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MINOR ARISTOLOCHIC ACIDS FROM *ARISTOLOCHIA ARGENTINA* AND MASS SPECTRAL ANALYSIS OF ARISTOLOCHIC ACIDS

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Key Word Index—*Aristolochia argentina*; Aristolochiaceae; aristolochic acids; aristoloside; mass spectrometry of aristolochic acids.

Abstract—The minor aristolochic acids isolated from *Aristolochia argentina* were identified as 6,7-dimethoxy, 6-hydroxy-7-methoxy, 2-hydroxy-8-methoxy and 7-hydroxy-8-methoxy disubstituted derivatives of the 3,4-methylenedioxy-10-nitro-1-phenanthroic acid, respectively. *A. argentina* also contains the previously reported aristoloside. The mass spectra of the aristolochic acids, their esters and decarboxylation products have been examined. A number of successive fragmentation processes leading to the formation of aromatic hydrocarbons were observed. Cleavage of the nitro group is a prominent process in the mass spectra of the aristolochic acids and their esters. Evidence is presented that the formation of the $[M - NO_2]^+$ ion occurs by an intramolecular aromatic substitution reaction with participation of the CO_2R group. The different behaviour of the decarboxylated aristolochic acids is also discussed. A mechanism is proposed for the favourable loss of CH_2O in the 8-methoxy isomer.

INTRODUCTION

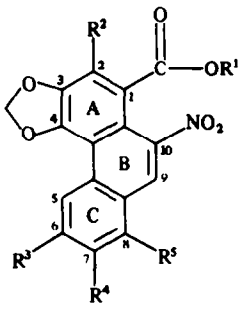
The roots of *Aristolochia argentina*, popularly known as 'charrua' or 'charruga', are used in Argentinean folk medicine as an emmenagogue and in the treatment of arthritis, poisoning and pruritus [1]. The aristolochic acids (AAs) of *A. argentina* were first examined by Hesse who, in 1895, described the isolation from the roots of aristinic acid (AA I), aristidinic acid and the phenolic aristolic acid (AA IVa) [2]. A re-investigation of this species indicated that the acidic fraction consists of a complex mixture of acids, seven of which could be identified as AAs I (**1**), II (**2**), III (**3**), IV (**4**), IIIa (**7**), IVa (**8**) and Ia (**6**) [3]. The isolation and characterization of the minor AAs V (**5**), Va (**9**), VIa (**10**) and VIIa (**11**), and aristoloside (**24**) are now reported for *A. argentina*. AA VIIa (**11**) (7-hydroxyaristolochic acid A) was previously isolated from *A. debilis* by Chen *et al.* [4] and from *A. tagala* by Ding *et al.* [5], and aristoloside, the 6-*O*-β-glucopyranoside of AA IVa (**24**), has been found in *A. manshuriensis* by Nakanishi *et al.* [6]. The physical properties, biogenesis and pharmacology of known AAs have been reviewed [7].

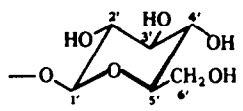
RESULTS AND DISCUSSION

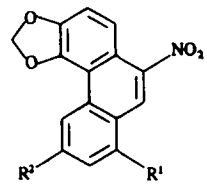
By application of the counter-current distribution technique, the crude mixture of AAs was first resolved into a non-phenolic and a phenolic fraction. The subsequent analysis showed that the former consisted of AAs I (**1**), II (**2**), III (**3**), IV (**4**), V (**5**) and traces of IVa (**8**), whereas the phenolic fraction contained the AAs Ia (**6**), IIIa (**7**), IVa (**8**), Va (**9**), VIa (**10**) and VIIa (**11**), as well as the non-phenolic acid V (**5**). Both fractions consisted of a complex mixture of compounds. From the fraction of non-phenolic acids the aristololactams DII (**28**) and DIII (**29**) were isolated [8] and important amounts of *p*-coumaric, vanillic and ferulic acids were found in the phenolic acid fraction. The latter also contained traces of aristoloside (**24**) but it was better isolated from the more polar extracts of the plant, as described in the Experimental.

AA VIa (**10**), $C_{17}H_{11}NO_8$ ($[M - H_2O]^+$, 339), was obtained as free acid. Its phenanthroid nature was revealed by the UV spectrum, whereas the shift of the peaks towards the longer wavelength region on addition of ammonia supported the presence of one phenolic group. The ^1H NMR spectrum of AA VIa (**10**) (Table 1) as a whole resembles that of AA I (**1**); however, the sharp singlet of H-2, which consistently appears at δ7.71–7.81 in the remaining AAs, is absent. The presence of the nitro group and that the hydroxyl group is located *ortho* to the carboxyl group was indicated by its mass spectral fragmentation (Scheme 3). The favoured eliminations of hydroxyl and water, the latter being typical of *o*-hydroxybenzoic acids, do not allow the molecular ion peak to be observed. These findings support the 2-hydroxy-3,4-methylenedioxy-8-methoxy substitution pattern for AA VIa (**10**). This acid exhibits a reduced affinity for silica gel in adsorption chromatography (see systems 8 and 11, Table 2), even when compared with the non-phenolic acids. Presumably, as in salicylic acid [9], intramolecular hydrogen bonding between the hydroxyl and carboxyl groups disrupts the strong interaction capacity of the latter towards the surface hydroxyls of the adsorbent.

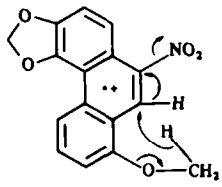
The AAs Va (**9**) and VIIa (**11**) could not be obtained in completely pure form. However, UV and chromatographic evidence showed them to be phenolic acids. AA Va (**9**) was characterized as a methyl ether methyl ester (**16**), $C_{19}H_{15}NO_8$ ($[M]^+$, 385), and an ethyl ether ethyl ester (**23**), $C_{21}H_{19}NO_8$ ($[M]^+$, 413), and AA VIIa (**11**) as a methyl ether methyl ester (**18**), $C_{19}H_{15}NO_8$ ($[M]^+$, 385). The location of the oxygenated functions was inferred from the signal multiplicities of their hydrogen resonances (Table 1). Compounds **16** and **23** show two singlets for the ring C protons indicating that the two oxy-substituents



Compound	R^1	R^2	R^3	R^4	R^5
1	H	H	H	H	OMe
2	H	H	H	H	H
3	H	H	OMe	H	H
4	H	H	OMe	H	OMe
5	H	H	OMe	OMe	H
6	H	H	H	H	OH
7	H	H	OH	H	H
8	H	H	OH	H	OMe
9	H	H	OH	OMe	H
10	H	OH	H	H	OMe
11	H	H	H	OH	OMe
12	Me	H	H	H	OMe
13	Me	H	H	H	H
14	Me	H	OMe	H	H
15	Me	H	OMe	H	OMe
16	Me	H	OMe	OMe	H
17	Me	OMe	H	H	OMe
18	Me	H	H	OMe	OMe
19	Et	H	OMe	OMe	H
20	Et	H	H	H	OEt
21	Et	H	OEt	H	H
22	Et	H	OEt	H	OMe
23	Et	H	OEt	OMe	H
24					
	H	H		H	OMe



25 R^1 = OMe, R^2 = H
26 R^1 = H, R^2 = OMe



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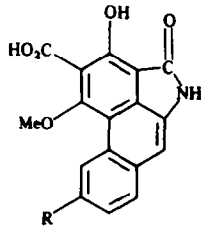


28 R = H
29 R = OMe

are *ortho*-located at positions C-6 and C-7, whereas compound **18** exhibits two doublets, one of them at low field (H-5), with characteristic *ortho*-coupling ($J_{5,6}$ = 8.5 Hz), consistent with a 7,8-dimethoxy compound. The assignments of the relative position of the hydroxyl/methoxyl substituents in the isomeric AAs Va (**9**) and VIIa (**11**) was tentatively made on the basis of the respective shifts of the long wavelength band in the UV spectrum. AA Va (**9**), as well as the acids Ia (**6**), IIIa (**7**) and IVa (**8**), exhibits a strong bathochromic shift of the long wavelength band in alkaline medium and its yellow spot on TLC plates becomes red under ammonia vapour. Behaviour which may be explained in terms of the contribution of *ortho*- and *para*-quinoid structures due to conjugation between the hydroxyl group at C-6 or C-8 and the nitro group at C-10. In contrast, AA VIIa (**11**) shows a less pronounced bathochromic shift and fails to give red spots when exposed to ammonia vapour, therefore not compatible with a phenolic group at C-8. On the basis of the above evidence, AAs Va (**9**) and VIIa (**11**) might be suggested to be 6-hydroxy-7-methoxy and 7-hydroxy-8-methoxy substituted, respectively.

