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# ARISTOFOLIN-A, A DENITRO-ARISTOLOCHIC ACID GLYCOSIDE AND OTHER CONSTITUENTS FROM ARISTOLOCHIA KAEMPFERI

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**Key Word Index**—*Aristolochia kaempferi*; *A. liukiunesis*; flowers; Aristolochiaceae; denitro aristolochic acid; flavonoid; amino acid.

**Abstract**—A denitro-aristolochic acid derivative, aristofolin-A, together with ten known compounds, were isolated from the fresh flowers of *Aristolochia kaempferi*. The structures of these compounds were determined by spectral analysis. © 1998 Elsevier Science Ltd. All rights reserved

## INTRODUCTION

The genus *Aristolochia* comprises ca. 400 species found in tropic to temperate zones. Five species are native to Taiwan, namely *A. curcurbitifolia*, *A. foveolata*, *A. heterophylla*, *A. zollingeriana* and *A. kaempferi*. Some species have been used in folk medicine as anodynes, antiphlogistics and detoxicants in Taiwan. *Aristolochia kaempferi* (*A. liukiunesis*) is distributed in the southern Ryukyu islands and Taiwan [1] and its chemical constituents have been investigated [2–4]. A new denitro-aristolochic acid glycoside and ten known compounds were isolated from the fresh flowers of *A. kaempferi*. The present paper deals with the structural elucidation of aristofolin-A (**1**).

## RESULTS AND DISCUSSION

Aristofolin-A (**1**) was obtained as pale yellow powder. It exhibited a $[M-1]^+$ at *m/z* 473.1082, corresponding to a quasi-molecular formula of $C_{23}H_{21}O_{11}$, by negative HR-FAB mass spectrometry. The UV absorptions at 223.8, 243.6(sh), 259.4, 295.2, 316.2, 329.6, 360.4 and 379.6 nm coupled with an IR absorption band at 1685 $cm^{-1}$ (C=O) and the lack of $NO_2$ band led to the suggestion of the existence of a typical phenanthrene derivative [5]. By the comparison of the $^1H$ NMR spectrum of **1** with that of aristolochic acid-I, a set of doublet signals at $\delta$ 8.77 and 7.69 (*d*, $J$ = 9.4 Hz) was assigned to H-10 and H-9, respectively. Two meta-coupling signals at $\delta$ 8.29 and 6.93 (*d*, $J$ = 1.4 Hz) were assigned to H-5 and H-7 and a singlet signal at $\delta$ 7.47 (1H, *s*) was attributed to H-2. One methoxyl at $\delta$ 3.96 (3H, *s*) was attached to C-8. The methylenedioxy group, observed as two singlets at $\delta$ 6.25 and 6.19, was fused to phenanthrene-1-carboxylic acid with a bond between C-3 and C-4. The negative FAB-mass spectrum gave a fragment peak at *m/z* 311 due to the loss of 162 amu, revealing the presence of a hexose in the molecule. An anomeric proton resonating as a doublet at $\delta$ 5.11 (1H, *d*, $J$ = 6.0 Hz) indicated the presence of a $\beta$-glucoside and a negative value ($[\alpha]_D$ −10.0°) of the optical rotation indicated that glucose would be the D-form. To confirm the connecting position of the glucosyl group and the phenanthrene-1-carboxylic acid unit of aristofolin-A, a NOESY experiment was conducted. This result showed NOE correlation between the anomeric proton ($\delta$ 5.11) and H-5 ($\delta$ 8.29) and H-7 ($\delta$ 6.93). Therefore, the sugar moiety and the methoxyl group should be located at C-6 and C-8, respectively. Based on the above data, the structure of **1** was assigned for aristofolin-A.

