eluted with $CHCl_3$–MeOH (6 : 1) to obtain **35** (8 mg) and **44** (10 mg). Fraction 8 was treated with the same manner as fraction 6 to give **4** (1 mg), **5** (1.3 mg) and **6** (0.9 mg). Fraction 9 was also rechromatographed on silica gel and eluted with $CHCl_3$–MeOH–$H_2O$ (20 : 7 : 1) to gain **33** (22 mg) and **36** (5.3 mg). Fraction 11 was chromatographed on silica gel and eluted with $CHCl_3$–MeOH (4 : 1) to obtain **37** (8 mg). Fraction 12 was treated in the same manner as fraction 11 to give **17** (6.4 mg), **24** (5 mg), **30** (2 mg), **31** (3 mg) and **37** (10 mg), successively. Fractions 13 and 14 were filtered to give **10** (20 mg) and **9** (0.7 mg), respectively. Fraction 16 was chromatographed on silica gel and eluted with $CHCl_3$–MeOH (5 : 1) to obtain **16** (8 mg), **18** (2 mg), **20** (2 mg) and **34** (0.6 mg).

Aristolochic Acid-VII Methyl Ester (**1**): Yellow syrup. HR-EI-MS: Calcd for $C_{19}H_{15}NO_8$, *m/z*: 385.0798 $[M]^+$. Found: 385.0800. UV $\lambda_{max}$ nm: 227, 273, 313, 373. IR $\nu_{max}$ cm$^{-1}$: 1734, 1649, 1556, 1541, 1458, 1380, 1258. EI-MS *m/z* (rel. int.): 385 ($[M]^+$, 38), 339 (100), 324 (41), 309 (25), 296 (16), 281 (10), 166 (25), 97 (11), 83 (13), 69 (18), 57 (29). $^1$H-NMR ($CDCl_3$) $\delta$: 8.85 (1H, d, $J$=9.6 Hz, H-5), 8.67 (1H, s, H-9), 7.70 (1H, s, H-2), 7.46 (1H, d, $J$=9.6 Hz, H-6), 6.37 (2H, s, –$OCH_2O$–), 4.06 (3H, s, $OCH_3$), 4.04 (3H, s, $OCH_3$), 3.87 (3H, s, $OCH_3$).

Aristolide-C (**2**): Colorless oil, $[\alpha]_D$ −20.6° ($c$=0.075, $CHCl_3$). HR-EI-MS: Calcd for $C_{18}H_{14}O_6$, *m/z*: 326.0790 $[M]^+$. Found: 326.0791. UV $\lambda_{max}$ nm: 219 (sh), 270, 295, 327 (sh). IR $\nu_{max}$ cm$^{-1}$: 1768, 1647, 1537, 1514, 1463, 1271, 1033, 974. EI-MS *m/z* (rel. int.): 326 ($[M]^+$, 100), 297 (7), 255 (9), 240 (11), 239 (21), 126 (9), 71 (8), 69 (7), 57 (14), 55 (8). $^1$H-NMR ($CDCl_3$) $\delta$ : 7.65 (1H, d, $J$=8.4 Hz, H-5), 6.93 (1H, d, $J$=8.4 Hz, H-6), 6.25 (1H, d, $J$=1.2 Hz, –$OCH_2O$–), 6.16 (1H, d, $J$=1.2 Hz, –$OCH_2O$–), 5.33 (1H, dd, $J$=13.6, 6.4 Hz, H-10ax.), 3.95 (1H, dd, $J$=13.6, 6.4 Hz, H-9eq.), 3.92 (3H, s, $OCH_3$), 3.83 (3H, s, $OCH_3$), 2.48 (1H, t, $J$=13.6 Hz, H-9ax.).

