ARISTOLOCHIC ACIDS IN *Aristolochia chilensis* AND THE *Aristolochia*-FEEDER *Battus Archidamas* (*Lepidoptera*)

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The larval stages of *Battus archidamas*, the only Papilionoid butterfly living in Chile, feed on the aerial parts of *Aristolochia chilensis*. The food material contains aristolochic acids, and so do the insects, which retain these compounds after metamorphosis. The compositions of the acid fractions of plant and herbivore extracts are different, however, indicating selective uptake and/or metabolic transformation by the insect. Aristolochic acids I (*I*) and Ia (*II*) are major components of the leaves and tender stems of *A. chilensis*, while aristolochic acids I (*I*) and (probably) IVa (*III*) appear to be the main acid components of *B. archidamas* imagines. The selective sequestration of aristolochic acids by *B. archidamas* and other *Aristolochia*-feeders is discussed.

Aristolochia chilensis MIERS is a summer-deciduous low creeping perennial herb ranging southwards from Caldera in Nothern Chile to beyond the latitude of Santiago. It is known by the names "oreja de zorro" (fox ear, a reference to the shape and color of its flowers) and "hierba de la Virgen María" (Virgin Mary's herb)[1], and decoction of its roots was taken at least until the second half of last century to reduce abundant lochia (puerperal secretions)[2]. This species is the larval food-plant of *Battus archidamas* BOISD., the only member of the family *Papilionoideae* (*Leptidotera*) living in Chile[3]. The butterfly is found within the range of *A. chilensis*, and can be seen fluttering in large numbers between September and January (spring and early summer) in places where the plant grows[4,5]. The females lay clusters of about nine eggs on the leaves of *A. chilensis*, which are consumed completely with the tender stems by the brown, orange-tubercled caterpillars. At their last instar the larvae observed near Santiago emigrate to dense spiny shrubs such as *Trevoa trinervis*, and pupate.

As a first contribution to the study of the chemical relationship between *B. archidamas* and *A. chilensis*, this paper reports the isolation of aristolochic acids from both species.

An ethanol extract of the defatted aerial parts of *A. chilensis* was processed to afford an acid fraction (0·025% dry weight) which yielded identified as aristolochic acids I (*I*) and I (*II*).

A methanol extract of live *B. archidamas* imagines (both sexes) was processed to afford an acid fraction (about 0·2 mg/insect) which yielded two colored solids designated CAB'-1 and CAB'-2, whose mass spectra showed that aristolochic acids *I* and *IVa* (*3*), respectively, are probably the major components.

EXPERIMENTAL

Melting points were determined in a capillary and are uncorrected. Ultraviolet spectra were recorded on a Zeiss DMR-22 spectrophotometer. Infrared spectra were determined on a Perkin–Elmer 237 spectrophotometer. Proton magnetic resonance spectra were recorded at 90 and 200 MHz using Bruker HW-20 and HW-200 instruments, with hexadeuteriodimethyl sulfoxide as solvent and tetramethylsilane as an internal standard. Mass spectra were recorded with a Kratos MS-50 spectrometer. Thin layer chromatography was performed on silica gel 60 F_{254} chromatofoils using benzene–methanol–acetic acid (85 : 10 : 5) (S1) and ethyl acetate–light petroleum (2 : 1) (S2). Paper chromatography was carried out on Whatman No 1 and No 3 papers using 1-propanol–1-butanol–water–25% ammonia (6 : 2 : 2 : 1) (S3).

Biological material. The aerial parts of *A. chilensis* MIERS were gathered near the summit of Lo Prado Pass (Santiago) in November, 1978. Herbarium samples are deposited in the Museo Nacional de Historia Natural, Santiago. Male and female imagines of *B. archidamas* were collected in the same locality one year later.

