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Direct *in vitro* and *in vivo* monitoring of destruxins metabolism in insects using internal surface reversed-phase high-performance liquid chromatography

I. Behaviour of E destruxin in locusts

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ABSTRACT

High-performance liquid chromatography with internal surface reversed-phase packing provides an analytical tool for studying the *in vitro* and *in vivo* metabolism of A and E destruxins in the haemolymph and various organs of male adults of *Locusta migratoria*. A slight amount of injected E destruxin is shown to be hydrated into E-diol destruxin in the haemolymph. The rest of the toxin is recovered unchanged in the fat-body, pericardial tissues and Malpighian tubules, and some further E-diol destruxin formation occurs in these organs. Because E-diol destruxin is only weakly toxic, this appears to be a detoxication process.

INTRODUCTION

Metharrhizium anisopliae fungus produces cyclodepsipeptides, named destruxins [1,2]. These compounds are composed of five amino acids and an α-hydroxy acid and exhibit a wide range of biological properties, such as cytostatic or cytotoxic effects on mouse leukemia cells [3], immunodepressive activity toward insect models [4] and insecticidal effects [5]. Moreover, in *Galleria mellonella* larvae, the tetanic paralysis triggered by destruxin ingestion was demonstrated as reversible,

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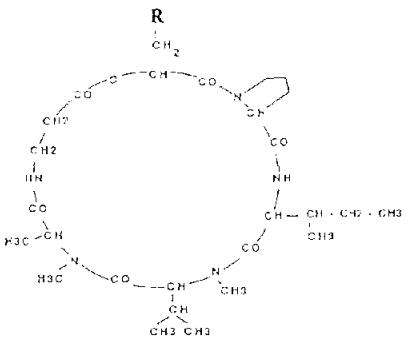
so a metabolic pathway of detoxication was suggested [6,7]. In locusts, the possible sites of detoxication are the haemolymph, because of the presence of various enzymes, or organs that are able to capture and/or damage xenobiotics [8,9]. To validate this detoxication hypothesis, it is necessary to examine the behaviour of destruxins in insect haemolymph or organs.

In vivo behaviour of natural [10] or synthesized compounds [11] exhibiting pesticidal activity were studied in arthropod haemolymph. To avoid the pretreatment of the biological fluids, xenobiotics were monitored directly by different analytical techniques such as high-field proton nuclear magnetic resonance (^{1}H NMR) [12,13], fast-atom bombardment mass spectrometry (FAB-MS) [14,15] and internal surface reversed-phase (ISRP) high-performance liquid chromatography (HPLC) [16,17]. The combined use of size-exclusion chromatography and reversed-phase chromatography allows the separation of the small molecules as analytes, while the large molecules are eluted in the void volume of the column. The ISRP method, initially designed for the direct study of various drugs and of their metabolites in protein matrices, such as human serum or plasma [18,19], was transposed to the direct *in vitro* routine analysis of insecticides in insect haemolymph [16], of fungicides in fungi [17], and later for an *in vitro* study assisted by FAB-MS [14] of E destruxin in the haemolymph of the locust *Schistocerca gregaria*.

Destruxins obtained from *Metarrhizium anisopliae* fungi after multi-stage purification and extraction procedures, have been previously analysed by reversed-phase HPLC. C_{18} packings require a significant proportion of organic modifier in the eluent, justifying the pretreatment of the samples prior to chromatographic injection. This procedure allowed the detection of microgram amounts of destruxins in fungal culture filtrate and insect blood [20]. However, this approach has several disadvantages, such as the rather laborious protocol and possible poor extraction yields in the case of most polar destruxins [21].

ISRP was chosen for its simplicity and rapidity, and also for the possible low detection limit for xenobiotics in biological media, despite the poor resolution usually resulting from this packing. This paper describes the direct and rapid monitoring of the A and E destruxins (Fig. 1) in haemolymph and in various organs of *L. migratoria* (fat-body, pericardial tissues and Malphighian tubules, [8,9]), by ISRP-HPLC. The first results of the monitoring of these destruxins in locust biological media, without any extraction or purification prior to HPLC injection, are reported. The principal aims were to test the feasibility of this approach, to define procedures for the biological samples and conditions for chromatographic separations, and to get qualitative insights into the *in vivo* behaviour of destruxins in different locust organs.



Destruxin	R
A	-CH=CH$_2$
E	-CH-CH$_2$ (epoxide)
E-diol	-CH(OH)-CH$_2$(OH)

Fig. 1. Structures of A and E destruxin and E-diol destruxin.

