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Preparative separation of stereoisomeric 1-methyl-4-methoxymethylcyclohexanecarboxylic acids by pH-zone-refining counter-current chromatography

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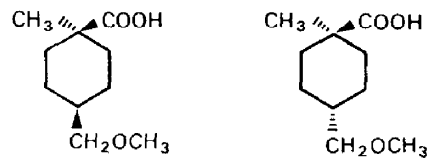
Abstract

The application of pH-zone-refining counter-current chromatography (CCC) to the preparative separation of stereoisomeric acids is described. The separation was accomplished on the basis of the difference in acidity of the two stereoisomers. pH-Zone-refining CCC of 400 mg of a crude synthetic mixture of stereoisomeric 1-methyl-4-methoxymethylcyclohexanecarboxylic acids yielded 49.5 and 40 mg of the pure *Z*- and *E*-stereoisomers, respectively. The two-phase solvent system consisted of hexane–ethyl acetate–methanol–water (1:1:1:1). Trifluoroacetic and octanoic acids were used as retainer acids. The eluent base was aqueous ammonia. The eluted fractions were monitored by gas chromatography–mass spectrometry.

1. Introduction

As part of an ongoing investigation of stereochemical effects in the fragmentation of organic gas-phase ions on chemical ionization and electron ionization [1,2], pure methyl esters of *Z*- and *E*-1-methyl-4-methoxymethylcyclohexanecarboxylic acids (**Z-1** and **E-1**, respectively, Fig. 1) were needed. The synthetic preparation of these compounds resulted in a crude mixture of methyl esters of **Z-1** and **E-1**. This mixture was considered appropriate to subject to a preparative separation technique recently developed in our laboratories, pH-zone-refining counter-current chromatography (CCC) [3,4], which enables effective separations of organic acid components of multigram mixtures [3–8]. This technique requires the addition of a retainer acid [e.g.,



Z-1 **E-1**

Fig. 1. Structures of 1-methyl-4-methoxymethylcyclohexanecarboxylic acids: **Z-1** (*cis* isomer) and **E-1** (*trans* isomer).

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trifluoroacetic acid (TFA)] to the sample solution or stationary phase to enhance partitioning of the analytes into the stationary organic phase. Isocratic elution with an eluent base (e.g., aqueous ammonia) elutes the acid components as well-resolved rectangular peaks in the order of their pK_a values and hydrophobicities [3]. In addition, a spacer acid (e.g., octanoic acid) is sometimes used to aid the separation. Although UV detection (which produces a series of broad rectangular peaks) may not indicate that separations have occurred, monitoring the pH of the eluted fractions results in a series of plateaus that correspond to the separated components. Because equatorial and axial carboxyl groups in cyclohexanecarboxylic acids differ in acidity by approximately 0.5 pK units [9,10], **Z-1** and **E-1** were considered appropriate compounds to be separated by pH-zone-refining CCC.

2. Experimental

2.1. Materials

Hexane, ethyl acetate, methanol and water were chromatography grade. TFA (Sigma, St. Louis, MO, USA), octanoic acid (Eastman-Kodak, Rochester, NY, USA) and aqueous ammonia (>25% NH_3 in water, Fisher Scientific, Pittsburgh, PA, USA) were used as received. The crude mixture of **Z-1** and **E-1** was prepared by the following route: 1,4-cyclohexanedimethanol (*cis–trans* mixture, Aldrich, Milwaukee, WI, USA) was partially methylated to 1-hydroxymethyl-4-methoxymethylcyclohexane [11]. Oxidation of the latter compound to 4-methoxymethylcyclohexanecarboxylic acid ($KMnO_4$, NaOH in water) followed by methylation with iodomethane and lithium diisopropylamide (LDA) in tetrahydrofuran [12] yielded the crude mixture of the methyl esters of **Z-1** and **E-1**. These esters were hydrolyzed to **Z-1** and **E-1** for the purpose of the present separation. The detailed procedure will be reported elsewhere [2].

