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N-acylaziridines as potential proinsecticides of carboxylic acids Part VI. Direct HPLC monitoring of the metabolism in insect tissues☆

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Abstract

To determine the reversible masking potential of carboxylic acids afforded by the *N*-acyl structure in a proinsecticide perspective, the hydrolysis of monosubstituted *N*-acylaziridines and unsubstituted *N*-acylpyrrolidine was studied by reversed-phase high-performance liquid chromatography (HPLC) during in vitro assays conducted in the presence of insect tissues or of α-chymotrypsin. Chromatographic analysis of unextracted biological samples so-called "the direct injection approach" was simpler and more accurate than the "extraction approach" because it avoids problems associated with extraction. Thus, periodical injections of samples of biological insect tissues or of α-chymotrypsin incubated with *N*-acyl substrates were performed on packings allowing direct injection: a wide-pore column or a monolithic column. Moreover, to allow the simultaneous monitoring of the carboxylic acids and of the parent substrates, ion-pairing was used. In these conditions, it was shown that *N*-acylpyrrolidine is not hydrolyzed whatever the enzymatic conditions or the pH. On the other hand, the unmasking of the carboxylic acid is the preponderant mode of hydrolysis of *N*-acylaziridines in the presence of α-chymotrypsine and the exclusive one in the presence of locust fat-body, which establishes the convenience of this structure in our proinsecticide perspective. Due to the enzymatic character of the unmasking of the carboxylic acid during biological hydrolysis of *N*-acylaziridines, the research of possible chiral recognitions was undertaken. Thus, the enantiomeric composition of these substrates was analysed at the stage of their approximative half hydrolysis using a chiral α-AGP column. It appeared that locust fat-body hydrolyses preferentially the (R)-isomers of *N*-acylaziridines while the reverse is observed when α-chymotrypsine is used.

Keywords: *N*-acylaziridine; Proinsecticide; Direct injection; Metabolism; Chirality

1. Introduction

For some time, we have been interested in developing proinsecticides masking reversibly acids and/or β-ethanolamines considered as active principles. Concerning the masking structures, we restricted our choice to molecules designed to have hydrolysis as the "activation mode", such as fluorinated esters [1], enol esters [2], oxazolines [3–7] and thiazolines [4,8]. More recently, we have also examined the potential of particular amides represented by the *N*-acylaziridine structure [4,6,8,9].

The study of the "unmasking" of the active principle, or "activation" step, is of most importance in this approach. With fluorinated substrates, $^{19}F[^{1}H]$ NMR method constitutes a very rapid and convenient analytical tool to explore the potential of new structures by in vitro assays [1,2,5,9] or ex vivo assays [2]. On the other hand, chromatography is more suited for non-fluorinated candidate molecules, and the basic method needed for the monitoring of xenobiotics in biological fluids is obviously the reversed-phase partition. Nevertheless,

☆ See Refs. [2,3,5,7,9] for parts I–V, respectively.
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the direct injection of proteinic fluids (plasma, serum, urine, hemolymph, etc.) on classical C_{18} or C_8 packings entails the rapid increase of the pressure, the decrease of the performances, especially of the efficiency, with a concomitant peak-broadening, and finally the clogging of the column. The problem is yet increased when eluting lipophilic compounds which need high concentrations of organic solvents in the eluents resulting in the denaturation of the proteins [10]. As these samples matrixes are not directly compatible with LC or LC–MS analysis [11], sample pretreatment using protein precipitation, liquid–liquid extraction or solid-phase extraction (SPE) [12] has been the solution frequently adopted. Nevertheless, as analysis of unextracted biological tissues is simpler, faster and avoids the problems associated with extraction of ionizable compounds, numerous solutions allowing the direct injection have emerged. Most of them can be considered as multidimensional chromatographic techniques (MDC) [13]. Multicolumn techniques represent a first MDC approach and many investigators have demonstrated the advantage of using a precolumn with large particulate alkyl-bonded phase silicas or ion exchangers, placed in-line with high-performance ODS column via switching valve arrangements ([13] and references therein, [14]). Another type of MDC strategy is based on a single packing designed for providing simultaneously several mode of separation which prototype is the Internal Surface Reversed-Phase concept developed by Hagestam and Pinkerton with the GFF packing (Gly-Phe-Phe) [15]. Based on the same or on a similar principle, a second generation of ISRP column GFFII [16] and Restricted Access Media (RAMs) [17–19] have been developed later. With the same objective of "direct injection", Shihabi and co-workers [20,21] have successfully used large-pore packings (300 Å) for the analysis of drugs and their metabolites by direct injection of plasma samples. The inconvenient of the lower number of plates resulting from the larger pores is compensated to some extent by smaller particle size (5 μm and even 2 μm) [22]. Taking into account both the importance of the equipment required for the column switching technique and also the cost of GFFII packings, we have adopted since several years the approach of 300 Å packings for direct injection of various complex matrices using either home-made or commercial C_1, C_4 or even C_{18} wide-pore columns [4,6,23–28].

