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Fate of Ingested Aristolochic Acids in *Battus archidamas*

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Key Word Index—*Battus archidamas*; Papilionidae; Lepidoptera; aristolochic acids; sequestration; excretion; metabolism.

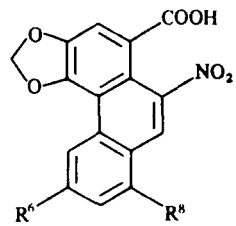
Abstract—*Battus archidamas* larvae reared on *Aristolochia chilensis* sequester aristolochic acids selectively and independently of lipophilicity. A considerable proportion of the ingested acids can be recovered from the excreta, but the amounts which remain unaccounted for suggest that metabolic detoxification is the principal mean of disposing of these substances by the insects.

Introduction

It has recently been shown that wild imagos of the swallowtail butterfly *Battus polydamas* Linn. contain much larger amounts of aristolochic acids than butterflies obtained from caterpillars reared on *Aristolochia elegans,* which is a poor source of these compounds [1]. The wild imagos proceeded from larvae which might have fed on several different *Aristolochia* species of unknown chemical composition. For any conclusions to be reached regarding the absorption and disposal of these substances by *Aristolochia*-feeders, the insects must be reared on a diet containing known amounts of aristolochic acids, large enough for significant incorporations to be measurable. This condition was met by feeding *B. archidamas* Boisd. larvae on their natural foodplant *A. chilensis* Miers, the edible parts of which are fairly rich in aristolochic acids [2].

Results and Discussion

Quantitative analysis of the leaves and stems of *A. chilensis* showed that they contained 0.028, 0.010, 0.006, 0.006 and 0.003 per cent of the aristolochic acids I (**1**), II (**2**), IIIa (**3**), IVa (**4**) and Ia (**5**), respectively (dry weight basis). Seventy eight second- and third-instar *B. archidamas* caterpillars feeding in the wild on *A. chilensis* were transferred to the laboratory and reared on fresh *A. chilensis* until they pupated. The live pupae, weighing 96.2 g, were frozen in liquid nitrogen and lyophilized to give 22.7 g of a powder which was analysed by the same method as the plant material. The dried frass weighed 109 g and was analysed similarly. The results are shown in Table 1 together with estimates of the amounts of aristolochic acids ingested and with the experimentally determined lipophilicities of the acids [3].



	R^6	R^8
1	H	OMe
2	H	H
3	OH	H
4	OH	OMe
5	H	OH

The results show that the overall sequestration of dietary aristolochic acids by *B. archidamas* larvae only amounts to about two per cent of the total dose. Nearly a quarter of the acids consumed is excreted, and a much larger proportion remains unaccounted for. It may be inferred that most of this 'lost' material has been metabolised to unidentified compounds. It must be remembered that efficient metabolic inactivation of phototoxic furanocoumarins underlies

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TABLE 1. SEQUESTRATION AND EXCRETION OF DIETARY ARISTOLOCHIC ACIDS BY *BATTUS ARCHIDAMAS* LARVAE*

Acid†	Ingested‡	Sequestered	Excreted	Lost§	$K_{0.50}$¶
AA–I (1)	37.0	0.55	7.56	28.8	0.152
AA–II (2)	14.0	0.05	7.03	6.9	−0.061
AA–IVa (4)	8.0	0.53	0.77	6.7	−0.252
AA–IIIa (3)	8.0	0.09	0.28	7.6	−0.495
AA–Ia (5)	4.5	0.23	1.40	2.9	−0.770
Total	71.5	1.56	17.04	52.9	—

*mg per 78 larvae from second/third instar to pupation.

†AA = Aristolochic acid (see Formulae).

‡Estimated from the added dry weights of *B. archidamas* pupae and faeces and from the composition of edible parts of *A. chilensis*.

§Lost = ingested—(sequestered + excreted).

¶Logarithm of chromatographic capacity factor determined as described in ref. [3].

the ability of other Papilionid caterpillars to thrive on plants protected by these compounds [4]. Our present results suggest that insects feeding exclusively on *Aristolochia* may similarly depend on enhanced detoxification mechanisms to exploit this ecological niche.

