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Delta(2)-oxazolines-1,3 and *N*-acylaziridines as potential proinsecticides of carboxylic acids V. Direct thin-layer chromatography monitoring of the metabolism in locust tissues[☆]

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Abstract

Modern thin-layer chromatography (TLC) was used for the evaluation of Δ^2-oxazolines-1,3 **I** and *N*-acylaziridine **VII** structures, as potential proinsecticides of carboxylic acids **III**. Thus the unmasking[2] of the active principles **III** from Δ^2-oxazolines-1,3 **Ia–c** and *N*-acylaziridine **VIIc** was monitored by spotting aliquots directly onto RP-18 TLC plates, without any sample pretreatment during in vitro assays performed in concentrated locust tissues. To achieve a good separation of carboxylate **IIIa** from endogenous components of the tissues, a short preliminary development with methanol or ion-pairing was necessary. From UV–TLC chromatograms (densitograms) it appeared that in a phosphate buffer at pH 7.4, the oxazoline **Ia** with a C_2 substituent devoid of α-ramification or α,β-insaturation hydrolysed slowly into the corresponding β-hydroxylamide **VIa** and intermediate aminoester **Va**. Significantly, locust mesenteron (or fat body) efficiently triggered the unmasking of **IIIa**, a transformation which corresponds to the expected proinsecticide behavior of **Ia**. Conducting TLC monitoring in the same locust tissues also revealed that the oxazolines **Ib** and **Ic** with an α-ramification and an α,β-insaturation, respectively, cannot be considered as proinsecticides of the corresponding carboxylic acids **IIIb** and **IIIc**. In contrast, the *N*-acylaziridine **VIIc** appeared as a convenient proinsecticide structure for masking the carboxylic acid **IIIc**.

Keywords: Δ^2-Oxazolines-1,3; *N*-Acylaziridine; Carboxylic acids

1. Introduction

The potential of the Δ^2-oxazolines-1,3 structure **I** for the reversible masking of carboxylic acids has been extensively used by Meyers and co-workers [1–3] for the enantioselective α-ramification of carboxylic acids. Also, in the prodrugs field, Vorbrüggen et al. [4,5] have retained structure **I** for the elaboration of NSAIDs (non-steroidal anti-inflamma-

[☆] See Refs. [8], [9], [32] and [12] for respective parts I–IV.

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[1] Part of thesis.

[2] Unmasking or activation of proactive chemicals: the release of the active principle(s).

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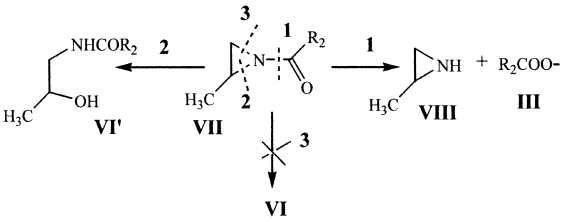
tory drugs) based upon carboxylic acids as the active principles.

For several years we have been working on the proinsecticde behavior of such heterocycles, looking for their capability to achieve a reversible masking of carboxylic acids **III** and/or β-ethanolamines **II** selected as the active principles. This hypothesis was confirmed by biological testings and in vitro metabolization studies carried out in biological insect tissues capable of triggering enzymatic hydrolysis. To avoid any sample pretreatment other than simple centrifugation, we have focused our work on spectroscopic or chromatographic techniques that allowed a direct analysis of the substrates and metabolites in the biological media. ^{1}H Nuclear magnetic resonance (NMR) [6] and high-performance liquid chromatography (HPLC) monitorings with internal surface reversed-phase (ISRP) packing [7,8] thus revealed that the hydrolysis of simple commercial Δ^2-oxazolines-1,3 **I** occurred in diluted locust haemolymph. Then, a series of lipophilic oxazolines **I** bearing various substituents in the C_2, C_4 and C_5 positions, and designed as contact insecticides were studied. In the case of the fluorinated oxazoline **Ia**, a ^{19}F-NMR monitoring [9,10] showed that an efficient unmasking of the biologically active oxazoline **Ia** [11] actually occurred during in vitro assays conducted in concentrated locust fat body and mesenteron, (cf. Scheme 1) [11,12].

In view of these findings it was of interest to look at the behavior of non-fluorinated species under similar in vitro conditions, but such investigations were difficult to envision through ^{1}H-NMR. Also,

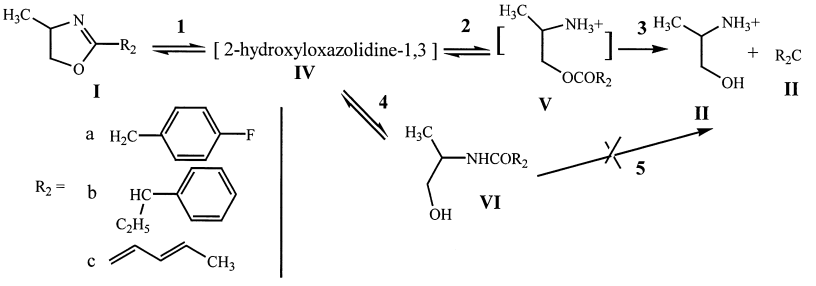


Scheme 2. Hydrolysis paths of the *N*-acylaziridines **VII**. Paths 1 and 2 result from enzymatic and pH effects, respectively. Path 3 has never been observed ([32], this work).