AA V (**5**) is a minor component of the mixture of non-phenolic acids from which it could be isolated as an ethyl ester (**19**), $C_{20}H_{17}NO_8$ ($[M]^+$, 399). Its ^1H NMR spectrum (Table 1) showed that AA V (**5**) is the *O*-methyl ether of AA Va (**9**). AA V (**5**) was also found in roughly equal amount in the fraction of phenolic AAs, being likewise identified as the ethyl ester (**19**). As compared with the remaining non-phenolic acids, AA V (**5**) exhibits an increased affinity for the polar phase under counter-current distribution conditions, a behaviour also reflected in partition chromatography, e.g. see system 1 (Table 2).

A total of 12 AAs are known, although other acids have been isolated [7] whose definitive structures remain to be determined. Apart from the 11 AAs described herein for *A. argentina*, the 7-methoxyaristolochic acid A (AA VIIa methyl ether) is known to occur in *A. debilis* [4]. Several naturally occurring derivatives of AAs have also been reported. The methyl esters of AAs I (**12**) and II (**13**) occur in *A. argentina* [3] and the former was also found in *A. indica* [10], whereas the methyl ester of AA IV (**15**) is a component of *A. kwangsiensis* [11] and *A. championi* [12]. The β-D-glucoside of AA IVa (**8**), aristoloside (**24**), previously isolated from *A. manshuriensis* [6], is now reported for *A. argentina* and AA II as the *N*-acyl derivative of alanine is produced by *A. longa* [13].

A particular feature of AAs is that they all possess a 3,4-methylenedioxy group and the hydroxyl and methoxyl substituents are preferentially situated at C-6 and C-8. The AAs are usually accompanied by small amounts of aristololactams [14], probably their biogenetic precursors, which exhibit the same structural characteristics but several of them are also 3,4-dimethoxy or 3-hydroxy-4-methoxy substituted. Although labelled stephanine has been found to be incorporated into AAs [15], the occurrence of 3-hydroxy-4-methoxy aristololactams sug-

Table 1. ^{1}H NMR chemical shifts of aristolochic acids and derivatives

Compound	Solvent	H-2	H-5	H-6	H-7	H-8	H-9	CH_2O_2	MeO-6	MeO-7	MeO-8
1	DMSO-d_6	7.77	8.45 *dd*	7.73 *t*	7.23 *dd*	—	8.48	6.46	—	—	4.04
20*	$CDCl_3$	7.73	8.57 *dd*	7.62 *t*	7.02 *dd*	—	8.79	6.31	—	—	—
2	DMSO-d_6	7.81	9.07 *m*	7.80 *m*	7.80 *m*	8.22 *m*	8.46	6.45	—	—	—
3	DMSO-d_6	7.77	8.52 *d*	—	7.45 *dd*	8.20 *d*	8.52	6.43	3.99	—	—
7	DMSO-d_6	7.76	8.48 *d*	—	7.29 *dd*	8.10 *d*	8.48	6.49	—	—	—
21†	$CDCl_3$	7.73	8.46 *d*	—	7.26 *dd*	7.80 *d*	8.23	6.33	—	—	—
4	DMSO-d_6	7.75	7.99 *d*	—	6.87 *d*	—	8.42	6.43	3.94	—	4.03
8	DMSO-d_6	7.79	8.06 *d*	—	6.82 *d*	—	8.50	6.51	—	—	4.02
22‡	$CDCl_3$	7.73	8.05 *d*	—	6.68 *d*	—	8.74	6.33	—	—	4.02
16§	DMSO-d_6	7.75	8.55	—	—	7.81	8.55	6.49	3.98	4.01	—
19‖	$CDCl_3$	7.73	8.58	—	—	7.31	8.28	6.37	4.08	4.11	—
23¶	DMSO-d_6	7.73	8.52	—	—	7.77	8.52	6.46	—	3.97	—
23**	$CDCl_3$	7.71	8.49	—	—	7.23	8.22	6.33	—	4.01	—
10	DMSO-d_6	—	8.29 *dd*	7.82 *t*	7.49 *dd*	—	8.19	6.66	—	—	3.95
18††	DMSO-d_6	7.75	8.83 *d*	7.79 *d*	—	—	8.52	6.51	—	4.02	4.04
24‡‡	DMSO-d_6	7.79	8.35 *d*	—	7.13 *d*	—	8.50	6.50 6.43	—	—	4.08

δ, ppm from TMS. J (Hz): 5,6 = 6,7 = 7,8 = 8.5; 5.7 = 2.5. Singlets not denoted.
* δ1.36 and 1.55 (3H, *t*) and 4. 20 and 4.34 (2H, *c*), (OCH_2Me).
† δ1.37 and 1.64 (3H, *t*) and 4. 19 and 4.33 (2H, *c*) (OCH_2Me).
‡ δ1.36 and 1.62 (3H, *t*) and 4.20 and 4.36 (2H, *c*) (OCH_2Me).
§ δ3.76 (3H, *s*, COOMe).
‖ δ1.41 (3H, *t*) and 4.36 (2H, *c*) (OCH_2Me).
¶ δ1.31 and 1.49 (3H, *t*) and 4.23 (4H, *c*) (OCH_2Me).
** δ1.36 and 1.57 (3H, *t*) and 4.28 and 4.35 (2H, *c*) (OCH_2Me).
†† δ3.77 (3H, *s*, COOMe).
‡‡ δ5.12 (1H, *d*, J = 5 Hz, H-1'), 3.20–4.10 (6H, *m* Hs-2'–6').

Table 2. TLC chromatographic data for aristolochic acids and derivatives

	R_f values (× 100) in system*												R_f values (× 100) in system*		
Compound	1	2	3	4	5	6	7	8	9	10	12	Compound	13	14	15
1	73	68	64	—	—	61	47	56	49	56	54	**12**	60	68	75
2	70	66	55	—	—	54	51	63	45	53	54	**13**	69	80	91
3	61	56	55	—	—	50	47	56	45	53	43	**14**	54	60	59
4	66	61	64	—	—	57	43	49	49	56	32	**15**	43	47	33
5	35	41	55	—	—	57	39	42	45	53	16	**16**	21	27	20
6	42	37	—	61	60	—	—	26	—	29	—	**17**	54	68	91
7	32	32	—	54	52	—	—	24	—	30	56	**18**	39	44	59
8	33	33	4	49	45	22	25	19	23	32	49	**19**	22	—	—
9	—	21	—	44	37	—	—	—	—	—	—	**20**	64	77	81
10	50	49	31	—	—	—	—	96	—	46	—	**21**	58	68	75
11	**47**	**44**	—	—	—	—	—	**32**	**25**	**34**	—	**22**	47	54	50
24	23	37	4	81	92	—	—	0	11	26	—	**23**	26	34	37
28	57	52	21	24	20	52	54	71	24	37	21				

* Cellulose: (1) *n*-BuOH–*n*-PrOH–H_2O–25% NH_3 (100:60:50:1); (2) *iso*-PrOH–H_2O–25% NH_3 (10:4:1); (3) $CHCl_3$–MeOH–25% NH_3 (20:7:1.5); (4) HOAc–H_2O (3:7), two developments; (5) C_5H_5N–H_2O–HCO_2H (4:100:1), three developments; (6) C_6H_6–Me_2CO–H_2O–25% NH_3 (25:110:20:1); silica gel: (7) C_6H_6–Me_2CO–HCO_2H (100:15:1); (8) as (7) (100:10:1), three developments; (9) $CHCl_3$–MeOH–25% NH_3 (10:4:1), two developments; (10) as (9) (20:13:5), two developments; (11) $CHCl_3$ [AA VIa (**10**), R_f 0.31]; (13) C_6H_6, two developments; Mg silicate: (12) *n*-BuOH–*n*-PrOH–H_2O–25% NH_3 (100:60:50:1), two developments; (15) 1° $CHCl_3$, 2° $CHCl_3$–EtOH (99:1); Al_2O_3: (14) C_6H_6, three developments.

gests that the CH_2O_2 group may originate in such compounds or in a more advanced precursor, i.e. during the last steps of the biosynthetic route to AAs. While aristololactams were also encountered in some members of other families [14], the AAs and their derivatives are restricted to the Aristolochiaceae and to butterflies whose larvae feed on such species [16].

