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BIOMEDICAL APPLICATIONS

Non-extractive metabolism study of E and A destruxins in the locust, *Locusta migratoria L.*

III. Direct high-performance liquid chromatographic analysis and parallel fast atom bombardment mass spectrometric monitoring

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Abstract

To study the behavior of the toxins E and A destruxin in biological media, a method was developed with direct injection on to a liquid chromatographic (LC) column. Either slightly lipophilic C_1 and C_4 "wide-pore" packings or the column-switching approach with a guard column were used. To confirm the results of the direct LC analysis, a "classical approach" with pretreatment prior to injection on to a C_8 packed column was also developed. Further, parallel fast atom bombardment MS monitoring in the negative-ion mode was carried out on the same biological samples, to obtain complementary information on the destruxin metabolism in locusts. Thus, the behaviours of E and A destruxin were examined *in vivo* in different organs of locusts. For E destruxin, several detoxication processes could be observed, such as hydrolysis and conjugation with glutathione.

1. Introduction

The entomopathogenous fungus *Metarhizium anisopliae* produces a series of toxic substances named destruxins (DTXs) [1–3], which are cyclodepsipeptides constituted by five amino acids and an α-hydroxy acid [3] (Fig. 1).

In addition to biological properties such as cytotoxic and immunodepressive actions [4,5] and antimicrobial and antiviral effects [6], DTXs also exhibit insecticidal activity [7]. Thus, after ingestion of DTXs, *Galleria mellonella* larvae show a tetanic paralysis [8]. As the paralysis was reversible, a detoxication process was suggested. Thus, the study of the *in vivo* behaviour of toxins in insects appeared necessary.

For some years we have been interested in metabolic pathways concerning various xenobiotics, proinsecticides or pesticides in locusts [9–13]. To examine the metabolism of unlabeled

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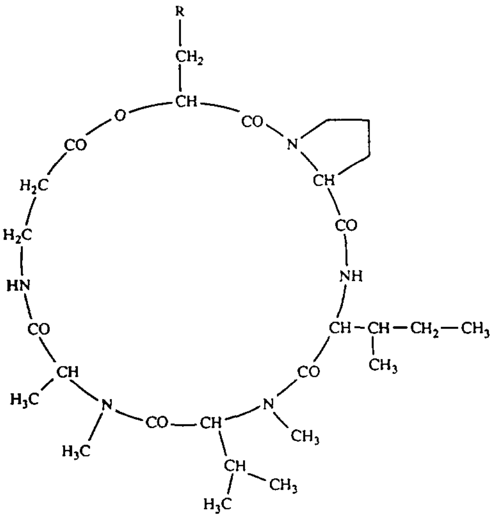


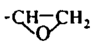
	R	Compound
a	$-CH{=}CH_2$	A DTX
b		E DTX
c	$-\underset{OH}{CH}-\underset{OH}{CH_2}$	E diol DTX
d	$-\underset{OH}{CH}-\underset{SG}{CH_2}$ / $-\underset{SG}{CH}-\underset{OH}{CH_2}$	ESG conjugate
e	$-\underset{OH}{CH}-\underset{SCys}{CH_2}$ / $-\underset{SCys}{CH}-\underset{OH}{CH_2}$	ECys conjugate

Fig. 1. Structures of destruxins and E DTX metabolites.

xenobiotics, we chose to apply or develop direct analytical techniques to avoid the disadvantages of sample clean-up before analysis: laborious protocol or possibly poor extraction yields of polar metabolites. *In situ* monitoring of various pesticides in animal or vegetable media has been performed without preliminary purification, by fast atom bombardment mass spectrometry (FAB-MS) with positive- [9] or negative-ion [10] detection and by direct injection of biological media on to the LC column [11–13]. Using the internal surface reversed-phase (ISRP) approach [14] for monitoring the *in vivo* behaviour of E and A DTXs in locusts by direct LC injection, we observed the disappearance of both toxins in several organs [12]. For E DTX, hydrolysis of the epoxide moiety leading to the E diol DTX has been demonstrated. However, owing to the low performance of the packing (low efficiency), the simultaneous observation of both toxins and polar metabolites was not possible by isocratic elution. An eluent of lower strength was necessary for monitoring of E diol DTX [12].

The aim of this work was the direct LC monitoring in locusts organs of especially E diol DTX and other possible polar metabolites in order to obtain information concerning detoxication processes in insects. Concerning the disappearance of A DTX during incubation, the aim was to elucidate which transformations were implied: either the A DTX→E DTX transformation followed by E DTX metabolization, or intrinsic A DTX metabolization.

Thus, improvements to direct LC techniques were necessary in order to monitor the *in vivo* behaviour of E and A DTXs in insects with simultaneous detection of their metabolites. We report our recent results concerning the locust *Locusta migratoria* L. selected as a non-target insect with several potential sites of detoxication (haemolymph, fat body and Malpighian tubules), using direct LC techniques for toxin monitoring assisted by FAB-MS assays.

2. Experimental

2.1. Biological samples

Mature males of the African migratory locust *Locusta migratoria* migratoriales (R and F) were grown in crowded conditions [12] with a 10-h light and 14-h dark cycle and fed on grass and bran.

2.2. Reagents and chemicals

Standards of E, E diol and A destruxin were purified from the entomogenic fungus *Metarhizium anisopliae* as described previously [3].

Glutathione reduced form (98%) and Trizma base (99.9%), employed without further purification, were supplied by Sigma (Saint-Quentin Fallavier, France). L-Cysteine (>99%) was purchased from Fluka (Mulhouse, France), and anhydrous sodium heptanesulphonate (99%) from Interchim (Montluçon, France).

