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ARISTOLOSIDE, AN ARISTOLOCHIC ACID DERIVATIVE FROM STEMS OF *ARISTOLOCHIA MANSHURIENSIS*

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Key Word Index—*Aristolochia manshuriensis*; Aristolochiaceae; stem; aristolochic acid derivatives; aristolochic acid glucoside; aristoloside; aristolochic acids I, IV, and -D.

Abstract—Aristoloside, a new companion aristolochic acid derivative isolated from stems of *Aristolochia manshuriensis* has been shown to be 6-*O*-β-D-glucopyranoside of aristolochic acid-D on chemical and physicochemical evidence. Three known acids, aristolochic acids I, IV (both as their corresponding methyl esters), and -D have also been characterized from stems of the plant.

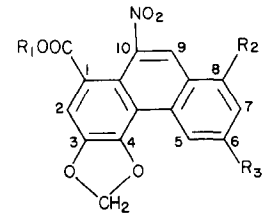
INTRODUCTION

Aristolochic acids, derivatives of 3,4-methylenedioxy-10-nitro-1-phenanthroic acid occur widely in many plants of the Aristolochiaceae. Pailer *et al.* [1–8] and Kupchan *et al.* [9–12] have established the structures of representative acids, i.e. aristolochic acids I (**1**), II (**2**), III, -C(IIIa)†, IV (**3**), and -D(IVa)† (**4**), aristolochic acid-D methyl ether lactam, and aristololactam β-D-glucoside, isolated from *Aristolochia clematitis*, *A. fanchi*, *A. debilis*, and/or *A. indica*. Some of these known acids have been also identified from other plants of the genera *Aristolochia* and *Asarum* [13–18]. Stems of *Aristolochia manshuriensis* are widely used as a crude drug, Kwan-Mu-Tong (in Chinese) in China and Korea. The constituent aristolochic acids I (**1**) and II (**2**) have so far been identified only on the basis of mass spectrometric evidence [17]. In our detailed study on aristolochic acid components contained in stems of the plant, a new companion named aristoloside, together with aristolochic acids I (**1**), IV (**3**), and -D (**4**), has been isolated from the methanol extracts. The structural elucidation of the new acid (**5**) and the identification of the known acids are dealt with in the present paper.

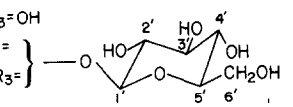
RESULTS AND DISCUSSION

Three acids (two of them as the corresponding esters) were isolated and identified as aristolochic acids I methyl ester (**1a**), IV methyl ester (**3a**), and -D (**4**) as described in detail in the Experimental.

Aristoloside (**5**), $C_{23}H_{21}NO_{13}$ $[M]^+$, *m/z* 519 (base peak) (FDMS spectroscopy) had spectral properties [IR, UV, and ^1H NMR (Table 1)] typical of aristolochic acids. On methylation with excess CH_2N_2 the acid (**5**) was transformed to the corresponding methyl ester (**5a**), $C_{24}H_{23}NO_{13}$ $[M]^+$, *m/z* 533 (base peak) (FDMS spectroscopy). The IR spectrum showed the presence of an ester carbonyl function in place of the carboxy carbonyl of **5** and the FD–mass spectrum gave an abundant fragment peak at *m/z* 371 (*m/z* 357 in **5**), due to the loss of one hexose unit $[M-162]^+$; this indicates that **5a** (also **5**) is assigned to a monoglycoside with a hexose moiety. Thus the glycoside (**5a**) was refluxed with HCl–MeOH (1:4) to afford quantitatively an aglycone and a methyl glucoside which was identified by GC after trimethylsilylation. The aglycone was in agreement with the methyl ester (**4a**) previously derived from **4** and already characterized.



1 R_1=H, R_2=OMe, R_3=H
1a R_1=Me, R_2=OMe, R_3=H
2 R_1=R_2=R_3=H
3 R_1=H, R_2=R_3=OMe
3a R_1=Me, R_2=R_3=OMe
4 R_1=H, R_2=OMe, R_3=OH
4a R_1=Me, R_2=OMe, R_3=OH
5 R_1=H, R_2=OMe, R_3=
5a R_1=Me, R_2=OMe, R_3=



†Pailer's group [4, 5, 8] termed these acids as aristolochic acids IIIa and IVa instead of aristolochic acids-C and -D used by the Kupchan group [11, 12]. Both groups independently established their structures. In this paper, we describe these acids as aristolochic acids-C and -D.