Isolation of Aristolochic Acids from *A. Chilensis*

A 1·7 kg sample of air-dried and ground plant material was extracted successively with light petroleum and ethanol at room temperature. Concentration of the ethanol extract *in vacuo* afforded a residue which was partitioned between 2 l of dilute ammonia (pH 9) and chloroform until fresh chloroform extracted no colored substances. The basic solution was adjusted to pH 3 with concentrated hydrochloric acid and extracted three times with 250 ml portions of chloroform–ethanol (3 : 2). The pooled chloroform extracts of the acid solution were concentrated under reduced pressure and extracted with three 100 ml portions of 5% sodium hydrogen carbonate. This aqueous solution was adjusted to pH 3 with concentrated hydrochloric acid, and a greenish–yellow solid was collected by filtration (90 mg). This material, designated AA-1, showed a single intense chromatographic spot in S1, S2 and S3. The pooled chloroform–ethanol extracts were likewise concentrated and extracted with 5% sodium hydrogen carbonate. Acidification as before yielded an orange–yellow precipitate (346 mg) which in S1, S2 and S3 appeared as a complex mixture. Two apparently homogeneous fractions were isolated from this precipitate by preparative thin layer chromatography (S1): a second portion of AA-1 (41·1 mg), and AA-2 (53·2 mg).

Isolation of aristolochic acids from B. Archidamas. Fifty-eight live male and female butterflies were killed by immersion in methanol (300 ml) and ground in a blendor. The solid matter was separated by filtration and refluxed twice for 5 h each time with 300 ml portions of methanol. The pooled methanol extracts were concentrated *in vacuo* to a volume of 100 ml, and diluted with water (25 ml). This solution was defatted with light petroleum, diluted again with water, basified with 5% sodium carbonate to pH 11, and extracted twice with 50 ml portions of chloroform. The basic solution was adjusted to pH 3 with 5% sulfuric acid and extracted with another two 50 ml portions of chloroform, which were dried and concentrated under reduced pressure affording the acid components of *B. archidamas* (10·9 mg). This material was subjected to preparative thin layer chromatography (S2), and two fractions were separated and designated

CAB′-1 (1·5 mg) and CAB′-2 (1·4 mg) which migrated together with AA-1 and AA-2, respectively, solated from *A. chilensis*.

AA-1: The material isolated from the chloroform extract, washed with hot methanol and recrystallized from ethanol–dimethylformamide (5 : 1), yielded yellow needles (34 mg), m.p. 273 to 275° (decomposes) [lit.[6] 275–277°, lit.[7] 283–285°]; UV spectrum (ethanol): λ_{max} 221 nm (log ε 4·47), 250 (4·51), 317 (4·05), 388 (3·78). ^{1}H NMR spectrum: δ 4·04 (s, 3 H, OCH_3); 6·48 (s, 2 H, OCH_2O); 7·35 (d, J = 8 Hz, 1 H, H-7); 7·81 (s, 1 H, H-2); 7·83 (t, J = 8 Hz, 1 H, H-6); 8·56 (s, 1 H, H-9); 8·62 (d, J = 8 Hz, 1 H, H-5). Mass spectrum: see Table I. This substance gave identical IR spectrum and TIC R_f values as those of a reference sample of aristolochic acid I. The mixture m.p. gave no depression.

AA-2: This fraction, purified by preparative paper chromatography (S3) and recrystallized from methanol, afforded an orange-colored solid (7·1 mg), m.p. 278° (decomposes) (lit.[8] 280°). UV spectrum (ethanol): max 221, 255, 283, 312, 389 nm; (ethanol-NaOH): max 241, 305, 340,

TABLE I

Characteristic mass-spectral signals of aristolochic acids and aristolactams

Sample		AA-1		AA-2		CAB′-1		CAB′-2	
		Int. %	Dev.[a]	Int. %	Dev.[a]	Int. %	Dev.[a]	Int. %	Dev.[a]
Z = H									
$M^{+\cdot}$	311·0429	15·6[c]	−0·6[c]	–		–		–	
$(M-NO_2)^+$	265·0501	48·9[c]	−0·9[c]						
Lactam	263·0581	76·3[c]	−0·6[c]						
Z = OH									
$M^{+\cdot}$	327·0739	trace[b]	~	25·9	−0·4	–		8·4	−0·9
$(M-NO_2)^+$	281·0450			100	−0·5			31·2	−0·2
Lactam	279·0531			50·0	−1·1			34·6	0
Z = OCH_3									
$M^{+\cdot}$	341·0535	35·2	+0·4	trace	−[b]	26·0	−0·2		
$(M-NO_2)^+$	295·0606	100	−1·2			100	+1·0	–	
Lactam	293·0688	13·9	−0·4			24·9	+1·7		
Z = OH + OCH_3									
$M^{+\cdot}$	357·0484			trace	−[b]			27·7	+0·7
$(M-NO_2)^+$	311·0556	–				–		89·3	−0·4
Lactam	309·0636							100	−0·4
Z = 2 OCH_3									
$M^{+\cdot}$	371·0641	trace	−[b]			0·5	−2·4	3·5	−1·7
$(M-NO_2)^+$	325·0712			–		4·9	+0·8	14·4	+0·2
Lactam	323·0793					1·8	−0·8	6·8	−1·0

