Enantiomerization kinetics studied by dynamic enantioselective liquid chromatography: Solvent, temperature and stationary phase effects on the rate of *N*-benzyl-1,3,2-benzodithiazole 1-oxide enantiomer interconversion

Joakim Oxelbark and Stig Allenmark *

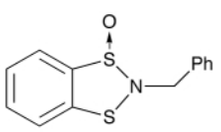
Department of Chemistry, Göteborg University, SE-41296 Göteborg, Sweden

Received (in Cambridge) 15th March 1999, Accepted 10th June 1999

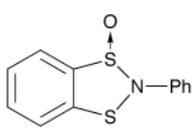
Enantiomerization of **1** was found to proceed *ca.* 10 times faster in hexane than in methanol. This has been attributed to the difference in $\Delta S^{\ddagger}$ found in the respective solvents. The presence of a chiral HPLC stationary phase (Whelk-O1) did not affect the rate of enantiomerization to any significant extent. The previously determined enantiomerization (by inversion of configuration) barrier of **2** using DHPLC (80 kJ mol^{-1}), is thus considered correct, and not biased by the presence of the stationary phase.

Introduction

In our earlier investigations of the stereochemical properties of the 1,3,2-benzodithiazole *S*-oxide ring system, we have found that the barrier to inversion of the sulfoxide configuration is remarkably low, and strongly dependent on the specific ring system.[1] While the *N*-benzyl substituted compound (**1**) displays ready racemization at room temperature, and an inversion barrier of about 95 kJ mol^{-1}, changes in the ring system, as well as further oxidation to the *S*,*S*′-bisoxide, yield compounds that are configurationally stable even at elevated temperatures.[2] The inversion barrier of the *N*-phenyl substituted compound (**2**) was determined[3] to be 80 kJ mol^{-1} using dynamic HPLC (DHPLC),[4–7] a technique similar to dynamic NMR (DNMR), although applicable within a different time scale.



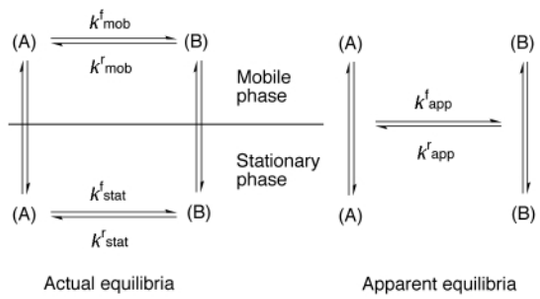
1



2

During enantioselective chromatography of **2**, the rapid interconversion of the enantiomers will produce a plateau composed of racemized material between the two peaks. At an interconversion rate fast enough, peak coalescence is observed. This process can be studied by computer simulation, thus yielding apparent rate constants ($k^{f}_{app} \neq k^{r}_{app}$), which are weighted means of the different enantiomerization rates in the mobile phase and the stationary phase. Since the mobile phase is an achiral environment $k^{f}_{mob} = k^{r}_{mob}$ holds, but due to the diastereomeric complexes formed from the analyte and the chiral selector $k^{f}_{stat} \neq k^{r}_{stat}$. Scheme 1 presents the different equilibria, where A and B denote the respective enantiomers. An aim of this study has been to examine to what extent the stationary phase might influence the rate of racemization.

The rate of enantiomerization in the mobile phase can be determined independently, *e.g.* by the use of polarimetric methods. The rate in the stationary phase can then be obtained from eqn. (1),[5] where k'_{A} and k'_{B} denote the retention factors ($k' = (t_R - t_0)/t_0$) of the early and late eluting peaks. The superscript f refers to the forward enantiomerization reaction, meaning the direction in which early eluting enantiomer is converted into late eluting enantiomer.



