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Kinetic study on the epimerization of trityloxymethyl butyrolactol by liquid chromatography

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Abstract

The epimerization of trityloxymethyl butyrolactol has been investigated using dynamic chromatography and an approximation function introduced by Trapp and Schurig that is based on stochastic and theoretical plate models. The epimerization rate constants and Gibbs activation energies of epimerization are directly calculated from chromatographic peak parameters, i.e. retention times of the interconverting species, peak width at half height and the relative plateau height by using the approximation function. The relationships between peak shape and chromatographic conditions, such as flow-rate, temperature and pH are investigated.

Keywords: Epimerization kinetics; Rate constants; Gibbs activation energy; Lactols

1. Introduction

Investigations into dynamic molecular processes and determination of their kinetic parameters are frequently performed through the utilization of dynamic spectroscopic techniques (NMR, ESR, IR) [1–5]. These kinetic parameters can also be determined chromatographically, specifically for two species that can interconvert on a chromatographic time scale and can be separated chromatographically [6–19]. This chemical interconversion can occur during sample preparation or on-column and may be influenced by the mobile phase or the stationary phase. The types of reactions that may occur include acid or base catalyzed reactions and isomerization [20–27].

Chromatographic retention of a species, in its simplest form, is a factor of the equilibrium constant for its distribution between the mobile and stationary phase. Interconversion of species however leads to the establishment of a secondary equilibrium. When an eluate is subjected to a secondary equilibrium, its retention is a weighted average of the two species [28]. If the rate of interconversion is slow compared to the chromatographic process two resolved peaks are observed due to the occurrence of little or no interconversion. If the rate of interconversion is fast compared to the chromatographic process, only one peak is observed due to the extensive interconversion. However, if the interconversion is on a similar time scale to that of the chromatographic process, band spreading and peak distortion may be observed. On-column interconversion of a species is usually characterized by tailing of the less retained peak and fronting of the more retained peak. Tailing of the less retained peak occurs as this species is converted to the more retained species. Conversely fronting of the more retained peak occurs as this species is converted to the less retained species. The two peaks may be joined by an elevated baseline (see Fig. 1). This elevated baseline represents species which have undergone at least one interconversion cycle.

The determination of rate constants for interconversion in chromatographic processes is generally undertaken with computer simulations using primarily three different models. The continuous flow model [7,8,29,30,31] is derived from chemical engineering principles. It utilizes a dimensionless Damköhler number (Da) representing the ratio of time constants for bulk mass transport and chemical reaction. The Damköhler number is derived from the first moment (center of mass) and second moment (variance) of the peak and the rate constants are subsequently obtained. The theoretical plate model [15,16,32,33] considers each theoretical plate as a distinct discrete reactor. At each plate the

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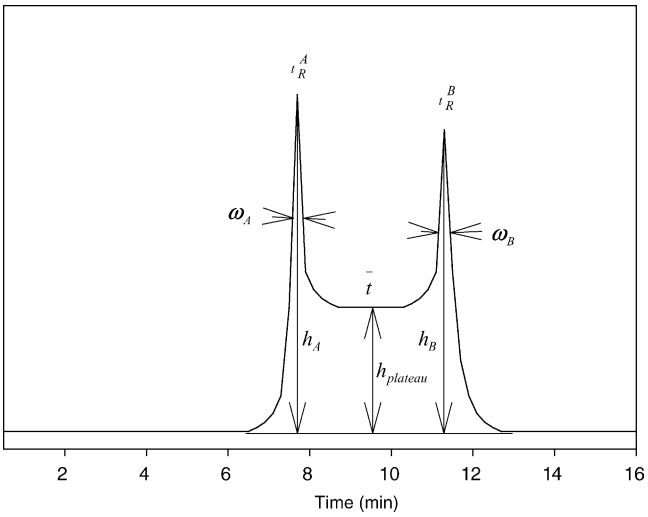


Fig. 1. Example of an elution profile of an interconverting species with the experimental parameters needed for calculation of rate constants and Arrhenius' constant.

species interconvert and are distributed between the mobile and stationary phase. The mobile phase is then shifted onto the next plate. The stochastic model [13,34,35] describes the chromatographic process using time dependent distribution functions. The elution profile is a summation of the distribution functions of the non-interconverted species and the probability density functions of the interconverted species.