HPLC analysis based on the use of classical C_{18} packing was precluded because they require preliminary precipitation/extraction sequences which proved to be unsuitable for the present purposes owing to the ionic character of the expected metabolites **III**, **II** or **VIII** (cf. Schemes 1 or 2).

By contrast, sophisticated packings including the above-mentioned ISRP [7,13] and C_1 and C_4 wide-pore ones [14–18] would be interesting for direct injection of untreated biological samples as avoiding the problematic extraction of ionic metabolites, but they were rather dedicated to diluted media.

In this paper we report on our successful attempt to conduct in vitro assays in concentrated insect tissues using modern thin-layer chromatography (TLC), a method that has so far mostly been applied for monitoring drugs and metabolites after extraction from their biological media [19–24]. This work which allowed us new insights into the metabolism in locust tissues of Δ^2-oxazolines-1,3 **Ia–c** and *N*-



Scheme 1. Hydrolysis paths for Δ^2-oxazolines **I**. Paths 2, 3 and 4 are only effective for oxazoline **1a** ([11,12], this work), path 5 has never been deployed.

acylaziridine **VIIc** (cf. Schemes 1 and 2) adds to the few examples of systems [25–30] that have been directly investigated by TLC without pretreatment.

2. Experimental

2.1. Biological samples

The locust mesenteron (caeca) and the locust fat body were provided by Dr. A. Louveaux, Laboratoire de Biologie Évolutive et Dynamique des Populations (Université Paris-Sud, France). The esterase from hog liver immobilized on Eupergit was supplied by Fluka (Saint-Quentin Fallavier, France). One unit of the enzyme will hydrolyse 1.0 μmol of ethyl valerate per minute at pH 8 and 25°C.

In vitro experiments were performed using substrate concentrations of $5\cdot10^{-4}$, 10^{-3}, $2\cdot10^{-3}$ or $5\cdot10^{-3}$ *M* obtained by diluting a 0.1 *M* stock solution of **Ia–c** or **VIIc** with an adequate volume of biological medium or phosphate buffer. Five or 10% (v/v) CH_3CN was used as the organic solvent for substrate solubilization.

After dissection, locust tissues were stored at −80°C in several eppendorf tubes until their utilization. Typically a mixture of 260 mg of locust fat body and 400 μl of phosphate buffer (0.1 *M*, pH 7.4) was ground and centrifuged, resulting in three fractions differing in density: with a bottom one consisting of tissue fragments, an upper one consisting of the lipidic components and an intermediate aqueous one being the only fraction used as the biological sample. For assays with mesenteron, locust caeca was ground at room temperature just before utilization, then centrifuged without any dilution; then only the supernatant was used and incubated with the appropriate substrate. Periodically, aliquots were fourfold diluted (in a 0.1 *M* phosphate buffer, pH 7.4) just before application onto the chromatoplate.

2.2. Commercial enzyme

In situ enzymatic hydrolysis of **Va** and **Vb** were performed by adding 8 mg of esterase (3.6 units of enzyme) to a 40 μl aliquot of a phosphate buffer solution of the initial substrate **Ia** (10^{-3} *M*), or **Ib** ($5\cdot10^{-4}$ *M*) at pH 6.3. The enzymatic hydrolysis were achieved after complete conversion of **Ia** and **Ib** into the corresponding aminoester **Va** or **Vb** (15 or 72 h, respectively) (Scheme 1).

2.3. Chromatography

Merck analytical TLC aluminium sheets (20×20 cm) coated with 0.2 mm layers of RP-18 F_{254} sorbent (Merck, Nogent sur Marne, France) were used. Prior to chromatography, the plates were prewashed with MeOH and dried on a Camag (Muttenz, Switzerland*) TLC heater at 80°C for 5 min. Manual application (5 μl) of phosphate buffer standard solutions of **I**, **III**, **VI** or **VII**, or of incubated biological samples was performed with 5-μl graduated micropipettes (Hirschmann, Eberstadt, Germany) at 10 mm intervals and 10 mm from the lower edge. Plates were developed to a distance of ca. 6 cm by ascending development in a Camag chamber* in ca. 30 min, then dried in a stream of ambient air. The scanning was performed in the reflectance mode (UV absorbance with deuterium lamp, scanning speed: 5 mm s^{-1}, bandwidth: 20 nm, slit dimension: 4×0.45 mm, using a computer-controlled TLC Scanner (Camag TLC Scanner 3* with CATS software residing in an computer with Pentium III processor). Monitoring was carried out at optimized wavelengths (see Table 1 and the Discussion section for the elution conditions). The products were identified through comparison of their in situ UV-reflectance spectra and hRf with the corresponding data of reference standards (hRf=R_F×100). Semi quantitative TLC was used to estimate the % hydrolysis of **Ia** into **VIa**, assuming that the linear part of the calibration curve: area=f [**VIa**] is meaningful due to the low concentration range for standards and products.