[a] Deviation in milli mass units; [b] low resolution data from separate measurements; [c] from a less purified sample (341·0552 16·1%; 295·0611 72·0%; 293·0688 91·9%).

450 nm ^{1}H NMR spectrum: δ 6·44 (s, 2 H, OCH_2O); 7·20 (d, J = 8 Hz, 1 H, H-7); 7·66 (t, J = 8 Hz, 1 H, H-6); 7·72 (s, 1 H, H-2); 8·48 (s, 1 H, H-9); 8·53 (d, J = 8 Hz, 1 H, H-5); addition of D_2O to the hexadeuteriodimethyl sulfoxide solution shifted the 6·44, 7·20, 7·72, and 8·48 ppm resonances upfield with respect to the 7·66 and 8·53 ppm signals by about 0 05 ppm. Mass spectrum see Table I.

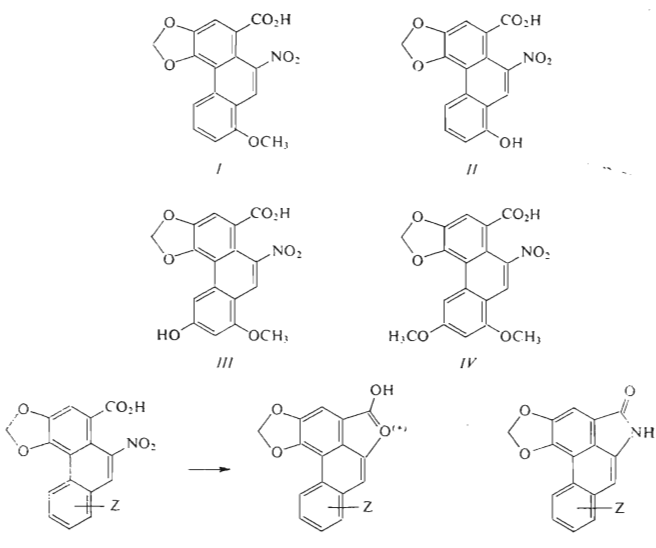
CAB'-1: Mass spectrum see Table I.

CAB'-2: Mass spectrum see Table I.

DISCUSSION

The identity of AA-1 was established by UV, IR, ^{1}H NMR, and high resolution mass spectrometry, as well as by comparison with a reference sample of aristolochic acid I (*I*) (R_F, IR, mixture m.p.).

The UV absorption of AA-2, and the batchochromic shift observed upon the addition of aqueous alkali, suggested a phenolic aristolochic acid structure[9]. It ^{1}H NMR



I *II* *III* *IV*

SCHEME 1

spectrum in hexadeuteriodimethyl sulfoxide showed a two-proton singlet at 6·44 pm corresponding to an aristolochic acid methylenedioxy group[10]. The singlets at 7·72 and 8·48 ppm are assigned to $C_{(2)}$—H and $C_{(9)}$—H, respectively (10). The remaining signals at 8·53 (d, J = 8 Hz, 1 H), 7·66 (t, J = 8 Hz, 1 H) and 7·20 ppm (d, J = 8 Hz, 1 H), are indicative of the presence of three adjacent aromatic ring protons. As their chemical shifts correlate well with those for aristolochic acid I, the phenolic group should be located at $C_{(8)}$, and AA-2 should thus be aristolochic acid Ia (*II*). The *meta* coupling expected between $C_{(5)}$—H and $C_{(7)}$—H is barely observable as a broadening of the corresponding doublets, and the same is true for the ^{1}H NMR spectrum of aristolochic acid I.

