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Short Communication

Endogenous alkaloids in man

XI.* Analysis of glyoxylate-derived 1,3-thiazolidines by ion-pair-assisted reversed-phase chromatography

Gerhard Bringmann*, Christiana Hesselmann and Doris Feineis

Institute of Organic Chemistry, University of Würzburg, Am Hubland, 8700 Würzburg (Germany)

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ABSTRACT

Reversed-phase ion-pair chromatography, using cetyltrimethyl ammonium hydrogensulphate as detergent, has been applied to the analysis of the highly polar glyoxylate-derived 1,3-thiazolidines **1**, **2** and **3**. On the base of this high-performance liquid chromatographic separation of the diastereomeric compounds **1a/b** and **3a/b** was achieved. Removal of the hydrophobic couterion by precipitation as its insoluble iodide, followed by an extraction with chloroform, seems to be a promising first step to establish ion-pair-assisted chromatography as a preparative high-performance liquid chromatographic method for the isolation of polar compounds.

INTRODUCTION

Binucleophilic amino acids, such as L-cysteine and D(−)-penicillamine, as well as their biogenic amines, such as cysteamine, rapidly undergo a cyclo-condensation reaction with glyoxylic acid, leading to the 1,3-thiazolidines **1a/b**, **2** and **3a/b** (Fig. 1), respectively, without formation of side-products [1,2].

The deliberate formation of such extremely polar, alkaloid-type heterocycles by a spontaneous, *i.e.* non-enzymatic, reaction is part of our therapeutic concept for the treatment of glyoxylate-induced oxalurias, with the aim of achieving chemical and thus physiological deactivation of toxic glyoxylic acid [2,3]. A drastic reduction in the glyoxylate level of the organism is necessary, especially for the treatment of the inherited metabolic disease hyperoxaluria [4], which is characterized by a fatal overproduction of oxalic acid and thus calcium oxalate. Also for ethylene glycol intoxications [5] and metabolic problems resulting from transurethral prostatectomy [6], the harmless scavenging of glyoxylic acid must be the goal of a specific therapy, since in both cases symptoms can be observed that indicate a direct action of glyoxylic acid on the central nervous system [7].

In this paper, we describe an analytical device that is not only suitable for the separation of the structurally similar thiazolidines **1**, **2** and **3**, but also allows the resolution of diastereomeric pairs of 2,4-thiazolidine-dicarboxylic acids **1a** and **1b** as well as **3a** and **3b** (see Fig. 2). With the assistance of ion-pair-forming detergent cetyltrimathyl ammonium

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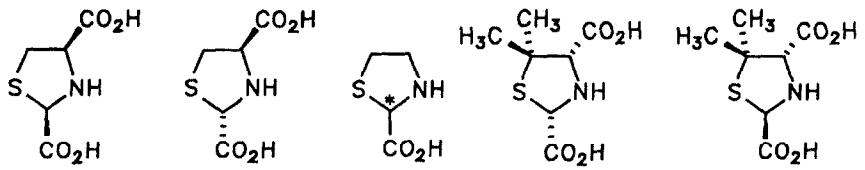

Fig. 1. Structures of glyoxylate-derived 1,3-thiazolidines.

hydrogensulphate, sufficient interaction between the hydrophobic substrate/counterion pair and the non-polar stationary C_{18} phase can be achieved. Using a coupled procedure, consisting of precipitation of the lipophilic cation (with iodide) and subsequent extraction (into chloroform), the detergent may furthermore be removed nearly quantitatively — an important precondition for a possible application of this method to preparative high-performance liquid chromatography (HPLC).

Ion-pair-assisted reversed-phase chromatography proved to be superior not only to thin-layer chromatography, but also to the method of ion suppression by the choice of the pH. Although classical chromatography of disubstituted thiazolidines on silica gel or on paper with special eluent systems adopted from amino acid analysis is possible and has been described for **1** [8], separation of the diastereomers could not be attained. Undesired effects on both the stationary phase and the dissolved molecule, caused by pH changes in ion-suppression chromatography, are limited in ion-pair chromatography, since it can be performed under neutral pH conditions.