2.4. Chemicals for chromatography

Solvents were of analytical grade for dioxane (Merck) and HPLC grade for methanol and acetonitrile (sodium dodceyl sulfate, SDS, Solvent, Documentation, Synthèse, Vitry, France). 18-MΩ deionized water obtained with a Milli-Q apparatus (Millipore, Saint-Quentin en Yvelines, France) was used

Table 1
hRf observed for the oxazolines **Ia–c**, *N*-acylaziridine **VIIc**, corresponding products **III**, **VI** and intermediate **V**, according to eluent and medium conditions

Medium	Conditions for elution[a]	md[b] (mm)	hRf[c]			
			Oxazoline **Ia** Log P=2.91			
			Ia	**IIIa**	**VIa**	**Va**
Phosphate buffer, pH 7.4	A	55	23	65	41	6
	B (cf. Fig. 1)	55	20	59	44	12
	C	60	25	38	44	19
	D	65	32	52	32	5
Phosphate buffer, pH 6.3	A (cf. Fig. 2A)	55	–	65	42	7
Commercial esterase	A (cf. Fig. 2B)	55	–	65	47	9
Locust mesenteron	A	55	[d]	[d]	–	[d]
	B (cf. Fig. 3A)	55	[d]	57	–	[d]
	C (cf. Fig. 3B)	60	[d]	37	–	[d]
Locust fat body	D	65	[d]	49	–	[d]
			Oxazoline **Ib**, log P=3.74			
			Ib	**IIIb**	**VIb**	**Vb**
Phosphate buffer, pH 7.4	E	65	32	54	64	–
Phosphate buffer, pH 6.3	E (cf. Fig. 4A)	55	27	54	64	10
Commercial esterase	E (cf. Fig. 4B)	55	–	55	–	16
Locust mesenteron	E (cf. Fig. 5)	65	29	–	–	–
			Oxazoline **Ic**, log P=3.97			
			Ic	**IIIc**	**VIc**	**Vc**
Phosphate buffer, pH 7.4	E	65	34	66	74	
Locust mesenteron	E	65	32	–		
			N-Acylaziridine **VIIc**, log P=2.27			
			VIIc	**IIIc**	**VIc′**	
Phosphate buffer, pH 7.4	F (cf. Fig. 6a)	65	31	54	62	
Locust mesenteron	F (cf. Fig. 6b)	65	25	50[e]	–	

[a] The different conditions of elution were as follows: A: water–acetonitrile (50:50, v/v); B: migration over 10 min with methanol, then and elution A; C: preliminary dipping in a methanolic solution of 2 m*M* cetyltrimethylammonium bromide (CTMA Br) followed by drying, deposite and development with phosphate buffer (0.05 *M*, pH 7.4)–acetonitrile (50:50, v/v); D: water–methanol (50:50, v/v); E: water–acetonitrile–dioxane (40:30:30, v/v); F: water–acetonitrile–dioxane (50:25:25, v/v).
[b] md=Migration distance.
[c] When two successive developments were performed, the indicated hRf concerns the md between the deposite and the last spot position.
[d] Possible overlapping of the peak with endogenous components of the biological medium (BM).
[e] Overloading with **IIIc**.

for the mobile phases and the preparation of the phosphate. Monobasic and dibasic potassium phosphates were supplied by Acros Organic France (Noisy-Le-Grand, France), cetyltrimethylammonium bromide (CTMA Br) by Aldrich. Solvents and water were filtered through a 0.45-μm filter (Pall, Ann Arbor, MI, USA).

*2.5. Preparation and structural characterization of the oxazolines **I**, the precursor N-acylaziridines **VII**, and the hydroxylamides **VI***

Oxazoline **Ia** [12,31] and β-hydroxylamide **VIa** [32] have been previously described. Oxazolines **Ib–c** were obtained by rearrangement of the corresponding

N-acylaziridines **VII** [33]. *N*-(2,4-Hexadienoyl)-2-methylaziridine **VIIc** was obtained by condensation of aziridine **VIII** (1 equiv.) with carboxylic acid **IIIc** (1 equiv.) in the presence of dicyclohexyl carbodiimide (DCC, 1 equiv.) in CH_2Cl_2. Hydroxylamides **VIb–c** and **VIc′** were obtained by condensation of 1 equiv. of **II** for **VIb–c**, and **II′** for **VIc′**, with 1 equiv. of **IIIb** (for **VIb**) or **IIIc** (for **VIc–c′**), using 1 equiv. of DCC in CH_2Cl_2.

For these new compounds, elemental analysis or high-resolution mass spectrometry (HRMS), IR and NMR data, agree well with the proposed structures; they will be described elsewhere [34].

3. Results and discussion

3.1. Checking the TLC method

3.1.1. Stationary phase

The choice of the reversed-phase as the chromatographic method was related to our objective of conducting a direct metabolism monitoring in biological fluids. Among the RP-TLC stationary phases currently available (C_8, C_{18}, diol, CN, or NH_2 layers), we chose the C_{18}-bonded silica, due to the ionic character of the compounds **II**, **III**, **V** and **VIII**. These were expected to form from the hydrolysis of oxazolines **Ia–c** and *N*-acylaziridine **VIIc** (cf. Schemes 1 and 2 concerning the metabolic fates established for **Ia** [12] and **VIIa** [32]).

3.1.2. Detection

The scanning was performed in the UV-reflectance mode. Due to the absence of chromophore in the β-ethanolamine **II** and aziridine **VIII** structures, the monitoring was focused on the substrates **Ia–c** and **VIIc**, the corresponding carboxylates **IIIa–c** and the hydroxylamides **VIa–c** and **VIc′**. Considering the absorbance in the UV-reflectance mode on RP-18 layers for standards of these latter products, the wavelength detection was optimized at 200 and 262 nm for [**Ia–b**, **IIIa–b** and **VIa–b**] and [**Ic**, **VIIc, IIIc** and **VIc,c′**], respectively.