The ^{1}H NMR data of AAs isolated from *A. argentina*

and/or their derivatives are collected in Table 1. Most of these compounds are differentiated by the arrangement of the oxygenated functions around the positions C-6, C-7 and C-8 of the phenanthrene skeleton that can be inferred from the splittings of the ring C protons as indicated in Table 1. One of these protons, H-5, because of its disposition out of the plane of the aromatic ring system, resonates at low field. The aromatic region usually shows two singlets corresponding to H-2 and H-9. The former exhibits a narrow resonance range (δ7.71–7.81) and often affords a sharper signal than H-9. The latter singlet shows some broadening which may arise from unresolved inter-ring coupling to H-5. Long-range couplings between H-5 and H-9 (0.6–0.7 Hz) have been observed in phenanthrene derivatives [17] and in AAs themselves [6]. Strongly deshielded by the nitro group, H-9 appears at low field, but its resonance position is influenced by the presence of substituents at C-8, probably due to *peri* interaction. Pronounced proton shifts, particularly on H-8 and H-9, also occur on changing the solvent from $CDCl_3$ to DMSO-d_6.

The mass spectra of AAs I, II, III, IIIa, IV and IVa obtained in free form in this work show an intense molecular ion peak, while the base peak is associated with the loss of 46 mu corresponding to the elimination of the nitro group. The relative abundance of $[M]^+$ in the spectra of AAs I, III and IIIa exhibits a narrow range of 38.3–38.9%. In the case of AAs IV and IVa, $[M]^+$ is 41.1–41.2% but is only 32.4% for AA II. The figures suggest the general trend towards a decrease of the $[M^+ - NO_2]/[M]^+$ ratio on introduction of methoxyl or hydroxyl groups at C-6 and C-8 that may be due to a lower ionization potential of the molecule and/or to a substituent effect by enhancing the double-bond character of the C–N bond. The factors which govern the daughter/-parent ion ratio have been discussed [18].

The facile loss of the nitro substituent is assisted by the presence of the *peri*-carboxyl group which participates in the elimination, i.e. the expulsion of the nitro radical from $[M]^+$ proceeds like an intramolecular aromatic substitution reaction (Scheme 1). The operation of this reaction with formation of cyclic oxonium ion in the AAs and their esters is supported by the following evidence:

(1) The rupture of the nitro group in AAs and their esters is the most favoured cleavage and essentially the only primary fragmentation, in contrast to the expected fragmentation order CO_2R, OMe, OH, NO_2 based on the appearance potentials of mono- and disubstituted benzenes [18].

(2) The carboxyl group of AAs decomposes through loss of two CO molecules, and eventually H, with concomitant abstraction of one C atom from the aromatic ring system (Scheme 1), whereas typical fission reactions around this group, e.g. elimination of OH, occur only to a small extent or are undetectable. In the same way, the elements of the CO_2Me and CO_2Et groups of the esters are lost as Me or C_2H_4 and CO, rather than as OMe, OEt, CO_2Me, etc.

(3) When the 1-CO_2H group is absent, the elimination of the NO_2 radical is less pronounced. In the decarboxylated AAs, $[M]^+$ constitutes the base peak whereas the relative abundance of the $[M - NO_2]^+$ ions is low (1–34%) and other primary fragmentations increase in importance. The same behaviour was encountered in isomeric nitronaphthoic acids [19, 20]. The main cleavage of 8-nitro-1-naphthoic acid, having both substituents in the *peri*-position, consists of elimination of NO_2, whereas its 1,5-isomer gives a large number of weak primary fragments.

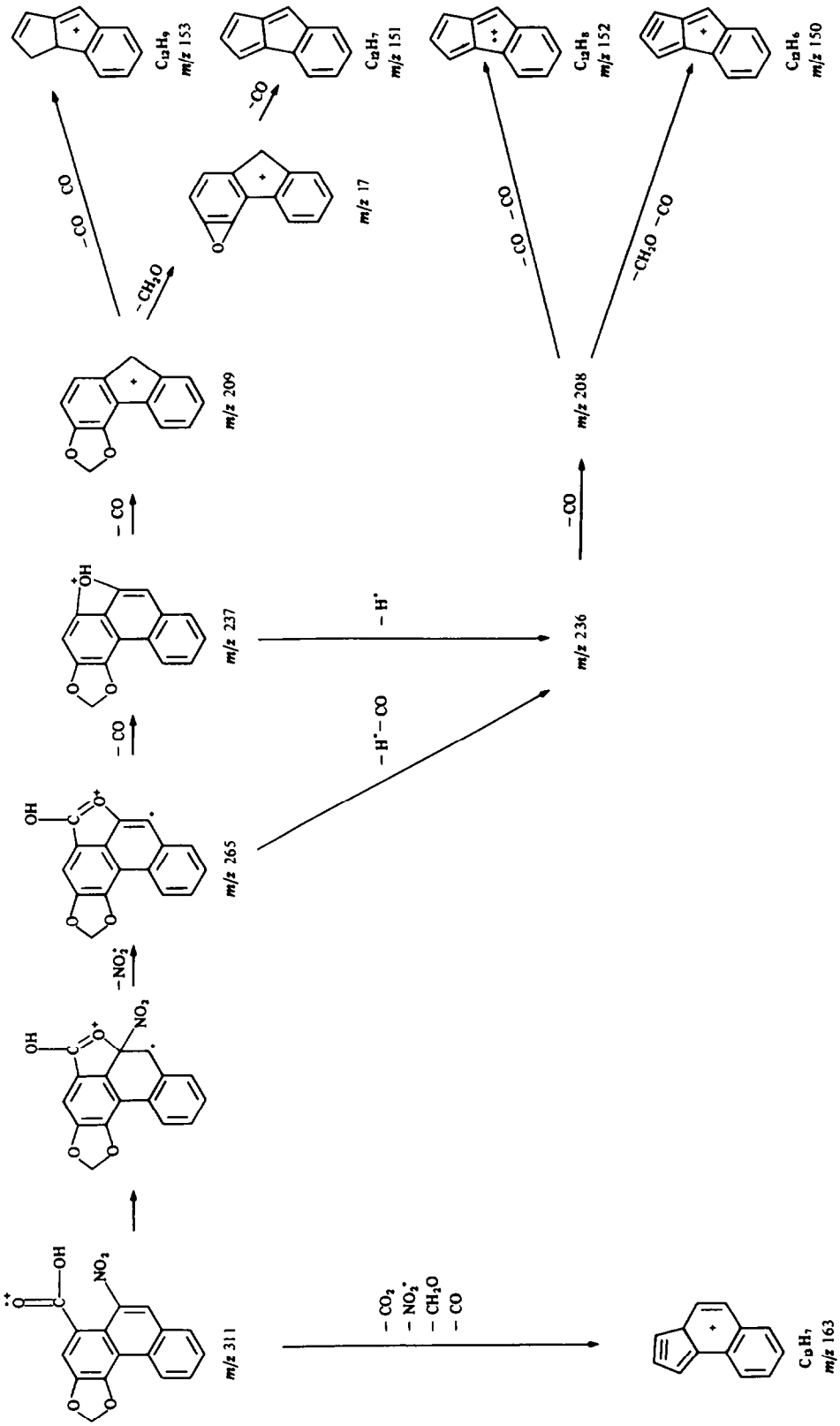
The ejection of the nitro group from $[M]^+$ in the AAs and their esters bears a close analogy to the cyclization process observed in substituted naphthoic [19] and cinnamic [21] acids.

