On-Flow Gas Chromatographic Method for the Determination of the Enantiomer Interconversion Energy Barrier

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Abstract

A novel on-flow gas chromatographic (GC) method is developed for the determination of the kinetic rate constants and interconversion energy barrier of thermally labile enantiomers. The validity of the developed method is approved by the study of interconversion of 1-chloro-2,2-dimethylaziridine enantiomers on an achiral column. The overall experiments are performed in a series of three columns placed in two independently heated GC ovens. The racemate of the 1-chloro-2,2-dimethylaziridine is injected and separated in the first chiral column at 60°C in which the interconversion of enantiomers is suppressed. Separated enantiomers are then transferred into the achiral column, where the enantiomers are interconverted at a selected temperature under the current carrier gas flow. Effluent from this column is transferred into the second chiral column, where the native enantiomers and those originated by the on-flow interconversion on an achiral column are again separated at 60°C. Chromatograms obtained by monitoring the effluents from the second chiral column are used to determine the peak areas of the original and the newly interconverted enantiomers. The corresponding peak areas and the interconversion times are used to calculate the interconversion rate constants and energy barriers of the 1-chloro-2,2-dimethylaziridine enantiomers. The apparent energy barriers of the enantiomers of 1-chloro-2,2-dimethylaziridine are equal for both enantiomers within a 95% confidence interval and independent of the polarity of the stationary phase of the column in which the interconversion of enantiomers occur.

Introduction

The stability of enantiomers of thermally labile chiral compounds is a function of the energy barrier to their interconversion. This process, in which the individual enantiomers of chiral molecules undergo inversion of their respective stereogenic elements, referred to often as enantiomerization, may be studied by several methods (1,2).

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Interconversion of enantiomers in static systems

The interconversion of enantiomers (*R* and *S*) in static systems can be described by the scheme:

$$R \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} S \qquad \text{Scheme 1}$$

where k_1 and k_{-1} are the rate constants of the $R \rightarrow S$ and $S \rightarrow R$ interconversion, respectively. Interconversion of enantiomers in static systems is considered as a reversible reaction, which can be described by the first order kinetic equation. For reversible first-order reactions (assuming that the rate constants $k_1 = k_{-1} = k$ and no *S* enantiomer is present prior to interconversion) the following expression has been derived for the rate constant *k*[3]:

$$k = \frac{1}{2t} \ln \frac{c_{R0}}{2c_R - c_{R0}} \qquad \text{Eq. 1}$$

where c_{R0} is the initial concentration of enantiomer *R* and c_R is its concentration at time *t*.

The reversible interconversion of individual enantiomers in static systems[3] leads to equilibrium with a constant (*K*):

$$K = \frac{[S]}{[R]} = \frac{k_1}{k_{-1}} \qquad \text{Eq. 2}$$

where [*R*] and [*S*] are the equilibrium concentrations of enantiomers *R* and *S*, respectively.

If $k_1 = k_{-1}$, then the reversible interconversion of the enantiomers in a static system leads to a racemic mixture ($K = 1$), and this type of interconversion is known as racemization. Therefore, if enantiomers in a racemic mixture are reversibly interconverted in static systems, the equilibrium concentration of both enantiomers is constant ([*R*] = [*S*]) and independent of the time and temperature of interconversion.

Kinetic data and energy barriers to interconversion of conformationally or configurationally labile compounds have been investigated both by dynamic NMR and chirooptical methods, as

well as by a combination of classical kinetics studies with enantioselective separation methods and stop-flow or dynamic chromatographic and electromigration methods, respectively (1,2).

Combination of a classical kinetic study of interconversion with an enantioselective separation

In this procedure, interconversion of a pure enantiomer is performed in a static system outside of the enantioselective separation system (off-line) at specific temperatures and for selected times. Samples of the reaction mixture of enantiomers are then separated by an enantioselective method at temperatures at which the interconversion of enantiomers is suppressed. Because achiral chromatographic detectors give equal responses for both enantiomers, the rate constants may be determined directly from the corresponding peak areas. As it follows from the previous text, in classical kinetic studies, the interconversion is performed in a stationary system, and the forward and backward rate constants are equal ($k = k_1 = k_{-1}$). Thus the rate constant k can be calculated directly from the peak areas using modified equation 1, for the interconversion of pure *R*-enantiomer:

$$k = \frac{1}{2t}\, ln\, \frac{A_R + A_S}{A_R - A_S} \qquad \text{Eq. 3A}$$

or pure *S*-enantiomer:

$$k = \frac{1}{2t}\, ln\, \frac{A_R + A_S}{A_S - A_R} \qquad \text{Eq. 3B}$$

where t is the time of the interconversion, and A is the peak area of the considered enantiomer prior to ($A_R + A_S$) and after the interconversion ($A_R - A_S$ or $A_S - A_R$), respectively.

Stop-flow methods

If pure enantiomers are not accessible for a kinetic study, they can be produced online by an enantioselective column separation of a racemate at a sufficiently low temperature. The separated enantiomers are then interconverted for a certain time at the desired temperature by stopping the mobile phase flow in the chiral or the achiral column (4,5). Original and interconverted enantiomers are then separated on chiral column. Thus, the applicability of this method requires that enantiomerization is suppressed during the chromatographic separation process. Such interconversion studies are known as stop-flow techniques. The stop-flow methods can be realized in a single chiral column or in a column series operated under multidimensional conditions (4,5).

