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Liquid Chromatography on Triacetylcellulose, 4[1]

Determination of Enantiomeric Purity in Spite of Incomplete Chromatographic Separation

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A novel procedure for the measurement of enantiomeric purity P is derived, whereby the mixture to be analyzed is subjected to liquid chromatography (LC) on an *optically active sorbent.* Extensive peak overlap does not prohibit the application of the method, whereas the complete absence of separation does. In addition, the mixture is subjected to LC on an *achiral sorbent.* The same photometer and polarimeter detections are used for both experiments. At the same time, an x/y recorder plots the rotation angle α *versus* the absorbance A, thus generating the slopes C_m and C_+ in Fig. 1 which result in $P = C_m/C_+$. – Optically active samples of **1**, **2**, **3**, and **4** were analyzed for P. The findings agree satisfactorily with the results of other methods.

Flüssigkeits-Chromatographie an Triacetylcellulose, 4[1]

Neuartige Bestimmung der enantiomeren Reinheit durch Flüssigkeits-Chromatographie trotz intensiver Peak-Überlappung

Eine neuartige Methode für die Messung der enantiomeren Reinheit P wird abgeleitet, wobei die zu analysierende Mischung der Flüssigkeits-Chromatographie (LC) an einem *optisch aktiven Sorbens* unterworfen wird. Die Anwendung des Verfahrens wird durch intensive Peak-Überlappung nicht verhindert, wohl aber durch vollständiges Fehlen einer Trennung. Außerdem wird die Mischung der LC an einem *achiralen Sorbens* unterworfen. Für beide Experimente wird dieselbe photometrische und polarimetrische Detektion verwendet. Ein x/y-Schreiber trägt dabei den Drehwinkel α gegen die Extinktion A auf, wobei die Steigungen C_m und C_+ in Fig. 1 und damit $P = C_m/C_+$ erhalten werden. – Optisch aktive Proben von **1**, **2**, **3** und **4** wurden im Hinblick auf P analysiert. Die Befunde stimmen zufriedenstellend mit Ergebnissen anderer Methoden überein.

The enantiomeric purity P of chiral substrates is of interest for several purposes: Calculation of chiroptical data, *i. e.* $[\alpha]$ and $\Delta\varepsilon$, of the pure enantiomers, which are needed for understanding such data; evaluation of educts and products of reactions, the mechanisms of which are under study; evaluation of the products of enantioselective syntheses; characterization of substrates to be checked for biological effects.

It is worthwhile to strive for new techniques of P determination because none of the known methods[2] is universally applicable. E. g., conversion of enantiomers to diastereoisomers and subsequent analysis of the latter require a functional group in the substrate and rely on complete reaction, the absence of racemization, and the knowledge of P of the reagent[2]. Absolute methods[2] do not need a standard for P, *i. e.*, they analyze the mixture of enantiomers by association with an optically active auxiliary, the enantiomeric purity of which does not enter the

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calculation of P. Chromatography on optically active sorbents is one of these absolute methods. In this respect, gas chromatography has advanced considerably, but requires elevated temperatures, whereas liquid chromatography (LC) can be performed at room temperature, thus reducing the rates of decompositions or racemizations. If a baseline separation of enantiomers is obtained, the areas under their peaks permit direct calculation of P. Unfortunately, the overall effort to achieve this by LC of a given substrate is frequently considerable, mainly because only few optically active stationary phases can be bought. Therefore, the variation of sorbent properties in a given laboratory is normally limited.

We have therefore chosen an alternative way to determine enantiomeric purity by LC: We try to develop methods applicable *in spite of peak overlap or even peak fusion* (e.g., Fig. 1, lower left part). One possibility is to collect the pure enantiomer(s) after the beginning or (and) towards the end of elution and to measure the specific rotation separately[3]. Our first procedure[1] determines the areas under two detection curves and has meantime proven successful in several cases[1,4-6]. However, we had mentioned[1] that improvements avoiding manual area determinations should be strived for. In our opinion, such an improvement is provided by the novel procedure described in this paper.