Table 1. ^{1}H NMR data of aristolochic acid derivatives [δ relative to TMS; multiplicity and coupling constant (Hz) in parentheses]

Compound	Solvent	1-COOMe	2-H	$-O\diagdown\diagup CH_2$ $-O$	5-H	7-H	8-OMe	9-H	Other
1a*	$CDCl_3$	3.87(*s*)	7.76(*s*)	6.36(*s*)	8.71(*ddd*, 8.6; 0.7; 0.7‡)	7.11(*dd*, 8.2; 0.7)	4.06(*s*)	8.82(*d*, 0.7‡)	7.71(1H, *dd*, 8.6; 8.2, 6-H)
3a*	$CDCl_3$	3.86(*s*)	7.73(*s*)	6.33(*s*)	8.13(*dd*, 2.1; 0.7‡)	6.70(*d*, 2.1)	4.01(*s*)	8.73(*d*, 0.7‡)	3.98(3H, *s*, 6-OMe)
4†	DMSO-d_6	—	7.78(*s*)	6.51(*s*)	8.11(*d*, 2)	6.85(*d*, 2)	4.04(*s*)	8.53(*s*)	—
4a*	C_6D_6–DMSO-d_6	3.76(*s*)	7.57(*s*)	6.24(*s*)	7.97(*dd*, 1.8; 0.6‡)	6.61(*d*, 1.8)	3.91(*s*)	8.67(*d*, 0.6‡)	—
5†	DMSO-d_6	—	7.78(*s*)	6.49(*s*) 6.44(*s*)	8.35(*d*, 2)	7.13(*d*, 2)	4.07(*s*)	8.50(*s*)	5.12(1H, *d*, 7, 1′-H) 3.2–4.2[6H, *m*, (2′–6′)-Hs]
5a*	$CDCl_3$–CD_3OD (7:3)	3.86(*s*)	7.73(*s*)	6.37(*d*, 1.2) 6.40(*d*, 1.2)	8.45(*dd*, 2.1; 0.6‡)	6.99(*d*, 2.1)	4.06(*s*)	8.73(*d*, 0.6‡)	5.15(1H, *d*, 7.3, 1′-H) 3.45–3.70[4H, *m*, (2′–5′)-Hs] 3.98(*dd*, 12; 2) 3.81(*dd*, 12; 5) 6′-H_2

*At 200 MHz.
†At 90 MHz.
‡Long-range coupling between 5- and 9-Hs.

Accordingly, **5a** is the 6-*O*-glucoside of **4a**. The ^{1}H NMR assignments for **5a** (Table 1) effected by the presence of a long-range coupling ($J = 0.6$ Hz) between 5- and 9-Hs and of a NOE enhancement (18%) at 7-H on irradiation of the 8-OMe function, coupled with the IR and UV data, are in favour of the structure inferred above.

The large coupling constant ($J = 7.3$ Hz) of the anomeric proton doublet at δ 5.15 (Table 1) establishes the *trans*-diaxial relationship between 1′- and 2′-Hs, suggestive of the presence of a β-D-glucopyranoside ($^4C_1$ conformation) in **5a**. Both 5(δ 8.45)- and 7(δ 6.99)-Hs showed respective NOE enhancements (20 and 9%) on irradiation of the anomeric proton, indicative of the presence of a 6-*O*-glycosidic linkage in **5a**. The optical rotations of **5** ($[\alpha]_D^{15} - 69.5°$) and **5a** ($[\alpha]_D^{15} - 59.4°$) both showed negative values. Analogously, some recent examples reported for β-D-glucopyranosides of achiral aglycones (syringin [19], 3,5-dihydroxy-4′-methoxystilbene 3β-D-glucopyranoside [20] and aloenin [21]) all exhibited negative rotations. Therefore the D-configuration is indicated for the glucose moiety in **5** and **5a**. The combined evidence defines the structures **5** and **5a** for aristoloside and its methyl ester respectively.