Interconversion of enantiomers in a chromatographic column is accomplished both in the mobile and stationary phase. As the rate of interconversion in these phases may differ, the data obtained by enantioselective chromatographic methods are considered as apparent. The apparent rate constant (k^{app}) can be the calculated from the modified equations 3A or 3B for the pure *R*-enantiomer:

$$k^{app} = \frac{1}{2t}\, ln\, \frac{A_R + A_S}{A_R - A_S} \qquad \text{Eq. 4A}$$

or pure *S*-enantiomer:

$$k^{app} = \frac{1}{2t}\, ln\, \frac{A_R + A_S}{A_S - A_R} \qquad \text{Eq. 4B}$$

Interconversion of enantiomers in dynamic systems

If the interconversion of enantiomers is not too fast and performed during a separation process, then the direct separation of a racemic or enriched mixture of thermolabile enantiomers can be considered as a reactive enantioselective separation. Because chromatographic and electromigration methods are dynamic systems in which the original and the interconverted enantiomers are separated, interconversion $R \rightarrow S$ and $S \rightarrow R$ looks irreversible (6) and can be described by an equation derived for the rate constant of the irreversible first order reactions (3).

If the enantiomers are successfully separated in an enantioselective system, the residence times of both enantiomers in this system differ and the apparent rate constants are supposed to be different:

$$k^{app}_{R \rightarrow S} = \frac{1}{t_{R,R}}\, ln\, \frac{A_R + A_S}{A_R} \qquad \text{Eq. 5A}$$

and

$$k^{app}_{S \rightarrow R} = \frac{1}{t_{R,S}}\, ln\, \frac{A_R + A_S}{A_S} \qquad \text{Eq. 5B}$$

where A is the corresponding peak area, k^{app} is the apparent rate constant, and $t_{R,R}$ and $t_{R,S}$ are residence (retention) times of the *R*- and *S*-enantiomer in the separation system, respectively[3].

The on-column interconversion of a racemic mixture during the separation process yields a peak cluster consisting of two peaks of nonconverted enantiomers and plateau formed by interconverted species. The peak characteristics (retention times, areas, heights, widths, and shapes) and the height of the plateau depend both on the enantiomer interconversion kinetics (temperature) as well as separation system (type of chiral selector and the mobile phase flow rate) (1,2,7). The following procedures have been used to calculate kinetic data and interconversion energy barriers from chromatograms obtained by the separation of enantiomers that interconverted during the separation process (1,2): (*i*) methods based on a computer assisted simulation of chromatograms, (*ii*) stochastic methods, (*iii*) methods based on approximation functions, and (*iv*) deconvolution methods.

Determination of the interconversion energy barrier

The energy barriers to $R \rightarrow S$ and $S \rightarrow R$ interconversion can be found from the apparent rate constants for the R→S (k^{app}_1) and S→R (k^{app}_{-1}) enantiomerization using the Eyring equation (3):

$$-\Delta G^{app}_{R \rightarrow S} = RT.ln\left(\frac{h \cdot k^{app}_{R \rightarrow S}}{\kappa \cdot k_b \cdot T}\right) \qquad \text{Eq. 6A}$$

or

$$-\Delta G^{app}_{S \rightarrow R} = RT.ln\left(\frac{h \cdot k^{app}_{S \rightarrow R}}{\kappa \cdot k_b \cdot T}\right) \qquad \text{Eq. 6B}$$

where R is the universal gas constant, T is the temperature in K, κ is transmission coefficient (κ–0,5 for the interconversion of conformational enantiomers), k_b is the Boltzmann constant, and h is Planck's constant.

Equations 6A and 6B may be used to calculate energy barriers to enantiomerization from the chromatographic data. If enan-

tiomers are separated and interconverted on a chiral column simultaneously, interconversion times of both enantiomers are different. This is supposed to be the reason for different kinetic activation parameters and interconversion energy barriers for enantiomers.

Determination of the thermodynamic kinetic activation data

The dependence of the apparent enantiomerization energy barrier ($\Delta G^{app}_{R\rightarrow S}$, $\Delta G^{app}_{S\rightarrow R}$) on temperature can be used to calculate the apparent activation enthalpy ($\Delta H^{app}_{R\rightarrow S}$, $\Delta H^{app}_{S\rightarrow R}$) and entropy ($\Delta S^{app}_{R\rightarrow S}$, $\Delta H^{app}_{S\rightarrow R}$) using the Gibbs–Helmholz equation (3):

$$\Delta G^{app}_{R\rightarrow S} = \Delta H^{app}_{R\rightarrow S} - T\Delta S^{app}_{R\rightarrow S} \qquad \text{Eq. 7A}$$

or

$$\Delta G^{app}_{S\rightarrow R} = \Delta H^{app}_{S\rightarrow R} - T\Delta S^{app}_{S\rightarrow R} \qquad \text{Eq. 7B}$$

From the mentioned equations, it follows that the enantioselectivity of a chiral selector is responsible for differences in the thermodynamic parameters for both enantiomers. The difference in the activation energy is proportional to the enantioselectivity (α), as per the following equation (2):

$$\Delta(\Delta G^{app}) = \Delta G^{app}_{S\rightarrow R} - \Delta G^{app}_{S\rightarrow R} = -RT\,ln\,\frac{k_S}{k_R} = RT\,ln\,\alpha \qquad \text{Eq. 8}$$

From equation 8 it follows that the more enantioselective a separation system, the higher is the apparent enantiomerization barrier for the compound represented by the second eluted peak. This is why the data calculated for the first eluted peak are less affected by the experimental conditions and to a first approximation they can be tabulated and compared with those data found by classical methods.