More recently Trapp and Schurig [36] have been able to obtain rate constants without the use of computer simulations. They utilized an approximation function to directly calculate interconversion rate constants and Gibbs activation energies. The input parameters were retention times of the two species, their peak widths, and the relative plateau height between the two peaks. Trapp's method has been utilized here for a kinetic study of the epimerization of trityloxymethyl butyrolactol. It is known that lactols can undergo ring opening and closing in solution. This phenomenon has been observed in carbohydrates such as glucose. Ring opening of trityloxymethyl butylrolactol in solution leads to inversion at the stereogenic center where the hydroxyl group is located (see Fig. 2). Under appropriate chromatographic conditions the resulting interconversion is manifested through peak broadening, plateau formation, and peak coalescence. Like typical interconversion of stereoisomers, this epimerization constitutes a reversible first order reaction [32,37]. Rate constants and the activation energy for interconversion were determined at varied flow-rates, temperatures, and chromatographic conditions. Additionally optimal conditions were identified to achieve either improved separation of the interconverting species or to have them co-elute as one peak.

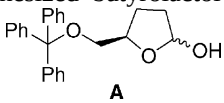
2. Experimental

2.1. Synthesis

S(+)-γ-(Trityloxymethyl)-γ-butyrolactone, purchased from Sigma–Aldrich, was reacted with DIBAL at −78 °C in methylene chloride to generate the corresponding butyrolactol. After one hour the reaction was quenched with aqueous ammonium chloride. The organic layer was collected and the methylene chloride evaporated off. The butyrolactol was purified by prep-HPLC.

2.2. NMR

The ^{1}H NMR spectra of the synthesized butyrolactol



A

was recorded on a 400 MHz Bruker spectrometer for verification. Compound **A** (mixture of two diastereomers, ∼1:1). ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.48–7.00 [m, 30 H, (15H for each diastereomer, same below), Ph-H], 5.62 (br d, $J = 3.6$ Hz, 1H, CHOH), 5.32 (br d, $J = 3.1$ Hz, 1H, CHOH), 4.45 (m, 1H, $CH_3CH_2OCPh_3$), 4.27 (m, 1H, $CHCH_2OCPh_3$), 3.30 (dd, $J = 3.8$ and 9.8 Hz, 1H, $Ph_3COCH_aH_b$), 3.22 (dd, $J = 4.8$ and 9.8 Hz, 1H, $Ph_3COCH_aH_b$), 3.11 (d, $J = 4.8$ Hz, 2H, $Ph_3COCH_aH_b$), and 2.15–1.95 (m, 8H, CH_2CH_2). ^{13}C NMR (100 MHz, $CDCl_3$), δ (mixture of ∼1:1 diastereomers) 144.14, 143.88, 128.87, 128.80, 127.89, 127.82, 127.14, 127.00, 99.09, 98.86, 79.46, 77.79, 66.85, 66.24, 34.31, 32.96, 29.75, 26.02, and 25.20.

Additionally the distribution constant for the two epimers of butyrolactol in solution were determined by NMR for the –CHOH group. The ratio is 1.0 for the peaks of CHOH at chemical shift 5.62 and 5.32.

2.3. Chromatographic conditions

All chromatographic studies were performed on a Hewlett Packard 1100 HPLC system equipped with a photo diode array UV detector. Data acquisition and treatment was per-

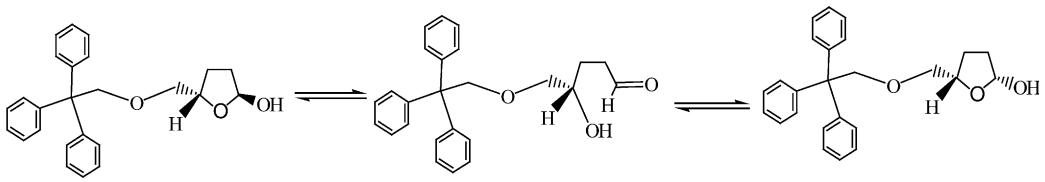


Fig. 2. Epimerization of butyrolactol.

formed with a Perkin-Elmer Turbochrom system. The reported chromatographic data is the average of triplicate determinations at a detection wavelength of 210 nm. The column dead volume was determined as per Knox and Kaliszan [38] by using deuterated methanol as a dead volume marker.