Derivation of an Expression for the Measurement of Enantiomeric Purity P

The absorbances A_+ and A_- of a mixture of enantiomers (+ prevailing) are given by Beer's law, the rotation angles α_+ and α_- by Biot's law[7] (cf. Fig. 1b of Ref.[1]):

$$\begin{aligned} \varepsilon b c_+ &= A_+ \\ \varepsilon b c_- &= A_- \\ \hline \varepsilon b (c_+ + c_-) &= A_+ + A_- \equiv A \end{aligned} \tag{1}$$

$$\begin{aligned} +[\alpha] l c_+ &= \alpha_+ \\ -[\alpha] l c_- &= \alpha_- \\ \hline [\alpha] l (c_+ - c_-) &= \alpha_+ + \alpha_- \equiv \alpha \end{aligned} \tag{2}$$

$A(v)$ and $\alpha(v)$ represent chromatograms, e.g., Fig. 1, left part. The factor $\alpha/A \equiv C$ at an actual volume v of eluate is obtained from eqs. (1) and (2):

$$C \equiv \frac{\alpha}{A} = \frac{[\alpha] l (c_+ - c_-)}{\varepsilon b (c_+ + c_-)} \tag{3}$$

We apply[8] $c_+ = \mathrm{d}n_+/\mathrm{d}v$ and $c_- = \mathrm{d}n_-/\mathrm{d}v$ to eq. (3), where $\mathrm{d}n_+$ and $\mathrm{d}n_-$ are the numbers of mols travelling in the volume element $\mathrm{d}v$:

$$C \equiv \frac{\alpha}{A} = \frac{[\alpha] l (\mathrm{d}n_+ - \mathrm{d}n_-)}{\varepsilon b (\mathrm{d}n_+ + \mathrm{d}n_-)} = \frac{[\alpha] l}{\varepsilon b} P' \tag{4}$$

$(\mathrm{d}n_+ - \mathrm{d}n_-)/(\mathrm{d}n_+ + \mathrm{d}n_-) \equiv P'$ represents the actual enantiomeric purity[2] in the element $\mathrm{d}v$. This derivation presumes α and A to be measured simultaneously. When monitoring these parameters sequentially[1], we will, therefore, pass the eluate through the detectors for A, then for α, and again for A (cf. Experimental Part).

If an *optically active sorbent* is used and enrichments of the enantiomers occur, the eluate cannot contain a relevant amount of the less abundant − enantiomer[1] in the Δv region of Fig. 1. Therefore, in this region $P' = 1$ and eq. (4) degenerates to

$$C_+ = \frac{\alpha_+}{A_+} = \frac{[\alpha] l}{\varepsilon b} \tag{5}$$

C_+ refers to the pure + enantiomer and can be measured from an $\alpha(A)$ diagram (Fig. 1, lower right part) by determining the slope at low volumes[9].

If an *achiral sorbent* is used, no enrichments of the enantiomers can occur. Therefore, P' in eq. (4) is constant during passage and equals the overall P of the mixture:

$$C_m = \frac{\alpha_m}{A_m} = \frac{[\alpha]l}{\varepsilon b} P \tag{6}$$

Thus, C_m is also constant (index m for „mixture") and can be determined by another $\alpha(A)$ diagram (Fig. 1, upper right part).

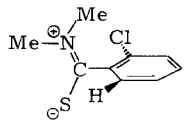
Eqs. (5) and (6) combine for

$$P = C_m/C_+ \tag{7}$$

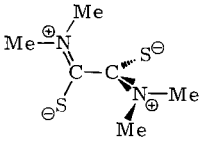
if the same l and b, *i. e.* the same detection systems, are used for the applications of the two sorbents. P is therefore obtained by measuring the two slopes of Fig. 1 (right part).

Results of Applications

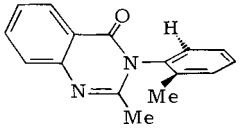
We have used eq. (7) for the P determination of several non-racemates obtained by semipreparative LC[5,10,11] on triacetylcellulose. All of them showed serious overlap of the peaks for the two enantiomers under our normal conditions[10]. None of the optically active samples of **1**, **2**, **3**, and **4** showed significant racemization at the temperature of analysis. The enantiomeric compositions of such samples were of interest in order to judge the efficiency of preparative work and to calculate the chiroptical data of the pure enantiomers by $[\alpha] = [\alpha_m]/P$ and $\Delta\varepsilon = \Delta\varepsilon_m/P$. $[\alpha]$ and $\Delta\varepsilon$, in turn, will be useful for understanding such data and for measuring the optical purities of future non-racemates from their specific rotations $[\alpha_m]$.