3.1.3. Elution conditions

Preliminary optimization of eluent was achieved for each substrate by testing different organic cosolvents in order to obtain (i) a good separation of the substrated **I** or **VII** from the corresponding carboxylate **III**, hydroxylamide **VI** and **VI′**, (ii) the best separation of these entities from the endogenous components of the biological media (which exhibit both, low and high hRf, see below). As expected on the basis of the lipophilicity expressed by log *P* [35], the required amount of organic cosolvent was greater for **Ib–c** than for **Ia**, cf. Table 1. The hRf values resulting from a development in the ascending mode were in the range 7–74 for standards of substrates **Ia–c**, **VIIc**, and their possible metabolites **IIIa–c**, **VIa–c** and **VIc′**. The separation of carboxylate **IIIa** from endogenous components of the mesenteron was unsuccessful under the elution conditions referred to as A in Table 1 and it appeared that a short preliminary elution (10 mm) with MeOH was necessary (B conditions in Table 1). The same result was achieved using ion-pairing with cetrimide bromide and the impregnation technique [36] (C conditions in Table 1). With locust fat-body as biological fluid, the best separation for **IIIa** resulted from the D elution conditions (cf. Table 1).

3.1.4. Qualitative TLC and metabolites identification

This work was focused on the locust mesenteron and fat body due to their important content in hydrolases [12,32], but the automated application on the chromatoplates was not possible with such viscous tissues (even after fourfold dilution just before application). Therefore, manual application was used, preventing rigorous calibration and quantitation due to difficulties in reproducibility. However, in the ''proinsecticide perspective'' every information, even qualitative, about the unmasking resulting from a TLC monitoring, is quite sufficient for the screening of new molecules. However, such a qualitative monitoring implies the rigorous identification of substrates **I** or **VII** and corresponding metabolites **III,** using their hRf and in situ UV-reflectance spectra in the biological tissues.

*3.2. TLC study of the Δ^2-oxazoline-1,3 **Ia***

*3.2.1. Behavior of **Ia** as a function of pH*

Assays performed with **Ia** in a phosphate buffer (pH 7.4) solution over a 4 h period (blank) using the

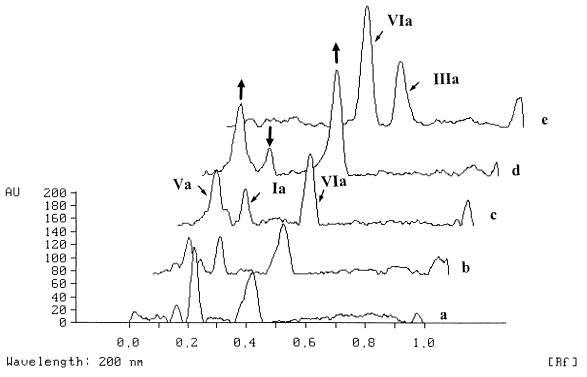


Fig. 1. Thin-layer chromatography monitoring of the behavior of the Δ^2-oxazoline 1,3 **Ia** in a phosphate buffer at pH 7.4 (blank at pH 7.4). C_{18} TLC plate. Manual deposite: 5 μl of a phosphate buffer, pH 7.4 0.1 *M*–acetonitrile (95:5, v/v) **Ia** solution (initially at $[\mathbf{Ia}]_0=2\cdot10^{-3}$ *M*). Elution: B conditions. cf. Experimental for other details. a "t_0", b "$t_{1\,h}$", c "$t_{2\,h}$", d "$t_{4\,h}$", e standards **IIIa** and **VIa** (10^{-3} *M* in CH_3CN–phosphate buffer, 5:95, v/v). The compounds were identified by their hRf (cf. Table 1) and their in situ UV-reflectance spectra (cf. Appendix A).

B elution conditions for the monitoring made it evident the progressive hydrolysis of **Ia** into the hydroxylamide **VIa** (cf. Fig. 1a–d,). Besides the diminishing peak of **Ia** (hRf=20), as early as the "t_0" densitogram an increasing peak was actually observed at the same hRf as the one of hydroxylamide **VIa** (hRf=44, cf. Fig. 1e). A comparison of the peak area for **VIa** in Fig. 1d and e indicated a concentration of about $7.5\cdot10^{-4}$ *M* in the **Id** case, i.e., ~38% hydrolysis for **Ia** after 4 h standing at this pH. Comparison of this semi-quantitative TLC result with the one obtained via ^{19}F monitoring, which indicated only ~20% hydrolysis [11,12], suggests a slight contribution of the C-18 layer to **Ia** hydrolysis. This hypothesis is reasonable, taking into account that RP-TLC materials are not end-capped. There was no significant formation of the carboxylate **IIIa** (hRf=59, cf. Fig. 1e), but an additional and time-increasing peak noted **Va** was observed at low hRf (hRf=12, Fig. 1a–d). These results agree with previous reports that 2-methyl-2-oxazoline [37–39] and the fluorinated oxazoline **Ia** [12], undergo conversion into hydroxylamides **VI** at basic pH. The reactions proceed via the hydroxyoxazolidine **IV** and aminoester **V** intermediates (Scheme 1). Accordingly, it is reasonable to assume that an aminoester structure is associated with the lower hRf entity **Va** (cf. Fig. 1 and see below for structural arguments).