Chromatographic separation was performed on a 3 μm YMC-pack ODS-AM column (150 mm × 4.6 mm). The eluent was HPLC grade acetonitrile and HPLC grade water. HPLC grade acetonitrile was purchased from Sigma–Aldrich Co. HPLC grade water was obtained from a Picotech water purifying system. Chromatographic runs were performed at 5, 10, 15, 20, and 25 °C. At each temperature point, five flow-rates were studied to ensure accuracy and precision.

3. Results and discussion

3.1. Peak shape approximation model

Trapp and Schurig [36] derived an approximation function, based on the stochastic model, which allows for the calculation of rate constants (k_1) of enantiomerization for a racemic mixture directly from chromatographic parameters, such as retention times of both A and B forms (t_R^A and t_R^B), peak widths at half height (w_A and w_B) and the relative plateau height ($h_{plateau}$) (Fig. 1). No computer simulation was required. The epimerization of butyrolactol is also a reversible first order reaction and the same data treatment can be employed to calculate the rate constants for this process. The elution profile $P(t')$ for interconverting epimers during the separation process can be expressed by the following equation:

$$P(t') = \Phi_A(t') + \Phi_B(t') + \Psi_A(t') + \Psi_B(t') \quad (1)$$

where, $\Phi_A(t')$ and $\Phi_B(t')$ are the two distribution functions of the non-interconverted epimers, and $\Psi_A(t')$ and $\Psi_B(t')$ are the probability density functions of the interconverted epimers. $\Phi_A(t')$ and $\Phi_B(t')$ can be expressed by ideal Gaussian distribution function and $\Psi_A(t')$ and $\Psi_B(t')$ can be calculated by modulated Gaussian function. After mathematical simplification, the approximate reaction rate constant can be calculated by the following equation [36]:

$$k_1^{approx} = -\frac{1}{t_R^A}\ln\left[\frac{(c_A^0+c_B^0)}{(t_R^A-t_R^B)}\left(1-\frac{h_{plateau}}{100}\left(0.5+\frac{1}{\sqrt{2\pi N}}\right)\right)\right] + \frac{1}{t_R^A}\ln\left[\frac{(c_A^0+c_B^0)}{(t_R^A-t_R^B)}\left(1-\frac{h_{plateau}}{100}\left(0.5+\frac{1}{\sqrt{2\pi N}}\right)\right)\right] + c_A^0\frac{0.01h_{plateau}-e^{-(t_R^B-t_R^A)^2/8\sigma_A^2}}{\sigma_A\sqrt{2\pi}} + c_B^0\frac{0.01h_{plateau}e^{-(t_R^A-t_R^B)^2/2\sigma_B^2}-e^{-(t_R^A-t_R^B)^2/8\sigma_B^2}}{\sigma_B\sqrt{2\pi}}\Bigg] \quad (2)$$

where, t_R^A and t_R^B are the retention times; c_A^0 and c_B^0 are the initial concentration of interconversion species A and B; $h_{plateau}$ is the height at the middle of the elevated base line; σ is defined as $\sigma_i = w_i/\sqrt{8\ln 2}$ and w_i is the half peak width for interconversion species A and B.

This approximation function of k_1^{approx} was validated [36] and found to give an average error of ±11.7% for the approximated rate constant, k_1^{approx}. The deviation of the Gibbs activation energy was ±0.11RT.

3.2. Determination of k_1^{approx} and the Arrhenius constant

From Eq. (2), the on-column epimerization rate constant can be estimated by using simple chromatographic data, such as retention time, peak width, and relative height of the plateau. Five temperature points were taken for the measurements of k_1^{approx} and five flow-rate points were taken for each temperature. The retention parameters, such as t_R^A, t_R^B, and $h_{plateau}$ are directly measured from the chromatograms of butyrolactol. Since the two epimers are present at equal concentrations in the diluent, as shown earlier by NMR data, c_A^0 and c_B^0 are assumed to be 0.5. Butyrolactone was also injected in the chromatographic runs for each condition, and its theoretical plate number was used for the calculation of k_1^{approx}. There are two reasons for using butyrolactone's theoretical plate number instead of butyrolactol's. First, this compound has very similar structure and functional groups as butyrolactol. Secondly, it does not undergo interconversion in either diluent or on-column and thus peak broadening due to interconversion is excluded from the non-reactive broadening [29]. The results for k_1^{approx} are listed in Table 1. Since the ring structure of butyrolactol is the similar to that of carbohydrates, the ring opening and closing reaction rates for both should be similar. Reported isomerization rates of common carbohydrates are comparable to the rates measured in this study [39]. A previous study of interconversion of muramyldipeptide [40] also reported similar results.