EXPERIMENTAL

Materials

Locusts were kept in crowded conditions [22] with a 10-h light and 14-h dark cycle, and fed on grass. Either male *Schistocerca gregaria* (Vth larval instar) of the African migratory locust or *L. migratoria* migratoïdes (Vth larval instar and three-week-old adults) were used. Fresh and dry locust weights are given in Table I.

Reagents and chemicals

The standards of A, E and E-diol destruxins were purified as previously described [23]. The positive- and negative-ion FAB mass spectra (P-FAB and N-FAB) were obtained with a quadrupolar mass spectrometer, NERMAG R-10-10-C, equipped with a Spectral-30-data system and an M-SCAN atom gun (<Xe> 8 keV, 200 μA, glycerol matrix) [24,25]

For A destruxin: P-FAB = MH^+ 578 (30), 590 (3), 552 (3), 550 (1), 465 (10),

TABLE I

ORGAN WEIGHTS OF ADULT LOCUSTS

Total weight of a set of three locusts was 4540 mg; total volume of their haemolymph was estimated as 3 × 200 μl [9]. The ratio (haemolymph/total weight) × 100 is *ca.* 13%.

Extracted organ (three locusts)	Fresh weight, *p* (mg)	Dried weight (mg)
Fat-body	90	60
Pericardial tissues	20	10
Malpighian tubules	20	10
Residue	4010	2500

437 (5), 224 (3); N-FAB = $[M-H]^-$ 576 (50), 496 (10), 399 (1), 286 (2), 173 (10), 88 (20).

For E destruxin: P-FAB = MH^+ 594 (50), 566 (3), 481 (3), 453 (8), 368 (3), 340 (3), 255 (3); N-FAB = $[M-H]^-$ 592 (100), 496 (35), 399 (10), 286 (35), 173 (20), 88 (20).

For E-diol destruxin: P-FAB = MH^+ 612 (100), 594 (20), 584 (10), 527 (3), 499 (40), 481 (30), 414 (12), 402 (10), 386 (30), 358 (10), 289 (60), 212 (18), 199 (60), 114 (10); N-FAB = $[M-H]^-$ 610 (100), 496 (65), 399 (10), 286 (30), 173 (70), 88 (10).

The ^1H NMR spectra of destruxins have been described previously [23].

Columns

The HPLC columns, a Regis Pinkerton GFF ISRP 5-μm (50 mm × 4.6 mm I.D.) analytical column, a Regis Pinkerton GFF ISRP 5-μm (150 mm × 4.6 mm I.D.) analytical column and a Regis Pinkerton GFF ISRP 5-μm (10 mm × 3 mm I.D.) guard-column were manufactured by Regis and supplied by Touzart et Matignon (Vitry sur Seine, France). HA- and FH-type filters (0.45 and 0.5 μm, respectively) were supplied by Millipore (Molsheim, France). Organic solvents of chromatographic grade were supplied by SDS (Vitry sur Seine, France) and filtered through a 0.5-μm FH Millipore membrane; 18-MΩ water was deionized (Waters Milli Q apparatus) and filtered through an HA Millipore membrane.

In vitro experiments

Plasma samples. Samples from *S. Gregaria,* male Vth larval instar, were obtained by centrifuging (500 *g*, 5 min) the crude haemolymph sample drawn off from the neck area of the locust [12]. The supernatant was used immediately and diluted three-fold, either in phosphate buffer (0.1 *M*, pH 7.4) for the blank, or in an E destruxin phosphate buffer solution (final concentration $1.5 \cdot 10^{-3} M$).

Crude haemolymph samples. Samples from *L. migratoria* locusts, male Vth larval instar or male adult, were obtained as previously. The final destruxin concentrations in crude haemolymph half-diluted in phosphate buffer were $2.50 \cdot 10^{-3}$ and $6.67 \cdot 10^{-3}$ *M* for A and E destruxins, respectively. Haemolymph solutions were centrifuged just before chromatographic injection.

In vivo experiments

Preparation of the biological samples. The sample (x µl, Table II) of aqueous destruxin solution was syringe-injected into a homogenous group of Vth larval instars or adult males between two abdominal pleurits. The locusts were then divided into three sets. Each set was left at room temperature for different periods, then their haemolymph or organs were sampled or dissected.

Haemolymph sampling. The samples (y µl) of haemolymph drawn-off from the neck area of three locusts with a graduated microcapillary were rapidly diluted in water or buffer (z µl, Table II). Haemolymph solutions were centrifuged just before chromatographic injection.