It is reasonable to assume that some of the aristolochic acids may be interconverted metabolically by the insect and/or its gut symbionts. In particular, aristolochic acid II (**2**) could be hydroxylated to aristolochic acids Ia (**5**) and/or IIIa (**3**) and aristolochic acids I (**1**) could be demethylated to aristolochic acid Ia (**5**). With this caveat in mind, it is noteworthy that about half of the ingested aristolochic acid II (**2**), which is not oxygenated in ring C and therefore probably less susceptible to attack by detoxifying enzymes, is excreted unchanged. At the same time, this acid is retained by *B. archidamas* to a lesser extent than any of the others. This, finding together with the low proportion of aristolochic acid I (**1**) sequestered as such by the insects, show that in this series of compounds lipophilicity does not favour retention, which is therefore not heavily dependent on passive transport through biological membranes. Further support for this conclusion can be found in the fact that aristolochic acid IIIa (**3**) is retained much less than either aristolochic acid IVa (**4**) or Ia (**5**) although its lipophilicity is intermediate between those of the latter compounds. The sequestration of aristolochic acids by *B. archidamas* must therefore depend primarily upon structural aspects not directly related to partition between aquaeous and lipidic phases.

Wild *B. polydamas* imagoes were found to contain much larger amounts of aristolochic acids I (**1**), II (**2**) and IIIa (**3**) than the *B. archidamas* pupae analysed in the present work [1]. Similarly, *Pachlioptera aristolochiae* butterflies only afforded aristolochic acid I (**1**) [5] along with a trace of aristolochic acid II (**2**) [1], suggesting that other analogues, if present, were less abundant. On the other hand, *Zerynthia polyxena* butterflies reared on *A. clematitis,* a plant which is known to contain mainly aristolochic acid I (**1**) in its leaves and stems [5], only gave aristolochic acids Ia (**5**) and IIIa (**3**) [6], the latter containing some aristolochic acid IVa (**4**) [1]. It will be interesting to determine the relative importance of diet and biochemical differences between Papilionid species in causing these variations.

Experimental

Materials. Green parts of *Aristolochia chilensis* were collected twice a week by Lo Prado Pass near Santiago, Chile (33° 10′ S, 71° W), between November 1984 and January 1985. The plant material used for the quantitative determination of aristolochic acids was immediately dried in an oven at 50°.

Second- and third-instar *Battus archidamas* larvae (78) were collected together with their food at the same location in November, 1984, and transferred immediately to 11 gauze-covered transparent plastic boxes. The boxes had a hole that allowed the leafy stems destined as food for the caterpillars to be kept fresh by dipping their cut ends in flasks containing tap water.

The faeces produced by the larvae were collected twice daily and dried in an oven at 50° until the insects pupated. The pupae (96.2 g) were frozen in liquid nitrogen and lyophilized to give a powder (22.7 g). The dried frass weighed 109 g. Authentic samples of aristolochic acids I (**1**), II (**2**), IIIa (**3**), IVa (**4**) and Ia (**5**), isolated previously from *A. chilensis* [2] and *A. argentina* [7], were used as chromatographic standards.

General. Analytical and prep. TLC were carried out on 20×20 cm A1 foils or glass plates (Merck silica gel 60 F-254, 0.2 and 0.5 mm thickness, respectively). Chromatograms were developed 5–6 times with $CHCl_3$–MeOH (85:15) or $CHCl_3$–MeOH (2:1), saturating the chamber with NH_3 in the latter case. HPLC was carried out on a Waters μBondapak C-18 column, 30×0.45 cm, eluting isocratically with MeOH–0.1 M ammonium phosphate buffer, pH 6.60 at 20° (1:1). The aristolochic acids isolated from the different materials, were identified by its spectroscopic properties, and comparison with authentic samples, as it had been previously described [1, 2].

Extraction and quantification of aristolochic acids. Dry, powdered *Aristolochia* and *Battus* materials were defatted with pentane, air-dried, and exhausted with EtOH (Soxhlet). The EtOH extracts were concd to dryness under red. pressure, the residues were partitioned between 3% $NaHCO_3$ and $CHCl_3$, the basic solutions were adjusted to pH 3 with conc HCl and extracted first with $CHCl_3$ and then with $CHCl_3$–EtOH (3:2). The crude acid fractions from *A. chilensis* were separated by CC on silica gel followed by prep. TLC. The individual compounds

were quantified spectrophotometrically. The plant material (160 g) afforded aristolochic acids I (1) (45.6 mg), II (2) (16.3 mg), IIIa (3) (9.5 mg), IVa (4) (9.6 mg) and Ia (5) (5.3 mg). The acids of *B. archidamas* pupae and faeces were subjected directly to preparative TLC and quantified spectrophotometrically and by HPLC (photometric detection at 254 nm). The results are shown in Table 1.

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