2.2. Apparatus

The separation was performed using a commercial high-speed CCC centrifuge (P.C. Inc., Potomac, MD, USA) that holds an Ito multilayer-coil separation column and a counterweight whose centers revolve 10 cm around the centrifugal axis. A multilayer column was constructed by one of us (Y.I.) from polytetrafluoroethylene tubing (ca. 165 m × 1.6 mm I.D., with a total capacity of approximately 325 ml). The β value (a centrifugal parameter) [13] ranged from 0.5 at the internal terminal to 0.85 at the external terminal. The column consisted of 16 coiled layers. Similar columns are commercially available from P.C. Inc., Pharma-Tech Research Corp. (Baltimore, MD, USA) and Shimadzu (Kyoto, Japan).

2.3. Solvent system, sample solution and separation procedure

The two-phase solvent system consisted of hexane–ethyl acetate–methanol–water (1:1:1:1). The solvent system was thoroughly equilibrated in a separatory funnel and the two phases were separated shortly before use. To the lower phase was added aqueous ammonia (3.4 mmol NH_3/1000 ml, pH 9.89). To the upper phase (500 ml) were added 100 μl of TFA (1.3 mmol) and 100 μl of octanoic acid (0.63 mmol).

For the preparative separation, 400 mg of the crude mixture containing **Z-1** and **E-1** was dissolved in 3 ml of the above solvent system (2.5 ml of unacidified upper phase and 0.5 ml of unbasified lower phase).

The separation was initiated by filling the column with the stationary (upper) phase by using a metering pump (Accu-Flo pump; Beckman, Palo Alto, CA, USA). The sample solution was injected through the sample port into the column by syringe. The mobile (lower) phase was then pumped into the column at a flow-rate of 3 ml/min while the column was rotated at 800 rpm in the head-to-tail mode. The column effluent was monitored with a UV detector (Uvicord S; LKB Instruments, Stockholm,

Sweden) at 206 nm, to which was attached an LKB 6-channel strip-chart recorder at a chart speed of 1 cm/20 min with a full-scale response of 2 absorbance units. Fractions (6 ml) were collected using a fraction collector (Ultrorac, LKB Instruments). The pH of each eluted fraction was measured with a pH meter (Accumet 1001; Fisher Scientific, Pittsburgh, PA, USA). All fractions of interest were brought to dryness by freeze-drying and were analyzed by gas chromatography–mass spectrometry (GC–MS).

2.4. Gas chromatography–mass spectrometry

The system used consisted of a Hewlett-Packard 5890 Series II gas chromatograph interfaced with an HP-5971 mass-selective detector. Full scan data were acquired. The gas chromatograph was equipped with a Supelcowax 10 fused-silica capillary column, 30 m × 0.25 mm I.D. and 0.25 μm film thickness (Supelco, Bellefonte, PA, USA). The initial column temperature was 40°C, and the solvent delay was 7 min. The column temperature was increased from 40 to 200°C at 15°C/min and from 200 to 270°C at 5°C/min. The injector temperature was 225°C.

A small portion of the dry residue from each fraction of interest was redissolved in ethyl acetate and injected (1 μl) into the GC–MS system.

3. Results and discussion

GC–MS analysis of the crude mixture used in the present work showed that the *Z* and *E* isomers were well separated along with several other components (Fig. 2); 400 mg of this mixture were preparatively separated by pH-zone-refining CCC. UV detection and pH monitoring of the eluted fractions from the CCC separation produced the counter-current chromatogram shown in Fig. 3a. The solvent front (first fraction containing mobile phase) emerged at fraction 13. Retention of the stationary phase, calculated from the volume of stationary phase collected from the column after the separation,

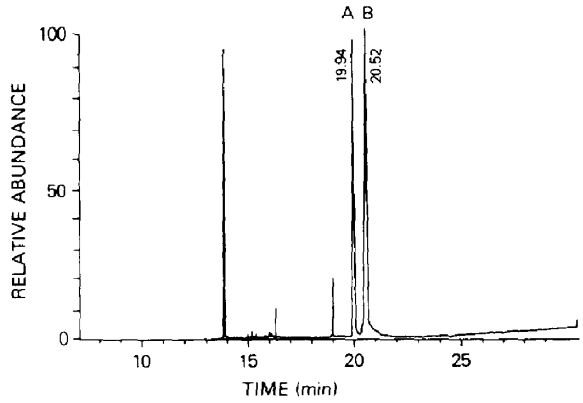

Fig. 2. GC–MS analysis of the crude synthetic mixture of **Z-1** (peak A) and **E-1** (peak B).

was 70.7%. The chromatogram obtained by UV detection has a broad rectangular shape. The low, flat absorbance line (following a sharp peak which represents impurities eluting between the TFA and octanoic acid, respectively, in Fig. 3a) represents saturation of the detector. This saturation is due to the strong absorbance at 206 nm of ethyl acetate, a component of the solvent system used. The two hatched areas correspond to the two descending pH plateaus. Each hatched area represents elution of a pure compound. The eluates corresponding to these areas were collected in fractions 42–63 and 69–76.