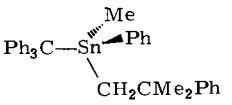
(*P*)[13]-**1**



(*P*)-**2**



(*P*)-**3**



(*R*)-**4**

A non-racemic mixture of the thiobenzamides (+)- and (−)-**1**[12] was analyzed (Fig. 1) by the novel procedure outlined above. The quantities applied were no more than 0.7 mg on a silica column and 0.6 mg on a triacetylcellulose column. The time intervals from the injections until complete elutions were 30 and 66 min, respectively, at pressures around 2 bar. From the slopes $C_m = 0.018°$ and $C_+ = 0.039°$ (Fig. 1, right part) we calculate $P = 0.47$ which means $[\alpha]^{22}_{436} = 656°$ ml g^{-1} dm^{-1} (ethanol/water,

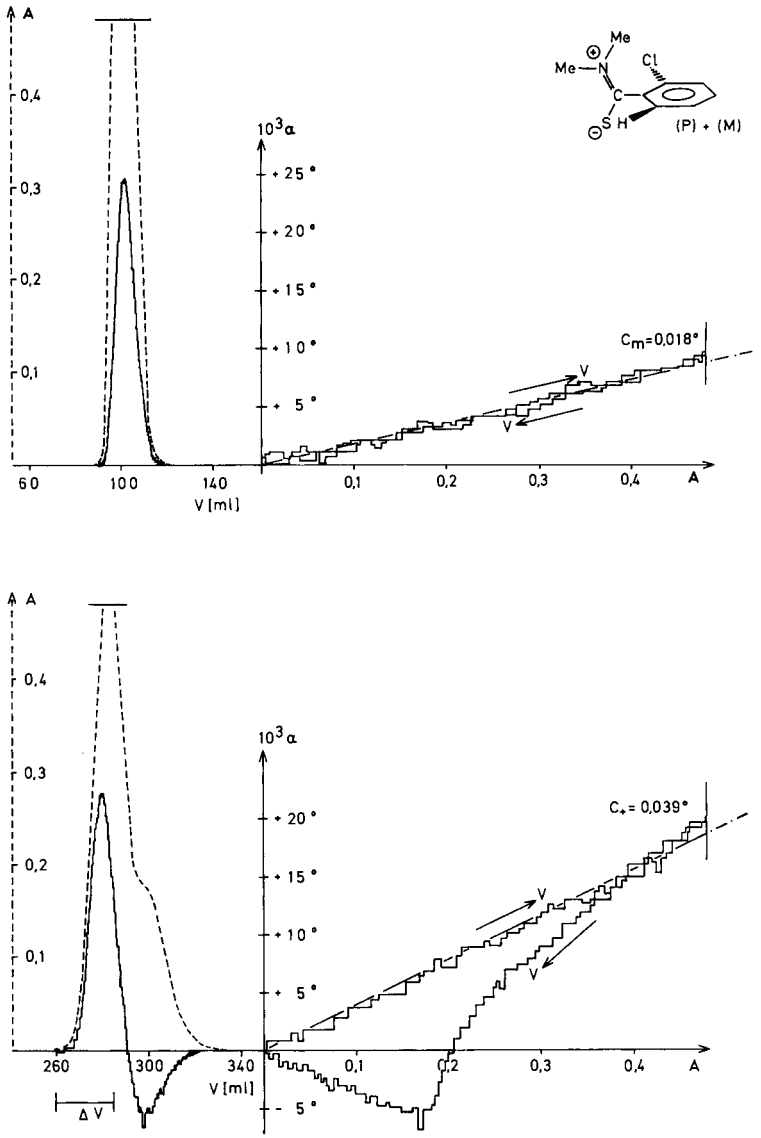


Fig. 1. Chromatograms $A(v)$ and $\alpha(v)$ (left) and corresponding diagrams $\alpha(A)$ (right) for a mixture of (+)- and (−)-**1**, $[\alpha_m]^{22}_{436} = +306 \pm 20°$ ml g^{-1} dm^{-1}. The periodic steps originate from the automatic averaging measurements of the polarimeter at intervals of 1 s. v: Actual volume of eluate. Δv: Region of v, where the eluate does not contain a relevant amount of the less abundant (−)-**1**. α: Rotation angle of eluate at 436 nm. A: Absorbance of eluate at 254 nm; the limits of A indicated are those of the photometer's linearity. The polarimeter is linear in the corresponding region. Above: After passage through a silica column (0.7 mg of **1**, flow 4 ml/min, pressure 1.8 bar). Below: After passage through a triacetylcellulose column (0.6 mg of **1**, flow 5.3 ml/min, pressure 2.0 bar). $P = C_m/C_+ = 0.47$

96:4) for the pure enantiomers. We have developed an alternative method[12] for the calculation of $[\alpha]$ from the measurement of C_+ (or C_-) without any preparative enrichment. In the case of (±)-**1** we found[12] $[\alpha]_{436}^{22} = 704°$ ml g^{-1} dm^{-1} (ethanol/water, 96:4) for the pure enantiomers. The determinations of errors of both LC methods must be deferred until more results are available. The value of 656° ml g^{-1} dm^{-1} is subject to an additional substantial error, which cannot be avoided: the error of polarimetric measurement. When all three sources of error are taken into consideration, the agreement of the above $[\alpha]$ results is satisfactory.

