Phytochemistry, Vol. 22, No. 12, pp. 2745–2747, 1983.
Printed in Great Britain.

0031–9422/83 $3.00 + 0.00

AN ARISTOLOCHIC ACID DERIVATIVE FROM *ARISTOLOCHIA LONGA*

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(*Received* 21 *March* 1983)

Key Word Index—*Aristolochia longa*; Aristolochiaceae; aristolochic acid derivatives; 2-(phenanthro[3,4-*d*]-1,3-dioxole-6-nitro-5-carboxamido)propanoic acid; aristolochic acids I, II, III and IV.

Abstract—2-(Phenanthro[3,4-*d*]-1,3-dioxole-6-nitro-5-carboxamido)propanoic acid, a new aristolochic acid derivative, was isolated as the methyl ester from roots of *Aristolochia longa*. The known aristolochic acids III (as its corresponding methyl ester), I, II and IV were also characterized, the latter being isolated for the first time in the free form.

INTRODUCTION

Aristolochic acids, derivatives of phenanthro[3,4-*d*]-1,3-dioxole-6-nitro-5-carboxylic acid, occur widely in many plants of the Aristolochiaceae and belong, in addition to chloramphenicol and a few other natural compounds, to substances rarely found in nature, containing a nitro group [1, 2], all of them with biological activity. The structures of aristolochic acids I (**1**) [3], II (**2**) [4], III (**3**) [5], III-a (**4**) [5], IV (**5**) [5], IV-a (**6**) [5], 7-hydroxyaristolochic acid I (**7**) [6] and 7-methoxyaristolochic acid I (**8**) [6] have been established.

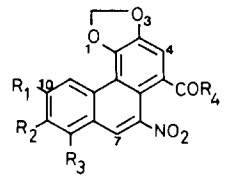
Aristolochic acids increase phagocytosis of leucocytes and exhibit tumour inhibitory activity [7, 8]. Up to the present time, all attempts to synthesize them have failed in providing an efficient method which could be used on an industrial scale, and the only source of these substances is the plant itself.

Aristolochia longa has been used in folk medicine in the Mediterranean area [9]. In our study on aristolochic acid components present in the roots of the plant, a new companion 2-(phenanthro[3,4-*d*]-1,3-dioxole-6-nitro-5-carboxamido)propanoic acid (**9**) (isolated as its methyl ester **9a**), together with aristolochic acid IV (**5**) (isolated for the first time) and aristolochic acids I (**1**), II (**2**) and III (**3**), was identified from the benzene extracts.

RESULTS AND DISCUSSION

The plant was extracted successively with hexane and benzene. The solid which separated out after leaving the benzene extract to stand overnight at room temperature was chromatographed on silica gel, and two fractions, F_1 and F_2, were separated. F_1 was pressure-chromatographed on silicic acid–Celite, yielding three components, **1**, **2** and **5**, which were purified by crystallization. Compounds **1** and **2** were identified by comparison of their mp and spectroscopic properties as aristolochic acids I and II, respectively [10, 11]. The spectroscopic properties of their methyl esters also agreed with these structures, **1a** and **2a** [11]. The IR spectrum of the more polar **5** [3300–2400, 1680 (COOH); 1530, 1350 (NO_2)] also corresponded to an aristolochic acid. According to the molecular formula $C_{18}H_{13}NO_8$, $[M]^+$ *m/z* 371, and the presence in the ^{1}H NMR spectrum of two methoxyls and four aromatic protons, two in the *meta* position (J = 2 Hz), it was assigned the structure aristolochic acid IV (**5**). The mp and spectroscopic properties (UV, IR, ^{1}H NMR) of its methyl ester agreed with those described for the methyl ester of aristolochic acid IV (**5a**) [5, 10] also described in *A. manshuriensis* [12] and *A. clematitis* [5].