EXPERIMENTAL

Chemicals

High-purity Milli-Q water (Millipore, Bedford, MA, USA) and chloroform of analytical-grade quality were used. Sodium dihydrogenphosphate dihydrate and disodium hydrogenphosphate monohydrate were obtained from Merck (Darmstadt, Germany). Cetyltrimethyl ammonium bromide was purchased from Fluka (Buchs, Switzerland) and converted into the hydrogensulphate by recrystallization from 0.5 *M* aqueous sulphuric acid. The aqueous mobile phase (pH 7.4) was prepared by dissolving 3.52 g (9.2 mmol) of cetyltrimethyl ammonium hydrogensulphate, 7.14 g (40 mmol) of disodium hydrogenphosphate dihydrate and 2.07 g (15 mmol) of sodium dihydrogenphosphate monohydrate in 1 l of HPLC-grade water. The organic mobile phase was HPLC-grade methanol. The mobile phase components were filtered through an HV 0.45-μm pore membrane filter (Millipore) and deaerated by sonication.

Synthesis

The thiazolidines **1** and **2** were prepared by dissolving a 1:1 molar ratio of glyoxylate (Merck) and of the aminothiols cysteamine or L-cysteine (Fluka) in ethanol. The solutions were adjusted to pH 6.5–7 with 2 *M* sodium hydroxide. The resulting crystalline heterocycles **1** and **2**, respectively, were isolated according to the procedure described by Fourneau *et al.* [9]. Compound **3** was synthesized by adding an equimolar amount of D-penicillamine (Degussa, Germany) to an aqueous solution of glyoxylic acid and isolated by freeze-drying. In principle, these syntheses have already been published in the literature [9–12], but without a full characterization of the diastereomeric products. Complete elucidation of the structure of the thiazolidines **1a/b**, **2** and **3a/b** by spectroscopy and by X-ray crystallography will be reported in a separate paper.

High-performance liquid chromatography

All experiments were carried out on a modular HPLC system, consisting of two M 510 pumps (Waters), a U6K injection valve (Waters) and a Lambda-Max Model 481 spectrophotometer (Waters). Chromatographic analyses were performed on a reversed-phase C_{18} column (Waters μBondapak C_{18}, 30 cm × 3.9 mm I.D., particle size 10 μm) using various mixtures of an isocratic mobile phase of methanol–phosphate buffer (pH 7.4) with 0.35% cetyltrimethyl ammonium hydrogensulphate as ion-

pair reagent. The UV absorption wavelength was set at 265 nm. The flow-rate was maintained at 2 ml/min. An HPLC manager PC control system (Waters, Maxima System with Interface I-200) in combination with Maxima evaluation software 820 was used for data acquisition and processing.

Calibration graphs and detection limits

Stock solutions of the thiazolidines **1**, **2** and **3** were prepared by dissolving 9.71 mg of **1**, 9.18 mg of **2** and 13.99 mg od **3** in 1 ml of Milli-Q water. Various aliquots of the undiluted (40, 20 μl) and of the ten-fold diluted stock solution (80, 60, 40, 20 μl) were subjected to HPLC in the system mentioned above to obtain data for calibration graphs and for detection limits. In each instance, calibration curves were constructed by plotting the integrated peak area against the corresponding thiazolidine standard concentration.

Detergent extraction procedure

A 166-mg aliquot (10 mmol) of potassium iodide was added to 100 ml of a solution of 3.52 g (9.2 mmol) of cetyltrimethyl ammonium hydrogensulphate in 1 l of Milli-Q water. After extraction with chloroform (3 × 33 ml), the organic layer was made up to 100 ml. Three aliquots of 10 ml were evaporated to dryness. The amount of detergent extracted was obtained by weighing the residue and calculating the average recovery.

RESULTS AND DISCUSSION

Choice of a suitable detergent

Sufficient interaction between highly polar substrate molecules and non-polar stationary phase can be achieved only by using distinctly lipophilic counterions dissolved in the mobile phase. Because of the presence of only one amino group, but two carboxyl functions, the amphoteric thiazolidines **1**, **2** and **3** were chromatographed in an anionic form, under addition of a positively charged detergent. In this way, up to two detergent molecules can be bound to the dicarboxylic acids at appropriate pH values.