An interpretation of the mass spectrum of AA II (**2**) is given in Scheme 1. Cleavage of the stable CH_2O_2 group usually takes place at the end of the degradation routes and consists of successive loss of CH_2O and CO or two CO molecules with the eventual migration of one or two H atoms on the ring system, a process also observed in simpler methylenedioxy compounds [22]. Rather stable (10–47% relative abundance) hydrocarbon species of type $C_{12}H_{6-9}$ are produced by AA II (**2**). A $C_{13}H_7$ cation (21%) accompanied by the $\pm$H ions is also noticeable. Elimination of CO_2 from $[M]^+$ (or thermal decarboxylation of the acid) would provide a pathway to C_{13} hydrocarbons and account for the peaks at *m/z* 267 and 191.

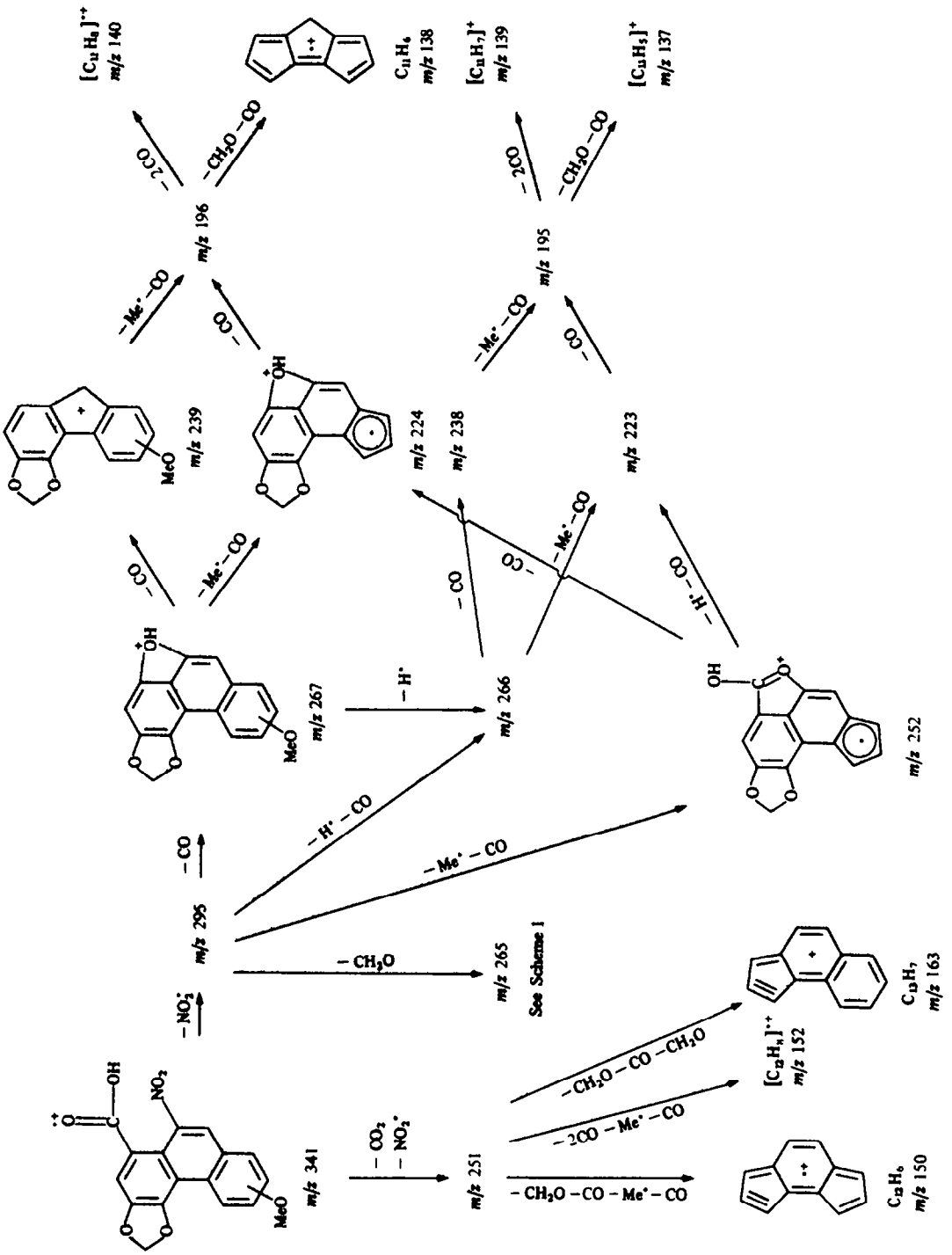
The mass spectra of the isomeric AAs I (**1**) and III (**3**) are qualitatively similar (Scheme 2), the principal decomposition modes of the methoxyl substituent being the sequential elimination of Me and CO or the direct expulsion of CH_2O. Intermediate ions at *m/z* 295, 267, 239, etc. may lose CH_2O (not shown in Scheme 2) to give fragments also produced by AA II. Thus, C_{11}, C_{12} and C_{13} hydrocarbon series result as the final products.

In the mass spectrum of AA IIIa (**7**) the principal ions below *m/z* 281 $[M-46]^+$ are derived from cleavages around the oxonium cycle and the hydroxy function. Degradation of the latter proceeds as usual with the initial expulsion of CO followed by loss of H or by direct ejection of CHO. $C_{11}H_{7-9}$ are observed as the main hydrocarbons. An alternative route leading to $C_{12}H_{7-9}$ through *m/z* 283, 237, 207–209 and 179–181 intermediate ions is also evident. The AAs IV (**4**) and IVa (**8**) follow the fragmentation pattern outlined for AAs I, III and IIIa. They also afford small amounts of C_{10} hydrocarbons due to double contraction of ring C.