TABLE II

SAMPLING OF HAEMOLYMPH AND TISSUES DURING THE *IN VIVO* STUDY OF E DESTRUXIN IN LOCUSTS

Instar of locust	Destruxin type	[Destruxin] · 10^2 (M)	Injected volume, x (μl)	Mixtures analysed[a]
Haemolymph				
Vth	A	1.1	10	y = 80 μl + z = 80 μl buffer
Vth	E	1.1	10	
Adult	E	4.4	20	y = 60 μl + z = 40 μl water
Tissues and organs				
Adult	A	1.1	10	p + 200 μl buffer
Adult	E[b]	4.42	20	p + 100 μl water

[a] y is the volume of haemolymph drawn off and diluted immediately; z is the volume of water or buffer added to y; p is the weight of organ or tissues listed in Table I.

[b] The same conditions were used for the study of E-diol destruxin.

Organ sampling. The dissected issues or organs of each set of insects (p mg) were pooled in water or buffer (z μl), then sonicated for 5 min and centrifuged. With the Malpighian tubules and the pericardial tissues, the supernatant was used for injection. With the fat-body, the injected phase corresponded to the fraction of intermediate density between the lipidic supernatant (top) and the tissues fragments (bottom) (Table II).

Instrumentation and chromatographic conditions

The Beckman HPLC system consisted of a Model 112 pump with a Model 421 monitor and of an Altex valve equipped with a 5-μl loop as the injector. The UV detector was a Model 165 and was set at 230 nm, with sensitivities corresponding to the range 0.5–0.02 a.u.f.s.

The various destruxins (A, E and E-diol) were identified in the chromatograms of the various biological media by comparison with authentic samples. Owing to the uncertainties arising from the biological samples, precise quantitation of the destruxins was not attempted in haemolymph or the organs. Rough estimates of concentrations were obtained by comparison with external standards (surface method).

RESULTS AND DISCUSSION

Chromatographic conditions and the biological medium

As expected, because of the weak lipophilic character of the GFF-type ISRP

packing compared with conventional C_8 or C_{18} packings, low percentages (2–5%) of an organic modifier were required for the elution of A and E destruxins to obtain their correct retention times (up to 50% was used by previous workers [20]). So, analysis of the destruxins in the presence of proteins is made possible, with a minor risk of denaturation since the recommended limit is less than 20%.

The blank plasma chromatograms of *S. gregaria* over a 24-h period (Fig. 2A) demonstrate the stability of the biological liquid medium, except the modification of retention concerning the more lipophilic components (L peak: lipids and lipoproteins [8]).

In the locust haemolymph, quantitative and qualitative fluctuations (composition and retention) were observed within a given species, as well as inter-species differences (*L. migratoria* or *S. gregaria*) for the lipid (L) and for the protein (P) constituents. Such differences in locust haemolymph composition have also been observed during comparative ^{1}H NMR analysis [13].

Behaviour of destruxins in plasma of S. gregaria

There is no significant evolution of the A and E destruxins in plasma, (Fig. 2B for the E case) within a period of 24 h. This result has been confirmed by FAB-MS experiments [14,15].

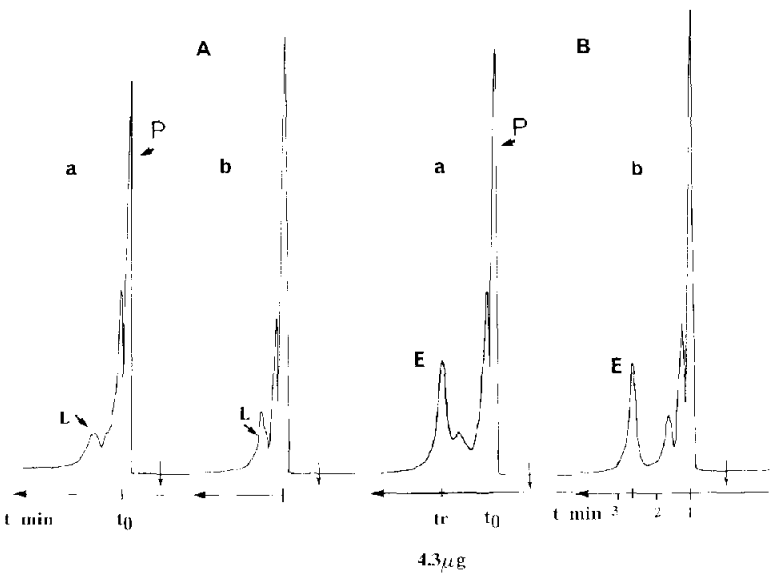


Fig. 2. Behaviour of E destruxin in plasma of Vth larval instar locusts (*Schistocerca gregaria*). (A) Blank plasma: (a) time 0; (b) at 24 h. (B) The same plasma with $1.5 \cdot 10^{-3}$ *M* destruxin added: (a) time 0; (b) at 24 h. t_R and t_0 are retention times of destruxin and unretained components, respectively. Peaks P and L are the protein and lipid constituents of plasma. Chromatographic column: Pinkerton analytical column (5 µm, 50 × 4.6 mm I.D.) equipped with a 5 µm Pinkerton guard-column, 10 × 3 mm I.D. (column set A). Eluent: 2% THF- 2-propanol (6:10, v/v) in phosphate buffer (0.1 *M*, pH 7.4), flow-rate 0.6 ml/min. UV detector set at 230 nm and 0.2 a.u.f.s. HPLC injection, after centrifugation, of 5-µl aliquots of plasma or incubated plasma.

