Preparative and Analytical Enantiomer Separation of Some Δ^4-1,3-Thiazoline-2-thiones on Swollen Microcrystalline Triacetylcellulose (TAC)

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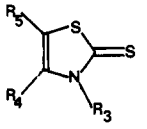
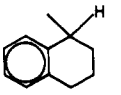
A number of thiazoline-2-thiones have been resolved by chromatography on swollen microcrystalline triacetylcellulose (TAC) with ethanol as eluent. It was found that the substitution pattern (R_4 and R_5) and the



R_4, R_5 = H, alkyl, or phenyl

R_3 = $CH_3\overset{|}{C}HCOOH$, $CH_3\overset{|}{C}HCOOCH_3$, $CH_3\overset{|}{C}HPh$, or 

conformation of R_3 of the thiazoline-2-thione ring had a great influence on the enantiomeric resolution. When R_3 has a phenyl group the *R* configuration is always eluted first. However, the elution order of the esters depends on the substituents (R_4 and R_5) of the thiazoline-2-thione ring. When R_3 had a carboxylic acid residue no resolutions were obtained. Three compounds (**2a**, **2b**, and **2c**) were resolved into their enantiomers in a preparative scale. In spite of relatively small R_s values, completely pure enantiomers could be obtained by a recycling technique.

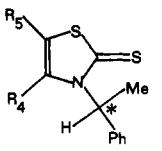
Liquid chromatographic separation on chiral stationary phases of underivatized enantiomers has developed rapidly during the last years.[1-3] Many different classes of compounds have successfully been resolved on analytical as well as on preparative scale. Especially swollen microcrystalline triacetylcellulose (TAC) as chiral stationary phase has proved to be very useful for preparative enantiomer resolutions, mainly due to the relatively high capacities of such columns.[4] A wide variety of racemates of organic compounds including materials lacking aryl groups or even functional groups, e.g., hydrocarbons, have been resolved into enantiomers on TAC columns.[5]

According to Hesse and Hagel,[6] swollen TAC obtained by heterogeneous acetylation of microcrystalline cellulose is similar in structure to the crystalline precursor. That is also confirmed in a study of the molecular and crystal structure of TAC by Stipanovic and Sarco.[7] They found that TAC, as native cellulose, has a laminar chain structure.

In the chromatographic process the solute is supposed to be included between these laminae or, as is proposed, in some kind of chiral cavities[8] between these laminae. If TAC is first dissolved and then precipitated the resolution power is more or less completely lost due to the loss of the crystalline structure.[9]

According to Dalgliesh's so-called "three points rule"[10] there must be at least three stereospecific interactions between the chiral phase and the solute to achieve enantiomer resolution. As far as TAC is concerned it is not known in detail what these three interactions are. It is,

Table I



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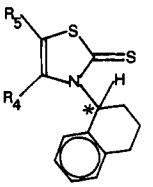

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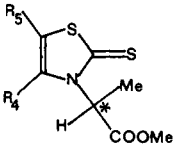

3

cmpd	R_4	R_5[a]	k_1[a]	k_2[a]	α[a]	R_s[b]	first eluated enantiomer (confign)
1a	H	H	1.13	1.79	1.59	2.7	*R*
1b	CH_3	H	0.92	1.44	1.56	1.9	*R*
1c	*i*-C_3H_7	H	0.57	0.64	1.12	0.3	*R*
1d	CH_3	CH_3	0.66	0.72	1.09	0.3	*R*
1e	C_2H_5	CH_3	0.49	0.62	1.27	0.6	*R*
1f	*i*-C_3H_7	CH_3	0.43	0.59	1.35	0.8	*R*
1g	C_6H_5	H	1.79	2.27	1.27	1.0	*R*
1h	H	C_6H_5	0.84	1.17	1.39	1.2	*R*
1i	CH_3	C_6H_5	0.72	2.15	2.98	2.3	*R*
1j	C_6H_5	CH_3	0.70	1.17	1.68	1.5	*R*
2a	H	H	1.24	1.43	1.15	0.6	*R*
2b	CH_3	H	0.74	0.93	1.26	0.8	*R*
2c	C_6H_5	CH_3	0.60	0.74	1.23	0.5	*R*
3a	H	H	1.49	1.76	1.18	0.8	*S*
3b	CH_3	H	1.34	1.53	1.14	0.5	*R*
3c	*i*-C_3H_7	H	0.86	0.92	1.08	0.2	*S*
3d	*t*-C_4H_9	H	1.03	1.23	1.18	0.7	*S*
3e	C_2H_5	CH_3	0.65	0.81	1.26	0.8	*R*
3f	*i*-C_3H_7	CH_3	0.78	1.33	1.71	2.2	*R*
3g	C_6H_5	CH_3	0.79	2.88	3.63	5.1	*S*

[a] Capacity factors and separation factors are defined according to ref 18. [b] Resolutions are defined according to ref 19.