*3.2.2. Structural arguments for the ester character of the **Va** entity*

The behavior of **Ia** was tested in a phosphate buffer at pH 6.3 since the formation of the aminoesters **V** which is known to be favored under acidic hydrolysis of oxazolines **I** [11,12,37–39]. The densitograms of a **Ia** solution after 15–19 h standing at pH 6.3 (cf. Fig. 2Aa–d) clearly indicated the complete

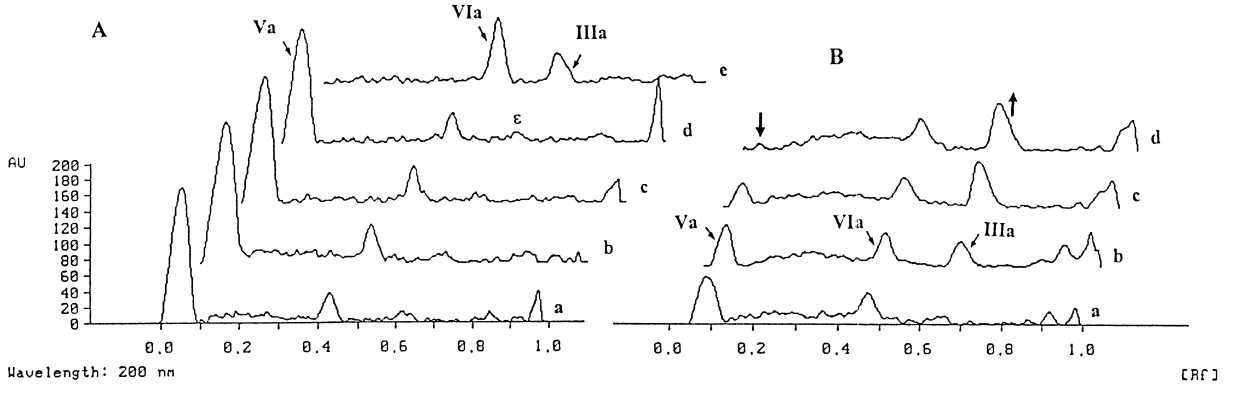


Fig. 2. Assays focused on the intermediate aminoester **Va** production. (A) Behavior of **Ia** after 15 h standing in a phosphate buffer at pH 6.3 (blank). Manual deposite: 5 μl of a solution of **Ia** ($[\mathbf{Ia}]_0=10^{-3}$ *M*) transformed into **Va**. Elution: A conditions. a "t_0" (=15 h standing at pH 6.3), b "$t_{1\,h}$", c "$t_{2\,h}$", d "$t_{4\,h}$", e standards **IIIa** and **VIa** (10^{-3} *M* in CH_3CN–phosphate buffer, 5:95, v/v). (B) Evolution of **Va** after addition of a supported esterase. Deposite: 5 μl of $[\mathbf{Va}]_0\sim10^{-3}$ *M*, cf. sample "t_0" in (A). Elution: A conditions. a "t_0", b "$t_{1\,h}$", c "$t_{2\,h}$", d "$t_{4\,h}$". Identification of compounds: cf. Fig. 1.

disappearance of **Ia** and the presence of an important peak at a low hRf (hRf=7.0), previously assigned to **Va**. The formation of the hydroxylamide **VIa** (hRf=42) was less pronounced than at pH 7.4. In addition, the formation of a slight amount of the carboxylate **IIIa** (hRf=65) was perceptible (cf. Fig. 2Ad–e).

To an aliquot of a **Ia** solution standing 15 h at pH 6.3 (phosphate buffer), was added a commercial esterase. This addition resulted in a rapid increase of the carboxylate **IIIa** (hRf=65), and a concomitant decrease in the low hRf peak assigned to **Va** (hRf=9), while the hydroxylamide **VIa** seemed to be nearly constant with time at this pH (cf. Fig. 2Ba–d). A previous ^{19}F monitoring [11,12] has made it evident that **Ia** undergoes complete conversion into an intermediate at pH 6.3, itself rapidly transformed into carboxylate **IIIa** by addition of a supported esterase. The $^{13}C[^{1}H]$ spectrum of a **Ia** concentrated solution ($8\cdot10^{-2}$ *M*) recorded at this pH revealed the appearance of a new series of signals ascribable to **Va**, notably a ^{13}C resonance typical of a carbonyl group of an ester function.

These results nicely confirm the ester character for **Va** intermediate.

*3.2.3. Behavior of **Ia** in the presence of locust tissues*

The densitogram of the locust mesenteron (Fig. 3A1a, B conditions of elution) indicates that important low and high-hRf-signals corresponding to endogenous components made the substrate monitoring more difficult than in phosphate buffer, due to the masking of **Ia** and **Va** signals. Concerning the in vitro behavior of **Ia** in the concentrated locust mesenteron (Fig. 3A1b–e), the densitograms show as early as ''t_0'' time clear differences as compared with the monitoring conducted in a phosphate buffer (pH 7.4), i.e., the absence of the hydroxylamide **VIa** and a gradual increase of the carboxylate **IIIa** (hRf=57). After 4 h, the hydrolysis rate of **Ia** was estimated to about 70% on the basis of the compared integration of **IIIa** and standard signals (Fig. 3A1f). Due to the low absorptivity ϵ of **Ia** at 200 nm, a high initial concentration was used ($[\mathbf{Ia}]_0=5\cdot10^{-3}$ *M* in mesenteron–CH_3CN, 95:5). This high substrate concentration can account for the partial unmasking of **IIIa** observed under the TLC monitoring conditions while ^{19}F-NMR monitoring ($[\mathbf{Ia}]_0=5\cdot10^{-4}$ *M* [11,12]) indicated instead a complete hydrolysis in 3 h. Indeed, a partial inhibition of the enzymatic process by excess substrate is susceptible to occur in the TLC monitoring conditions.