In vitro behaviour of A and E destruxins in crude haemolymph of L. migratoria

This absence of evolution might be imputed to the haemolymph pretreatment. So, subsequent assays were done on crude locust haemolymph instead of plasma. We also exchanged *S. gregaria* for *L. migratoria* as the model insect by supposing a more important enzymic activity in the latter case, based on ^{1}H NMR observations concerning esterasic activities [13].

Subsequently, *in vitro* monitoring was performed with crude haemolymph of *L. migratoria*. Within a period of 3 h, there was no significant evolution in the chromatograms corresponding to the A and E destruxins (Fig. 3).

These experiments show the feasibility of direct monitoring of destruxins in haemolymph without the need for extraction or purification, and it may be deduced that the enzymic systems in haemolymph were not mainly concerned in the detoxication of the destruxins. It seemed necessary to perform *in vivo* monitoring in injected locusts, since important differences of metabolism in haemolymph have been previously observed between *in vivo* and *in vitro* experiments [13].

In vivo behaviour of A and E destruxins in haemolymph of injected locusts (L. migratoria)

With Vth larval instar, the concentration is time-dependent for the two de-

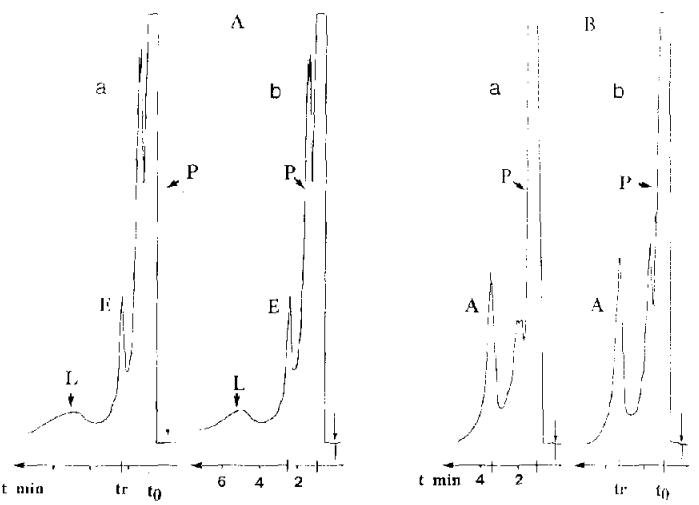


Fig. 3. *In vitro* behaviour of destruxins in crude haemolymph of Vth larval instar locusts (*L. migratoria*) half-diluted in phosphate buffer (0.1 *M*, pH 7.2). (A) E destruxin $6.67 \cdot 10^{-3}$ *M*: (a) at time 0; (b) at 3 h. (B) A destruxin $2.50 \cdot 10^{-3}$ *M*: (a) at time 0; (b) at 3 h. Chromatographic column: Pinkerton analytical column (5 μm, 50 mm × 4.6 mm I.D.) equipped with a 5-μm Pinkerton guard-column (10 mm × 3 mm I.D.) (column set B). UV detector set at 230 nm and 0.05 a.u.f.s. HPLC injection, after centrifugation, of 5-μl aliquots of haemolymph or incubated haemolymph. Eluents: (A) 2% THF–2-propanol (6:10, v/v) in phosphate buffer (0.1 *M*, pH 7.2), flow-rate 0.5 ml/min; (B) 5% THF–2-propanol (6:10, v/v) in phosphate buffer, flow-rate 0.6 ml/min. t_R and t_0 are retention times of destruxin and unretained components, respectively. Peaks P and L are the proteic and lipid constituents of haemolymph. Capacity factors: $k'_E = 1.63$; $k'_A = 2.36$.

TABLE III

BEHAVIOUR OF A DESTRUXIN IN INJECTED Vth LARVAL INSTAR LOCUSTS

Estimation of the change of the concentration of A over time. Injection of 10 μl of $1.1 \cdot 10^{-2}$ *M* phosphate buffer solution of A into a set of three locusts. Assuming a total volume of 200 μl of haemolymph for each locust and the confinement of A destruxin to this fluid, the [A] in haemolymph would be $5.5 \cdot 10^{-4}$ *M* at the beginning, assuming also perfect and rapid diffusion throughout the haemolymph.