Scheme 1

$$k^{f}_{app} = (k^{f}_{mob} + k'_{A}k^{f}_{stat})/(1 + k'_{A}) \quad (1a)$$

$$k^{r}_{app} = (k^{r}_{mob} + k'_{B}k^{r}_{stat})/(1 + k'_{B}) \quad (1b)$$

DHPLC is applicable to reactions with a Gibbs free energy of activation of about 70–100 kJ mol^{-1}. DNMR demands the presence of a chiral solvating agent or a chiral lanthanide shift reagent, and requires temperatures above 80 °C when the barrier is higher than 80 kJ mol^{-1}, which means that thermal stability of the analyte (which is not quite sufficient in our case) as well as high boiling solvents are necessary in this case.

In contrast to the common polarimetric methods, DHPLC and DNMR are both applicable to compounds with low or no optical activity. In addition, DHPLC has the advantage of requiring only very small amounts of analyte.

Results and discussion

Racemization kinetics measured using CD spectroscopy

The activation parameters of the enantiomerization reaction of **1** in free solution have been determined in different solvents, using CD spectroscopy to monitor the loss of optical activity of enantiomerically enriched samples. The data are listed in Table 1. All parameters refer to the reversible enantiomerization reaction, which means that a factor of 0.5 has been applied to the observed rate constants (k_{obs}) of the irreversible racemization reaction determined by CD spectroscopy (eqn. (2)).

$$(R) \underset{k_r}{\overset{k_f}{\rightleftharpoons}} (S) \quad k_f + k_r = k_{obs} \quad (2)$$

Table 1 Enantiomerization data obtained by CD spectroscopy

	Solvent				
	MeOH	MeCN	CH_2Cl_2	Hexane	Mixture[a]
$\Delta H^{\ddagger}$[b]	97.0 ± 0.9	104.9 ± 3.2	103.0 ± 2.4	98.9 ± 2.7	103.5 ± 2.2
$\Delta S^{\ddagger}$[c]	0.4 ± 3.1	26.9 ± 11.7	25.2 ± 8.2	26.1 ± 9.5	28.4 ± 7.6
$\Delta G^{\ddagger}$[d]	96.9 ± 0.5	96.9 ± 0.5	95.6 ± 0.5	91.5 ± 0.4	95.1 ± 0.5
No.[e]	16	16	14	16	30
Temp. range[f]	20	33	27	24	35
Mean T[g]	295	296	294	285	295
k_{298}[h]	1.0	1.0	1.8	10	2.1

[a] Hexane (45%) and methanol (1%) in dichloromethane, also used as mobile phase in DHPLC experiments. [b] In kJ mol^{-1}. [c] In J K^{-1} mol^{-1}. [d] In kJ mol^{-1}. [e] Number of measurements used for the determination of activation parameters. [f] Temperature range (in K) of the measurements used for the determination of activation parameters. [g] Mean temperature (in K) of the range used. [h] Relative rate constant calculated at 298 K.

Table 2 Results of DHPLC simulations

Temp.[a]	$\Delta G^{\ddagger}_{mob}$[b]	$10^3 k_{mob}$[c]	$10^4 k^{f}_{stat}$[d]	$10^4 k^{r}_{stat}$[e]	$\Delta G^{\ddagger f}_{stat}$[f]	$\Delta G^{\ddagger r}_{stat}$[g]	$\Delta G^{\ddagger}_{stat,\,mean}$[h]	$\Delta G^{\ddagger}_{app,\,mean}$[i]
308.9[j]	94.70	0.625	3.64	2.71	96.14	96.92	96.50	95.45
312.9[j]	94.59	1.06	5.29	3.98	96.39	97.13	96.74	95.49
315.0[j]	94.53	1.38	7.05	5.26	96.29	97.06	96.65	95.42
317.9[k]	94.45	2.01	7.52	5.65	97.04	97.80	97.39	95.53
320.7[k]	94.39	2.84	10.5	8.01	97.02	97.76	97.37	95.49