An ion-pairing technique using cetrimide bromide (Fig. 3B1, C elution conditions) led to the same conclusions concerning the **Ia** behavior, i.e., an efficient unmasking of **IIIa**. However, major differences were observed between the densitograms obtained under the B and C elution conditions. A first difference is the inversion of the spots concerning **IIIa** and **VIa**, since **IIIa** becomes more lipophilic under ion-pairing conditions. A second difference deals with the high-R_F region of the mesenteron densitogram. Not surprisingly, the ion-pairing modified both the retention of ionic endogenous components and that of residual silanols. This last effect accounts for the hRf increase observed for **Va** when changing the elution conditions (B for C, Table 1, Fig. 3).

Thus, it appears that the TLC and NMR monitorings lead to the same conclusions, i.e., the very efficient hydrolysis of **Ia** into the corresponding carboxylate without significant formation of the hydroxylamide **VIa**. With concentrated locust fat-body, a very similar behavior was observed. Due to its biological properties and metabolism the oxazoline **Ia** is obviously a proinsecticide of carboxylate **IIIa**.

*3.3. TLC study of Δ^2-oxazoline-1,3 **Ib** and **Ic***

*3.3.1. Behavior of **Ib** and **Ic** as a function of pH*

Since there is no evolution of **Ib–c** into **Vb–c**, **VIb–c** or **IIIb–c** in a phosphate buffer at pH 7.4, TLC monitorings are not represented while standards hRf are listed in Table 1.

In contrast, after 3 days in a phosphate buffer at pH 6.3 the oxazoline **Ib** is almost completely and selectively transformed into the corresponding aminoester **Vb**, without significant production of the hydroxylamide **VIb** (cf. Fig. 4A and Table 1). The ester character for **Vb** was evidenced by the finding that addition of a commercial esterase induces the disappearance of **Vb** and the concomitant formation of the carboxylate **IIIb** (cf. Fig. 4B and Table 1). It should be emphasized that, both the formation of **Vb**