2′-Hydroxyethyl 4-hydroxybenzoate (**3**): Colorless oil. HR-EI-MS: Calcd for $C_9H_{10}O_4$, *m/z*: 182.0579 $[M]^+$. Found: 182.0579. UV $\lambda_{max}$ nm: 258. IR $\nu_{max}$ cm$^{-1}$: 3400, 1710, 1280. EI-MS *m/z* (rel. int.): 182 ($[M]^+$, 6), 149 (9), 139 (21), 138 (22), 121 (100), 93 (13), 69 (16), 65 (18), 57 (17). $^1$H-NMR ($CDCl_3$) $\delta$: 7.98 (2H, d, $J$=8.6 Hz, H-2, 6), 6.86 (2H, d, $J$=8.6 Hz, H-3, 5), 5.60 (1H, br s, OH), 4.44 (2H, t, $J$=4.8 Hz, H-1′), 3.95 (2H, t, $J$=4.8 Hz, H-2′).

Ariscucurbin-A (**4**): Colorless oil. HR-EI-MS: Calcd for $C_{12}H_{14}O_5$, *m/z*: 238.0841 $[M]^+$. Found: 238.0843. UV $\lambda_{max}$ nm: 216, 234, 297, 326. IR $\nu_{max}$ cm$^{-1}$: 3300, 1700, 1640, 1600, 1510, 1270, 1160. EI-MS *m/z* (rel. int.): 238 ($[M]^+$, 99), 194 (59), 177 (100), 145 (41). $^1$H-NMR ($CDCl_3$) $\delta$: 7.67 (1H, d, $J$=16.0 Hz, H-3), 7.20 (1H, d, $J$=2.0 Hz, H-5), 7.08 (1H, dd, $J$=8.2, 2.0 Hz, H-9), 6.82 (1H, d, $J$=8.2 Hz, H-8), 6.40 (1H, d, $J$=16.0 Hz, H-2), 4.23 (2H, t, $J$=5.0 Hz, H-1′), 3.90 (3H, s, $OCH_3$), 3.80 (2H, t, $J$=5.0 Hz, H-2′).

Ariscucurbin-B (**5**): Colorless oil. HR-EI-MS: Calcd for $C_{11}H_{12}O_4$, *m/z*: 208.0736 $[M]^+$. Found: 208.0738. UV $\lambda_{max}$ nm: 227, 300 (sh), 312. IR $\nu_{max}$ cm$^{-1}$: 3440, 3200, 1710, 1610, 1210, 1160, 830. EI-MS *m/z* (rel. int.): 208 ($[M]^+$, 35), 164 (46), 147 (100), 119 (29), 91 (23), 65 (15), 57 (10). $^1$H-NMR ($CDCl_3$) $\delta$: 7.69 (1H, d, $J$=16.0 Hz, H-3), 7.48 (2H, d, $J$=8.8 Hz, H-5, 9), 6.80 (2H, d, $J$=8.8 Hz, H-6, 8), 6.36 (1H, d, $J$=16.0 Hz, H-2), 4.24 (2H, t, $J$=5.0 Hz, H-1′), 3.80 (2H, t, $J$=5.0 Hz, H-2′).

Ariscucurbin-C (**6**): Colorless oil. HR-EI-MS: Calcd for $C_{11}H_{12}O_4$, *m/z*: 208.0736 $[M]^+$. Found: 208.0735. UV $\lambda_{max}$ nm: 226, 300 (sh), 312. IR $\nu_{max}$ cm$^{-1}$: 3440, 3200, 1710, 1250, 1170, 820. EI-MS *m/z* (rel. int.): 208 ($[M]^+$,

31), 164 (32), 147 (100), 121 (22), 71 (23). $^1$H-NMR ($CDCl_3$) $\delta$: 7.65 (2H, d, $J$=8.8 Hz, H-5, 9), 6.88 (1H, d, $J$=12.5 Hz, H-3), 6.75 (2H, d, $J$=8.8 Hz, H-6, 8), 5.81 (1H, d, $J$=12.5 Hz, H-2), 4.19 (2H, t, $J$=5.0 Hz, H-1′), 3.76 (2H, t, $J$=5.0 Hz, H-2′).

**Acknowledgments** The authors are grateful for the financial support of a National Science Council of the Republic of China Grant (NSC 84-2331-b006-074).

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