[a] Column temperature in K. [b] $\Delta G^{\ddagger}$ of the enantiomerization reaction calculated from $\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger}$ as determined in the mixed solvent 45% hexane + 1% methanol in dichloromethane using CD spectroscopy. [c] Rate of enantiomerization in the mobile phase (in s^{-1}) as calculated from $\Delta G^{\ddagger}_{mob}$. [d] Rate of forward enantiomerization in the stationary phase (in s^{-1}) as determined by simulation and eqn. (1). [e] Rate of reverse enantiomerization in the stationary phase (in s^{-1}) as determined by simulation and eqn. (1). [f] Free activation energy of forward enantiomerization in the stationary phase in kJ mol^{-1} (from k^{f}_{stat}). [g] Free activation energy of reverse enantiomerization in the stationary phase in kJ mol^{-1} (from k^{r}_{stat}). [h] Gibbs free energy of activation associated with the mean of the forward and reverse rate constants in the stationary phase (in kJ mol^{-1}) as determined by simulation. [i] Gibbs free energy of activation associated with the mean of the forward and reverse apparent rate constants (in kJ mol^{-1}) as determined by simulation. [j] Flow rate 0.5 ml min^{-1}; $N = 3600$ theoretical plates used for the simulation. [k] Flow rate 1.0 ml min^{-1}; $N = 3000$ theoretical plates used for the simulation.

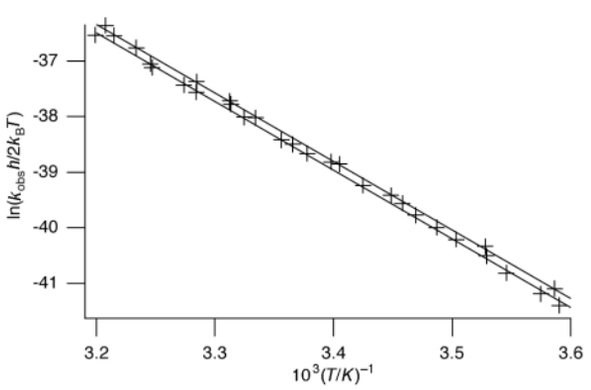


Fig. 1 Eyring plot of the racemization of **1** in hexane (45%) and methanol (1%) in dichloromethane, monitored by CD spectroscopy. Upper line: (*S*)-**1**, lower line: (*R*)-**1**.

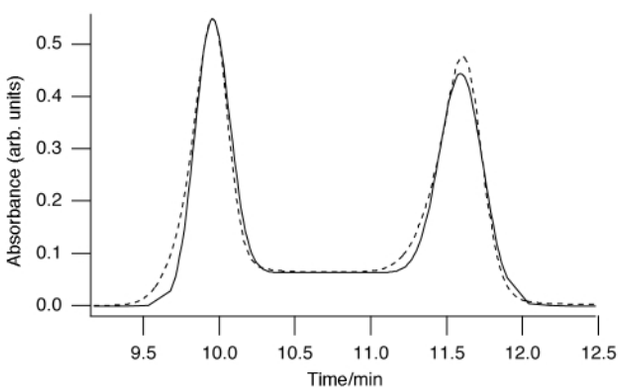


Fig. 2 Simulated (solid line) and experimental (broken line) chromatogram of (±)-**1**. Whelk-O1 (200 × 4.6 mm); hexane (45%) and methanol (1%) in dichloromethane as mobile phase; flow rate: 0.5 ml min^{-1}; column temp.: 36 °C; parameters used for simulation: $N = 3600$, $t_0 = 5.2$.

The rate of enantiomerization was found to clearly depend on the solvent. In the series methanol–acetonitrile–dichloromethane–hexane the relative rates of enantiomerization were found to be 1 : 1 : 1.8 : 10. These rate ratios may not only reflect the change in solvent polarity, since dichloromethane is much closer to hexane than to methanol on a polarity scale, but might also indicate an effect from the solvent on the mechanism of the enantiomerization. It is noteworthy that the difference in rate found in hexane, acetonitrile and dichloromethane resides entirely in $\Delta H^{\ddagger}$, while the low rate found in methanol, is to a large extent dependent on a low $\Delta S^{\ddagger}$-value.