Organic solvents (chromatographic grade; BDH Hypersolv and Merck) were filtered through an FH filter (0.5 μm) (Millipore, Molsheim, France) and 18-MΩ deionized water (obtained with a Waters Milli-Q apparatus) was filtered through an HA filter (0.45 μm) (Millipore).

2.3. *HPLC columns*

Two analytical columns (75 × 4.6 mm I.D. and 150 × 4.6 mm I.D.) were packed by the Société Française de Chromato Colonne (SFCC–Shandon, Eragny, France) with 5-μm C_1 Nucleosil wide-pore packing (300 Å). A Regis Pinkerton 5-μm GFF ISRP (10 × 4.6 mm I.D.) guard column, manufactured by Regis Chemical and supplied by Touzart et Matignon (Vitry sur Seine, France), was placed before the columns. A 5-μm C_8 Kromasil analytical column (125 × 4.6 mm I.D.) (100 Å) was supplied by Touzart and Matignon. The analytical column was preceded by the Regis Pinkerton 5-μm GFF ISRP (10 × 4.6 mm I.D.) guard column. A 5-μm C_4 Nucleosil wide-pore (300 Å) packed column (150 × 4.6 mm I.D.) and a C_4 Nucleosil wide-pore guard column (10 × 4.6 mm I.D.) were supplied by Interchim.

2.4. *Chromatographic instrumentation*

A Waters HPLC system (Millipore, Saint-Quentin en Yvelines, France) consisting of a Model 625 LC pump, a UV 486 absorbance detector and a Model 746 data integrator module was used. Injections were made with a Rheodyne Model 9125-080 valve equipped with a 5-μl loop. A Rheodyne Model 7230 manual switching valve was supplied by Touzart et Matignon.

A Merck HPLC system consisting of an L 6200 intelligent pump system, an L 4000 UV detector and a D-2500 chromato-integrator was used. Injections were made with a Rheodyne Model 7125 valve equipped with a 5-μl loop.

2.5. *Mass spectrometric analysis*

Mass spectrometry was performed with a Nermag R-10-10-C quadrupole spectrometer–Spectral 30 data system (Delsi-Nermag, Quad Service, Nanterre, France). FAB mass spectra were obtained with an MS-Scan atom gun (M-SCAN, Ascot, Berkshire, UK) delivering 8-keV xenon atoms ⟨Xe⟩ under a 200-μA arc current. The FAB target consisted of a copper probe of 3 mm^2 area, with an incidence angle of 45° relative to the primary beam. Preparation of samples was carried out by spotting 3 μl of supernatant on the FAB probe covered with glycerol. Blanks of haemolymph, fat body and Malpighian tubules did not give any anionic signal in negative-ion FAB-MS [10].

2.6. *Preparation of biological samples (Table 1)*

Samples (20 μl) of aqueous destruxin solution (each experiment is explained in Table 1) were syringe injected (time t_0) into a homogeneous group of locusts between two abdominal segments. The insects were divided into several sets of locusts. Each set was left at room temperature and then dissected at different incubation times. Their haemolymph or organs were sampled and diluted in water. Blank tissues were obtained from sets of untreated locusts.

2.7. *Haemolymph sampling*

Samples of haemolymph (*ca.* 20–30 μl per locust) withdrawn from the neck area with a graduated microcapillary were pooled, rapidly completed with deionized water and then centrifuged for 5 min at 4000 *g* before chromatographic analysis or liquid–solid extraction.

2.8. *Organ sampling*

The dissected organs were pooled in deionized water (Table 2), sonicated and centrifuged for 5 min at 4000 *g*. The supernatant was used directly for injection except with the fat body, where the injected phase was the fraction of intermediate

Table 1
Preparation of biological samples for *in vivo* studies of E and A DTXs

Figure	Concentration of injected solution ($\times 10^2$) (M)	Sets of x locusts	x: number of locusts	Incubation period
E DTX				
2	3.37	4	3	10 min, 1, 2, 5 h
3	3.37	4	3	10 min, 1, 2, 5 h
4	4.4	1	10	1.5 h
5	2.09	3	4	10, 30 and 90 min
A DTX				
6	1.84	4	3	10 min, 1, 2, 5 h
7	1.73	1	5	1 h
8	2.02	1	11	1.5 h

density between the lipidic supernatant (top) and the tissues fragments (bottom).

2.9. Liquid–solid extraction of contaminated haemolymph

E DTX

Extraction was performed using Sep-Pak Light C_{18} (0.3 ml) cartridges (Waters), which were prepared by flushing with 10 ml of MeOH, then with 10 ml of water. The haemolymph sample was loaded (500 μl), washed with 6 ml of water, 6 ml of H_2O–MeOH (75:25, v/v), 6 ml of H_2O–MeOH (50:50, v/v) and then MeOH. The extracts were evaporated and/or lyophilized and dissolved in 100 μl of H_2O–CH_3CN (70:30, v/v).

A DTX

The same procedure as used with E DTX was applied except that water was replaced with H_2O–CH_3CO_2H (97.5:2.5, v/v).

2.10. Synthesis of ESG and ECys conjugates

The conjugates were prepared by reaction of GSH (5 equiv.) or cysteine (5 equiv.) with E DTX (1 equiv.) in H_2O–CH_3CN–Et_3N (89:10:1, v/v/v) for 16 h at room temperature under

Table 2
Organ sampling

Figure	Organ studied	Mass of organ (mg)	Volume of water (μl)
E DTX			
2A	Fat body	120	300
2C	Malpighian tubules	30	300
A DTX			
7	Corpora alata gland	15	150

nitrogen. Isolation and purification were carried out by collecting the appropriate fractions from repeated injections (10 × 50 μl of the crude reaction mixture) on the analytical C_1 LC column used (Fig. 2).