From the Arrhenius Equation, which represents the relationship between the rate constant k and the activation energy of the reaction, the activation energy of this reaction can be calculated.

$$\ln k = -E_a/RT + \text{constant} \quad (3)$$

where E_a is the activation energy and R is the gas constant. The plot of $\ln k$ versus the reciprocal of the absolute temperature is a straight line with a slope of E_a/R. This plot is

Table 1
The calculated k_1^{approx} value

Temperature (°C)	k_1^{approx} ($\times 10^{-4}$ s^{-1})	S.D. ($\times 10^{-4}$ s^{-1})
25	2.54	0.02
20	2.42	0.01
15	2.22	0.01
10	1.98	0.01
5	1.77	0.02

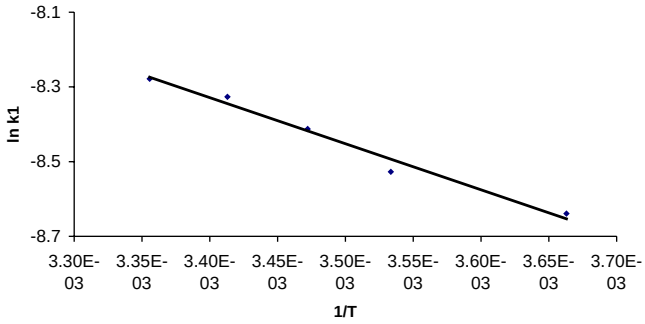


Fig. 3. The Arrhenius plot for trityloxymethyl butyrolactol (ODS-AM 4.6 mm × 150 mm × 3.5 μm column).

shown in Fig. 3. The correlation coefficient for this straight line is 0.990 and the calculated activation energy for this isomerization reaction is 10.3 kcal/mol.

3.3. Peak shape studies

For an inter-converting compound, it is desirable to obtain a single sharp peak for overall impurity and quantitative analysis. Chromatographic conditions have a large effect on the peak shape when on-column interconversion occurs. Peak resolution is observed if the rate of chemical conversion is slow compared to the chromatographic exchange process. If the rate of chemical conversion is fast relative to the chromatographic exchange process only one peak is observed. Between these two extremes fronting, tailing, and elevated baselines are observed. Parameters such a flow-rate, pH of the mobile phase and temperature can be varied to obtain the ideal peak shape.

3.4. Flow-rate

Chromatograms of butyrolactol obtained at the flow-rates of 0.2, 0.4, 0.6, 0.8, and 1.0 ml/min with a mobile phase consisting of a mixture of water/acetonitrile (35/65, v/v) at 5 °C are shown in Fig. 4. At the lower flow-rate of 0.2 ml/min, a single wide peak is obtained, as the interconversion rate is fast relative to the chromatographic process. At higher flow-rates, a bimodal peak corresponding to the epimers was observed due to the fact that the interconversion rate is slower relative to the chromatographic process. This observation is in agreement with similar studies [30].

3.5. Temperature effect

The influence of temperature on the peak shape of butyrolactol was investigated at several column temperatures while keeping other parameters constant. Fig. 5 shows that the increase of temperature led to a significant improvement in the peak shape. This effect is attributed to an increase of the interconversion rate for the two epimers at higher temperature, eventually resulting in a single peak. The retention time for butyrolactol changed significantly while varying

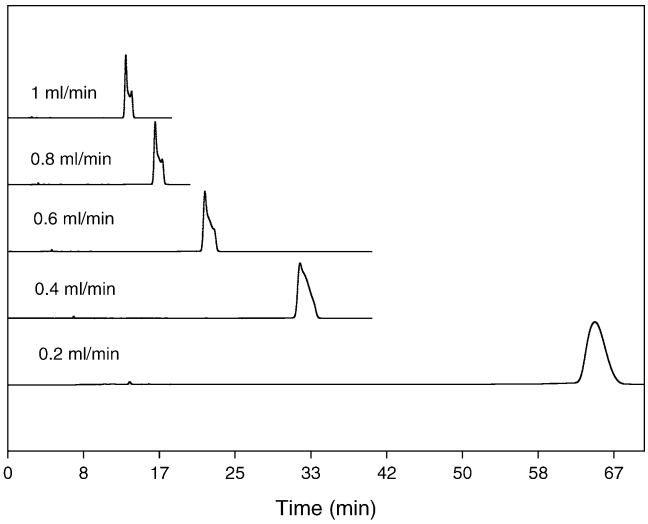


Fig. 4. Effect of flow-rate on the peak shape of trityloxymethyl butyrolactol (ODS 4.6 mm × 250 mm × 5 μm column, 35:65/water:acetonitrile, 5 °C).

the temperature. This phenomenon is different from that observed for proline-containing substances reflecting a larger enthalpic contribution to retention for butyrolactol [41].