The reason for the markedly lower error limits obtained in methanol, compared to the other solvents, is not entirely clear. To a very large extent, however, it depends on the slightly different enantiomerization rates obtained for the respective enantiomers in the less polar solvents. Fig. 1 shows an Eyring plot of the reaction in the mobile phase, where the regression analysis yields two slightly different (statistically significant) lines if the data points obtained from measurements on the (*R*)- and (*S*)-enantiomers are treated separately. The rate constants determined for the respective enantiomers at the same temperature differ by only about 6%, and separate regression analyses do not produce any qualitatively different activation parameters ($\Delta\Delta H^{\ddagger} = 0.1$ kJ mol^{-1}, $\Delta\Delta S^{\ddagger} = 1$ J K^{-1} mol^{-1}). We have not been able to detect any impurity, neither has any leakage of the chiral stationary phase from the column been observed.

Dynamic chromatography

When subjecting **1** to enantioselective chromatography, the compound behaves as a normal, stable racemate at room temperature. Heating the column above 35 °C, however, results

in a clearly visible plateau between the peaks, due to the fast enantiomerization of the respective enantiomers. In Table 2 data obtained from a set of experimental-simulated chromatograms, run in a temperature range of 35–50 °C, are given. The entries $\Delta G^{\ddagger}_{mob}$ and k_{mob} were calculated for the temperatures given in Table 2 from the activation parameters determined experimentally in the solvent mixture used for chromatography. Fig. 2 shows an example of an experimental chromatogram and its simulated counterpart. In the present case, the difference between the $\Delta G^{\ddagger}$-value determined using CD spectroscopy ($\Delta G^{\ddagger}_{mob}$) and the apparent $\Delta G^{\ddagger}$-value generated by the simulation procedure ($\Delta G^{\ddagger}_{app,\ mean}$), is only about 1 kJ mol^{-1}, where $\Delta G^{\ddagger}_{app,\ mean}$ is the average of $\Delta G^{\ddagger f}_{app}$ and $\Delta G^{\ddagger r}_{app}$ obtained from k^{f}_{app} and k^{r}_{app} respectively. This finding is important to our previously published work[2] on the related compound **2**. The structural similarity of the two compounds implies that the previously determined Gibbs free energy of activation of about 80 kJ mol^{-1} to enantiomerization of **2** using DHPLC is correct, and not to any significant degree biased by the presence of the chiral selector. DHPLC has proven to generate results that are similar to the ones obtained by an independent technique.[8]

Considering the fact that the presence of the chiral selector will affect the rate of enantiomerization, although the effect is relatively small, one notes that enantiomerization proceeds slower in the stationary phase than in the mobile phase (*i.e.* $\Delta G^{\ddagger}_{stat,\ mean} > \Delta G^{\ddagger}_{mob}$). The difference in Gibbs free energy of activation is only about 2 kJ mol^{-1}, which is of the order of accuracy of the method. The reproducibility has been estimated to ±5% of the rate constants, which corresponds to ±0.1 kJ mol^{-1} in $\Delta G^{\ddagger}$. Taking into account that complexation with the chiral selector means a stabilization of the ground state, it is not surprising that some extra energy is needed to reach the transition state, *e.g.* to partially break a hydrogen bond. This view has been put forward by others,[9] based on the finding that atropisomeric naphthamides show a barrier to enantiomerization increased by 1.3–5.5 kJ mol^{-1} on the Whelk-O1 column as compared to in free solution. The finding that the effect is smaller in our case could be explained by the much greater steric demands associated with enantiomerization of the naphthamides.