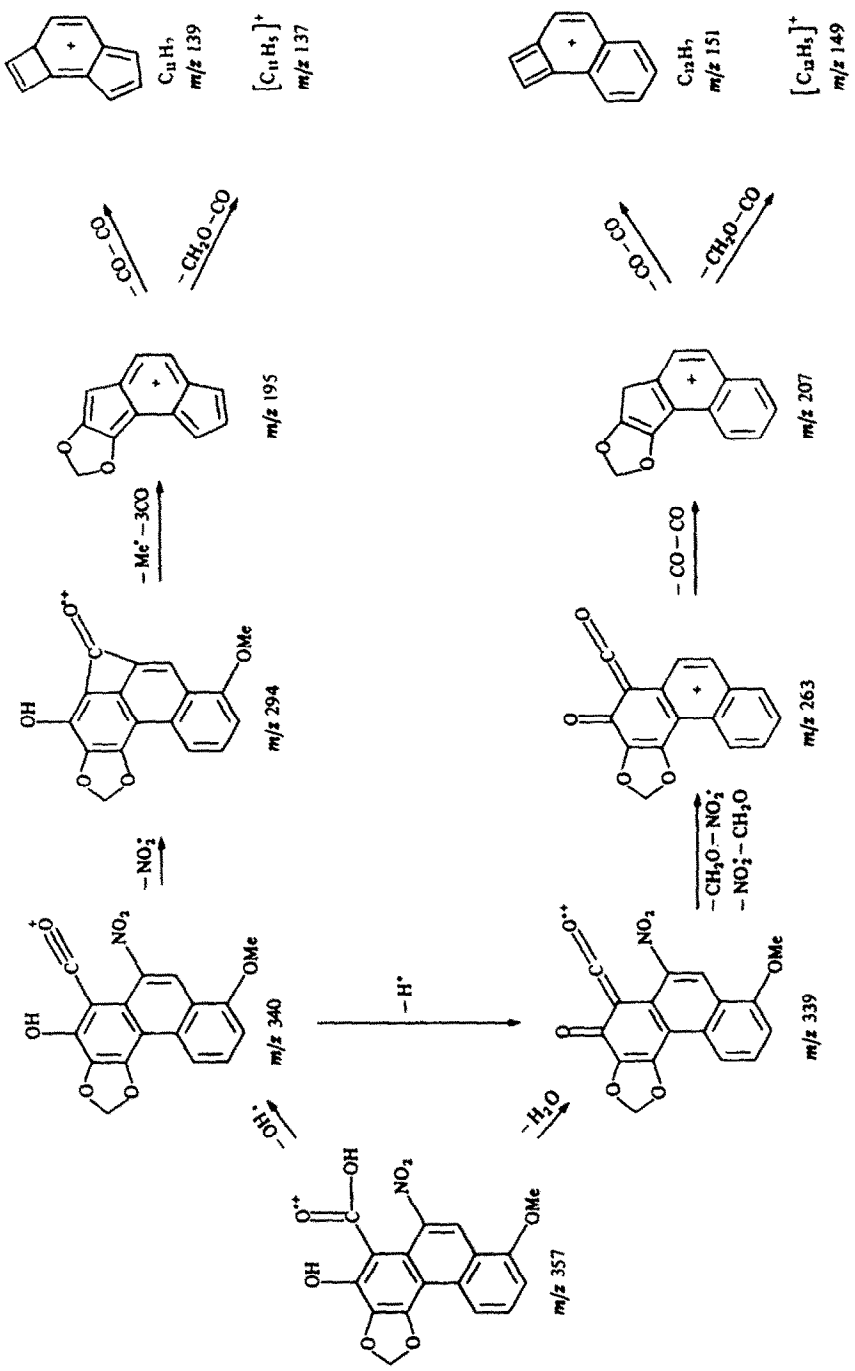
While the principal fragmentation processes of AA VIa (**10**) are also reflected in the mass spectra of ordinary AAs, fundamental changes occur when a hydroxyl group is located at the 2-position. No NO_2 loss from $[M]^+$ is encountered, but instead OH and H_2O eliminations become significant, as indicated in Scheme 3. Such fissions are so pronounced that the molecular ion peak is not recognizable. The ejection of a hydroxyl radical from the carboxyl group is also the primary fission in benzoic acids but it is not noted in the remaining AAs. In the case of AA VIa (**10**), the driving force for this reaction is probably the formation of an acyl ion stabilized by the electron-donating hydroxyl group. However, the $[M - OH]^+$ ion itself exhibits a low abundance ($< 15\%$) because of preferred subsequent elimination of the nitro substituent to give the *m/z* 294 ion which constitutes the base peak in the spectrum. The $[M - OH - NO_2]^+$ ion then suffers a facile loss of a methyl radical with production of the abundant (83.5%) even-electron *m/z* 279 ion. An alternative mode of breakdown of $[M]^+$ occurs through loss of water, a process which like salicylic acid can be formulated in terms of a six-membered ring transition state, yielding a stable *m/z* 339 ion (58.0%). The latter then losses CH_2O and NO_2 in a fragmentation behaviour that is rather reminiscent of that occurring in **25** (see below).



Scheme 1. Mass spectral fragmentation of aristolochic acid II.



Scheme 2. Mass spectral fragmentation of aristolochic acids I and III.



Scheme 3. Mass spectral fragmentation of aristolochic acid VIa.

As previously found for the methyl esters of the AAs I (**12**), II (**13**), III (**14**) and IV (**15**) [23], the mass spectra of the esters **16** and **18** show an intense molecular ion peak (38.4–38.9% relative intensity) and the most favoured cleavage is again the rupture of the C–N bond to provide the $[M-NO_2]^+$ ion responsible for the base peak. Compounds **16** and **18** exhibit only minor differences in the relative abundance of certain ions. From the expected fissions around the CO_2Me group, only the loss of OMe is observed but it is of much less importance as an alternative decomposition mode. The $[M-NO_2]^+$ ions suffer the preferred loss of Me to give abundant $[M-61]^+$ fragments (30–60% relative abundance) which then decompose as indicated for the AAs.

The $[M-NO_2]^+$ species of the ethyl esters of AAs show a more pronounced tendency to eliminate the alkyl moiety. The mass spectra of the ethyl esters **19–23** reveal abundant molecular (30–55%) and $[M-NO_2]^+$ (54–84%) ions, while the base peak remains at $[M-74]^+$ due to facile loss of a C_2H_4 molecule from the latter. The stable $[M-NO_2-C_2H_4]^+$ species is equivalent to the $[M-NO_2]^+$ ion afforded by the AAs. The favoured elimination of C_2H_4 from the CO_2Et group may proceed by α- or β-H rearrangement, cf. dialkyl phthalates [24], or through a McLafferty-type reaction. However, the latter mechanism appears unlikely since no peaks attributable to the stable ionized AAs due to direct ejection of C_2H_4 from the odd-electron molecular ion can be detected.

In the mass spectra of the decarboxylation products of AAs I (**25**) and III (**26**) the base peak is due to the molecular ion. The $[M-NO_2]^+$ ions are of less importance than in the AAs and their esters for the reasons mentioned above. The fragmentation modes of **25** and **26** follow closely those outlined for the AAs, i.e. $[M]^+$ suffers the usual successive loss of NO_2, Me, CH_2O and CO in several ways with production of $C_{13}H_{7-9}$ and $C_{12}H_{6-8}$ as the main hydrocarbons. However, significant differences in the relative abundance of certain fragment ions are evident. In the mass spectrum of the 8-methoxy isomer **25**, the C_{13} hydrocarbons predominate over the C_{12} ones, the opposite situation being observed with the 6-methoxy isomer **26**, and the *m/z* 178 and 236 ions are greatly reduced indicating that the cleavage of the methoxyl group through loss of CH_2O in the $[M-NO_2]^+$ ions is favoured in **25**, whereas in **26** cleavage largely proceeds by elimination of Me and CO. It is also noteworthy that **26** exhibits an abundant (34.4%) $[M-NO_2]^+$ ion, while such a species amounts to only 1.4% in the spectrum of **25**, behaviour which was previously observed by Pailer *et al.* [23]. The favoured expulsion of CH_2O and the low abundance of the $[M-NO_2]^+$ ion in the 8-methoxy isomer **25** may be rationalized in terms of a double H rearrangement process, see **27**. The $[M-NO_2]^+$ species may be stabilized by H· (or hydride) transfers from C-9 to C-10 and from OMe to C-9, the loss of CH_2O being triggered by the removal of one OMe hydrogen. The initial driving force for the reaction would be a 1,2-H shift to C-10, a process which is repressed in AAs, thus providing a radical (or electron-deficient) site at C-9. Because of the favourable *peri*-interaction, loss of CH_2O then occurs through a six- rather than by the four- and five-centred H-transfer mechanisms observed in substituted anisoles [25]. Concerted expulsion of NO_2 and CH_2O or rapid elimination of CH_2O from the $[M-NO_2]^+$ ion would account for the low abundance of this fragment in the mass spectrum of **25**.

EXPERIMENTAL

Mps were determined on a Kofler block and are uncorr. UV spectra were recorded in 95% EtOH and IR spectra in KBr discs. ^{1}H NMR spectra were measured at 60 or 80 MHz using TMS or the central resonance of DMSO-d_5 as internal reference. Mass spectra were recorded at 70 eV. For TLC separations of the AAs and derivatives, see Table 2. The following pairs of solvents were used for 2D-TLC on cellulose and silica gel: 1–2, 1–4, 1–5, 2–5, 4–5, 10–8. On TLC plates, the AAs and their esters give yellowish spots and they can also be detected as dark spots under UV light. When exposed to NH_3 vapour, the spots of the AAs Ia (**6**), IIIa (**7**), IVa (**8**) and Va (**9**) become red, whereas those of AAs VIa (**10**) and VIIa (**11**) give orange-red and intense yellow colours, respectively. CC separations were carried out on fine silica gel, fine Woelm Mg silicate or Merck microcrystalline cellulose with the aid of air pressure.