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(3) Davankov, V. A. *Adv. Chromatogr.* **1980**, *18*, 139.
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(6) Hesse, G.; Hagel, R. *Liebigs Ann. Chem.* **1976**, 996.
(7) Stipanovic, A. J.; Sarko, A. *Polymer* **1978**, *19*, 3.
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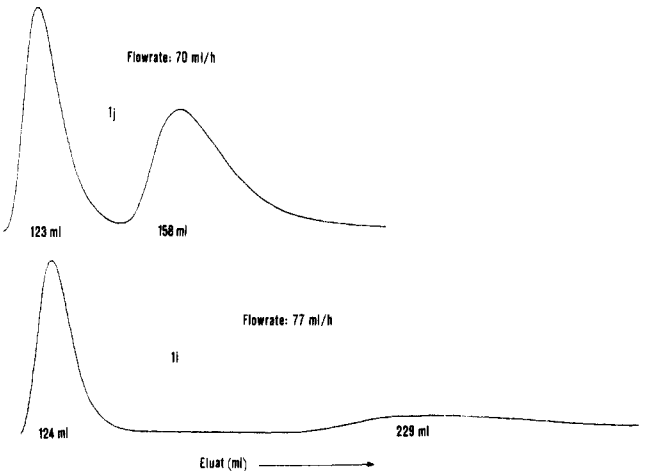


Figure 1. Chromatograms of compounds **1i** and **1j**.

however, empirically found that an unsubstituted aromatic ring adjacent to the asymmetric center is conducive for a separation. Bulky substituents, on the other hand, close to the asymmetric center very often leads to a decrease in the separation.

One of us has prepared[11,12] a series of Δ^4-thiazoline-2-thiones to study the effects of substitution at 4 and 5 positions on the conformation of the N substituent, the "rotor". The chiroptical properties of the title compounds were investigated by variable-temperature-dependent circular dichroism (CD) spectroscopy.[11,12] The barriers to rotation around the C–N bond were determined by use of NMR techniques.[11-13] These barriers were too low (13–15 kcal/mol) to give rise to atropisomers at ambient temperature.

The chiral compounds discussed below are shown in Table I. These compounds were prepared both in racemic and in optically active forms, except for compounds **2a**, **2b**, and **2c** which were prepared only in their racemic form.

The purpose of this work was (a) to separate compounds **2a–c** into enantiomers in a preparative scale for CD studies; (b) to investigate the influence of the substitution pattern of the thiazoline-2-thione ring on the separation; and (c) to compare the resolution of the subclasses of the title compounds **1**, **2**, and **3**.

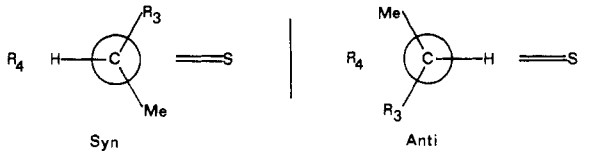
Results and Discussion

Analytical Separations. The chromatographic data of the analytical part are summarized in Table I.

As can be seen the substitution pattern of the thiazoline-2-thione ring has a great influence on the resolutions and on the capacity factors. An increase in the degree of alkyl substitution in positions 4 and 5 of compounds **1** and **2** leads to smaller capacity factors. When the bulkiness of the alkyl groups R_4 and R_5 increases in the series **1a** to **1f**, it seems as if the inclusion between the laminae is progressively more hindered from that side of the molecule. The most significant result of this is the decrease in the capacity factors (k).

The separation factors (α), however, do not show such a simple relationship. The α values within the series **1a–f** show a decrease in magnitude for compounds **1a**, **1b**, and **1c**, where there is a hydrogen atom in position 5, and a small increase for **1d**, **1e**, and **1f**, where there is a methyl group in position 5.

Scheme I. Conformations of the "Rotor" in the Thiazoline-2-thiones



In a mutual comparison of compounds which have a phenyl group in position 4 or 5, it can be seen that position 5 seems to be the most favorable to achieve a good separation. The great difference in capacity factors for both enantiomers between compounds **1g** and **1h** and for the last eluated enantiomers of **1i** and **1j** (Figure 1) is also noteworthy. The band broadening of the last eluated enantiomer of **1i** can most likely be ascribed to the mass transfer from the stationary phase to the mobile phase.[14]

The asymmetric center of compounds **2a–c** is locked in a tetrahydronaphthalene ring. The separation factors for these compounds are smaller than the corresponding values for compounds **1**. One simple explanation for this could be that the rigidity of the tetrahydronaphthalene ring does not allow the molecule to find a stereospecifically good position on the stationary phase.