Time after injection (h)	[A] · 10^4 *M* estimated by HPLC in haemolymph samples[a]	[A] · 10^4 *M* estimated in haemolymph of living locust[b]	Decrease (%)
0.5	0.9(5)	1.9	*ca.* 5
1	0.9	1.8	*ca.* 11
2	0.8	1.6	*ca.* 13
5	0.7	1.4	

[a] By comparing the peak surface of $3.67 \cdot 10^{-4}$ *M* A destruxin external standard with A destruxin solutions obtained by dilution in phosphate buffer (1:1, v/v) of the haemolymph sampled from the injected locusts.

[b] By taking into account the two-fold dilution before chromatographic injection (see also Table II).

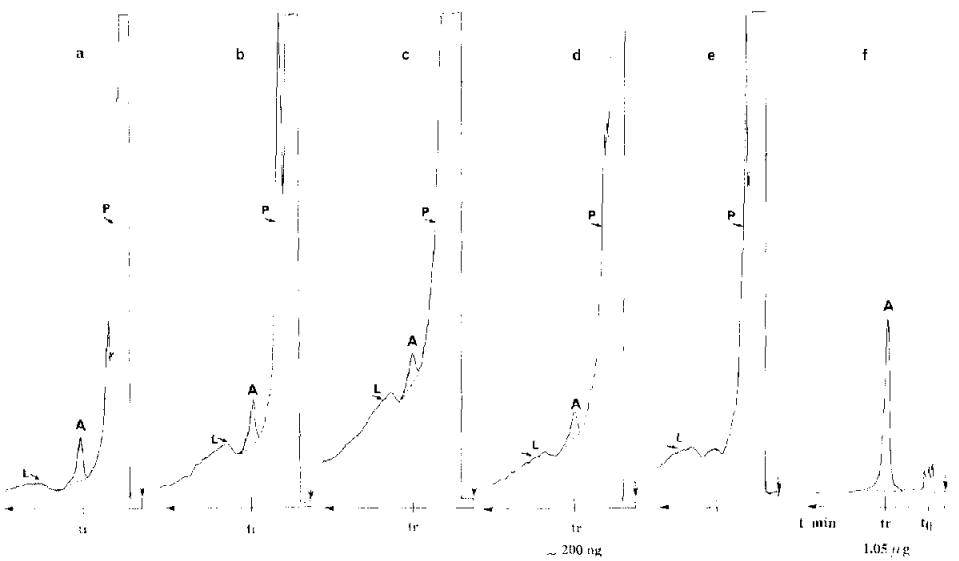


Fig. 4. Behaviour of A destruxin in injected Vth larval instar locusts (*L. migratoria*) (*cf.* Table II): (a) 0.5 h; (b) 1 h; (c) 2 h; (d) 5 h; (e) Blank haemolymph; (f) standard $3.67 \cdot 10^{-4}$ *M* in buffer (at 0 h and 5 h). Chromatographic conditions: column set as in Fig. 3; 5% organic modifier; 0.05 a.u.f.s.: HPLC injection, after centrifugation, of 5-μl aliquots of sample prepared according to Table II.

struxins. With A destruxin, there is a slow evolution at the beginning, and about a 30% decrease within 5 h (Table III, Fig. 4a–d). The A destruxin concentration in the 5-h sample was estimated as $7 \cdot 10^{-5}$ *M*, *i.e.* 200 ng per injection (Fig. 4d). This result is an indication of the possibilities of the method in comparison with the previous one described by Samuel *et al.* [20] which leads to a detection limit of 1 μg. Moreover, a systematic deficit in the amount of A destruxin (Table III) was observed, even at the beginning of the evolution. This may be explained by the low proportion of haemolymph in the total weight of locust (*ca.* 13%, Table I), and by possible diffusion toward various organs.

With E destruxin the decrease is more pronounced (15–20% between the 0.5-h and the 1-h samples, Fig. 5a and b).

With adult locusts, the decrease in E destruxin content is apparently of the same importance as in the previous case. However, the FAB-MS study [15] has revealed the intervention of a metabolic process as a reason for this decrease, since a new ion corresponding to the hydrated E molecule appears at *m/z* 612 in the mass spectrum of the 1-h sample.

The preceding results show differences between assays of samples from larvae and adults with the E destruxin. One interpretation would be that enzymes are more active in the adults.