Fractions 42–63 contained a single component (40 mg) whose GC retention time (Fig. 3b) was identical to that of peak B in Fig. 2. Fractions 69–76 contained a single component (49.5 mg) whose GC retention time (Fig. 3c) was identical to that of peak A in Fig. 2. The structural assignments (fractions 42–63: **E-1**; fractions 69–76: **Z-1**) were made by comparing the nuclear magnetic resonance (NMR) spectra of the two separated stereoisomers with the NMR spectrum of the *cis* isomer, **Z-1**, whose configuration was determined by X-ray crystallography [2]. The separation of these stereoisomers by pH-zone-refining CCC was possible because of the difference in acidity (pK_a) of axial- and equatorial-oriented carboxyl groups in cyclohexanecarboxylic acids [9,10]. The axial carboxyl group in **Z-1** interacts with the hydrogens in positions 1 and 3,

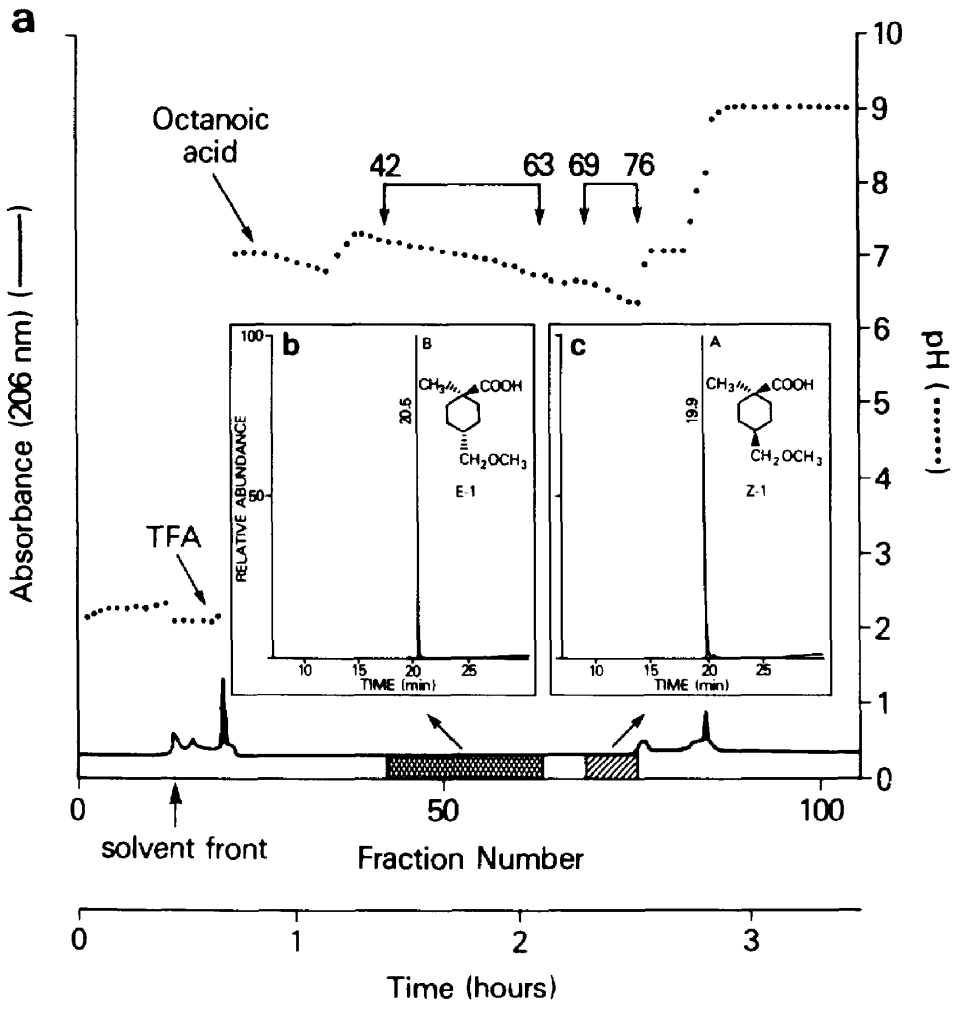

Fig. 3. pH-Zone-refining CCC of 400 mg of a synthetic mixture of **Z-1** and **E-1** (see Fig. 2). (a) Counter-current chromatogram and pH profile of the separation. (b) GC–MS analysis of combined fractions 42–63. (c) GC–MS analysis of combined fractions 69–76.

resulting in hindrance of solvation and hence acid weakening [10] (Fig. 4). In pH-zone-refining CCC, acids elute in descending order of acidity [3]. Thus in the present separation, *trans* isomer **E-1** with its equatorial carboxyl group elutes before the weaker acid, isomer **Z-1**.