2.11. Calibration graphs for the three destruxins

E, E diol and A DTX were identified in the chromatograms of various biological media by comparison with standard samples. The calibra-

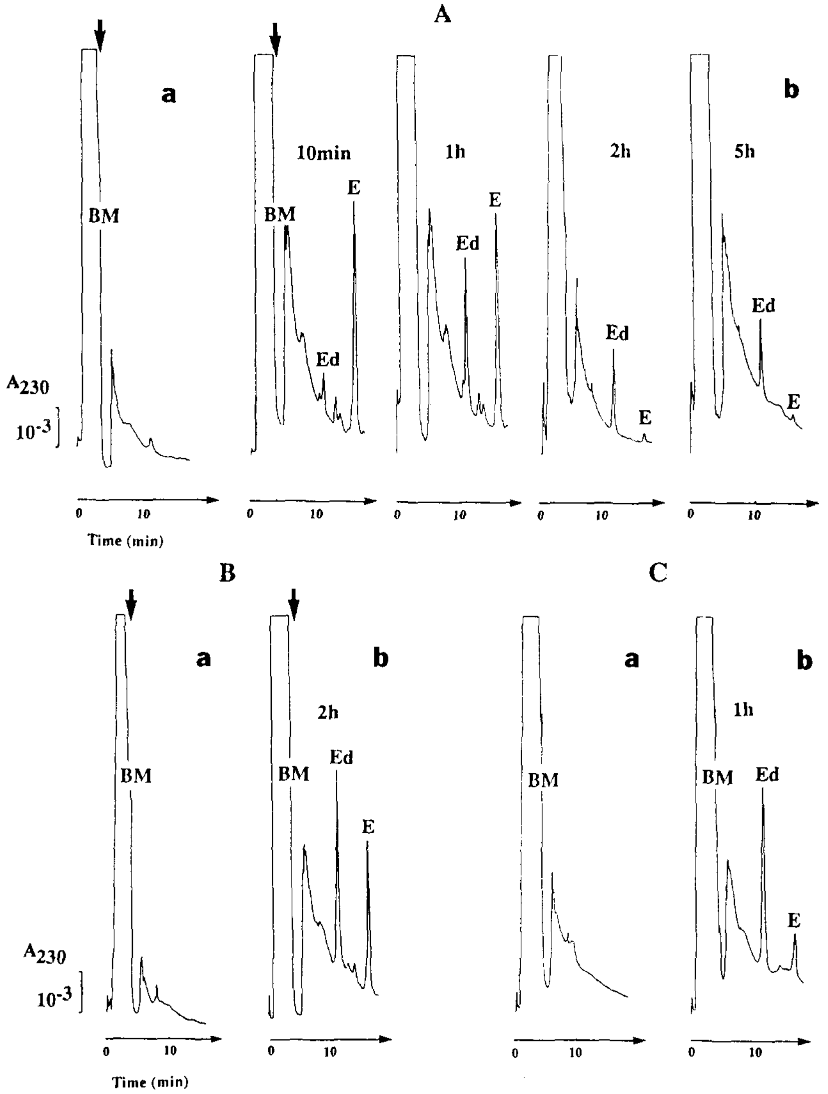


Fig. 2. *In vivo* hydrolysis of E DTX into E diol DTX in locusts *L. migratoria* after injection of E DTX (*cf.*, Table 3). (A) Fat body: (a) blank from untreated locusts; (b) contaminated organ at different incubation times. (B) Haemolymph: (a) blank; (b) 2 h after injection of E DTX. (C) Malpighian tubules: (a) blank; (b) 1 h after injection of E DTX. Chromatographic conditions: a Pinkerton ISRP GFF cartridge (5 μm, 10 × 3 mm I.D.) with a C_1 wide-pore column (Nucleosil, 5 μm, 300 Å, 75 × 4.6 mm I.D.) were used as guard columns. A C_1 analytical column (150 × 4.6 mm I.D.) was connected in-line at $t = 4$ min (indicated by an arrow) via a manual switching valve. Isocratic elution with H_2O-CH_3CN (75:25, v/v) at a flow-rate of 0.5 ml/min. Detection wavelength, 230 nm (0.01 AUFS). Injection, 5-μl aliquots after centrifugation. BM = biological media.

tion graphs obtained by plotting the concentrations C (M) of the three toxins against peak area P appeared to be linear within the range 2×10^{-6}–10^{-3} M covering the assays, with the equation $C = aP + b$. The toxins were quantified in the biological media by measuring the peak areas and using the calibration graphs.

3. Results and discussion

3.1. *E destruxin*

As a first improvement for direct LC injection of biological samples, we tried to transpose the C_1 300 Å wide-pore packing which is convenient for the direct determination of drugs in biological media [15], to an artificial mixture of E and E diol DTX in haemolymph. The slight hydrophobic character of this packing allows the use of a lower proportion of organic modifier in the eluent and prevents protein denaturation and precipitation on the column. Moreover, wide-pore particles (300 instead of 60 Å) diminish the obstruction of pores by proteins. A second improvement was brought about by coupling two C_1 columns via a switching valve and by using the first one as a guard column. With such a configuration, 25% of acetotrile as co-solvent and the switching occurring at 4 min, most of the proteins of haemolymph were discarded from the analytical column, while E and E diol DTXs were switched on to the analytical column. These LC conditions were applied to different organs of treated locusts (Fig. 2). Concerning E DTX, an important and rapid diffusion of the toxin towards the different organs studied was observed. Moreover, the E DTX concentrations decreased in all samples during incubation (*cf.*, Table 3 and also results in Fig. 2A from fat body). Simultaneously, E diol DTX was detected in all the organs studied from the beginning of the incubation (Fig. 2); its concentration reached a maximum after 1 h in haemolymph and fat body and then decreased (Table 3).