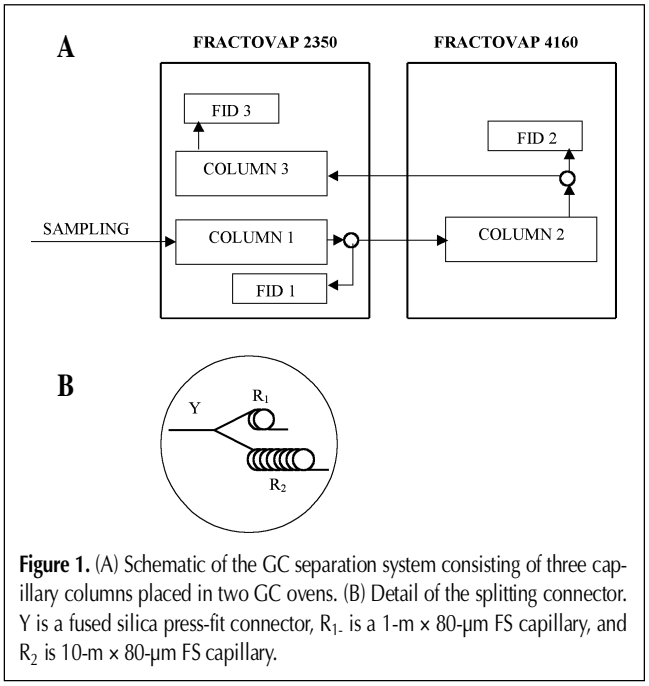

Figure 1. (A) Schematic of the GC separation system consisting of three capillary columns placed in two GC ovens. (B) Detail of the splitting connector. Y is a fused silica press-fit connector, R_1 is a 1-m × 80-µm FS capillary, and R_2 is 10-m × 80-µm FS capillary.

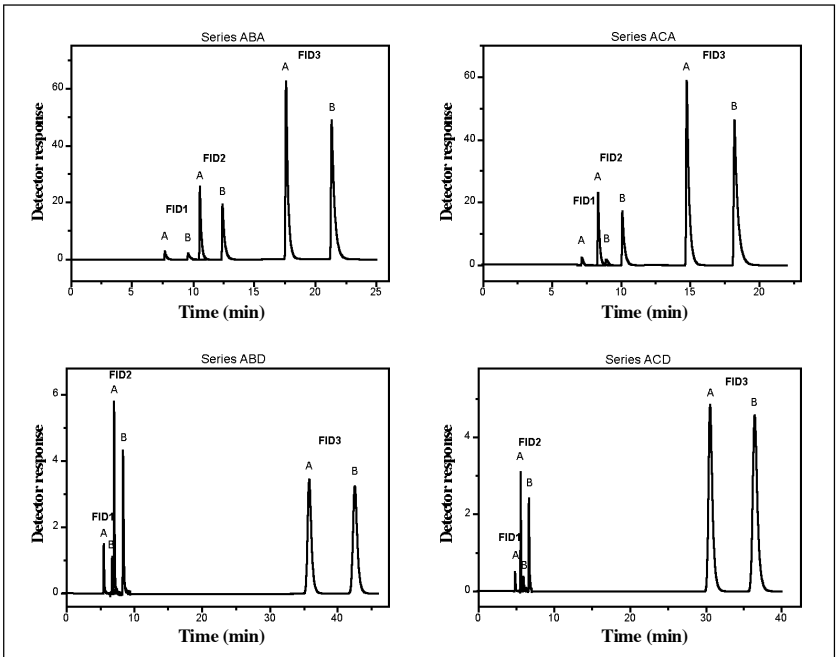

Figure 2. A chromatogram obtained by the separation of 1-chloro-2,2-dimethylaziridine enantiomers on a three column series detected with all three FIDs. For the figure, A and B label are the native enantiomers, and A′ and B′ are the interconverted ones. All columns coupled in series were operated at 60°C.

Experimental

Instruments

Two GC instruments, FRACTOVAP 4160 and FRACTOVAP 2350 (Carlo Erba, Milan, Italy), equipped with split–splitless injectors and flame ionization detectors (FID) were used. Helium was the carrier gas with a 20-cm/s mean flow rate. Air and hydrogen were used as the supplementary gases.

Measurements for the on-flow determination of interconversion energy barriers in the achiral column were performed in a series of three columns placed in two gas chromatographic (GC) ovens (Figure 1A). The first column contained a chiral stationary phase (CSP) and was placed in the Fractovap 2350 oven. The racemate of 1-chloro-2,2-dimethylaziridine was injected onto this column. Enantiomers of this analyte were separated at 60°C, at which the interconversion of enantiomers was suppressed. Effluent from the first column was split using a Y-piece (Figure 1B). The Y piece consists of a fused-silica Y press fit connector coupled to the end of the first capillary column and two different length of 50-µm-i.d. silanized fused silica capillaries. The effluent from the longer capillary was monitored with FID1 of the Fractovap 2350 GC. The effluent from the shorter capillary was transferred via a heated fused silica tube into the second achiral column, which was placed in the Fractovap 4160 oven. Enantiomers of the

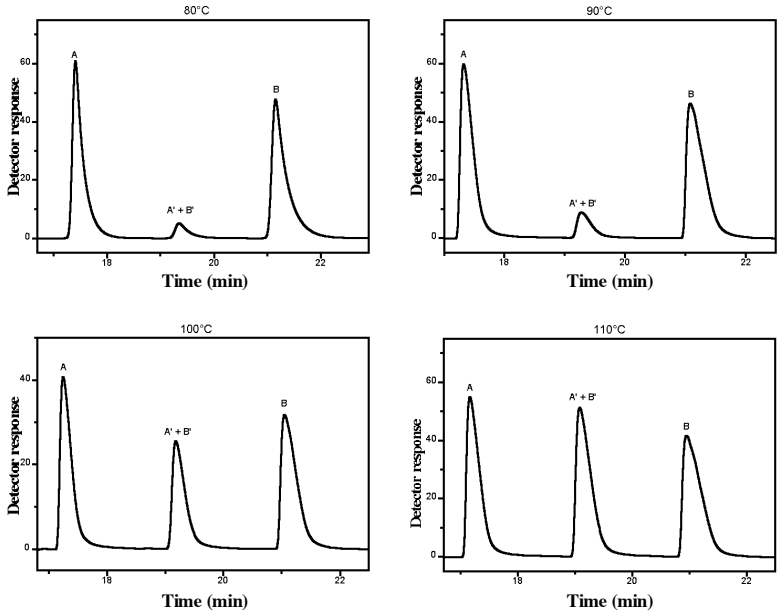

Figure 3. Fragments of chromatograms registered by FID3 for enantiomers of 1-chloro-2,2-dimethylaziridine in three column series (ABA). The first and the third columns (columns A) were heated at 60°C. On-flow interconversion of enantiomers occurred on the ULTRA 2 column (column B) at 80°C, 90°C, 100°C, and 110°C.