The absence of evolution in haemolymph for *in vitro* assays in contrast with *in*

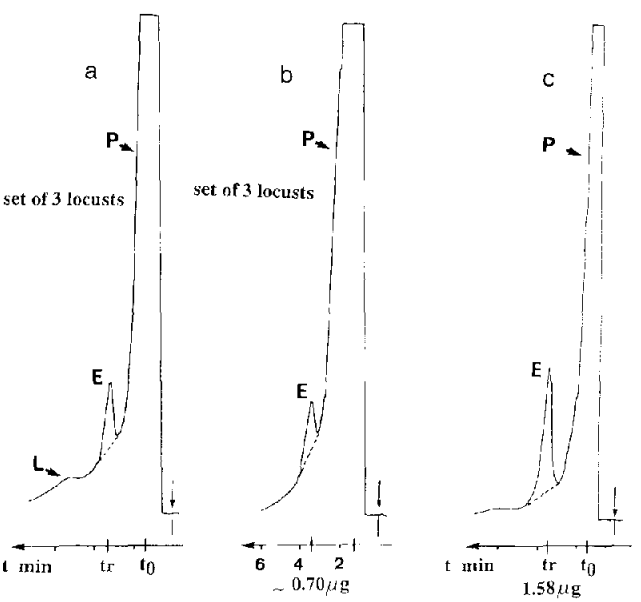


Fig. 5. Behaviour of E destruxin in injected Vth larval instar locusts (*L. migratoria*) (*cf.* Table II): (a) 30 min; (b) 1 h; (c) overloading of the blank ($[E]_{final} = 5.33 \cdot 10^{-4}$ *M*). Chromatographic conditions: same column set as in Fig. 3 and 4; same eluent as in Fig. 3A; flow-rate 0.5 ml/min; 0.1 a.u.f.s.; HPLC injection as in Fig. 4.

vivo assays, may be explained by considering that haemolymph acts as a simple carrier of the toxin towards various organs and tissues that trigger enzymic transformations. Subsequently, haemolymph may also act as a carrier of the toxin metabolites towards excretory organs.

Thereafter, the study was focused on the *in vivo* monitoring of E destruxin and its possible metabolites, in organs or tissues of the adult *L. migratoria*, in order to take into account both the best conditions for metabolism and the best insecticidal acitivity of the destruxin.

Behaviour of E destruxin in detoxication and/or excretion organs of L. migratoria adults

Monitoring of E destruxin in organs. A homogenous set of *L. migratoria* adults were injected with E destruxin solution. After 30 min and 1 h, the three insects of each set were killed, and their organs were dissected, pooled in water, sonicated and centrifuged. The analysis was carried out under the previous operating chromatographic conditions (2% of organic modifier).

E destruxin was detected in all the samples, and the amounts decreased with time (Fig. 6, Table IV). The fat-body showed the higher content of E destruxin and a greater decrease than the other organs or haemolymph (*e.g.* 30% in Fig. 6Aa and b and 15–20% in Fig. 5a and b).

The parallel *in vivo* FAB-MS study confirmed these observations. Moreover, it disclosed an enzymic hydrolysis of the epoxide function of E destruxin, leading to formation of the E-diol destruxin metabolite (Fig. 7) [15].

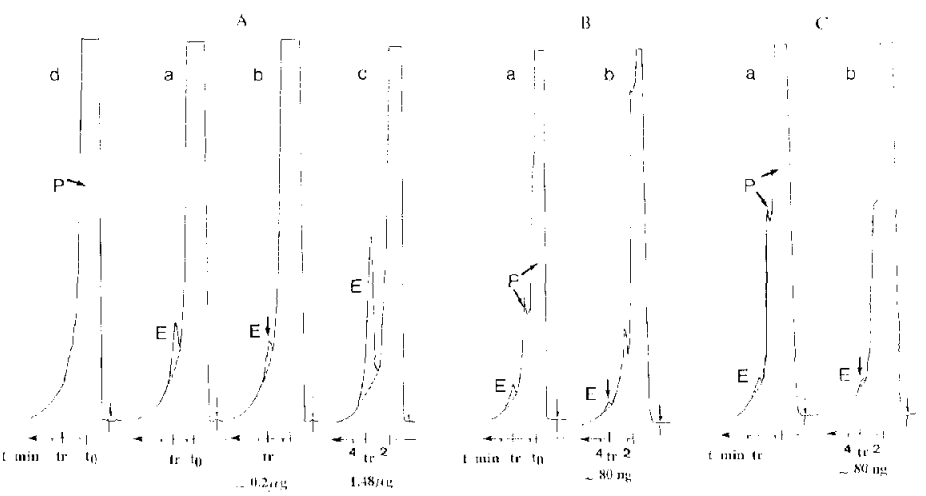


Fig. 6. Distribution of E destruxin in organs and tissues of injected locusts (*L. migratoria*) (*cf.* Table II). (A) Fat-body: (a) 30 min; (b) 1 h; (c) overloading of the blank ($[E]_{final} = 5 \cdot 10^{-4}$ *M*); (d) blank. (B) Malpighian tubules: (a) 30 min; (b) 1 h. (C) Pericardial tissues: (a) 30 min; (b) 1 h. Chromatographic conditions: column set, eluent and flow-rate as in Figs. 4 and 5; 0.1 a.u.f.s.; HPLC injection of 5-µl aliquots of sample prepared according to Table II.