The opposite situation, *i.e.* faster enantiomerization in the stationary phase, has also been reported.[4,5] The difference is only a few kJ mol^{-1}, except in one case, where the barrier to inversion of an aziridine was lowered about 10 kJ mol^{-1} by the nickel(II)-containing stationary phase.[8]

Experimental

2-Benzyl-1,3,2-benzodithiazole 1-oxide was prepared as described previously.[1] Before resolution into enantiomers, the racemate was purified on a silica HPLC column (Kromasil 250 × 20 mm, 50% *tert*-butyl methyl ether in hexane as mobile phase, with UV detection at 230 nm). Pure enantiomers were prepared by injecting 1 mg of (±)-**1** on a Whelk-O1 column (150 × 10 mm), using neat *tert*-butyl methyl ether as the mobile phase. The enantiomers were collected and immediately evaporated under reduced pressure without heating, and kept in the freezer (−20 °C) for less than one day. Racemization kinetics were measured by dissolving the enantiomer (≈0.5 mg) in the appropriate solvent (1–2 ml) and thermostating the sample in a temperature controlled (water flow-through) 1 cm quartz cell in the CD spectrometer (JASCO mod. J-715). The racemization was monitored at the low absorbing CD band around 340 nm and the temperature of the reaction mixture was measured during the run by the use of a Pt 100 : 1/10 DIN temperature sensor in the cell. The reaction was followed for less than two half lives. Except for a few kinetic runs performed at the same temperature, an even distribution was made over the temperature range investigated. Error limits of the activation parameters were estimated to ±2 standard deviations of the regression coefficients.

Dynamic HPLC was carried out using a (3*S*,4*R*) Whelk-O1 5μ sorbent (Regis Technologies, Inc., Morton Grove, IL) packed in a 200 × 4.6 mm column immersed in a thermostated water bath, as described previously.[3] The system was left to equilibrate thermally for at least 15 min before running chromatograms. The mobile phase was composed of 45% hexane and 1% methanol in dichloromethane.

Simulations of chromatograms were performed using a slightly modified † version[9] of the SIMUL[10,11] program, which is based on the discontinuous plate model.[4,5,7,12] Plate number, retention times and the void time of the column are determined experimentally. These figures together with approximate rate constants of the reaction in the mobile and stationary phases, are used as input parameters. Rate constants are then changed until the simulated and experimental chromatograms show the best possible correspondence.[3]

Acknowledgements

This work was supported by grants from the Swedish Natural Science Research Council (K-AA/KU 02508-324) and from the Knut and Alice Wallenberg Foundation (96–110). We also thank Professor F. Gasparrini and Professor D. Misiti, University of Rome, for kindly placing their simulation program and computer facilities at our disposal.

† Only user interface modifications.

References

1 S. Allenmark and J. Oxelbark, *Enantiomer*, 1996, **1**, 13.
2 S. Allenmark and J. Oxelbark, *Chirality*, 1997, **9**, 638.
3 J. Oxelbark and S. Allenmark, *J. Org. Chem.*, 1999, **64**, 1483.
4 M. Jung and V. Schurig, *J. Am. Chem. Soc.*, 1992, **114**, 529.
5 J. Veciana and M. I. Crespo, *Angew. Chem., Int. Ed. Engl.*, 1991, **103**, 85.
6 W. Bürkle, H. Karfunkel and V. Schurig, *J. Chromatogr.*, 1984, **288**, 1.
7 A. Eiglsperger, F. Kastner and A. Mannschreck, *J. Mol. Struct.*, 1985, **126**, 421.
8 F. Gasparrini, L. Lunazzi, D. Misiti and C. Villani, *Acc. Chem. Res.*, 1995, **28**, 163.
9 F. Gasparrini, D. Misiti, M. Pierini and C. Villani, *Tetrahedron: Asymmetry*, 1997, **8**, 2069.
10 QCPE Program No. 620.
11 M. Jung, *QCPE Bull.*, 1992, **12**, 52.
12 *cf.* W. Bürkle, H. Karfunkel and V. Schurig, *J. Chromatogr.*, 1984, **288**, 1; V. Schurig and U. Leyrer, *Tetrahedron: Asymmetry*, 1990, **1**, 865.

Paper 9/04649F