Table I. Temperature Dependence of Rate Constant* and Interconversion Energy Barriers† Found for Enantiomers of 1-Chloro-2,2-Dimetylaziridine on the ULTRA 2 PDMS Column‡

Temperature (°C)	$k^{app}_{A\to B}$ s^{-1}	$k^{app}_{B\to A}$ s^{-1}	$\Delta G^{app}_{A\to B}$ kJ/mol	$\Delta G^{app}_{B\to A}$ kJ/mol
80	0.000103	0.000426	111.9	107.7
85	0.000336	0.000561	110.0	108.5
90	0.000384	0.000535	111.2	110.2
95	0.001098	0.001537	109.5	108.5
100	0.001921	0.002444	109.3	108.6
105	0.003250	0.003874	109.2	108.6
110	0.003344	0.003923	110.6	110.1

* $k^{app}_{A\to B}$ and $k^{app}_{B\to A}$.
† $\Delta G^{app}_{A\to B}$ and $\Delta G^{app}_{B\to A}$.
‡ Column B, coupled in an ABA column series. The temperature of the first and the third column (columns A) was 60°C.

1-chloro-2,2-dimethylaziridine were interconverted in this achiral column at the selected temperature under the current carrier gas flow (on-flow). Effluent from the second column was again split in the 1:10 ratio. The composition of the smaller part of the effluent was monitored by the FID2 of the Fractovap 4160 GC. The larger part of the effluent from the second column was transferred via a heated fused-silica tube into the third column (that also contained the CSP), which was placed in the Fractovap 2350 oven. The enantiomers of the original (initial) 1-chloro-2,2-dimethylaziridine sample and those that originated from the on-flow interconversion on the achiral column were separated again at 60°C in the second CSP-containing column. The composition of the effluent from this column was monitored by the FID3 situated in the Fractovap 2350 oven. Injection port of the Fractovap 2350 GC was thermostated at 150°C. All FIDs were stored at 180°C. One microliter of diluted sample was introduced into the column by split injection with a split ratio of 100:1. Figure 2 shows chromatograms detected with all three FIDs for the separation of 1-chloro-2,2-dimethylaziridine enantiomers in which all columns were operated at 60°C. The signals from the FIDs were monitored and processed by a chromatographic integration software (9) CSW 32 and transfered into a Microcal Origin software (10).

Capillary columns

Column A

Two 30-m fused silica capillary columns with 0.25-mm i.d. coated with a 0.125-µm film of heptakis(2,6-di-*O*-pentyl-3-triflouroacetyl)-β-cyclodextrin (Chiral Dex B-TA) (ASTEC, Whippany, New Jersey).

Column B

A fused-silica capillary column (25-m × 0.25-mm i.d.) coated with a 0.17-µm film of ULTRA 2 polydimethylsiloxane (PDMS) (Hewlett Packard, Avondale, NJ) was used.

Column C

A fused silica capillary column (10-m × 0.32-mm i.d.) coated with a 0.20-µm film of a polyethyleneglycol (PEG) (CP WAX 52 CB, Chrompack, Middelsburg, the Netherlands) was used.

Column D

A capillary column (30-m × 0.32-mm i.d.) coated with a 0.25-µm film thickness of a mixed stationary phase [heptakis (-6-*O*-tert-butyldimethylsilyl-2,3-di-*O*-acetyl)–cyclodextrin (TBDMDAC) diluted in OV 1701 in the ratio 1:1] was used. The TBDMSDAC column has been prepared by Dr. W. Buda in the laboratory of Professor P. Sandra at the Department of Organic Chemistry, University of Ghent (Ghent, Belgium).

Sample

1-Chloro-2,2-dimethylaziridine was prepared from 2-methyl-2-amino-1-propanol and NaClO by Dr. I. Skacani at the Department of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology (Bratislava, Slovakia), using a procedure described by Coleman (11) and Graefer and Meyer (12) .

Results and Discussion

As discussed previously, the interconversion of 1-chloro-2,2-dimethylaziridine enantiomers by the on-flow technique was realized in an achiral column placed in a three-column series and heated independently in two ovens. A ChiralDex BTA column

(column A), placed in the Fractovap 2350 GC oven was used as the first column. After the sampling of the diluted racemate, the enantiomers of 1-chloro-2,2-dimethylaziridine were separated on this column at a temperature at which the interconversion was suppressed (60°C). The second column was an achiral ULTRA 2 (column B) or CP WAX 52 CB (column C) placed in the Fractovap 4160 GC oven. The interconversion of enantiomers was studied in these achiral columns at a selected temperature interval under the continuous carrier gas flow (on-flow technique). The enantiomers interconverted on the second column were then separated at 60°C on the third chiral column placed in the Fractovap 2350 GC oven. Chiral Dex BTA (column C) or TBDMSDAC (column D) were used as the third column in the three column series. The composition of the effluents from each column was monitored by three detectors (FID1, FID2, and FID3). The interconversion time (t_{int}) was equal to the residence time of enatiomers in the achiral (second) column. This time was determined as a difference of the corresponding retention times of enantiomers recorded by the FID2 and FID1 detectors. Because the pure enantiomers of 1-chloro-2,2-dimethylaziridine were not accessible, the first eluted enantiomer was labeled as A and second eluted enantiomer as B. Thus intercoversion times of the enantiomers were found from the equations: $t_{int}(A) = t_{R,A}(FID2) - t_{R,A}(FID1)$, $t_{int}(B) = t_{R,B}(FID2) - t_{R,B}(FID1)$, respectively. Experiments on the column series ABD moreover showed that the elution order of 1-chloro-2,2-dimethylaziridine enantiomers on the ChiralDex BTA (column A) and TBDMSDAC columns (column D) was the same. The FID3 detector was used to monitor the composition of effluent from the third (chiral) column. The FID3 records (chromatograms) were monitored and processed by chromatographic integration software (CSW 32) and exported into Microcal Origin software, where the peak fitting program was used to get the final peak areas of the enantiomers.