To confirm the previous results and also to detect other possible metabolites, the same bio-

Table 3
Hydrolysis of E DTX into E diol DTX in locust organs with LC monitoring (*cf.*, Fig. 2)

Compound	DTX concentration (M)[a]			
	Set 1[b]	Set 2	Set 3	Set 4
(A) Fat body				
E	$1.4 \cdot 10^{-4}$	$1.2 \cdot 10^{-4}$	$\sim 5 \cdot 10^{-6}$	$\sim 4 \cdot 10^{-6}$
E-diol	$2.0 \cdot 10^{-5}$	$8.5 \cdot 10^{-5}$	$4.8 \cdot 10^{-5}$	$4.3 \cdot 10^{-5}$
(B) Haemolymph				
E	$18.6 \cdot 10^{-4}$	$3.1 \cdot 10^{-4}$	$8.1 \cdot 10^{-5}$	$1.5 \cdot 10^{-5}$
E-diol	$8.7 \cdot 10^{-5}$	$11.4 \cdot 10^{-5}$	$9.3 \cdot 10^{-5}$	$8.4 \cdot 10^{-5}$
(C) Malpighian tubules				
E	$7.3 \cdot 10^{-5}$	$2.7 \cdot 10^{-5}$	$\sim 6 \cdot 10^{-6}$	$\sim 4 \cdot 10^{-6}$
E-diol	$3.6 \cdot 10^{-5}$	$9.4 \cdot 10^{-5}$	$3.0 \cdot 10^{-5}$	$6.7 \cdot 10^{-5}$

[a] For the reason below a precise quantification was not undertaken in this preliminary work. Concentrations [$C(M)$] were only estimated from 5-μl injected samples by comparison of peak areas (P) with those of external standards. Calibration graphs C $(M) = aP + b$ were found to be linear within the range $2 \cdot 10^{-6}$–$1 \cdot 10^{-3}$ M: E DTX, $a = 2.25 \cdot 10^{-10}$, $b = 1.1 \cdot 10^{-6}$, $r = 0.999$; E diol DTX, $a = 2.09 \times 10^{-10}$, $b = 1 \cdot 10^{-6}$, $r = 0.998$.

[b] Owing to inter-individual fluctuations in the composition of the locust biological media, the haemolymph or organs samples of each individual of a given set were pooled. Nevertheless, some differences remain between the chromatographic profiles of the biological samples corresponding to the different sets. Incubation times: 10 min, 1 h, 2 h and 5 h for sets 1, 2, 3 and 4, respectively.

logical samples were subjected to negative-ion FAB–MS. This method allows the desorption and ionization of analytes from biological media [9,10]. Thus, hydrolysis of E DTX into E diol DTX was also observed: the deprotonated molecules of E and E diol DTXs were located at *m/z* 592 and 610, respectively (Fig. 3a). Otherwise, the mass spectrum exhibited additional anions of higher mass at *m/z* 899, 713, 690 and 626 (compared with the blank from normal tissues). They can be explained by conjugation with entities present in the biological media. Thus, the intense peak at *m/z* 690 observed in treated haemolymph could correspond to the deprotonated molecule of phosphorylated or sulphated E DTX. The other anions can be explained by conjugation with the tripeptide glutathione (Glu–Cys–Gly). In fact, many detoxication pathways of xenobiotics involve conjugations with the glutathione via glutathione-S-transferase catalysis [16,17]. Moreover, glutathione conjugates usually lead to cysteine conjugates by enzymatic hydrolysis of both Glu and Gly residues [17]. To ascertain the formation of such