From the mixture of methyl esters obtained by careful esterification of F_2, first by silica gel CC and then by preparative TLC, **3a** (the methyl ester of aristolochic acid III [5]) and **9a** were obtained. The IR spectrum of this latter substance, with a formula of $C_{20}H_{16}N_2O_7$, $[M]^+$ *m/z* 396, showed the presence of –COOR (1750 cm^{-1}), –CONH– (1635, 1515 cm^{-1}) and nitro groups (1350 cm^{-1}). The ^{1}H NMR spectrum of **9a** ($CDCl_3$, Table 1),



	R_1	R_2	R_3	R_4
1	H	H	OMe	OH
1a	H	H	OMe	OMe
2	H	H	H	OH
2a	H	H	H	OMe
3	OMe	H	H	OH
3a	OMe	H	H	OMe
4	OH	H	H	OH
5	OMe	H	OMe	OH
5a	OMe	H	OMe	OMe
6	OH	H	OMe	OH
7	H	OH	OMe	OH
8	H	OMe	OMe	OH
9	H	H	H	NH–CH(CH_3)–COOH
9a	H	H	H	NH–CH(CH_3)–COOMe

Table 1. ^{1}H NMR spectral data of aristolochic acids and derivatives

(MHz)	**1*** (60)	**1a†** (200)	**2*** (60)	**2a†** (200)	**3a†** (200)	**5*** (60)	**5a†** (200)	**9a*** (60)	**9a†** (200)	
H-4	7.76 *s*	7.77 *s*	7.79 *s*	7.76 *s*	7.77 *s*	7.70 *s*	7.73 *s*	7.60 *s*	7.53 *s*	
H-7	8.48 *s*	8.83 *s*	8.50 *s*	8.31 *s*	8.60 *s*	8.32 *s*	8.74 *s*	8.45 *s*	8.22 *s*	
H-8	—	—	8.19*m*	7.97 *d* (7.5)	7.91 *d* (8.2)	—	—	8.17 *m*	7.93 *d* (7.2)	
H-9	7.22 *d* (8)	7.11 *d* (7.9)	7.78 *m*	7.75 *m*	7.34 *d* (8.2)	6.78 *d* (2)	6.70 *d* (2)	7.78 *m*	7.70 *m*	
H-10	7.70 *t* (8)	7.75 *dd* (9.7, 7.9)	7.78 *m*	7.75 *m*	—	—	—	7.78 *m*	7.70 *m*	
H-11	8.45 *d* (8)	8.71 *d* (9.7)	8.96 *m*	9.12 *d* (7.8)	8.31 *s*	7.84 *d* (2)	8.13 *d* (2)	9.02 *m*	9.08 *d* (7.9)	
OMe-8	4.04 *s*	4.06 *s*	—	—	—	3.97 *s*	4.01 *s*	—	—	
OMe-10					4.02 *s*	3.88 *s*	3.98 *s*			
OCH_2O	6.45 *s*	6.38 *s*	6.47 *s*	6.39 *s*	6.40 *s*	6.39 *s*	6.34 *s*	6.49 *s*	6.35 *s*	
5-COOMe	—	3.87 *s*	—	3.88 *s*	3.88 *s*	—	3.86 *s*	—	—	
Other								9.02 *m*	6.74 *d* (7.0)	-CONH-
								4.45 *m*	4.79 *m*	-CH-
								3.70 *s*	3.82 *s*	-COOMe
								1.45 *d* (7)	1.59 *d* (7.0)	CH_3-CH

*Recorded in DMSO-d_6.
†Recorded in $CDCl_3$.
Coupling constants (Hz) in parentheses.

which gave signals for $-O-CH_2-O-$ δ 6.35 (2H, *s*) and six aromatic protons, was similar to that of aristolochic acid II (**2**). Furthermore, it showed signals for –COOMe δ 3.82, CH_3–CH δ 1.59 (3H, *d*), –CONH– δ 6.74 (1H, *d*) and –CH– δ 4.79 (1H, *m*). By double-resonance of the signal at δ 4.79, the doublets at δ 6.74 and 1.59 were converted into singlets. Double-resonance of the doublet centred at δ 1.59 transformed the multiplet at δ 4.79 into a quartet (J = 7 Hz) and that of the doublet at 6.74 into a doublet (J = 7 Hz), revealing the presence of the grouping –CONH–CH(Me)–COOMe. The characteristic fragments of the mass spectrum (Scheme 1) confirmed the structure proposed for **9a** as the methyl ester of 2-(phenanthro[3,4-*d*]-1,3-dioxole-6-nitro-5-carboxamido)propanoic acid.