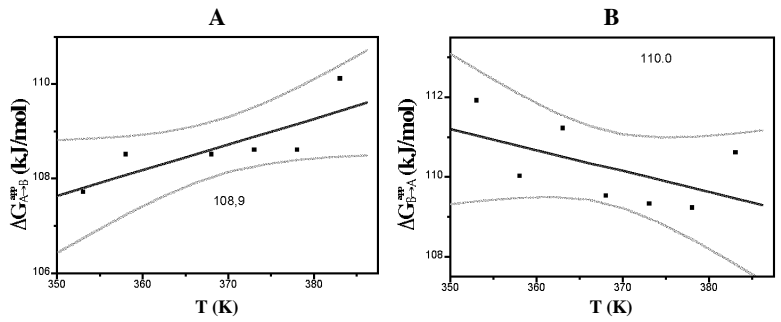

Figure 4. Temperature dependence of $\Delta G^{app}_{A\rightarrow B}$(A) and $\Delta G^{app}_{B\rightarrow A}$(B) found for enantiomers of 1-chloro-2,2-dimethylaziridine on the Ultra 2 PDMS column (column B) in the column series ABA.

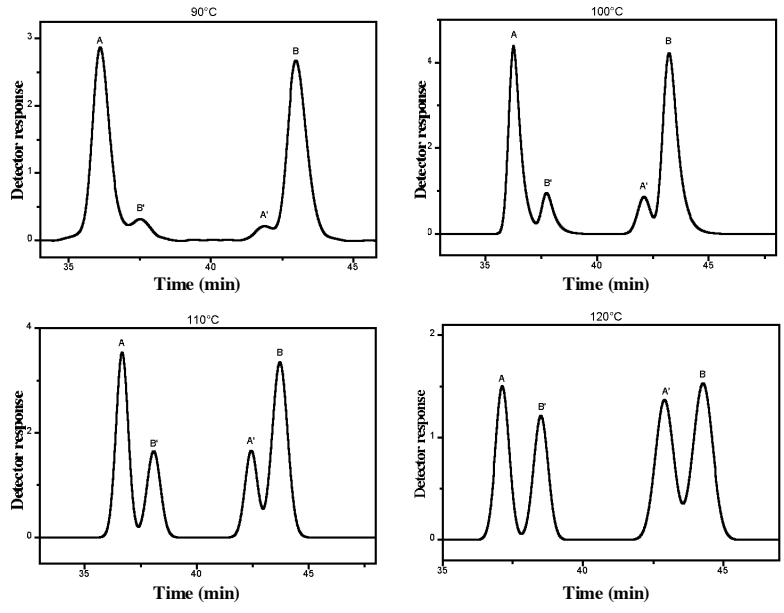

Figure 5. Fragments of chromatograms registered by FID3 for the enantiomers of 1-chloro-2,2-dimethylaziridine in a three-column series (ABD). The first and third columns (columns A and D) were heated at 60°C. On-flow interconversion of the enantiomers occurred on the ULTRA 2 column (column B) at 90°C, 100°C, 110°C, and 120°C.

On-flow interconversion of 1-chloro-2,2-dimethylaziridine enantiomers on the PDMS column

The on-flow kinetic study of the interconversion of 1-chloro-2,2-dimethylaziridine enantiomers on the ULTRA 2 PDMS column was performed in two column series (ABA and ABD). The enantiomerization times were determined from the corresponding retention times from the FID1 and FID2. The temperature of the achiral ULTRA 2 column was changed in the range of 60–150°C in 5°C increments.

Figure 3 shows the fragments of chromatograms recorded from the FID3 for the enantiomers of 1-chloro-2,2-dimethylaziridine in the three column series (ABA). In the Figure, three chromatograms three peaks are shown. The first and the third peak belong to the native (original sample) enantiomers A and B, respectively. The second peak consists of the enantiomers, A' and B', which originated from the separated native enantiomers A and B during the on-flow interconversion on the achiral ULTRA 2 column at various temperatures. Because the selectivities of the chiral columns A were practically the same, the interconverted spices were not separated on the ABA column series. Peak areas of the interconverted enantiomers A' and B' were found by the deconvolution of the middle peak (A' + B') by the peak-fitting program (10). Table I shows the rate constants ($k^{app}_{A\rightarrow B}$ and $k^{app}_{B\rightarrow A}$) and interconversion energy barriers ($\Delta G^{app}_{A\rightarrow B}$ and $\Delta G^{app}_{B\rightarrow A}$) found for the enantiomers of 1-chloro-2,2-dimethylaziridine on the ULTRA 2 PDMS column (column B) coupled in an ABA column series. Because the peaks of the interconverted enantiomers A' and B' were almost fully overlapped, there were difficulties in finding their corresponding peak areas by the deconvolution of overlapped peaks A' + B'.