In addition to **1**, aristolochic acid-I (**2**) [6], -IVa (**3**) [6], -C (**4**) [6], sodium aristolochate-1 (**5**) [7], -II (**6**) [7], kaempferol3-*O*-rutinoside (**7**) [8], quercetin-3-*O*-rutinoside (**8**) [9], $\beta$-sitosterol (**9**) [10], $\beta$-sitosterol-D-glucoside (**10**) [10] and asparagine (**11**) [11] were also isolated fiom the flowers of *A. kaempferi*. These known structures were characterized by the comparison of their spectral data with literature values.

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## EXPERIMENTAL

Mps: uncorr. $^1$H NMR (200 and 400 MHz): $CD_3OD$, TMS as int. standard, except where noted. UV: MeOH. IR: KBr, unless otherwise stated.

*Plant material*

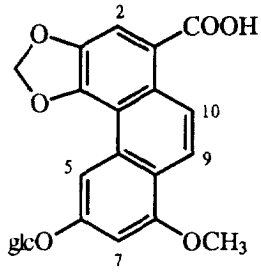
*Aristolochia kaempferi* was collected from Nantou, Taiwan, in April, 1994 and identified by Prof. C.S. Kuoh. A voucher specimen is deposited in the Herbarium of Cheng Kung University, Taiwan.

*Extraction and separation*

The fresh flowers (20 g) were extracted wilh MeOH (×6) at room temp. and concd to give a deep brown syrup (0.68 g). The MeOH extract was partitioned successively between $H_2O$ and $CHCl_3$. The $H_2O$ layer was concd *in vacuo* to give a brown soln (280 mg) which was filtered to give **11** (25.4 mg). The soln was chromatographed on Sephadex LH-20 and eluted with a gradient of $H_2O$ and MeOH to obtain **1** (1.5 mg), **3** (2 mg), **6** (1.6 mg), **7** (1.7 mg) and **8** (2.5 mg), successively. The $CHCl_3$ layer was chromatographed directly on silica gel and eluted with $CHCl_3$–MeOH (9:1) to give 9 frs. Fr. 1 was separated by prep. TLC (silica gel, $CHCl_3$) to obtain **9** (1 mg). Fr. 5 was treated in a similar way as fr. 1 to give **2** (2 mg). Fr. 6 was subjected to CC on silica gel and eluted with $CHCl_3$–MeOH (9:1) to give **5** (2 mg). Fr. 8 was treated in a similar manner to fr. 1 to obtain **3** (1 mg), **4** (1 mg) and **10** (3 mg), successively.

*Aristofolin-A* (**1**)

Pale yellow powder ($CHCl_3$–MeOH), mp > 280°. HRFABMS (neg.): calcd. for $C_{23}H_{21}O_{11}$, *m/z* 473.1084 $[M-1]^+$, found 473.1082. UV$\lambda_{max}$ nm: 223.8, 243.6(sh), 259.4, 295.2, 316.2, 329.6, 360.4. IR $\nu_{max}$ cm$^{-1}$: 3500–3300(0H), 1685, 1520, 1420, 1100, 723. FAB-MS(neg.) *m/z* (rel. int.): 473($[M-1]^+$, 19), 311(17), 287(18), 237(21), 213(23), 197(56), 139(42), 107(100), 105(38). $^1$H NMR: $\delta$ 8.77(1H, *d*, $J$ = 9.4 Hz, H-10), 8.29(1H, *d*, $J$ = 1.4 Hz, H-5), 7.69(1H, *d*, $J$ = 9.4 Hz, H-9), 7.47(1H, *s*, H-2), 6.93(1H, *d*, $J$ = 1.4 Hz, H-7), 6.25, 6.19(each 1H, *s*, $-OCH_2O-$), 5.38(1H, *br s*, OH), 5.11 (1H, *d*, $J$ = 6.0 Hz, anomeric-H), 5.19–4.99(2H, *m*, OH), 4.64(1H, *m*, OH), 3.96(3H, *s*, $OCH_3$), 3.73–3.22(6H, *m*, glucosyl-H).




**1**

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