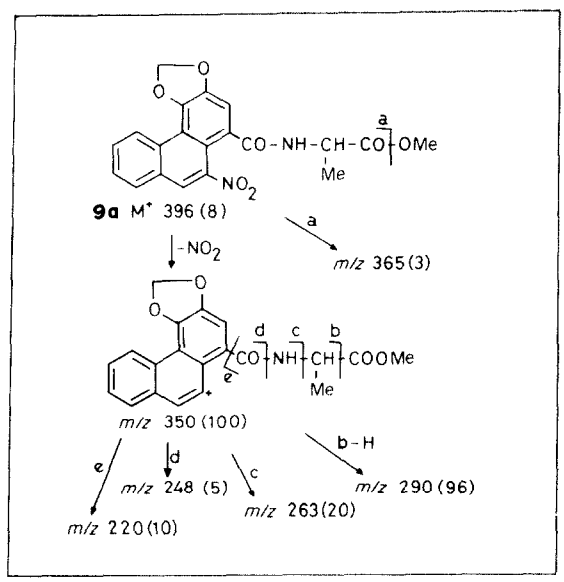
Comparison of the optical rotation of **9a** with that described in the literature [13] suggests the possibility of a derivative of L-(+)-alanine.

EXPERIMENTAL

General. Mps are uncorr. IR: KBr disc. ^{1}H NMR: 60 or 200 MHz, DMSO-d_6 and $CDCl_3$, TMS as int. standard. EIMS: heated inlet system, 70 eV. UV: EtOH. TLC, prep. TLC (0.75 mm) and CC: silica gel (Merck). Pressure CC: silicic acid (Mallincrodt) and Celite 545 (Fisher) 5:1. Rotations were determined in $CHCl_3$.

Plant material. Roots of *A. longa* were collected in La Bouza (Salamanca, Spain) in June and identified by Professor Casaseca Mena. A herbarium specimen has been deposited in the Dept of Botany, University of Salamanca.

Extraction and separation. The dried, ground material (3.5 kg) was extracted successively with hexane (10.1 g) and C_6H_6 (31.7 g). The hot C_6H_6 extract was left at room temp. overnight and the dark-brown powder which separated out (10.7 g) was filtered. This powder (5.01 g) was chromatographed over silica gel (248 g). Fraction F_1, eluted with $CHCl_3$–MeOH (4:1) (3.31 g), and fraction F_2 (7:3) (0.46 g) were obtained. F_1 (0.50 g) was rechromatographed over silicic acid–Celite (300 g) under pres. (3 kg/cm^2) and 750 ml fractions were collected: with $CHCl_3$–MeOH (99.5:0.5) fractions 3–20 and with $CHCl_3$–



Scheme 1. Mass spectral fragmentation of **9a**.

MeOH (97.5:2.5) fractions 21–23, to give **2** (fractions 3–7, 169 mg), **1** (fractions 10–19, 287 mg) and **5** (fraction 21, 59 mg).

F_2 (0.46 g) methylated with excess CH_2N_2 in Et_2O yielded 5 esters (revealed by TLC), **1a**, **2a**, **5a**, **3a** (3 mg) and **9a** (40 mg), which were separated by silica gel CC (eluted with $CHCl_3$) and prep. TLC (developed with $CHCl_3$ several times).