Table II. Temperature Dependence of Rate Constants* and Interconversion Energy Barriers† Found for Enantiomers of 1-Chloro2,2-Dimetylaziridine on the ULTRA 2 PDMS Column‡

Temperature (°C)	$k^{app}_{A\rightarrow B}$ s^{-1}	$k^{app}_{B\rightarrow A}$ s^{-1}	$\Delta G^{app}_{A\rightarrow B}$ kJ/mol	$\Delta G^{app}_{B\rightarrow A}$ kJ/mol
90	0.000462	0.001477	110.6	107.1
100	0.001946	0.002033	109.3	109.2
110	0.005483	0.005085	109.0	109.3
120	–	0.007842	–	110.8
130	0.016143	–		111.3
140	0.011850	0.016491	115.2	114.0

* $k^{app}_{A\rightarrow B}$ and $k^{app}_{B\rightarrow A}$.
† $\Delta G^{app}_{A\rightarrow B}$ and $\Delta G^{app}_{B\rightarrow A}$.
‡ Column B, coupled in an ABD column series. The temperature of the first and the third column (column A and D) was 60°C.

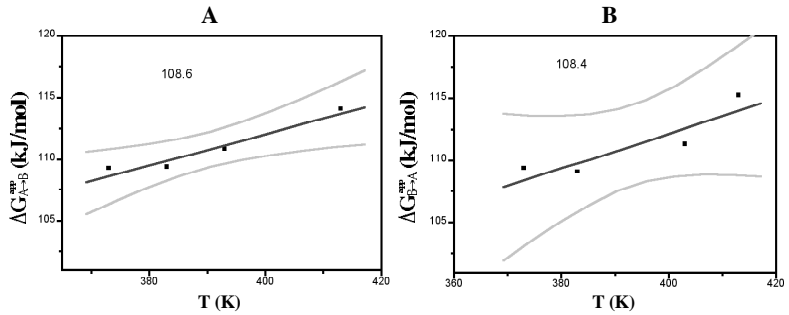

Figure 6. Temperature dependence of (A) $\Delta G^{app}_{A\rightarrow B}$ and (B) $\Delta G^{app}_{B\rightarrow A}$ found for the enantiomers of 1-chloro-2,2-dimethylaziridine on the Ultra 2 PDMS column (column B) in the column series ABD.

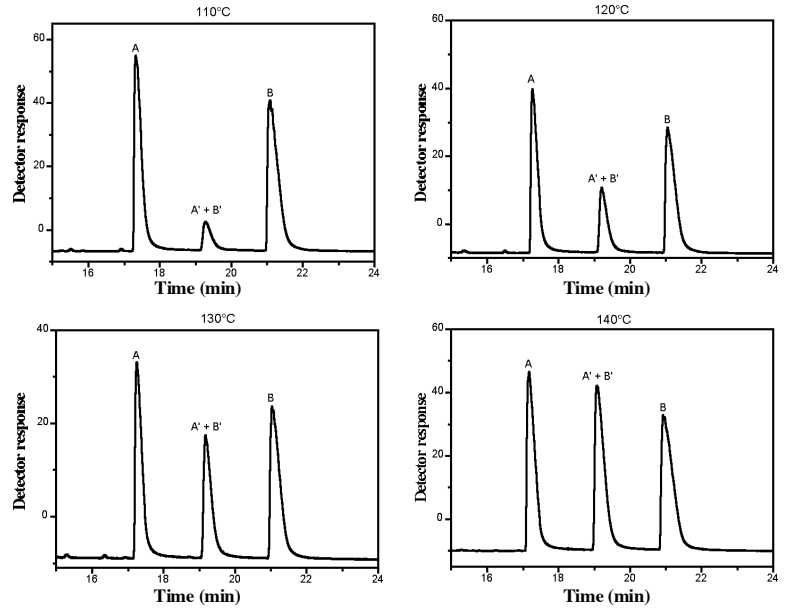

Figure 7. Fragments of chromatograms registered by FID3 for enantiomers of 1-chloro-2,2-dimethylaziridine in three column series (ACA). The first and the third columns (columns A) were heated at 60°C. On-flow interconversion of the enantiomers occurred on the CP WAX 52 CB column (column C) at 110°C, 120°C, 130°C, and 140°C.

As a consequence of this uncertainty, the use of the data listed in Table I in Figure 4 shows an unreal dependence of the apparent energy barrier on the temperature of the second eluted (B) 1-chloro-2,2-dimethylaziridine enantiomer. The upper and lower 95% confidence limits shown in Figure 4 demarcate the intervals in which the results can be expected with 95% probability.

The separation of the interconverted enantiomers of 1-chloro-2,2-dimethylaziridine could be obtained when using a three column series in which the polarity of the two chiral columns differed. Figure 5 shows the chromatograms produced from the column series ABD at different intercoversion temperatures. As the enantiomer selectivity of the first chiral column (ChiralDex B-TA), was less than that of the second CSP (TBDMSDA), a very good separation of the interconverted enantiomers was observed in Figure 5. The elution order of enantiomers in Figure 5 was confirmed by the selectivity factors of 1-chloro-2,2-dimethylaziridine enantiomers determined on single Chiral Dex B-TA and TBDMSDA columns, and in the ABD column series at 60°C.

The rate constants ($k^{app}_{A\rightarrow B}$ and $k^{app}_{B\rightarrow A}$) and interconversion energy barriers ($\Delta G^{app}_{A\rightarrow B}$ and $\Delta G^{app}_{B\rightarrow A}$) found for enantiomers of 1-chloro-2,2-dimethylaziridine on the ULTRA 2 PDMS column (column B) coupled in an ABD column series are listed in Table II. Because the peaks of enantiomers (A and B' as well as A' and B) are partially overlapped, the peak areas (A, B', A', and B) were found by the deconvolution of the overlapped peaks by the peak-fitting program (10).

Figure 6 shows the temperature dependence of $\Delta G^{app}_{A\rightarrow B}$(A) and $\Delta G^{app}_{B\rightarrow A}$(B) found for the enantiomers of 1-chloro-2,2-dimethylaziridine on the ULTRA 2 PDMS column (column B) in the column series ABD. Within the experimental error, the slopes of the plots (for the two enantiomers) are not significantly different. It should be noted that the upper and lower 95% confidence intervals are thinner for the first-eluted enantiomer.