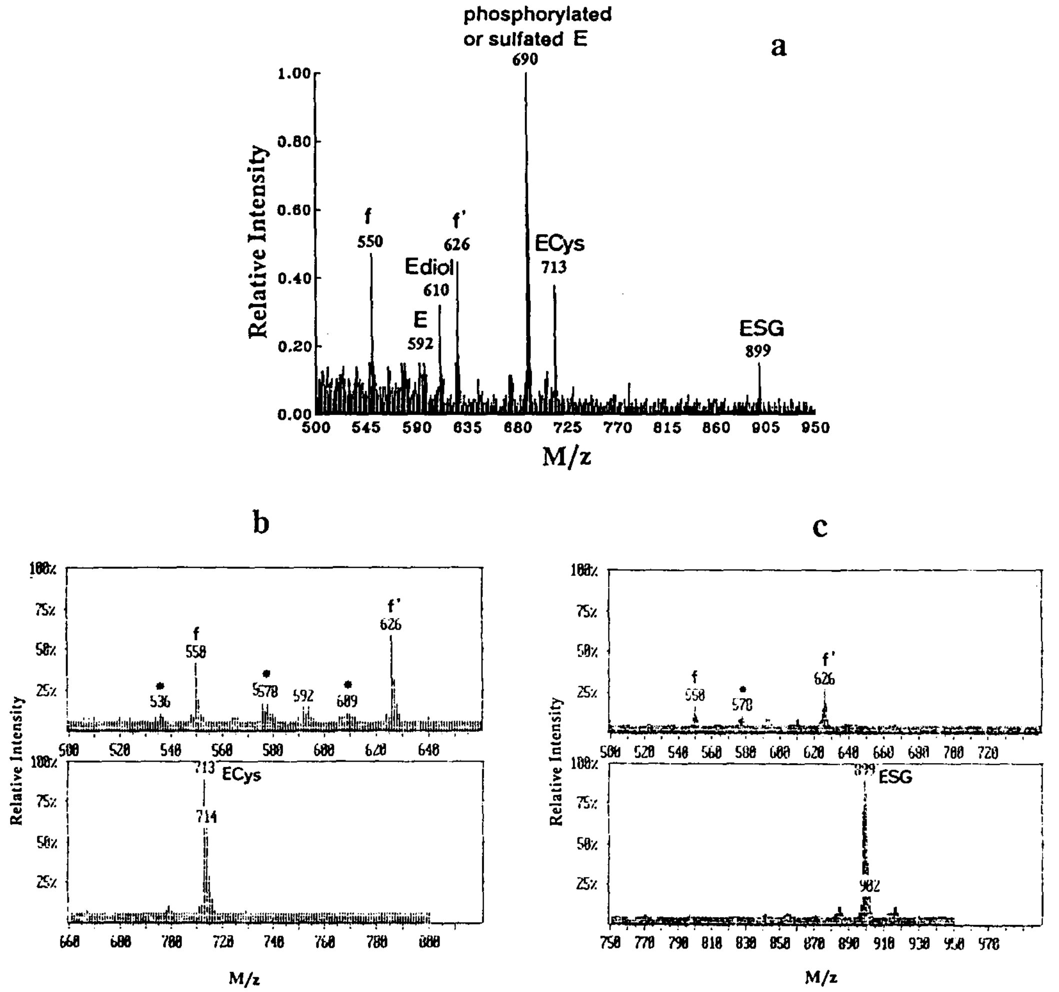


Fig. 3. Evidence for formation of E DTX conjugate. Negative-ion FAB mass spectra (⟨Xe⟩, 8 keV, 200 μA). (a) Locust haemolymph 5 h after injection of E DTX; (b) synthetic standard of ECys conjugate; (c) synthetic standard of ESG conjugate. Peaks: * = impurities from standards; f = fragment ion from E DTX; f′ = fragment ion from ESG and ECys.

conjugates, we prepared standard samples by submitting E DTX to glutathione and cysteine nucleophilic attack. In negative-ion FAB-MS the resulting entities exhibited deprotonated molecules (Fig. 3b and c) identical with those found in treated locust haemolymph (Fig. 3a) and corresponded to the $[M-H]^-$ anions of the glutathione–E DTX conjugate (ESG) and cysteine–E DTX conjugate (ECys).

Classical monitoring with metabolite extraction

By working with standards of E DTX, E diol DTX and of the previous E DTX conjugates, we optimized their simultaneous analysis using a classical reversed-phase LC packing (C_8-RPLC). We decided to try the eluent used by Hernandez *et al.* [18] in the separation of the two pairs of diastereoisomers of glutathione adducts of styrene oxide. Thus, using the isocratic mode with a Tris–phosphate buffer (pH 7.0) and 25% of acetonitrile, the four standards were separated within 22 min (Fig. 4a).

The same conditions allowed the analysis of the liquid–solid extract resulting from Sep-Pak C_{18} extraction of haemolymph from treated locusts. Ninety minutes after injection of the toxin, the chromatographic profile of the haemolymph extract indicated the presence of the four additional compounds (Fig. 4b) with respect to the blank haemolymph (Fig. 4d). While ESG, ECys and E DTX were identified without any ambiguity, the compound eluting at 10.8 min could be attributed to one of the E diol diastereoisomers. In fact, the non-enzymatic (*i.e.* unselective) hydrolysis of E DTX resulted in two well separated E diol diastereoisomers, as observed with the crude E DTX hydrolysis mixture (two peaks at 10.0 and 10.8 min in Fig. 4c). We noted that the E diol compounds produced by *Metarhizium anisopliae* (standard peak at 10.0 min in Fig. 4a) and by locusts (one peak at 10.8 min in Fig. 4b) corresponded to different diastereoisomers.

Direct LC injection for metabolite monitoring

To avoid the disadvantages of the previous "extraction approach", *i.e.* the need for large amounts of biological samples and tedious pretreatment, we used direct injection with wide-pore packings. We selected C_1 and C_4 instead of C_8 wide-pore packings in order to diminish the risk of protein precipitation on the column. Numerous trials led to the choice of a C_4 wide-pore packing and an optimized eluent composed of CH_3CN–MeOH–2-propanol (15:10:5, v/v/v) in aqueous 5 m*M* heptanesulphonate (adjusted to pH 3.4). The four compounds were separated from each other and from the endogenous haemolymph components in 20 min (Fig. 5a, b and c) using an artificial mixture of haemolymph with standards.

The change in the chromatographic profiles of haemolymph (Fig.5d–f) and organs as a function of the incubation time led to several conclusions:

(1) the disappearance of E DTX;

(2) an increase in E diol DTX after the beginning of the incubation in most of the organs; the present method allows the discrimination between both E diol diastereoisomers produced either in locusts organs [higher t_R, 10.9 (Fig. 5d–f)] or by the fungus *Metarhizium anisopliae* [lower t_R, 10.1 (Fig. 5b and c)];

(3) the appearance of ESG conjugate immediately after the incubation; and

(4) the amount of ECys conjugate becomes significant only after 30 min and then increases, particularly in haemolymph and Malpighian tubules.

As it was ascertained that the two conjugates did not result from E diol DTX injected into locusts, the results can be explained by two distinct transformations starting from E DTX:

E DTX → E diol DTX
E DTX → ESG conjugate → ECys conjugate

Inhibition assays of the glutathione-S-transferase activity in locusts are under investigation to confirm the hypothesis of the enzymatic conjugation with glutathione.