3.6. Effect of pH

The influence of phosphate buffer pH on the peak shape was investigated by using a mobile phase consisting of 10 mM phosphate buffer–acetonitrile (35/65, v/v) at temperature of 25 °C and flow-rate of 1 ml/min. Fig. 6 shows that at either a higher or lower pH environment, a single peak of butyrolactol was observed. At intermediate pH (∼4.5), two peaks of butyrolactol were observed. The reason for these phenomena is that both acid and base can accelerate the ring opening process [42], thus increasing the interconversion rate for the two epimers. A previous study [43] also

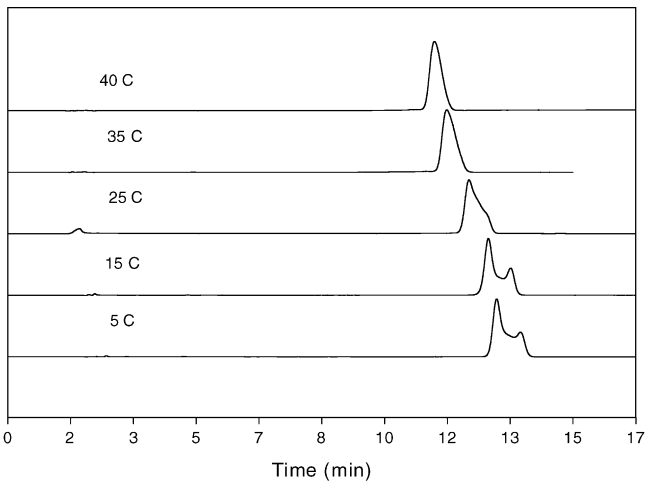


Fig. 5. Effect of temperature on the peak shape of trityloxymethyl butyrolactol (ODS-AM 4.6 mm × 250 mm × 5 μm column, 35:65/water:acetonitrile, 1 ml/min).

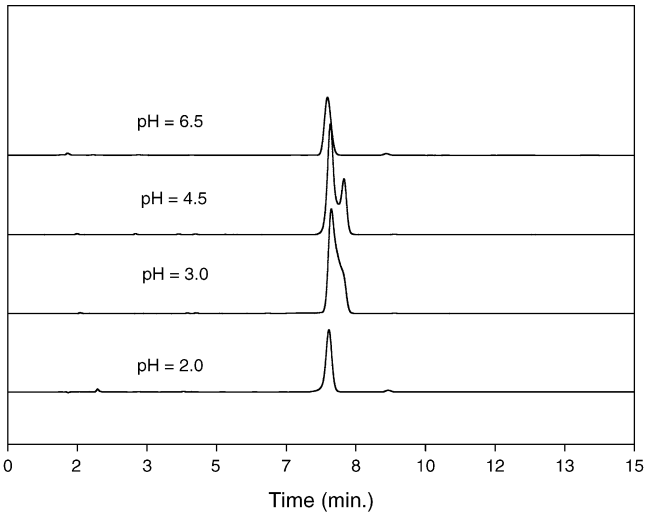


Fig. 6. Effect of pH on the peak shape of trityloxymethyl butyrolactol (ODS-AM 4.6 mm × 150 mm × 3 μm column, 35:65/water:acetonitrile, 1 ml/min, temperature 25 °C).

points out that this type of interconversion has the slowest reaction rate in the pH 3–5 region.

4. Conclusion

The approximation function developed by Trapp and Schurig was successfully used to directly calculate inter-conversion rate constants and Gibbs activation energies for the inter-conversion of butyrolactol on a chromatographic column. It was demonstrated that temperature, pH and flow-rate are major parameters that can influence peak shape of the inter-converting species. These parameters can be manipulated so that a single peak can be obtained for the inter-converting species to enhance accurate quantitation.

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