On-flow interconversion of 1-chloro-2,2-dimethylaziridine enantiomers on the PEG column

To study a dependence of the interconversion of 1-chloro-2,2-dimethylaziridine enantiomers on the polarity of the achiral column, data obtained on ULTRA 2 PDMS column (column B) were compared with that obtained on CP WAX 52 CB PEG column (column C) in the column series ACA and ACD.

Figure 7 shows the chromatograms registered by FID3 for the enantiomers of 1-chloro-2,2-dimethylaziridine in the three column series (ACA). In Figure 7, as in the Figure 3, three peaks are seen. The first and the third peak belong to the native enantiomers A and B, respectively. The

Table III. Temperature Dependence of Rate Constants* and Interconversion Energy Barriers† Found for Enantiomers of 1-Chloro-2,2-Dimetylaziridine on the CP WAX 52 PEG Column‡

Temperature (°C)	$k^{app}_{A\to B}$ s^{-1}	$k^{app}_{B\to A}$ s^{-1}	$\Delta G^{app}_{A\to B}$ kJ/mol	$\Delta G^{app}_{B\to A}$ kJ/mol
90	0.001049	0.000364	108.2	111.4
100	0.002069	0.001908	109.1	109.4
110	0.004815	0.005430	109.4	109.0
120	0.012563	0.012533	109.2	109.2
130	0.026926	0.028936	109.5	109.3
140	–	0.036879	–	111.3

* $k^{app}_{A\to B}$ and $k^{app}_{B\to A}$.
† $\Delta G^{app}_{A\to B}$ and $\Delta G^{app}_{B\to A}$.
‡ Column C, coupled in an ACA column series. The temperature of the first and the third column (columns C) was 60°C.

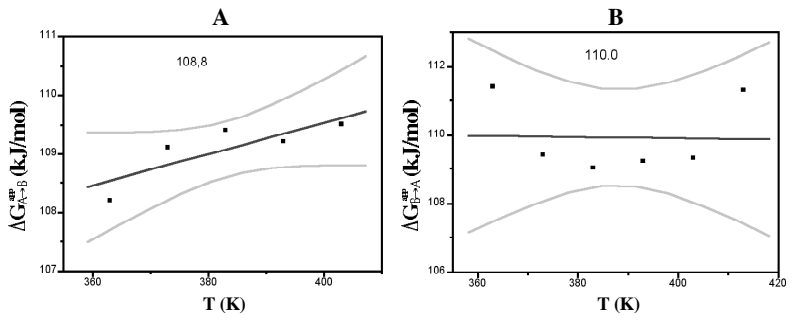

Figure 8. Temperature dependence of (A) $\Delta G^{app}_{A\to B}$ and (B) $\Delta G^{app}_{B\to A}$ found for the enantiomers of 1-chloro-2,2-dimethylaziridine on the CP WAX 52 CB column (column C) in the column series ACA.

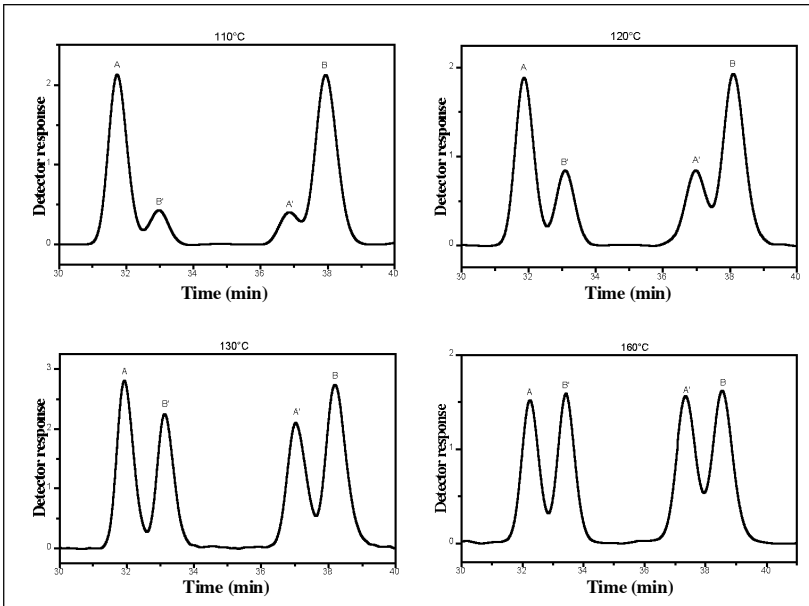

Figure 9. Fragments of chromatograms registered by FID3 for enantiomers of 1-chloro-2,2-dimethylaziridine in three column series (ACD). The first and the third columns (column A and D) were heated at 60°C. On-flow interconversion of enantiomers occurred on the CP WAX 52 CB column (column C) at 110°C, 120°C, 130°C, and 160°C.

second peak consists of enantiomers A' and B', which have been formed from the native enantiomers A and B during the on-flow interconversion on the achiral CP WAX 52 CB column at various temperatures. As stated previously, the selectivity of the chiral columns A were practically the same, and the interconverted spices were not separated on the ABA column series and the peak areas of the interconverted enantiomers A' and B' were found by the deconvolution of the middle peak (A' +B') by the peak-fitting program (10). Table III shows the rate constants ($k^{app}_{A\to B}$ and $k^{app}_{B\to A}$) and interconversion energy barriers ($\Delta G^{app}_{A\to B}$ and $\Delta G^{app}_{B\to A}$) found for enantiomers of 1-chloro-2,2-dimethylaziridine on a CP WAX 52 CB PEG column (column C) coupled in the ACA column series. Because the peaks of the interconverted enantiomers A' and B' are almost fully overlapped, there were once again difficulties in finding their corresponding peak areas using the deconvolution of overlapped peak A' + B'. The dependencies in Figure 8 show, similarly to those in Figure 4, slopes that differ substantially, and the separation of the upper and lower 95% confidence intervals are again thinner for the first eluted enantiomer.