3.2. A destruxin

Instead of the previous ISRP packing [12], we selected the C_1 wide-pore packing. Taking into account the more lipophilic character of A DTX

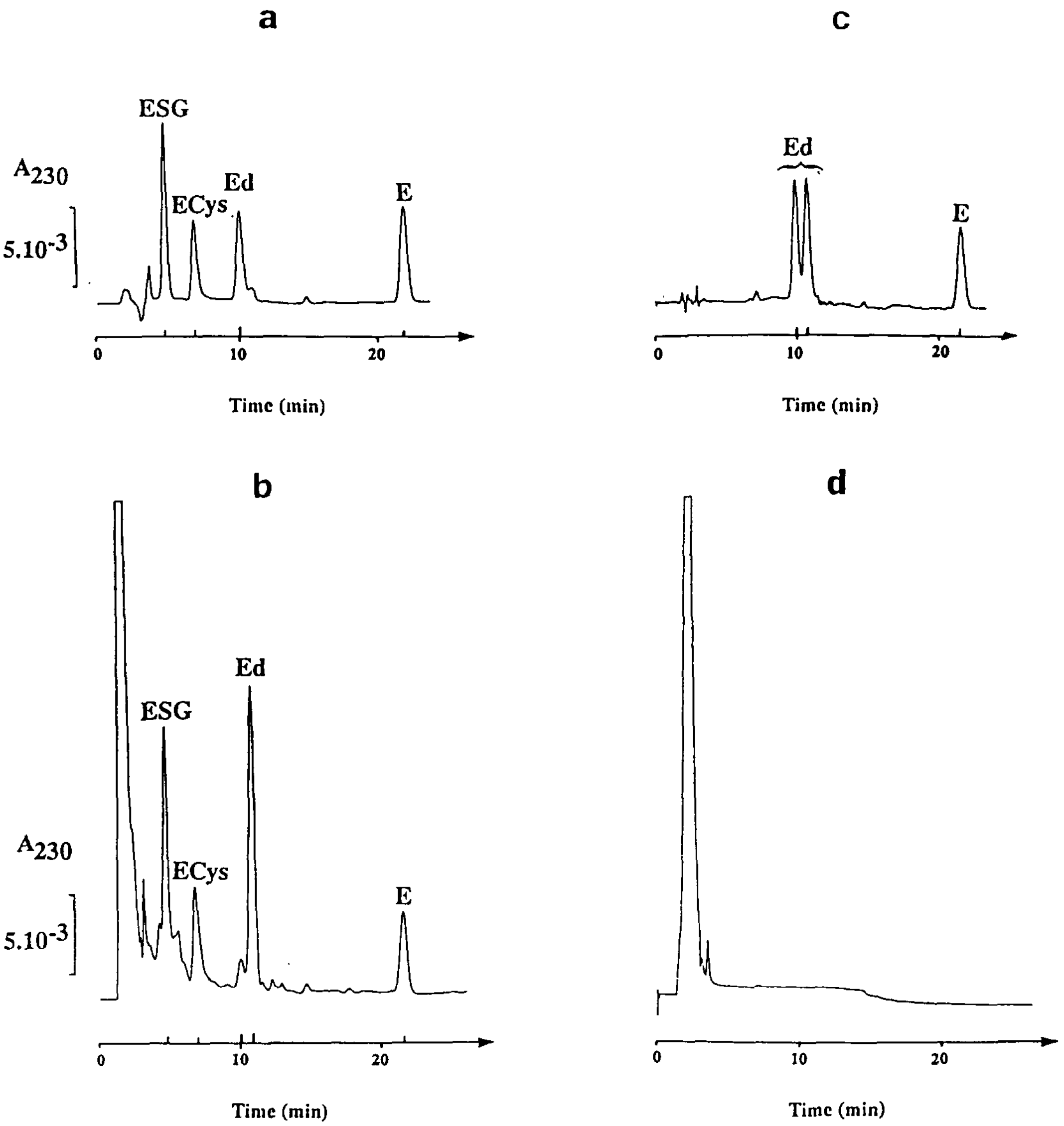


Fig. 4. Evidence for formation of E DTX conjugates. Chromatographic separations on a conventional C_8 column. (a) Standards of E, E diol DTXs (from *Metarhizium anisopliae*) and standards of synthetic ESG, ECys adducts; (b) extract from liquid–solid extraction of haemolymph (1.5 h after injection with E DTX); (c) standard of partly hydrolysed E DTX into E diol DTX diastereoisomers, (d) blank from normal haemolymph extract. Chromatographic conditions: C_8 analytical column (Kromasil, 5 μm, 100 Å, 125 × 4.6 mm I.D.) with the Pinkerton ISRP GFF cartridge (5 μm, 10 × 3 mm I.D.). Isocratic elution with Tris–phosphate buffer (pH 7.0)–CH_3CN (75:25, v/v), flow-rate 0.5 ml/min up to 11.5 min and then 0.8 ml/min. Detection wavelength, 230 nm (0.03 AUFS). Injection, 5-μl aliquots. Tris–phosphate buffer preparation has been described previously [18]. Retention times: ESG, 4.6; Ecys, 6.8; E diol, 10 and 10.8 (two diastereoisomers); E, 21.5 min.

than E DTX, we increased the proportion of acetonitrile from 25% to 30%. The chromatographic profiles obtained for treated tissues showed the presence of A DTX 10 min after injection, indicating rapid diffusion of the toxin in tissues (Fig. 6 for haemolymph), as already observed for E DTX. The progressive disappearance of A DTX in different biological samples