EXPERIMENTAL

General. Mps are uncorr. FDMS using Si emitters were performed under the following conditions (accelerating V, 3 kV; emitter current, 17–25 mA, chamber temp., room temp. – 100°); MS and accurate MS: at 75 eV. ^{1}H NMR spectra: see Table 1. GC with FID: 2 m × 3 mm packed with 1.5% SE-52. Si gel HF-254 and PF-254 (Merck) were used for TLC and prep. TLC, respectively.

Plant material. Stems of *A. manshuriensis* Komarov, a crude drug of Kwan-Mu-Tong (in Chinese), were imported from China and identified by K.Y.

Isolation of total acids. Dried stems (1 kg) were pulverized and extracted with MeOH at room temp. for 20 days and the solvent evaporated under red. pres. to give a thick brown syrup (19 g) which was dissolved in *n*-BuOH. The soln was extracted ×5 with saturated aq. $NaHCO_3$. The combined alkaline soln was acidified with 5% aq. HCl and again extracted with *n*-BuOH. The extracts were evaporated to dryness *in vacuo* to yield total acids (1.5 g) as a residue.

Separation of total acids. The acids (0.8 g) were dissolved in MeOH and subjected to prep. TLC [2 mm thickness; developed with $CHCl_3$–MeOH–H_2O (13:7:2, lower layer)] to give three major bands coloured yellow (R_f 0.60), wine-red (0.50), and orange (0.22), the respective scrapings from which afforded, after extraction with MeOH, three components, A-1 (112 mg), A-2 (96 mg), and A-3 (203 mg).

Methylation of A-1. Methylation of 112 mg with excess CH_2N_2 yielded two methyl esters (revealed by TLC), which were separated by prep. TLC [0.5 mm thickness; developed with C_6H_6–EtOAc (10:1); eluted with $CHCl_3$–MeOH (1:1)]. The more polar ester (51.3 mg), shiny yellow needles [$CHCl_3$–MeOH (1:1)] was identified by comparison of its mp and IR (KBr), UV (MeOH), and MS data with those [1, 2, 10, 18] for aristolochic acid I methyl ester (**1a**). The ^{1}H NMR data (Table 1) were also in agreement with the structure **1a** (Found: C, 60.50; H, 3.39; N, 3.90. Calc. for $C_{18}H_{13}NO_7$: C, 60.85; H, 3.68; N, 3.94). The other ester (37.2 mg), yellow needles (MeOH) had mp and IR (KBr), UV (MeOH) and MS data identical with those [4, 7, 12, 18] published for aristolochic acid IV methyl ester (**3a**). The ^{1}H NMR (Table 1) and the following NOE experimental results were consistent with structure **3a**. The 5- and 7-Hs showed respective NOE enhancements of 20.3 and 18.4% on irradiation of the 6- and 8-methoxy-methyls. Both methoxy-methyls were, in turn, enhanced on irradiation of 7-H. Contrary to this, only the 6-OMe signal was enhanced on irradiation of 5-H. (Found: C, 59.14; H, 3.66; N, 3.66. Calc. for $C_{19}H_{15}NO_8$: C, 59.22; H, 3.92; N, 3.64.)

Aristolochic acid-D (**4**) *and its methyl ester* (**4a**). The A-2 component was, after treatment with Dowex 50W × 8 (1 g), re-crystallized from MeOH to give bright wine-red crystals, the physicochemical data [mp and IR (KBr), UV (MeOH) and ^{1}H NMR (Table 1) spectra] of which agreed with those [8, 12] reported for aristolochic acid-D (**4**). FDMS *m/z* (rel. 57.15; H, 3.10; N, 3.92.) On exhaustive methylation with (Found: C, 57.01; H, 3.30; N, 3.75. Calc. for $C_{17}H_{11}NO_8$: C, 57.15; H, 3.10; N, 3.92). On exhaustive methylation with excess CH_2N_2, **4** yielded quantitatively its methyl ether methyl ester, identical with aristolochic acid IV methyl ester (**3a**) (by mmp, MS, TLC). **4** was methylated with CH_2N_2 with TLC monitoring (for 1 hr) to afford preferentially the corresponding methyl ester, orange crystals (MeOH). It had mp 249–253° identical to that [8] reported for aristolochic acid-D methyl ester (**4a**). The following additional data were consistent with the structure **4a**; UV λ_{max}^{MeOH} nm (log ϵ): 220 (4.41), 242 (4.53), 253 (4.53), 293 (sh; 4.07), 330 (4.02), 402 (4.0); IR ν_{max}^{KBr} cm^{-1}: 1718, 1603, 1518; ^{1}H NMR: see Table 1; a NOE enhancement (22.4%) of 7-H was observed on irradiation of 8-OMe; MS (measured as the corresponding TMSi ether) *m/z* (rel. int.): 443 $[M]^+$ (36.8), 397 $[M-46]^+$ (100), 382 $[M-61]^+$ (62.5) [Found: $[M]^+$ 443.1051. Calc. for $C_{21}H_{21}NO_8$ Si (the TMSi ether): 443.1046].