On adding deuterium oxide to the ^{1}H NMR sample solution of AA-2, the OCH_2O, $C_{(2)}$—H, $C_{(7)}$—H, and $C_{(9)}$—H signals are shifted with respect to the rest of the spectrum by about 0·05 ppm. Although this solvent effect is small, it is sufficient to make the $C_{(9)}$—H signal clearly distinguishable from the upfield $C_{(5)}$—H doublet component, with which it overlaps in hexadeuteriodimethyl sulfoxide.

All aristolochic acids exhibit strong molecular ions upon electron impact. Their fragmentation is characterized by the loss of NO_2, leading generally to the base peak in the mass spectra, followed by successive eliminaton of small units such as H, CH_3, CO, CHO, CH_2O, yielding ions of much lesser intensities. Additionally, in all the spectra the molecular ion peaks of the similarly substituted aristolactams can be observed, as the latter are produced as minor contaminants during the purification of the acids (Scheme 1).

Comparison of the high resolution mass spectra of AA-1, AA-2, CAB'-1, and CAB'-2 with the tabulated values for aristolochic acid and aristolactam molecular ions and for aristolochic acid $M-NO_2$ ions confirms that AA-1 is very nearly

TABLE II
Aristolochic acids in *A. chilensis* and *B. archidamas* fractions

Fraction	Aristolochic acid[a]			
A. chilensis				
AA-1	*I*	—	IV	—
AA-2	I	*Ia*	—	IVa
B. archidamas				
CAB'-1	*I*	—	IV	—
CAB'-2	—	Ia	IV	*IVa*

[a] The major components are printed in italics.

pure aristolochic acid I, while AA-2 consists almost exclusively of aristolochic acid Ia the major component of CAB'-1 is a monomethoxylated compound, most probably aristolochic acid I, but CAB'-2 contains mainly a hydroxy-methoxy derivative, probably aristolochic acid IVa (*III*). A study of the weaker peaks in the mass spectra of these fractions shows that AA-1 contains a small amount of a dimethoxylated aristolochic acid, presumably aristolochic acid IV (*IV*), which is also present as a minor component of CAB'-1 and -2. AA-2 seems to contain traces of aristolochic acids I and IVa, and aristolochic acid *Ia* seems to be present in CAB'-2. These tentative conclusions are summarized in Table II.

The sequestration of aristolochic acids by papilionid caterpillars is a frequent phenomenon[8,11,12] which has been interpreted as a result of some ancestral butterfly larva with the ability to store these metabolites obtaining immediate protection from predators[13]. As aristolochic acid I is bactericidal[13] and cytotoxic[6], but not acutely toxic to birds[11], it seems possible that the evolutionary advantage conferred by the ability to accumulate such substances may result from their action upon microbial and perhaps invertebrate parasites rather than vertebrate predators.

Although aristolochic acids have been isolated from well over twenty *Aristolochia* species, the aerial parts which serve as food for papilionid larvae have only been studied with positive results in *A. clematitis* (0·26% crude acids, dry wright basis)[11], *A. rotunda* (0·5%) (ref.[11]), *A. acuminata* (0·22%) (ref.[7]), *A. multiflora* (0·015%) (ref.[7]), and now *A. chilensis* (0·025%). The butterfly *Zerynthia polyxona*, reared on *A. clematitis*, contains about 0·15 mg perspecimen[8], an amount found in less than its own weight of fresh plant material. *B. archidamas*, reared on *A. chilensis*, stores approximately 0·2 mg per specimen although the concentration of aristolochic acids in this plant is fairly low, and is thus quite and efficient sequesterer.

Nothing is known so far about the uptake, storage, modification and excretion mechanisms or even the tissue distribution of aristolochic acids in lepidoptereans. In the case of the *B. archidamas* – *A. chilensis* relationship, the near-absence of aristolochic acid Ia in the imago contrasts with its fairly large (20%) contribution to the acid fraction of the leaves and stems, suggesting a high degree of selectivity. On the other hand, the insect stores similar amounts of monomethoxy and hydroxy-methoxy aristolochic acids in spite of the chromatographically obvious difference in lipophilicity. It remains to be seen if these discrepancies in composition reflect some metabolic activity in *Battus* leading to changes in the methylation status of the compounds present in its foodplant. The fact that only hydroxylated aristolochic acids were isolated from *Zerynthia polyxena*[8] reared on *A. clematitis*, whose leaves and stems contain[11] mainly aristolochic acid I, is another indication of selectivity and/or metabolic transformation in the insect.

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