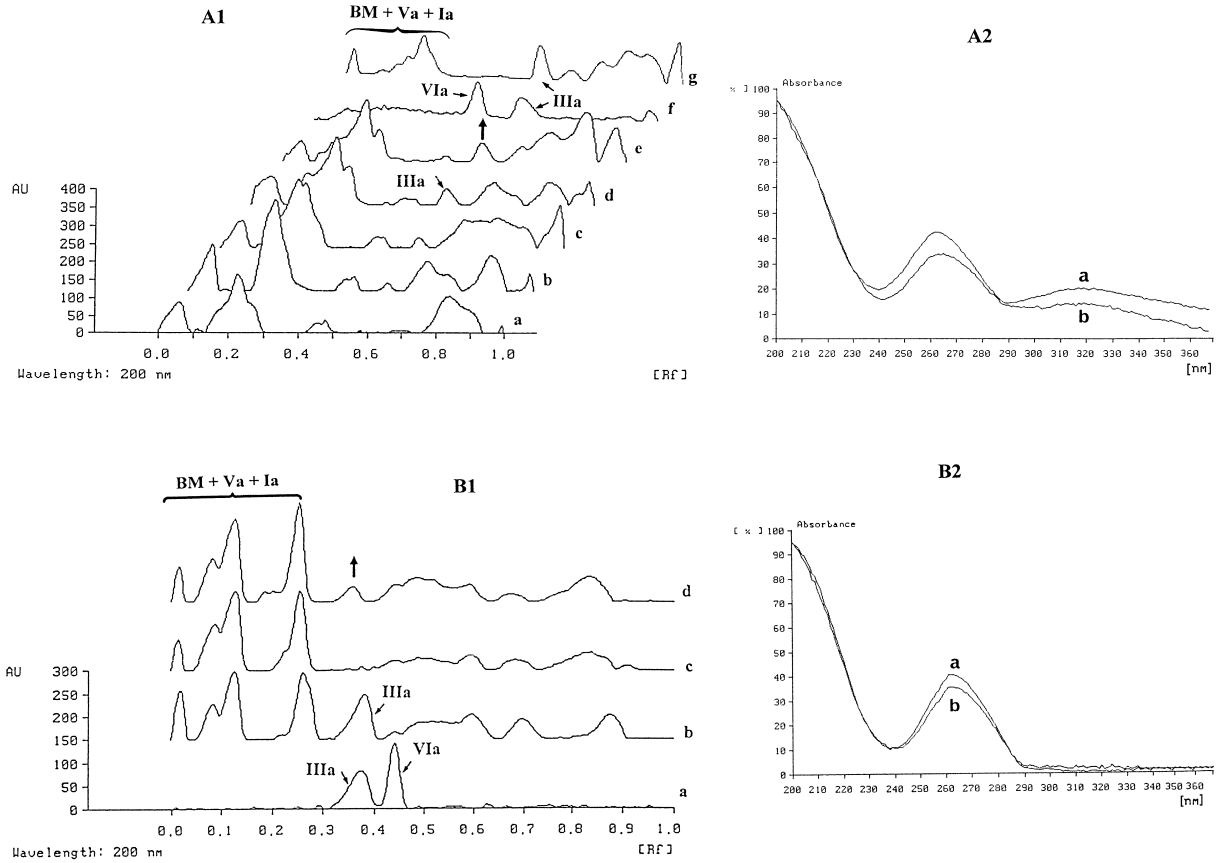


Fig. 3. Thin-layer chromatography monitoring of the metabolization of the oxazoline **Ia** in the presence of locust mesenteron. (A1) Densitograms resulting from B elution conditions. BM: Endogenous components of the biological medium. Deposite: 5 μl of $[\mathbf{Ia}]_0 = 1.25 \cdot 10^{-3}$ *M* (after a fourfold dilution). a blank=biological medium, b "t_0", c "$t_{1\,h}$", d "$t_{2\,h}$", e "$t_{4\,h}$", f standards **IIIa** and **VIa** (10^{-3} *M*), g standard IIIa $1.25 \cdot 10^{-3}$ *M* after fourfold dilution. (A2) In situ UV-reflectance spectra in locust mesenteron (B conditions). a carboxylate **IIIa**, b carboxylate **IIIa** resulting from **Ia** hydrolysis. (B1) Densitograms resulting from C elution conditions. Deposite and other conditions: cf. (A1) a standards **IIIa** and **VIa** (10^{-3} *M*), b standard **IIIa** in mesenteron ($1.25 \cdot 10^{-3}$ *M* after fourfold dilution, c blank=biological medium, d "$t_{3\,h}$". (B2) In situ UV-reflectance spectra in locust mesenteron (C conditions). a Carboxylate **IIIa**, b carboxylate **IIIa** resulting from **Ia** hydrolysis.

and its hydrolysis by an esterase seem to be more difficult than for **Va**. The aminoester **Vc** corresponding to **Ic** has never been observed.

*3.3.2. Behavior of **Ib** and **Ic** in concentrated locust biological media*

In the presence of concentrated locust mesenteron **Ib** showed the same stability as in a phosphate buffer at pH 7.4, without any significant transformation into **Vb** or **IIIb**, even after a period of several hours (Fig. 5). The same result was obtained with **Ic**.

So, by virtue of their great stability at physiological pH, which prevents both the formation of the aminoester **Vb,c** and the subsequent hydrolysis into the related carboxylates, the oxazolines **Ib–c** are not proinsecticides of these carboxylates **IIIb,c**.

The difference in behavior observed between **Ib,c** and **Ia** can be explained by considering that in Scheme 1, the first step leading to the formation of the 2-hydroxyl-oxazolidine **IV**, is disfavored in the **b,c** cases. As a matter of fact, due to either R_2-donor inductive or R_2-electronic-delocalization effects, the electrophilicity at the C_2 center is reduced for **Ib** and **Ic**, respectively. Also, steric effects may partially

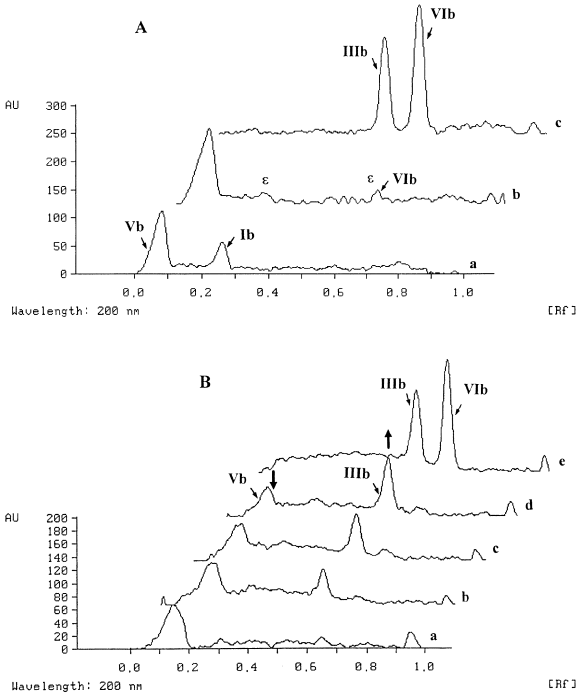


Fig. 4. Direct TLC monitoring of oxazoline **Ib** in a phosphate buffer. (A) Blank in a phosphate buffer at pH 6.3. Deposite of 5 μl of $[\mathbf{Ib}]_0 = 5 \cdot 10^{-4}$ *M*. Elution: E conditions. a after 1 day, b after 3 days, c standards **IIIb** and **VIb** ($5 \cdot 10^{-4}$ *M*). (B) Evolution of the previous solution after addition of a supported esterase. Deposite of 5 μl of $[\mathbf{Vb}]_0 = 5 \cdot 10^{-4}$ *M*. Elution: E conditions. a "t_0", b "$t_{1\,h}$", c "$t_{2\,h}$", d "$t_{4\,h}$", e standards **IIIb** and **VIb** ($5 \cdot 10^{-4}$ *M*) Identification of the compounds: cf. Fig. 1.

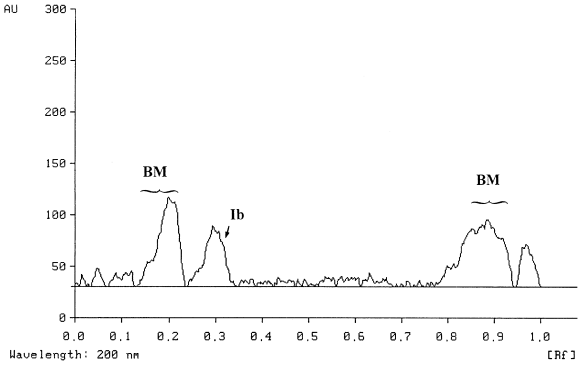


Fig. 5. Direct TLC monitoring of oxazoline **Ib** in the presence of locust mesenteron. Deposite of 5 μl of $[\mathbf{Ib}]_0 = 5 \cdot 10^{-4}$ *M* (after fourfold dilution). Elution: E conditions. "$t_{2\,h}$". Identification of the compounds: cf. Fig. 1.

explain the inhibition of paths 1 and 2 in the enzymatic and in the non-enzymatic processes involving **1b**.