TABLE IV

PRESENCE OF E DESTRUXIN IN ORGANS OF INJECTED ADULT LOCUSTS (FIG. 6)

Fig. 6	Fat-body		Malpighian tubules		Pericardial tissues	
	[E] · 10^4 M^a	Evolution (%)	[E] · 10^4 M^a	Evolution (%)	[E] · 10^4 M^a	Evolution (%)
(a) 0.5 h	1.03	*ca.* −30	0.3(3)	*ca.* −10	0.4(1)	*ca.* −30
(b) 1 h	0.7		0.2(9)		0.2(9) (*ca.* 80 ng)	
(c) Ref.	5 (1.48 μg)		5		5	

[a] Precise concentrations and (amounts) in case (c). Estimated concentrations and (amounts) in cases (a) and (b).

Monitoring of E-diol destruxin in organs. The HPLC experiments were extended to the monitoring of E-diol destruxin formation in the locust organs.

The eluent strength was optimized with an authentic sample of E-diol destruxin polar metabolite. Consequently, the buffer eluent was used without an organic modifier. These conditions entailed the retention of the E destruxin, and an effective presence of E-diol destruxin was detected in all the organs and tissues, especially in the Malpighian tubules and the fat-body (Fig. 8, Table V). The amount of this metabolite is almost constant in the latter, and seems to increase in pericardial tissues. Owing to the small amounts of the metabolite and to the superposition of other components, these HPLC observations required confirmation by FAB-MS analysis [15]. The behaviour of E destruxin in these two organs can be ascribed to a detoxication process; E destruxin is metabolized to E-diol, which has lower toxic activity in various insects [5].

Malpighian tubules contain significant amounts of E-diol (Fig. 8B, a and b). The decrease of this level with time may be interpreted as the excretory function of this organ.

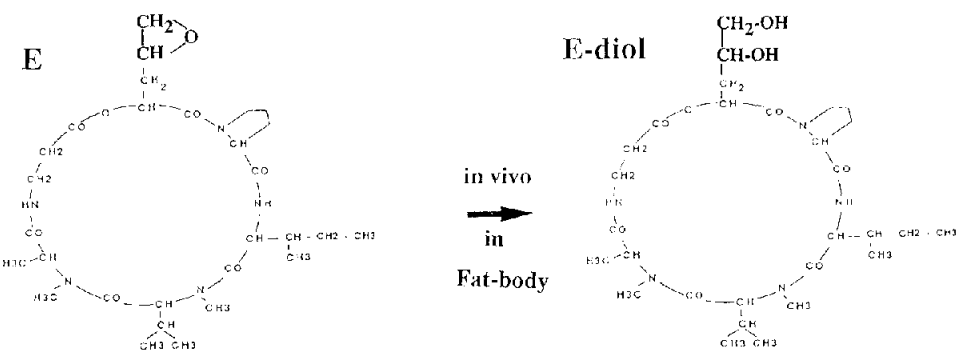


Fig. 7. Transformation of E destruxin into E-diol destruxin.