Because of the very high polarity of the examined heterocycles **1**, **2** and **3**, an important factor in the selection of an appropriate eluent is the choice of the ion-pair reagent. The first, orientating experiments aimed at finding a suitable detergent were performed under pH conditions (pH 9.4) that allow the complete double deprotonation of the substrate molecules, thus maintaining the pH value near to the point of destruction of a C_{18} phase for a short time. Initially, we tested tetrabutyl ammonium chloride under these pH conditions, using a water–acetonitrile-containing solvent — a system that has already been described in the literature — for the ion-pair chromatography of an *N*-formylated 1,3-thiazolidine from penicillin degradation [13]. However, for the highly polar 2,4-thiazolidine-dicarboxylic acids, sufficient retention could not be achieved. In contrast, the long-chain, distinctly more lipohilic cetyltrimethylammonium ion could be used far more successfully under identical analytical conditions.

Optimization of the eluent

For the elaboration of optimum chromatographic conditions, maximum ionization of the substrate molecules was required. However, the extreme pH sensitivity of the column material, particularly towards quaternary ammonium salts in alkaline media, had to be taken into account [14]. Fortunately, using cetyltrimethyl ammonium salts in water–methanol mixtures at pH 7.4, sufficient ionization of the substrate molecules could be achieved. As Fig. 2 shows, separation of the 1,3-thiazolidine **2** and the diastereomers **1a** and **1b**, as well as **3a** and **3b**, could be achieved in a single analytical step, using this column-preserving eluent system.

Reproducibility and linearity

For an examination of the reproducibility and linearity of the described analytical procedure, and for an estimation of the detection limit, as obtained by UV recording at the absorption maximum of the compounds at 265 nm, analysis functions $m = f(R)$, with m = mass of the analyte and R = detector response, were determined for **1**, **2** and **3**. For all these compounds, a linear correlation between the injected mass and the detector response was found over the whole concentration range (see Experimental section), with a correlation coefficient $r > 0.99$.

The detection limits for **1a** ($3.4 \cdot 10^{-6}$ g), **1b** ($1.3 \cdot 10^{-5}$ g), **2** ($1.6 \cdot 10^{-5}$ g), **3a** ($3.9 \cdot 10^{-6}$ g), and **3b** ($2.4 \cdot 10^{-5}$ g), which are in the microgram range for UV spectroscopic detection (265 nm), reflect the

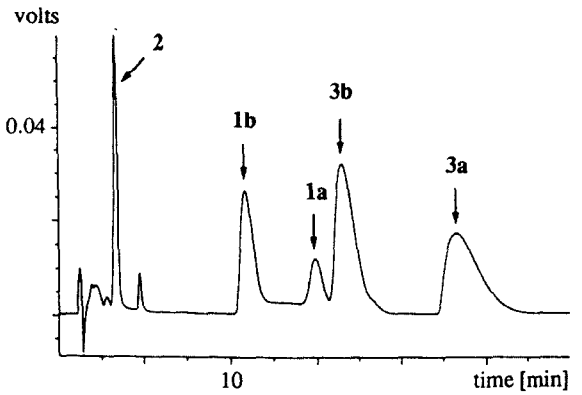

Fig. 2. Ion pair-assisted reversed-phase HPLC separation of the diastereomeric 1,3-thiazolidines **1a/b**, **2** and **3a/b**. Eluting solvent: aqueous mobile phase–methanol (50:50). Chromatographic conditions: see Experimental section.

low-intensity UV maximum of the small thiazolidine heterocycles, which moreover is located almost exactly at the UV cut-off of the utilized solvents. In consequence, although the practicability of the analytical procedure is restricted to samples whose content of **1**, **2** and **3** are not in the trace range, the possibility of a complete removal of the detergent hints at novel fields of application.

Removal of the detergent

The special problems of the extraction of aqueous, detergent-containing solutions with organic solvents (*i.e.* the formation of emulsions) and the problems of precipitation (*i.e.* adsorption effects) could be solved by a promising coupled procedure, by extracting the initially precipitated iodide and its aqueous mother liquor with chloroform. In this way, not only could substance inclusions eventually formed in the precipitation step be released, but also, astonishingly, practically no problems of emulsion formation occurred. Recovery of cetyltrimethyl ammonium hydrogensulphate was calculated to be 103%.

CONCLUSION

The method presented in this paper is a novel approach to the solution of the presented chromatographic problems. Not only is it applicable to analytical samples, but it also seems to be of value for exploitation even on a preparative scale. In this way, ion-pair-assisted reversed-phase chromatography, with its great chromatographic potential, might be admitted to the arsenal of preparative HPLC methods for the first time. This work is in progress.

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