Extraction of the aristolochic acids from Aristolochia argentina. Underground parts (4.7 kg, dry wt) of *A. argentina*, collected during 1969–1974 at Villa Allende (Córdoba, Argentina), were first extracted 2 × with boiling petrol. The plant material was then dried in air and extracted 3 × by boiling for 8 hr with EtOH. The EtOH extracts and washings (30 l.) were evaporated to a dark-brown oil (765 g) that was suspended in H_2O, adjusted to pH 9.3 with conc NH_3 and extracted with Et_2O. To the aq. soln, Et_2O and conc HCl to pH 3.0 were added when the AAs passed into the organic phase. The aq. phase was further extracted with Et_2O and reserved for the isolation of aristoloside. The combined ethereal extracts containing the AAs were washed with H_2O and, after addition of EtOH (1 l.), concentrated (up to 0.7 l.) The extraction procedure as described above was repeated 2 × with the remaining plant material (8.6 kg). The resultant EtOH soln (1.7 l.) (corresponding to 13.3 kg underground stems) was allowed to stand at 5° for 2 days. Yellow crystals of AAs (20.95 g), predominantly AAI (**1**), separated which were recrystallized 2 × (dioxane) to give a mixture (11.3 g) of AAsI (**1**) and IV (**4**) (approximate ratio 10:1). The AAs contained in the combined mother liquors (49.0 g) were taken up in Et_2O and passed first into 5% $NaHCO_3$ soln and then at pH 3.0 into a $CHCl_3$ phase. Evaporation of the $CHCl_3$ extracts gave a dark oil (42.1 g) of the crude mixture of acids which was submitted to countercurrent distribution as indicated below.

Separation of phenolic and non-phenolic aristolochic acids by countercurrent distribution. The separation was performed in twelve 1 l. separatory funnels with *n*-BuOH as the upper phase and aq. 5% $NaHCO_3$ adjusted to pH 10.0 with conc aq. NaOH as the lower phase. Both phases were brought into equilibrium with each other. The crude mixture of acids (42.1 g) was dissolved in 400 ml of the lower phase and 400 ml of the upper phase and charged to the first funnel. To the other 11 funnels was added 400 ml of the upper phase; this makes out the stationary phase (for practical reasons, the lower phases were transferred in this expt rather than the upper phases as in classical countercurrent method). When the phases had separated in funnel 1, the lower phase was transferred to funnel 2 and a new 400-ml vol. of the lower phase was added to the first funnel. The contents of the first two funnels were then equilibrated and the procedure was repeated. When the lower phase originally in funnel 1 had reached funnel 12, the separation of phenolic and non-phenolic AAs was then obvious from the appearance of the funnels. The upper phases of funnels 1–6 containing the non-phenolic AAs were very dark (usually yellow-coloured when working with pure acids), diminishing from 1 to 6, due to slow-moving impurities, whereas the red colour from the phenolic AAs dominated, with a maximum at funnel 10, in the lower phases of funnels 8–12. A total of 18 transfers were done, the lower phases containing

phenolic acids being collected at funnel 12. Since no extraction from the upper phases of the first funnels was apparent, the BuOH phases of funnels 1, 2, 3, 4 and 5 were removed from the system after transfers 9, 11, 13, 15 and 17, respectively. *Non-phenolic acids*: The contents of funnels 1–8 were jointly adjusted to pH 3.0 with conc HCl. The organic phase (3 l.) was separated, washed with H_2O and evaporated under red. pres. (N_2 current) to give a dark residue which was taken up in EtOH (100 ml). After the soln had been kept at 5° overnight, yellow crystals (10.75 g) were collected, which by recrystallization (dioxane) yielded a mixture (10.40 g) of AAs I (**1**), III (**3**) and IV (**4**) (ratio 10:1:2). Evaporation of the combined mother liquors gave an impure oil (14.1 g) in which AAs I (**1**), IV (**4**), IVa (**8**) (traces) and aristololactam DII (**28**) could be detected by TLC. *Phenolic acids*: The BuOH phases of funnels 11 and 12 were extracted with pH 10 buffer and the aq. extracts were combined with the lower phases of funnels 11–12 and those collected at funnel 12. The resultant aq. soln (7 l.) on slow acidification with conc HCl gave a red ppt. of phenolic AAs which, after standing at 5° overnight, was collected (5.82 g). Extraction of the remaining aq. soln with Et_2O and evaporation of the ethereal extracts afforded an oily residue (6.34 g) consisting mainly of *p*-coumaric, ferulic and vanillic acids, and traces of phenolic AAs and aristoloside. The intermediate fractions of funnels 9 and 10 were discarded.

Separation of non-phenolic aristolochic acids. The mother liquors (14.1 g) of crystallization of the main non-phenolic AAs were repeatedly chromatographed on cellulose columns (*n*-BuOH–*n*-PrOH–H_2O–25% NH_3, 100:10:10:1, 100:14:25:1, 100:24:30:1, etc. and $CHCl_3$–MeOH–25% NH_3, 100:9:1.5, 100:16:3, 100:21:4.5, etc.) in order to remove aristololactam DII (**28**) and impurities. Several fractions containing partially fractionated AAs were obtained.

Isolation of AAs I (**1**) *and II* (**2**). Enrichment of AA II from mixtures of these two acids was performed by the same chromatographic procedure. The fractions containing AA II were dissolved in $CHCl_3$–EtOH (4:1), applied to silica gel plates and developed (system 8) to give crystals ($CHCl_3$–*n*-PrOH) of AAs II (24.7 mg) and I (420 mg).

Isolation of AAs III (**3**) *and IV* (**4**). Some fractions containing mixtures of these acids were combined and further subjected to CC over cellulose ($CHCl_3$–MeOH–25% NH_3, 20:7:1.5). Yellow crystals (DMF) of AA IV (27.6 mg) were obtained from the first fractions, whereas the last fractions, a mixture of the two acids, afforded AA III (37.4 mg) upon recrystallization from DMF (AA III is less soluble than AA IV in most solvents).

Ethylation of non-phenolic aristolochic acids. Fractions containing AAs I and II were ethylated with ethereal $MeCHN_2$. The resultant mixtures of esters was dissolved in DMF and, after separation of AA I ethyl ester (235 mg) by crystallization, applied to silica gel plates. Once the solvent (DMF) was evaporated (60°, 12 hr, vacuum), the plates were developed (system 13). Elution ($CHCl_3$–MeOH) of the bands afforded yellow needles ($CHCl_3$–*n*-PrOH) of the ethyl esters of AAs I (63.7 mg) and II (55.0 mg). Similarly, several fractions rich in AAs III and IV were combined and processed as described above to give the ethyl esters of AAs III (20.7 mg), IV (17.3 mg), I (3.0 mg) and V (0.8 mg).

Isolation of aristolochic acid VIa (**10**). From the red solid ppt. of phenolic AAs (5.82 g), a portion of pure AA IVa (2.34 g) was removed by crystallization (dioxane). The mother liquor (3.40 g), a black oil containing mainly the AAs IVa and IIIa together with minor AAs and aristoloside (systems 1–10), was passed through a silica gel column (C_6H_6, $CHCl_3$, MeOH). The first fractions, in which AA VIa was detected, were combined and rechromatographed in the same way. Evaporation of the fractions containing this acid gave an oil from which orange needles separated upon standing. The crystals, after being suspended (C_6H_6), filtered and washed (C_6H_6), consisted (systems 1, 2, 3, 8, 10 and 11) of pure AA VIa (1.6 mg).

Methylation and ethylation of phenolic aristolochic acids. After separation of AA VIa, the fractions with phenolic AAs were partly (1.09 g) methylated with CH_2N_2 and partly (1.60 g) ethylated with $MeCHN_2$. The methylation product was worked up as indicated above for the ethyl esters of non-phenolic AAs to give the *O*-methyl methyl esters of AA IVa (**15**) (233 mg), IIIa (**14**) (85 mg), Va (**16**) (3.0 mg) and Ia (**12**) (2.4 mg). The mixture of ethylated acids (soluble in $CHCl_3$), after repeated prep. TLC (system 13), afforded the *O*-ethyl ethyl esters of AA IIIa (**21**) (420 mg), IVa (**22**) (338 mg) and Va (**23**) (19.1 mg), and the ethyl ester of the non-phenolic AA V (**19**) (0.8 mg). The upper yellow band (R_f 0.64) furnished crystals (28.5 mg) from which pure AA Ia ethyl ether ethyl ester (**20**) (7.6 mg) was obtained by recrystallization from $CHCl_3$–*n*-PrOH.