The first aim of this work was to rapidly study the eventual unmasking of carboxylic acids triggered by locust tissues for a series of *N*-acylaziridines **1** and for one *N*-acylpyrrolidine **1′**. Consequently, in the "direct monitoring approach", we used commercial C_4 300 Å columns to analyse untreated biological samples. Ion-pairing (IP) was required because of the presence of ionic metabolites as $\mathbf{3}^-$. We have also tested monolithic packings [29] said of the fourth generation of column [30] with C_{18} packings, taking into account recent reports concerning their application for the direct injection of bio-fluids [29–32].

Secondly, for *N*-acylaziridines demonstrated as presenting a significant enzymatic activation, we researched a possible chiral recognition during their hydrolysis. As corresponding enantiomers were not easily available, we indirectly analysed the enantiomeric excess for unchanged *N*-acylaziridines **1** resulting from an incomplete activation of the corresponding racemate. Therefore, after a solid-phase extraction stage, the unchanged *N*-acylaziridines **1** were univocally transformed into their corresponding α-*O*-substituted β-hydroxylamides **4** which enantiomeric excess (e.e.) was determined using a chiral α-AGP column [33,34].

2. Experimental

2.1. Reagents and chemicals

2-Methylaziridine, dicyclohexylcarbodiimide (DCC) and 2-aminopropanol (racemate or pure enantiomer) were supplied by Aldrich (Sigma–Aldrich Chimie, L'Isle d'Abeau Chesnes, Saint-Quentin Fallavier, France). CH_2Cl_2 was supplied by SDS (Solvent Documentation Synthèse, Vitry, France).

Racemates of N-acyl-2-methylaziridine ***1a–b*** were obtained by condensation of commercial racemate 2-methylaziridine **2** (1 equiv.) added at 0 °C to corresponding commercial carboxylic acid **3** (1 equiv.) in the presence of DCC (1 equiv.) in CH_2Cl_2. After 24 h standing at room temperature, the crude *N*-acylaziridines resulting from filtration of the dicyclohexylurea and evaporation of the solvent were distilled under vacuum.

N-p-fluoro-phényl-acetyl pyrrolidine ***1′*** was obtained according to the same protocol.

Hydroxylamides **4a–b** were obtained as racemates or as pure enantiomers by condensation of 1 equiv. of 2-aminopropanol (racemate or pure enantiomer) added at 0 °C, with 1 equiv. of carboxylic acid **3a–b** using 1 equiv. of DCC in CH_2Cl_2. After 24 h standing at room temperature, and filtering off the dicyclohexylurea and evaporation of the solvent, the crude hydroxylamides were purified by TLC (Silica Merck GF 254, eluent: ethylacetate/*n*-heptane/methanol: 57/41/2). All condensations in the presence of DCC were conducted with reactive and solvents free of water.

Elemental analysis or HRMS, IR and NMR data for **1a–b** and their corresponding hydoxylamides **4a–b** and **1′** agree well with the proposed structures. These new compounds will be completely described elsewhere [35].

2.2. Chemicals for chromatography

Acetonitrile and methanol were of HPLC grade (SDS, Solvent Documentation Synthèse, Vitry, France). Deionized water (18 MΩ) obtained with a Milli-Q apparatus (Millipore, Saint-Quentin-en-Yvelines, France) was used for the preparation of mobile phases and phosphate buffers by mixing appropriate volumes of dissolved monobasic and dibasic potassium phosphates which were supplied by Acros (Noisy le Grand, France). Tetrabutylammonium bromide