Aristolochic acid I (**1**). Yellow needles (DMF–H_2O), mp 276–281° (decomp.). IR ν_{max}^{KBr} cm^{-1}: 3400–2400, 1685 (COOH), 1595 (aromatic ring), 1525 and 1355 (NO_2), 1042 (ether). ^1H NMR: Table 1. EIMS 70 eV, *m/z* (rel. int.): 341 $[M]^+$ (19), 311 (19), 295 $[M-NO_2]^+$ (83), 280 (20), 265 (58), 83 (100). Esterification (CH_2N_2–Et_2O) of **1** gave aristolochic acid I methyl ester (**1a**). Yellow crystals ($CHCl_3$), mp 281–282°. IR ν_{max}^{KBr} cm^{-1}: 1712, 1590, 1515, 1372, 1135, 1038, 740. ^1H NMR: Table 1. EIMS 70 eV, *m/z* (rel. int.): 355 $[M]^+$ (18), 325 (38), 324 (3), 309 (36), 294 (31), 279 (100), 266 (13), 264 (79).

Aristolochic acid II (**2**). Orange–yellow crystals (Me_2CO), mp 270–272° (decomp.). IR ν_{max}^{KBr} cm^{-1}: 3400–2400 and 1690 (COOH), 1595 (aromatic ring), 1525 and 1355 (NO_2). ^1H NMR: Table 1. EIMS 70 eV, *m/z* (rel. int.): 311 $[M]^+$ (15), 295 (7), 265 (53), 208 (24), 163 (30), 151 (46), 83 (100). Esterification (CH_2N_2–Et_2O) of **2** gave aristolochic acid II methyl ester (**2a**). Yellow crystals ($CHCl_3$), mp 277°. IR ν_{max}^{KBr} cm^{-1}: 1715, 1600, 1530, 1355, 1050. ^1H NMR: Table 1. EIMS 70 eV, *m/z* (rel. int.): 325 $[M]^+$ (32), 294 (7), 279 (100), 264 (75), 208 (35).

Aristolochic acid III *methyl ester* (**3a**). Yellow crystals ($CHCl_3$), mp 265–266°. IR ν_{max}^{KBr} cm^{-1}: 1715, 1620, 1530, 1350. ^1H NMR: Table 1. EIMS 70 eV, *m/z* (rel. int.): 355 $[M]^+$ (35), 324 (8), 309 (100), 294 (98), 279 (53).

Aristolochic acid IV (**5**). Deep-red prisms (DMF–H_2O). mp 268–270° (decomp.). IR ν_{max}^{KBr} cm^{-1}: 3300–2400 and 1680 (COOH), 1600 (aromatic ring), 1530 and 1350 (NO_2), 1060 (ether). UV λ_{max}^{EtOH} nm: 223, 251, 317, 389. ^1H NMR: Table 1. EIMS 70 eV *m/z* (rel. int.): 371 $[M]^+$ (6), 341 (39), 325 (8), 311 (10), 295 (100), 280 (27). Esterification (CH_2N_2–Et_2O) of **5** gave aristolochic acid IV methyl ester (**5a**). Orange-brown crystals ($CHCl_3$), mp 238–240°. IR ν_{max}^{KBr} cm^{-1}: 1715, 1610, 1520, 1375, 1045. UV λ_{max}^{EtOH} nm: 223, 257, 397. ^1H NMR: Table 1. EIMS 70 eV, *m/z* (rel. int.): 385 $[M]^+$ (71), 354 (40), 339 (100), 324 (50), 309 (41).

2-(*Phenanthro* [3,4-*d*]-1, 3-*dioxole*-6-*nitro*-5-*carboxamido*)*propanoic acid methyl ester* (**9a**). Pale-yellow crystals (MeOH), mp 252–253° (decomp.). $[\alpha]_D$ −133° ($CHCl_3$; *c* 0.57). IR ν_{max}^{KBr} cm^{-1}: 3320 (NH), 1750 (COOR), 1635 and 1515 (CONH), 1350 (NO_2), 1053 (ether). UV λ_{max}^{EtOH} nm: 218, 251, 297. ^1H NMR: Table 1. EIMS 70 eV, *m/z* (rel. int.): 396 $[M]^+$ (8), 380 (1), 365 (3), 350 (100), 294 (25), 290 (96), 263 (20), 248 (5), 220 (10).

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