Not surprisingly, the strongest acid present (TFA) elutes immediately after the solvent front, followed by octanoic acid (Fig. 3a). The *trans* acid **E-1** elutes at the end of the pH plateau formed by octanoic acid. A sharp impurity peak appears at the point of transition between the respective pH plateaus of TFA and octanoic acid. Without addition of the octanoic acid, impurities that eluted immediately after TFA would have contaminated the early fractions of the first eluted isomer (**E-1**). Octanoic acid was chosen as a second retainer acid on the basis of preliminary experiments with retinoic acids that indicated similar hydrophobicities of retainer acid and analytes result in purified product even in the first eluted fractions [14]. Octanoic acid

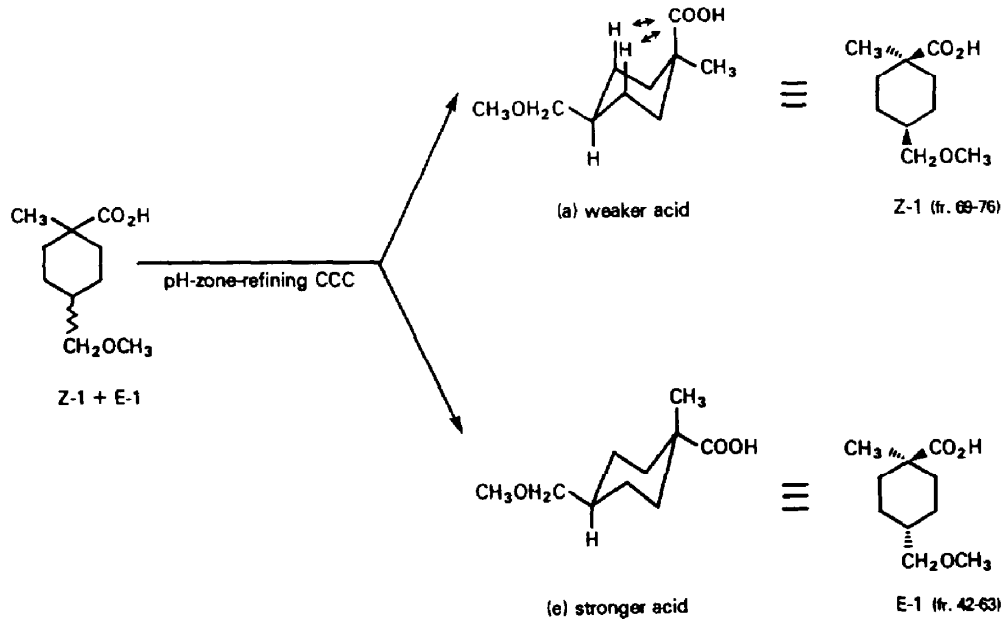

Fig. 4. Schematic diagram of the conformational isomers **Z-1** and **E-1**. In pH-zone-refining CCC the stronger acid (e) elutes before the weaker acid (a).

facilitates the purification of the analytes by acting as a spacer between the analytes and those impurities having comparable hydrophobicities.

Overall, the present study reveals the value of pH-zone-refining CCC for preparative-scale separation of stereoisomeric cyclohexanecarboxylic acids. The results presented here, in conjunction with previous work [7], suggest the great potential of this technique for separating other closely related organic acids.

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