An exchange of the phenyl group of the "rotor" for a carboxylic ester group (**3**) usually resulted, except for **3f** and **3g**, in a decrease of the separation factors. An NMR study of the esters[12,13] shows that these compounds can exist in two stable conformations at ambient temperature, syn and anti (Scheme I). Additional conformations are found for **3c** and **3f** due to the hindered rotation of the isopropyl group in position 4. For compounds **3a** and **3d** with R_5 = H, one of the conformations dominates, anti and syn, respectively. These compounds show a better separation than **3b** and **3c** where both stable conformations are significantly populated. When $R_5 = CH_3$, the separation is improved compared to the corresponding phenyl analogues, at least for **3f** and **3g**.

The carboxylic acids corresponding to the esters **3a**, **3b**, and **3d** have capacity factors in the range of 0.4–0.5 and show very small or no separation. The carboxylic acid group at the asymmetric center destroys all selectivity. The much smaller capacity factors of the acids compared to the corresponding esters indicate strong interactions between the mobile phase and the solute, most likely due to hydrogen bonding.

As the absolute configurations of the compounds studied were known, it was possible to compare the elution orders of the enantiomers. For compounds **1** and **2**, the R configuration was always eluated first. NMR studies of **1** and **2** show that only the anti conformation is significantly populated even when R_4 is as large as i-Pr. When R_4 = Ph, up to ca. 15% of the syn form exists at ambient temperature.[11]

The elution orders of the esters **3** are, however, not easily rationalized. It looks as if the position of the conformational equilibrium has an important influence on the chiral recognition mechanism. For compounds **3a** and **3d**, where only one of the stable conformations dominates, anti and syn, respectively, the same elution order between the enantiomers is found. As soon as several conformations are involved, the interaction between the stationary phase and the solute is more complex. It is found that the elution

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(12) Roschester, J.; Sandström, J.; Berg, U., to be published.
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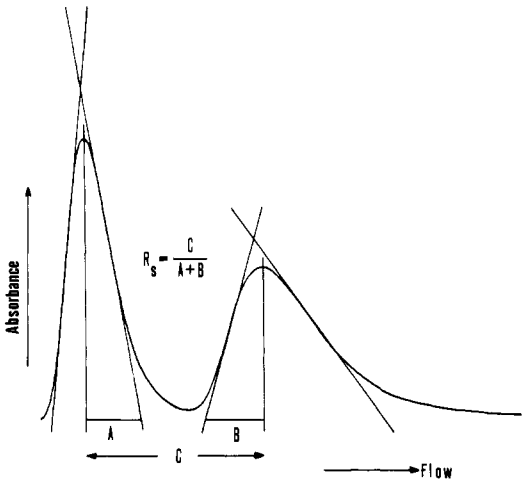

Figure 2. Definition of resolution (R_s).

orders in such cases could be reversed (Table I).

Conclusions

The preparative separation of enantiomers by liquid chromatography on TAC as stationary phase is a very useful method to obtain optically active material in smaller amounts, e.g., for chiroptical studies. Even in cases where the separation is small between the enantiomers, material with high optical purity can easily be obtained if recycling technique is used.

Caution should be exercised when absolute configurations of optically active compounds are determined by their elution order. The shape and conformation of the molecule is governed by the attached substituents on the thiazoline-2-thione ring. This has a profound influence on the stereoselectivity. The elution orders are of course associated with these interactions. The importance of the conformation of the solute for the chiral recognition process has also been pointed out by Pirkle.[15] A good knowledge of the separation mechanisms and the active sites of the stationary phase is therefore essential for such purposes.