*3.4. TLC study of the N-acylaziridine **VIIc***

We previously established that the *N*-acylaziridine **VIIa** (Scheme 2) is also a convenient structure for the reversible masking of **IIIa** [32]. Taking into account the irreversible masking of the carboxylate **IIIc** brought about the oxazoline **Ic** structure, it remained to determine the nature of the masking provided by the *N*-acylaziridine **VIIc**.

*3.4.1. Behavior of **VIIc** according to pH*

The blank performed at pH 7.4 (in a phosphate buffer, cf. Fig. 6-1a) indicated a slight hydrolysis of the substrate into the hydroxylamide **VIc**′ without any formation of **IIIc** or **VIc**: only path 2 of Scheme 2 [40] is efficient in these conditions.

*3.4.2. Behavior of **VIIc** in concentrated locust biological media*

In marked contrast with the above behavior, the proinsecticide behavior of **VIIc** was the unique evolution in the presence of concentrated locust mesenteron. As early as the "t_0" densitogram **VIIc** was almost fully hydrolyzed into **IIIc** (cf. Fig. 6-1b, -2, Scheme 2 path 1).

4. Conclusion

The major feature of this TLC method is that it provides the opportunity to perform in the same step the substrate and metabolite extraction and TLC affording very rapidly reliable information about the metabolism of new proinsecticide candidates.

Thanks to the fluorinated oxazoline **Ia** chosen as a model for its known metabolism, a TLC procedure allowing a direct monitoring in concentrated locust tissues has therefore been designed. Thus **Ia** was confirmed as being an efficient proinsecticide of the corresponding carboxylate **IIIa**. Both the TLC and ^{19}F-NMR approaches were mutually validated as analytical methods for the monitoring of this substrate. Then this method was applied to study new molecules. Therefore it was clearly made evident

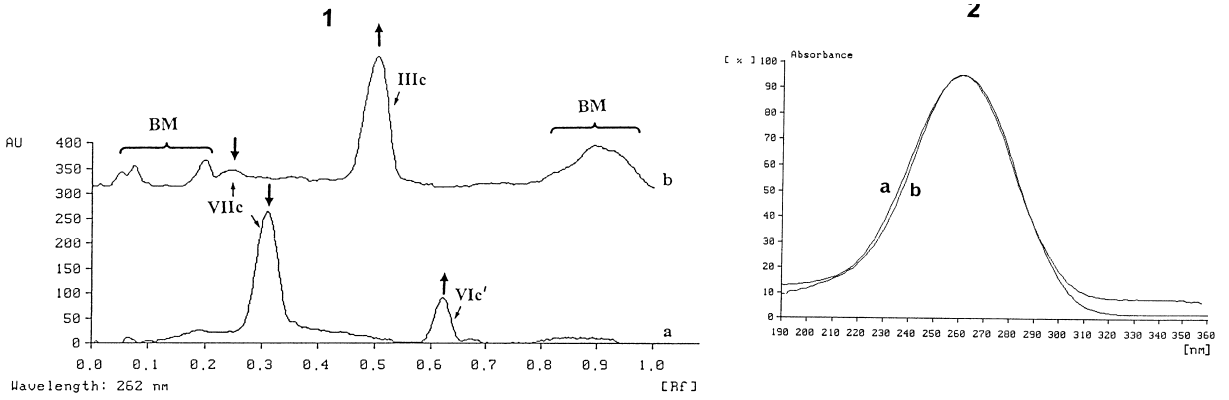


Fig. 6. Direct TLC monitoring of the behavior of the *N*-acylaziridine **VIIc**. (1) Densitograms. Application of 5 μl of $[\mathbf{VIIc}]_0 = 10^{-3}$ *M*. Elution: F conditions. a in the presence of phosphate buffer at pH 7.4 after 2 h, b in the presence of concentrated locust mesenteron at "t_0". (2) In situ UV-reflectance spectra in locust mesenteron. a carboxylate **IIIc**, b carboxylate **IIIc** resulting from **VIIc** hydrolysis.

that oxazolines **Ib** and **Ic** are not proinsecticides of the corresponding carboxylate **IIIb,c**, by contrast to *N*-acylaziridine **VIIc** which is very efficiently hydrolyzed by the enzymes of the locust tissues into corresponding carboxylate **IIIc**.

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Appendix A. Supplementary material

1. In situ UV-reflectance spectra of compounds in Fig. 1: a **Va**, b **Ia**, c **VIa** resulting from the hydrolysis of **Ia**, d **VIa**, e **IIIa**.
2. In situ UV-reflectance spectra of compounds in Fig. 2: a **Va**, b **VIa**, c **IIIa**, d **IIIa** resulting from the hydrolysis of **Va**.
3. In situ UV-reflectance spectra of compounds in Fig. 4: a **Vb**, b **IIIb**, c **IIIb** resulting from the hydrolysis of **Vb**, d **VIb**.
4. In situ UV-reflectance spectra of compounds in Fig. 5: a **Ib** in a phosphate buffer (pH 7.4), b **Ib** after 2 h elapsed in locust mesenteron.

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