Aristoloside (**5**). The A-3 component was further purified by prep. TLC [2 mm thickness; developed with $CHCl_3$–MeOH–H_2O (6:4:1); eluted with MeOH] and treatment with Dowex 50W × 8 to give aristoloside (**5**) in a pure form, orange prisms, mp 193–196° (MeOH), $[\alpha]_D^{15} - 69.5°$ (MeOH; *c* 0.23). UV λ_{max}^{MeOH} nm (log ϵ): 222 (4.41), 243 (4.51), 252 (4.52), 318 (4.07), 392 (3.93); IR ν_{max}^{KBr} cm^{-1}: 3380 (OH), 1695 (COOH), 1597 (aromatic ring), 1517 (NO_2), 1040 (ether); ^{1}H NMR: Table 1: FDMS *m/z* (rel, int.): 542 $[M+Na]^+$ (10), 519 $[M]^+$ (100), 489 $[M-30]^+$ (9), 357 $[M-162]^+$ (17.5) (Found: C, 52.00; H, 4.16; N, 2.68. $C_{23}H_{21}NO_{13} \cdot 1/2\ H_2O$ requires C, 52.28; H, 4.20; N, 2.65%.)

Aristoloside methyl ester (**5a**). The acid (**5**) in MeOH was methylated with excess CH_2N_2 to afford quantitatively the corresponding methyl ester (**5a**), orange crystals, mp 176–178°, $[\alpha]_D^{15} - 59.4°$ (MeOH; *c* 0.32). UV λ_{max}^{MeOH} nm (log ϵ): 222 (4.44), 243 (4.51), 253 (4.51), 324 (4.10), 395 (4.03); IR ν_{max}^{KBr} cm^{-1}: 3390 (OH), 1705 (COOMe), 1599 (aromatic ring), 1520 (NO_2), 1040 (ether); ^{1}H NMR: Table 1; FDMS *m/z* (rel. int.): 556 $[M+Na]^+$ (10), 533 $[M]^+$ (100), 503 $[M-30]^+$ (16), 371 $[M-162]^+$ (58). (Found: C, 52.52; H, 4.31; N, 2.64. $C_{24}H_{23}NO_{13} \cdot H_2O$ requires C, 52.27; H, 4.57; N, 2.54%). Alternatively, methylation of A-3 followed by prep. TLC purification [developed with $CHCl_3$–MeOH–H_2O (7:3:1, lower layer); eluted with MeOH] also gave **5a** in good yield.

Methanolysis of **5a**. A soln of **5a** (23.6 mg) in HCl–MeOH (1:4; 30 ml) was heated under reflux for 2 hr. The mixture was poured into ice–H_2O and extracted with *n*-BuOH. The *n*-BuOH was washed with H_2O and evaporation of the solvent under red. pres. gave an aglycone (14 mg), orange crystals (from MeOH), mp 250–254°, identical with **4a** by

mmp 249–253°, IR (KBr), UV (MeOH) and TLC. The acidic aq. layer was neutralized with Amberlite IR 45 (60 g) and concd *in vacuo* to yield a glyconic residue, which was trimethylsilylated with *N*, *O*-bis(trimethylsilyl)trifluoroacetamide and pyridine, and identified as the methyl glucoside (R_t, 34 and 38 min) by GC (column temp., 150°; N_2 at 38 ml/min).

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