Separation of phenolic aristolochic acids. A ppt. of phenolic AAs (3.6 g, corresponding to 10.79 kg plant material) obtained by countercurrent distribution, after removal of a portion of AA IVa (**8**) (2.07 g) by crystallization (dioxane), was chromatographed on a cellulose column (*iso*-PrOH–H_2O–25% NH_3, 10:1:1, 10:2:1, 10:4:1). Examination of the eluates by TLC (systems 1–2) indicated that preliminary purification and fractionation of the AAs were achieved. Fractions which showed identical TLC pattern were combined and submitted to prep. TLC (system 2). One major and four minor bands were scraped off the plates and eluted ($CHCl_3$–MeOH): R_f 0.49, R_f 0.40–0.47, R_f 0.37, R_f 0.32–0.33 and R_f 0.21 containing the AAs VIa (**10**), V (**5**) and VIIa (**11**), Ia (**6**), IIIa (**7**) and IVa (**8**), and Va (**9**), respectively. The major R_f 0.32–0.33 band and fractions of the column containing mixtures of AAs IIIa (**7**) and IVa (**8**) were subjected to prep. TLC (system 8). The red solid residue obtained by elution ($CHCl_3$–MeOH) of the upper bands was taken up in $CHCl_3$–MeOH (7:3), washed with H_2O and evaporated to give needles (DMF–$CHCl_3$) of AA IIIa (66.0 mg). The R_f 0.40–0.47 band consisted of a mixture of AAs V (**5**) and VIIa (**11**) which were resolved on silica gel plates (system 8) and the same procedure was used to isolate AA Ia (**6**), contaminated with AAs IIIa (**7**) and IVa (**8**), from the R_f 0.37 band. A significant reduction in the amount of these acids through the successive purifications was noticeable, presumably due to irreversible binding to the absorbents. Only a small amount of the minor AAs was obtained which was not completely free from impurities. Their identification was therefore accomplished through the corresponding esters. The AAs V (**5**), Ia (**6**) and Va (**9**) were treated with $MeCHN_2$, whereas the AAs VIa (**10**) and VIIa (**11**) were methylated with CH_2N_2. Purification of the methylated and ethylated acids by prep. TLC (system 13) afforded the *O*-ethyl ethyl esters of AA Ia (**20**) (1 mg) and Va (**23**) (3 mg), the ethyl ester of AA V (**19**) (1 mg) and the *O*-methyl methyl esters of AA VIa (**17**) (traces) and VIIa (**18**)(0.6 mg).

Aristolochic acid I (**1**). EIMS *m/z* (rel. int.): 342 (6.9), 341 $[M]^+$ (38.9), 297 (5.2), 296 (25.2), 295 (100), 281 (5.7), 280 (26.8), 267 (9.8), 266 (9.5), 252 (6.2), 239 (4.6), 237 (9.3), 224 (9.2), 209 (7.5), 196 (11.4), 193 (5.6), 152 (7.7), 151 (7.6), 150 (7.3), 139 (5.9), 138 (8.0), 126 (4.9), 105 (5.0). *Decarboxylation product* (**25**). Obtained by heating aristolochic acid I in quinoline with Cu powder as catalyst, cf. ref. [23]. EIMS *m/z* (rel. int.): 298 (25.7), 297 $[M]^+$ (100), 267 (5.9), 240 (10.6), 238 (7.6), 224 (5.6), 221 (9.7), 211 (8.7), 209 (6.5), 208 (5.1), 193 (22.5), 192 (5.0), 191 (5.4), 165 (23.3), 164 (7.2), 163 (26.2), 152 (7.3), 151 (7.3), 150 (17.2), 80 (8.1).

Aristolochic acid II (**2**). Mp 264°; UV λ_{max} nm (log ε): 249 (4.58), 296 (4.12), 362 (3.59), IR ν_{max} cm^{-1}: 1690, 1415, 1358, 1242, 1133, 1055; EIMS *m/z* (rel. int.): 311 $[M]^+$ (32.4), 267 (11.1), 266 (15.9), 265 (100), 264 (10.9), 263 (8.0), 237 (14.5), 236 (14.6), 209

(17.8), 208 (14.4), 207 (15.2), 181 (13.3), 179 (10.3), 163 (21.3), 153 (10.6), 152 (23.0), 151 (47.0), 150 (25.2), 119 (10.3), 71 (12.1), 69 (52.4), 57 (19.7), 44 (55.6), 43 (13.0).

Aristolochic acid III (**3**). Mp. 300°; UV λ_{max} nm (log ε): 245 sh (4.51), 255 (4.55), 276 sh (4.08), 299 (4.06), 370 (3.78); IR ν_{max} cm^{-1}: 1695, 1635, 1611, 1439, 1358, 1251, 1162, 1140, 1063, 1040; EIMS *m/z* (rel. int.): 342 (7.2), 341 $[M]^+$ (38.9), 297 (4.8), 296 (20.2), 295 (100), 281 (5.7), 267 (7.9), 266 (8.1), 252 (7.1), 239 (9.9), 238 (6.5), 237 (6.0), 224 (5.6), 193 (4.8). *Decarboxylation product* (**26**). Obtained as indicated for **25**. EIMS *m/z* (rel. int.): 298 (20.0), 297 $[M]^+$ (100), 267 (6.4), 252 (7.3), 251 (34.4), 239 (12.4), 237 (7.9), 236 (10.2), 224 (9.8), 221 (8.9), 211 (9.1), 193 (14.0), 185 (10.8), 178 (8.3), 152 (9.7), 151 (6.6), 150 (20.4), 81 (14.0), 71 (13.8), 69 (26.7), 57 (26.5).

Aristolochic acid IV (**4**). Mp 266°; UV λ_{max} nm (log ε): 244 sh (4.49), 251 (4.50), 285 sh (4.08), 315 (4.03), 364 (3.75), 393 (3.82); IR ν_{max} cm^{-1}: 1688, 1672, 1618, 1442, 1386, 1347, 1262, 1226, 1191, 1173, 1074; EIMS *m/z* (rel. int.): 372 (8.1), 371 $[M]^+$ (41.2), 341 (7.8), 327 (6.5), 326 (20.6), 325 (100), 324 (6.1), 323 (4.0), 310 (14.3), 309 (4.1), 297 (9.7), 296 (11.0), 295 (20.5), 294 (4.0), 281 (5.6), 280 (5.2), 269 (5.2), 267 (10.7), 251 (4.5), 239 (5.3), 223 (5.4), 73 (21.8), 40 (8.0).

Aristolochic acid V (**5**). *Methyl ester* (**16**). See aristolochic acid Va methyl ether methyl ester. *Ethyl ester* (**19**). Mp 290°; UV λ_{max} nm: 269, 306 sh, 382; IR ν_{max} cm^{-1}: 1716, 1701, 1626, 1606, 1391; EIMS *m/z* (rel. int.): 399 $[M]^+$ (29.8), 354 (21.3), 353 (79.7), 326 (21.8), 325 (100), 324 (22.5), 323 (11.1), 310 (9.0), 309 (16.5), 296 (15.3), 281 (9.1), 266 (10.1), 251 (12.3), 238 (8.4).

Aristolochic acid Ia (**6**). UV λ_{max} nm: 222, 255, 286 sh, 317, 390; UV $\lambda_{max}^{EtOH-NH_3}$ nm: 236, 305, 395, 450. *Ethyl ether ethyl ester* (**20**). See ref. [3].