Experimental Section

The TAC was prepared according to the method described by Hesse and Hagel.[9] The ground material was wind-sieved (Zick-Zack Sichter A 100 MZR, Alpine AG, Augsburg, FRG) into suitable fractions. The fraction with the particle size range of 15–25 μm was swollen in boiling ethanol/water (95/5; w/w) and slurry packed into two glass columns (600 mm × 10mm), keeping a constant flow rate during the packing. The pressure drop at the end of the sedimentation was about 9 bar. Ethanol/water (95/5; w/w) was used as eluent at ambient temperature. The dead volume of the column (72.8 mL) was determined by 1,3,5-tri-*tert*-butylbenzene, which is supposed to pass the column unretained.[5] The columns were connected to a switching valve (Rheodyne Model 7010) to be able to use the recycling technique.[4,16] Two detectors, coupled in series, were used, a polarimeter (Perkin-Elmer Model 241 MC) equipped with a flow cell (1 mL/10 cm; volume/length) and a UV detector (LKB Uvicord 2138 S) equipped with a standard cell (0.07 mL/0.5 cm; volume/length). The detectors were connected to a two-channel recorder (LKB Model 2210). The sample injections were made with an injection valve (Rheodyne Model 5020). Depending on the solubility of the solutes, the injection volumes had to be varied from 1 mL in the analytical runs up to 15 mL in the preparative runs. All samples were dissolved in ethanol/water (the eluent) without use of any modifier, in order not to disturb the equilibrium of the chromatographic system. In the preparative separations the compounds were isolated from the collected fractions by evaporation. The chemical as well as the enantiomeric purity of the eluted compounds was checked by NMR with shift reagents.[11,12] In a number of cases, compounds **2a** and **2b**, it was, however, not possible to determine the enantiomeric purity by this technique. Instead an analytical amount of the compound was recycled until a base-line separation was almost achieved. The enantiomeric purity was then determined directly from the chromatogram.[11] It was also found that neither racemization nor decomposition of the compounds occurred in the workup procedure.

In the calculation of the R_s values, computer simulations[17] of the chromatograms were carried out in order to get the pertinent chromatographic data. Due to the occasional long tailing of the second eluted enantiomer, the resolution R_s was defined in a somewhat modified way (Figure 2).[19]

Preparative Separations. The tetrahydronaphthalene compounds **2** were only prepared as racemates since the optically active starting materials were not available. Two different methods for the resolutions were used. Compounds **2a** and **2b** were separated on a 30-cm-long column with an inner diameter of 2.5 cm. Six injections, amounting to 50–60 mg in 3 mL of ethanol, were made. The first part of the eluted band was collected and reinjected. According to analytical chromatogram evaluation the optically pure enantiomers of **2a** and **2b** were obtained after only the second passage.

Compound **2c** was recycled. An injection volume of 15 mL (loop injection) was necessary to dissolve ca. 40 mg of the solute. No significant band broadening due to the large injection volume was noticed. The compound was cycled 6 times. After each cycle the compound was depleted of the second (−) enantiomer. The yield was ca. 60% of the completely pure (+) enantiomer.

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Registry No. **1a**, 96349-05-2; (*R*)-**1a**, 96393-05-4; **1b**, 96349-06-3; (*R*)-**1b**, 96393-06-5; **1c**, 96349-07-4; (*R*)-**1c**, 96393-07-6; **1d**, 96349-08-5; (*R*)-**1d**, 96393-08-7; **1e**, 96349-09-6; (*R*)-**1e**, 96393-09-8; **1f**, 96349-10-9; (*R*)-**1f**, 96393-10-1; **1g**, 96349-11-0; (*R*)-**1g**, 96393-11-2; **1h**, 96349-12-1; (*R*)-**1h**, 96393-12-3; **1i**, 96349-13-2; (*R*)-**1i**, 96393-13-4; **1j**, 96349-14-3; (*R*)-**1j**, 96393-14-5; **2a**, 96349-15-4; (*R*)-**2a**, 96393-15-6; **2b**, 96349-16-5; (*R*)-**2b**, 96393-16-7; **2c**, 96349-17-6; (*R*)-**2c**, 96393-17-8; **3a**, 96349-18-7; (*S*)-**3a**, 96393-18-9; **3b**, 96349-19-8; (*R*)-**3b**, 96393-19-0; **3c**, 96349-20-1; (*S*)-**3c**, 96393-20-3; **3d**, 96349-21-2; (*S*)-**3d**, 96393-21-4; **3e**, 96349-22-3; (*R*)-**3e**, 96393-22-5; **3f**, 96349-23-4; (*R*)-**3f**, 96393-23-6; **3g**, 96349-24-5; (*S*)-**3g**, 96393-24-7; TAC, 9012-09-3.

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(18) Snyder, L. R.; Kirkland, J. J. "Introduction to Modern Liquid Chromatography", 2nd ed.; Wiley: New York, 1979; p 22.

(19) Due to the long tailing of the second eluated enantiomer, found in some cases, e.g., compound **1i**, a somewhat modified definition of resolution (R_s) was adopted. $R_s = C/(A + B)$ (Figure 2).