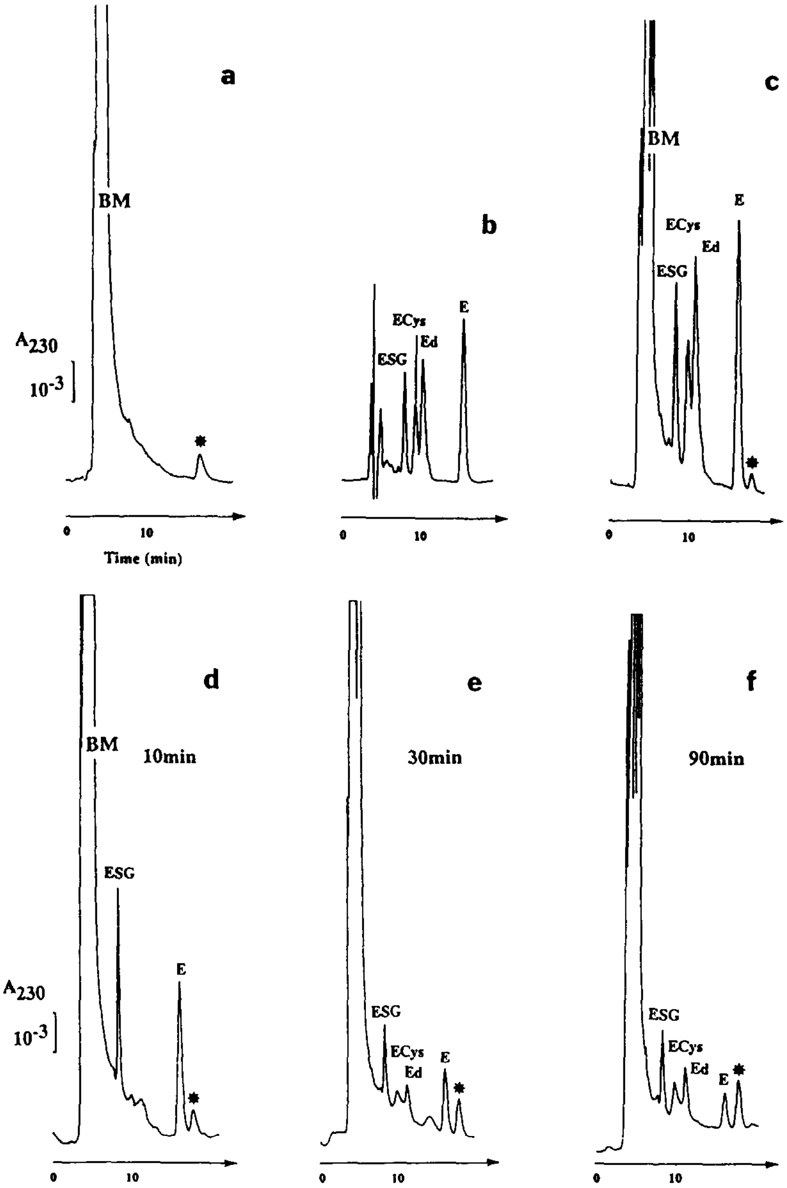


Fig. 5. Direct injection on to C_4 wide-pore packed column. (a) Blank from normal haemolymph; (b) standards of ESG, ECys, E diol and E DTXs previously encountered in Fig. 4a; (c) artificial mixture of the four standards in haemolymph; (d–f) *in vivo* LC profiles of E DTX in locusts haemolymph during incubation. Chromatographic conditions: C_4 analytical column (Nucleosil, 5 μm, 300 Å, 150 × 4.6 mm I.D.) with a C_4 cartridge guard column (10 × 3 mm I.D.). Isocratic elution with aqueous heptanesulphonate (5 m*M*, pH 3.4)–CH_3CN–MeOH–2-propanol (70:15:10:5, v/v); flow-rate, 0.5 ml/min. Detection wavelength, 230 nm (0.03 AUFS). Injection, 5-μl aliquots. Retention times: ESG, 7.8; ECys, 9.2; E diol, 10.1 and 10.9 (two diastereoisomers); E, 15.2 min. Peaks: BM = biological media; * = lipids.

was confirmed and the quantification showed that this was enhanced in haemolymph (Table 4).

These chromatographic conditions allowed the detection of the E DTX standard which would elute at 12.2 min. As this peak was not observed

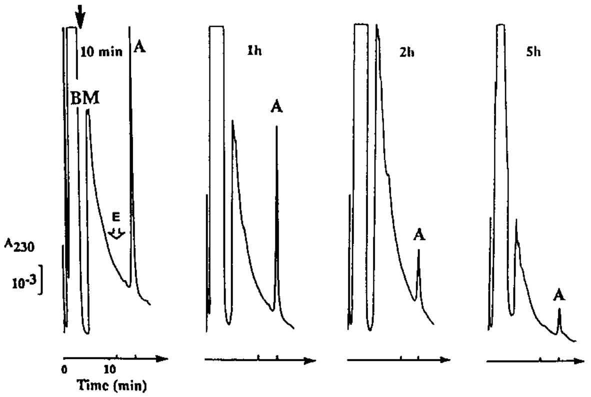


Fig. 6. *In vivo* behaviour of A DTX in locust haemolymph during incubation. Chromatographic profiles, see Table 4. Chromatographic conditions as in Fig. 2, except the proportion of organic modifier was 30% instead of 25%.

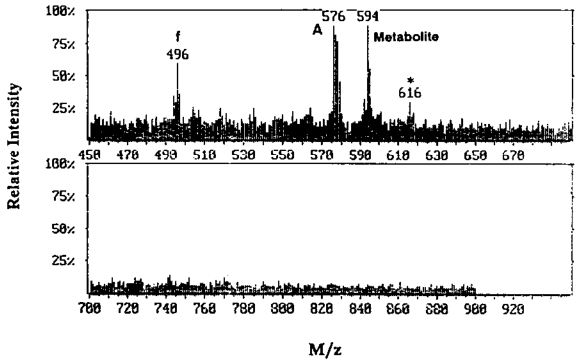


Fig. 7. Negative-ion FAB mass spectrum (⟨Xe⟩, 8 keV, 200 μA) of the liquid–solid extract from injected locust haemolymph. Peaks: f = fragment ion from A DTX; * = $[M + Na - 2H]^-$ anion of the metabolite from A DTX.

in the tissues studied, we focused the analysis on the organ susceptible to provide enzymatic epoxidation, namely the endocrine gland Corpora allata. As no E DTX was detected, we concluded that A DTX was not converted into E DTX in *L. migratoria*, so that metabolic pathways other than the E DTX transformations were responsible for the decrease in A DTX concentration.