The separation of the interconverted enantiomers of 1-chloro-2,2-dimethylaziridine was again, however, expected in the three column series when different polarity CSPs were used. Figure 9 shows the chromatograms for enantiomers registered in the effluent issued from the second chiral column by FID3 on the column series ACD at the different interconversion temperatures. As can be seen in Figure 5, a very good separation of the interconverted enantiomers can be observed in Figure 9.

The rate constants ($k^{app}_{A\to B}$ and $k^{app}_{B\to A}$) and interconversion energy barriers ($\Delta G^{app}_{A\to B}$ and $\Delta G^{app}_{B\to A}$) found for enantiomers of 1-chloro-2,2-dimethylaziridine on CP WAX 52 CB PEG column (column C) coupled in an ACD column series are listed in Table IV. The peaks of enatiomers (A and B' as well as A' and B) were again found by the deconvolution of the overlapped peaks by the peak-fitting program (10).

Figure 10 shows the temperature dependence of $\Delta G^{app}_{A\to B}$ (A) and $\Delta G^{app}_{B\to A}$ (B) found for enantiomers of 1-chloro-2,2-dimethylaziridine on the CP WAX 52 CB PEG column (column C) in the column series ACD. The slope of this dependence does not differ substantially, and the separation of the upper and lower 95% confidence intervals are, again, less for the second eluted enantiomer.

Table V lists the values of interconversion energy barriers ($\Delta G^{app}_{A\to B}$ and $\Delta G^{app}_{B\to A}$) found for the enantiomers of 1-chloro-2,2-dimethylaziridine on the PDMS ULTRA 2 (column B coupled in the ABA and ABD series) and PEG column CP WAX 52 (column C coupled in the ACA and ACD series) at 100°C. The temperature of the first and the third column (column A and D) was 60°C.

Conclusion

From the data in Table V, the following conclusions can be reached: (*i*) the apparent interconversion energy barriers of 1-chloro-2,2-dimethylaziridine enantiomers for the first-eluted (108.7 kJ/mol both for ULTRA 2 and CP WAX 52 columns) and second-eluted enanatiomer (109.2 and 109.5 kJ/mol for ULTRA 2 and CP WAX 52 column, respectively) differ slightly but do not depend on the achiral column polarity; and (*ii*) the apparent energy barriers for enantiomers of 1-chloro-2,2-dimethylaziridine are equal for both enantiomers within the 95% of confidence interval (the differences between the interconversion of enantiomers in the gas and liquid phase can be neglected) and are independent of the polarity of the stationary phase in the column, within which the interconversion of enantiomers occurred.

Table IV. Temperature Dependence of Rate Constants* and Interconversion Energy Barriers† Found for Enantiomers of 1-Chloro-2,2-Dimetylaziridine on the CP WAX 52 PEG Column‡

Temperature (°C)	$k^{app}_{A\to B}$ s^{-1}	$k^{app}_{B\to A}$ s^{-1}	$\Delta G^{app}_{A\to B}$ kJ/mol	$\Delta G^{app}_{B\to A}$ kJ/mol
100	0.001303	0.002109	110.5	109.0
110	0.004454	0.004481	109.7	109.7
120	0.012781	0.009861	109.2	110.0
130	0.016039	0.021061	111.3	110.4

* $k^{app}_{A\to B}$ and $k^{app}_{B\to A}$.
† $\Delta G^{app}_{A\to B}$ and $\Delta G^{app}_{B\to A}$.
‡ Column C, coupled in an ACD column series. The temperature of the first and the third column (column A and D) was 60°C.

Table V. Values of Interconversion Energy Barriers* Found for the Enantiomers of 1-Chloro-2,2-Dimetylaziridine on the PDMS ULTRA 2† and CP Wax 52 PEG Column‡

Column	Column series	$\Delta G^{app}_{A\to B}$ kJ/mol	$\Delta G^{app}_{B\to A}$ kJ/mol
ULTRA 2	ABA	108.9	110.0
	ABD	108.6	108.4
CP WAX 52	ACA	108.8	110.0
	ACD	109.9	109.1

* ($\Delta G^{app}_{A\to B}$ and $\Delta G^{app}_{B\to A}$).
† Column B, coupled in ABA and ABD series.
‡ Column C coupled in ACA and ACD series at 100°C. The temperature of the first and the third column (column A and D) was 60°C.

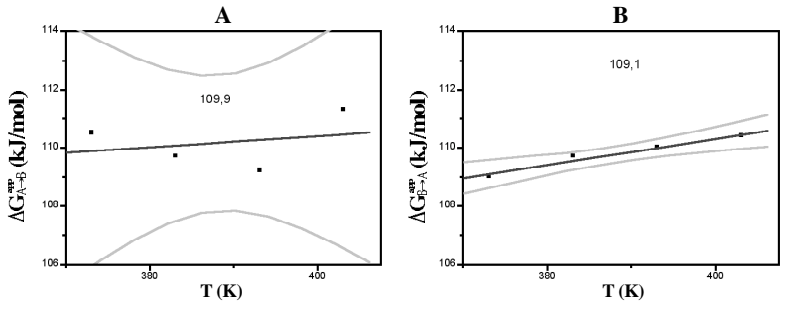

Figure 10. Temperature dependence of found for enantiomers (A) $\Delta G^{app}_{A\to B}$ and (B) $\Delta G^{app}_{B\to A}$ of 1-chloro-2,2-dimethylaziridine on the CP WAX 52 CB column (column C) in the column series ACD.

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