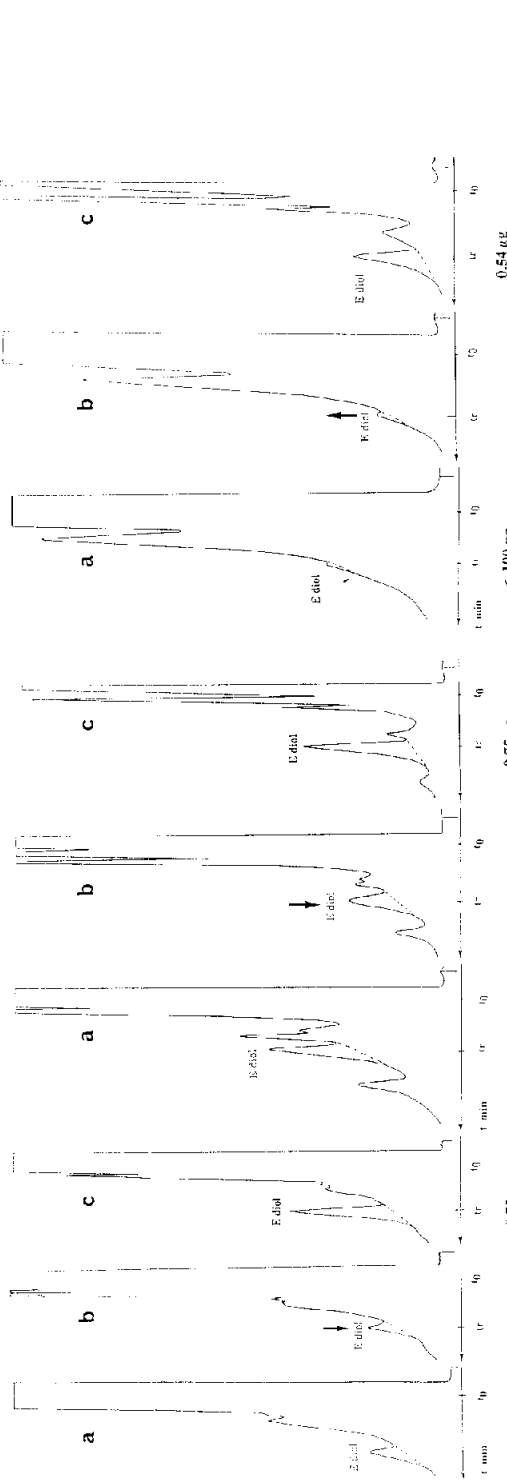


Fig. 8. Distribution of E-diol in organs and tissues of adult locusts (*L. migratoria*) injected with E destruxin (*cf.* Table II). (A) Fat-body: (a) 30 min; (b) 1 h; (c) overloading of the blank ($[E\text{-diol}]_{final} = 2.45 \cdot 10^{-4}$ *M*). (B) Malpighian tubules: (a) 30 min; (b) 1 h; (c) overloading of the blank ($[E\text{-diol}]_{final} = 2.45 \cdot 10^{-4}$ *M*). (C) Pericardial tissue: (a) 30 min; (b) 1 h; (c) overloading of the blank ($[E\text{-diol}]_{final} = 1.78 \cdot 10^{-4}$ *M*). Chromatographic conditions. Pinkerton analytical column (5 µm, 150 mm × 4.6 mm I.D.) equipped with a 5-µm Pinkerton guard-column 10 × 3 mm I.D. (column set C); eluent, phosphate buffer (0.1 *M*, pH 7.4); flow-rate, 0.6 ml/min; UV detector set at 230 nm and 0.02 a.u.f.s.; HLPC injection as in Fig. 6, *cf.* Table II. E destruxin is not eluted under these conditions.

TABLE V

FORMATION OF E-DIOL DESTRUXIN IN ORGANS OF INJECTED ADULT LOCUSTS (FIG. 7)

Fig. 7	Fat-body		Malpighian tubules		Pericardial tissues	
	[E] · 10^4 M^a	Evolution (%)	[E] · 10^4 M^a	Evolution (%)	[E] · 10^4 M^a	Evolution (%)
(a) 0.5 h	0.9	*ca.* −10	2.2(4)	*ca.* −40	0.1(6) (*ca.* 50 ng)	*ca.* +50
(b) 1 h	0.8		1.3(7)		0.3(5)	
(c) Ref.	2.45 (0.75 μg)		2.45		1.78 (0.54 μg)	

[a] Precise concentrations and (amounts) in case (c). Estimated concentrations and (amounts) in cases (a) and (b).

CONCLUSION

The results of this preliminary study, using direct ISRP-HPLC to monitor the behaviour of destruxins in locust organs and tissues, demonstrate the applicability of this method to biological media that are more difficult to sample than haemolymph, and confirm its advantages.

Thus, without any of the extraction and purification required in the conventional approach, amounts of 0.1 μg per injection have been directly detected by this method, even of the polar E-diol destruxin.

The ease and rapidity of the protocols permitted an initial evaluation of the importance of various biological media in the metabolism of E destruxin in the locust. Among the various organs assumed to possess storage, detoxication or excretion functions, the fat-body showed great affinity towards E-destruxin, resulting in storage and enzymic hydrolysis of E destruxin, providing an efficient detoxication route. Pericardial tissues also seem to exert a detoxication effect, with an increase in the E-diol content. The Malpighian tubules tissues contain both E destruxin and E-diol destruxin, and a decrease in the levels of these two compounds can be interpreted as an excretory function of these tissues.

Further investigations of the *in vivo* behaviour of these peptides in various biological media are presently being undertaken with a view to establishing the relative contribution of each organ and, in particular, the excretion routes. Other insects also are under investigation.

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