However, the negative-ion FAB-MS analysis of the different organs from treated locusts showed, in the neighbourhood of the $[M - H]^-$ anion of A DTX (*m/z* 576), a new anion (*m/z* 594), which could result from hydrolysis of A DTX (Fig. 7). Recently, an LC study concerning the behaviour of tritium-labelled A DTX in *Galleria mellonella* larvae demonstrated the ring opening of the toxin by hydrolysis [19]. Under our previous chromatographic conditions, the same highly polar resulting peptide would elute near the void volume with the endogenous proteins. LC analysis of such polar metabolites is under investigation using less organic modifier in the eluent and/or ion-pair reagents.

4. Conclusion

The present results concerning the *in vivo* behaviour of destruxins in locusts highlight the advantages of applying LC and MS techniques directly to biological samples, thus providing ease and rapidity without the inconvenience of

Table 4

Evolution of A DTX in locust organs with LC monitoring (*cf.*, Fig. 6 for haemolymph)

Organ	A DTX concentration after injection (*M*)[a]			
	Set 1[b]	Set 2	Set 3	Set 4
Haemolymph	$31.1 \cdot 10^{-5}$	$20.9 \cdot 10^{-5}$	$8.6 \cdot 10^{-5}$	$3.2 \cdot 10^{-5}$
Fat-body	$1.2 \cdot 10^{-5}$	$1.1 \cdot 10^{-5}$	$\sim 6 \cdot 10^{-6}$	$\sim 3 \cdot 10^{-6}$
Malpighian tubules	$\sim 8 \cdot 10^{-6}$	$\sim 6 \cdot 10^{-6}$	$\sim 5 \cdot 10^{-6}$	$\sim 5 \cdot 10^{-6}$

[a,b] See conditions in Table 3. Calibration graph $C\ (M) = aP + b$; was found to be linear within the range $2 \cdot 10^{-6}$–$1 \cdot 10^{-3}$ *M*: $a = 1.81 \cdot 10^{-10}$, $b = 2 \cdot 10^{-6}$, $r = 0.998$.

possible selective extraction. Thus, by avoiding the pretreatment sequence inherent in the conventional extraction approach, it was possible to reveal the metabolic pathways for E DTX in locust (hydrolysis and several conjugations) and also to demonstrate that the conjugates did not result from E diol DTX.

Concerning the progressive disappearance of A DTX in all the locust organs, we have ruled out the hypothesis of the transformation of A DTX into E DTX in favour of a cyclodepsipeptide hydrolysis, which remains to be proved.

Direct LC and MS techniques and liquid–solid extraction provided comparable results with DTX. However, to monitor more polar compounds, direct approaches would override liquid–solid extraction, avoiding the inconvenience of possible selective extraction. Another advantage is the application of the described approach to small samples.

5. References

[1] Y. Kodaira, *J. Fac. Text. Sci. Technol., Shinshu Univ., Ser. E*, 29 (1961) 59.
[2] S. Tamura and N. Takahashi, in M. Jacobson and O. Grosby (Editors), *Naturally Occuring Insecticides*, Marcel Dekker, New York, 1971, p. 499.
[3] M. Pais, B.C. Das and P. Ferron, *Phytochemistry*, 20 (1981) 715.
[4] J.M. Quiot, A. Vey and C. Vago, *Adv. Cell Cult.*, 4 (1985) 199.
[5] A. Vey, J.M. Quiot, C. Vago and J. Fargues, *C. R. Acad. Sci., Ser. III*, 300 (1985) 647.
[6] J.M. Quiot, A. Vey, C. Vago and M. Pais, *C.R. Acad. Sci., Ser. D*, 291 (1980) 763.
[7] P.H. Robert and J. Fargues, *C. R. Acad. Sci., Ser. III*, 303 (1986) 641.
[8] D.W. Roberts, *J. Invert. Pathol.*, 8 (1966) 222.
[9] C. Lange, C. Mulheim, J.C. Cherton, A Vey and M. Pais, *Rapid Commun. Mass Spectrom.*, 5 (1991) 169.
[10] C. Lange, C. Loutelier, J.C. Cherton, P. Cassier, A. Vey and M. Pais, *Rapid Commun. Mass Spectrom.*, 6 (1992) 28.
[11] J.C. Cherton, M.T. Chevassus-Clement, C. Lange, O. Convert, R. Fritz and P. Leroux, *J. Chromatogr.*, 566 (1991) 499.
[12] J.C. Cherton, C. Lange, C. Mulheim, P. Cassier, M. Pais and A. Vey, *J. Chromatogr.*, 566 (1991) 511.
[13] J.C. Cherton, C. Loutelier, C. Lange and P. Cassier, *Sci. Total Environ.*, 132 (1993) 381.
[14] H. Hagestam and T.C. Pinkerton, *Anal. Chem.*, 57 (1985) 1757.
[15] Z.K. Shihabi and R.D. Dyer, *J. Liq. Chromatogr.*, 10 (1987) 2383.
[16] E. Boyland, *Biochem. Soc. Trans.*, 2 (1974) 7.
[17] K.A. Hassall, *The Chemistry of Pesticides*, Verlag Chemie, Weinhein, 1982, p. 58.
[18] O. Hernandez, A.V. Bhatia and M. Walker, *J. Liq. Chromatogr.*, 6 (1983) 1475.
[19] A. Jegorov, V. Matha and H. Kradec, *Comp. Biochem. Physiol. C*, 103 (1992) 227.