Aristolochic acid IIIa (**7**). Mp 285°; UV λ_{max} nm: 250 sh, 256, 301, 358 sh, 371, 386 sh; UV $\lambda_{max}^{EtOH-NH_3}$ nm: 256, 301, 369 sh, 385, 418; IR ν_{max} cm^{-1}: 1692, 1631, 1450, 1352, 1271, 1228, 1160, 1140, 1068, 958; EIMS *m/z* (rel. int.): 328 (6.7), 327 $[M]^+$ (38.3), 283 (6.9), 282 (28.8), 281 (100), 280 (6.6), 253 (8.8), 252 (11.0), 225 (15.9), 224 (9.1), 223 (7.9), 207 (5.3), 195 (5.3), 179 (6.5), 167 (5.8), 139 (5.9), 80 (6.4), 44 (12.0). *Ethyl ether ethyl ester* (**21**). Mp 186°; UV λ_{max} nm (log ε): 257 (4.59), 281 sh (4.16), 304 (4.07), 354 (3.91), 370 (3.92), 385 (3.91); IR ν_{max} cm^{-1}: 1727, 1618, 1524, 1463, 1387, 1351, 1230, 1197, 1151, 1046; EIMS *m/z* (rel. int.): 384 (10.5), 383 $[M]^+$ (49.1), 338 (23.5), 337 (75.8), 310 (29.7), 309 (100), 308 (19.3), 281 (16.3), 280 (34.4) 279 (16.2), 252 (8.2), 251 (7.5), 224 (9.0).

Aristolochic acid IVa (**8**). Mp 254–259°; UV λ_{max} nm (log ε): 220 (4.37), 244 sh (4.46), 254 (4.47), 326 (3.91), 401 (3.92), UV $\lambda_{max}^{EtOH-NH_3}$ nm: 254, 388, 448; IR ν_{max} cm^{-1}: 1692, 1610, 1441, 1372, 1317, 1253, 1200, 1176, 1149, 1109, 1046, EIMS *m/z* (rel. int.): 357 $[M]^+$ (41.1), 313 (13.7), 312 (57.6), 311 (100), 310 (8.9), 297 (11.6), 296 (23.8), 283 (10.4), 282 (10.4), 267 (10.6), 209 (9.0), 44 (10.1). *Ethyl ether ethyl ester* (**22**). Mp 257–258°; UV λ_{max} nm (log ε): 243 (4.57), 255 (4.50), 326 (4.03), 402 (4.00); IR ν_{max} cm^{-1}: 1709, 1605, 1379, 1323, 1267, 1166, 1042; EIMS *m/z* (rel. int.): 414 (12.0), 413 $[M]^+$ (54.5), 368 (25.3), 367 (83.5), 340 (27.4), 339 (100), 338 (47.2), 324 (6.7), 311 (11.0), 310 (28.1), 309 (14.6), 296 (7.1), 295 (7.1), 281 (7.6), 267 (8.7), 266 (10.1).

Aristolochic acid Va (**9**). *Methyl ether methyl ester* (**16**). Mp 290–300°; UV λ_{max} nm (log ε): 270 (4.60), 305 sh (4.17), 385 (3.89); IR ν_{max} cm^{-1}: 1712, 1515, 1381, 1241, 1215, 1166, 1114, 1050, 1003; EIMS *m/z* (rel. int.): 386 (9.4), 385 $[M]^+$ (38.4), 340 (21.2), 339 (100), 325 (13.4), 324 (59.9), 310 (9.4), 309 (20.9), 296 (16.2), 281 (8.1), 253 (8.1), 251 (13.2), 238 (8.4), 162 (7.9). *Ethyl ether ethyl ester* (**23**). Mp 222–226°; UV λ_{max} nm (log ε): 270 (4.60), 304 sh (4.15), 383 (3.89): IR ν_{max} cm^{-1}: 1718, 1515, 1383, 1205, 1166, 1112, 1047; EIMS *m/z* (rel. int.): 413 $[M]^+$ (16.1), 368 (9.7), 367 (28.3), 340 (13.3), 339 (50.1), 310 (13.3), 85 (65.5) 83 (100), 57 (13.4), 48 (11.8), 47 (26.2); 44 (15.0).

Aristolochic acid VIa (**10**). Mp 280–282°; UV λ_{max} nm: 226, 232 sh, 254, 268 sh, 276 sh, 288 sh, 314 sh, 347 sh, 386, 401; UV $\lambda_{max}^{EtOH-NH_3}$ nm: 256, 331, 411, 447; EIMS *m/z* (rel. int.): 340 (15.0), 339 $[M - H_2O]^+$ (58.0), 309 (25.5), 295 (23.6), 294 (100), 280 (19.5), 279 (83.5), 263 (13.0), 251 (26.2), 207 (10.5), 195 (10.9), 151 (17.2), 150 (13.5), 149 (13.2), 147 (11.4), 139 (16.2), 138 (10.3), 137 (19.0), 84 (31.1), 71 (30.2), 69 (33.1), 57 (62.9), 55 (31.0), 43 (41.4).

Aristolochic acid VIIa (**11**). *Methyl ether–methyl ester* (**18**). Mp 251°; UV λ_{max} nm: 225, 274, 312, 373, EIMS *m/z* (rel. int.): 385 $[M]^+$ (38.9), 339 (100), 324 (30.3), 309 (39.7), 296 (19.2), 281 (19.0), 266 (26.9), 84 (12.5), 69 (13.3), 57 (13.2), 43 (12.3).

Based on the weight of crystals obtained, the content of AAs in *A. argentina* is estimated to be the following (mg/kg of dry plant material): I, 1500; IV and IVa, 300; III, 100; IIIa, 40; II, 10, Ia and Va, 3; V, VIa and VIIa, 0.2.

Aristoloside (**24**). The aq. soln (9 l., corresponding 13.3 kg plant material) remaining after extraction with Et_2O of the neutral and basic compounds at pH 9.3 and the acidic ones at pH 3.0 was extracted with $CHCl_3$–EtOH (3:2). The organic extracts were washed with H_2O and evaporated to dryness to give a black oil (25 g). Analytical TLC revealed the presence of a yellow spot (R_f 0.23, system 1). In order to purify this compound, the oil was subjected to countercurrent distribution in 15 × 1 l. separatory funnels with *n*-BuOH (400 ml) as the upper phase and 5% $NaHCO_3$ (400 ml) as the lower phase. After 14 transfers, the R_f 0.23 compound was contained in funnels 6–15, whereas slow-moving impurities, chiefly localized in the BuOH phases, remained in funnels 1–5. The total contents of funnels 6–15 were adjusted to pH 3 with conc HCl under agitation when the R_f 0.23 compound passed into the organic phase. The BuOH phase was then washed with H_2O, concentrated *in vacuo* and chromatographed on a cellulose column (*n*-BuOH–*n*-PrOH–H_2O–25% NH_3, 100:14:25:1, 100:24:30:1, 100:60:50:1). The last fractions (3.7 g) contained the R_f 0.23 compound which was further purified by prep. TLC on silica gel [$CHCl_3$–MeOH–HCO_2H (80:20:1), R_f 0.46] and cellulose [HOAc–H_2O (1:3), R_f 0.50] to give an orange solid (HOAc) (8.5 mg), the physicochemical data of which (mp, UV, IR, ^1H NMR) agreed with those reported for aristoloside **24** [6]. *A. argentina* contains about 1 mg/kg of this compound.

p-*Coumaric, ferulic and vanillic acids.* TLC of the fraction of H_2O-soluble acids (6.34 g) after separation of the phenolic AAs (see above) revealed the presence of the title compounds as the main components (system, R_f values: 1, 0.52, 0.41, 0.33; 2, 0.62, 0.55, 0.44; 8, 0.48, 0.61, 0.63; dark spot, blue and violet fluorescent spots, respectively). The mixture was first partially fractionated through a cellulose column (*iso*-PrOH–H_2O–25% NH_3, 10:1:1, 10:2:1, etc.). Prep. TLC [silica gel, C_6H_6–dioxane–HOAc (90:25:4) and system 2] of some fractions afforded crystals (MeOH–H_2O) of *p*-coumaric, ferulic and vanillic acids (mp, TLC, UV, IR). It is estimated that the content of each of these acids in *A. argentina* may attain 0.1 g/kg dry wt plant material.

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