Similarly, analytical chromatography of a non-racemic mixture of the dithiooxamides (+)- and (−)-**2** resulted in $P = 0.51$, which means $[\alpha]_{365}^{34.5} = 4527°$ ml g^{-1} dm^{-1} ($CHCl_3$) for the pure enantiomers. The same mixture was analyzed by ^1H NMR in the presence of $(+)_{589}$-1-(9-anthryl)-2,2,2-trifluoroethanol, the result[14] being $P = 0.50 \pm 0.01$.

A check of the chromatographic finding by ^1H NMR of the same optically active sample was also possible for the heterocyclic biaryl compound **3** (Table 1). Another sample of it showed, under different LC conditions, nearly complete separation of the two peaks for (+)- and (−)-**3**. Thus, the $A(v)$ curve was suitable for obtaining P (Table 1) *via* the weights of cut chromatogram copies in this case. Taking into account the above mentioned error situation, the agreement of the $[\alpha]$ values is still satisfactory.

Table 1. Specific rotation of the pure heterocyclic biaryl enantiomers (+)- and (−)-**3** in ethanol/water (95:5) *via* enantiomeric purities P. Cf. text

Method	P	$[\alpha]_{365}^{21}$ [grad ml g^{-1} dm^{-1}]
LC, present procedure	0.64	710
^1H NMR[a]	0.67 ± 0.01	675 ± 32[6]
LC, present procedure	0.35	634
LC[b]	0.36	616[6]

[a] In the presence of $(+)_{589}$-tris[3-(heptafluorobutyryl)-D-camphorato]europium(III). – [b] Direct evaluation of the areas under the $A(v)$ curve was made possible by nearly complete peak separation.

Table 2. Specific rotation of the pure tetraorganotin compounds (+)- and (−)-**4** in ether *via* enantiomeric purities P. Cf. text

Method	P	$[\alpha]_{589}^{20}$ [grad ml g^{-1} dm^{-1}]
LC, present procedure	0.98	205
LC, procedure described[1] earlier	0.94	214
LC, present procedure	1.0	199
LC, procedure described[1] earlier	0.62	209

The last example, the tetraorganotin **4**, does not allow an easy check of results by methods other than chromatographic ones because no heteroatoms useful for association or reaction are present. The same optically active sample was subjected to the

procedure presented in this paper and to the one described[1] earlier (P = 0.98 and 0.94, respectively, Table 2). Other samples (P = 1.0 and 0.62, Table 2) resulted in very similar [α] values.

The novel method was applied successfully to several other mixtures[6] which will be described elsewhere, because they did not permit tests of our method by independent ones.

Discussion

For the present determination of P, the absorbance A must be measurable at some wavelength; the same is true for the rotation angle α, taking into account sufficient transmission at the wavelength chosen. Our procedure depends upon *some* separation by the optically active sorbent which automatically generates some linear region in the $\alpha(A)$ diagram (Fig. 1, lower right part). However, our method does not at all depend upon a separation yielding a more or less *split* $A(v)$ chromatogram. Neither a *certain* peak shape nor *equal* peak shapes for the enantiomers are required.

The $\alpha(A)$ diagram of analytical chromatography on an optically active sorbent may represent a useful check during a preparative separation of enantiomers. In particular, a diagram consisting *only* of a straight line definitely indicates the absence of the second enantiomer within error limits.

The novel technique avoids the area determinations of our first procedure[1]. These are impossible if the linear range of the photometer is insufficient for recording the whole $A(v)$ curve (e.g. Fig. 1). The novel technique, in addition, needs LC on an achiral sorbent, i.e. some additional quantity of the mixture to be analyzed or its unchanged recovery from the eluate of LC on the optically active sorbent. However, for this latter experiment, the novel procedure can replace the mixture by the corresponding racemate, if separation by the optically active sorbent generates some sufficiently extended linear region(s) in an $\alpha(A)$ diagram.

Recently, the measurement of P by monitoring of A, ΔA (the circular dichroism), and $\Delta A/A$ after passage through an optically active sorbent has been proposed[15]. These authors stated P to be estimated with their detection system more readily than by our procedure[1] of monitoring A and α. This statement may refer to the simultaneity of measurements of the respective two parameters. We agree that $\Delta A/A$ detection[15] provides for exactly simultaneous monitoring, whereas we compensate for sequential monitoring[1] by passing the eluate through the detectors for A, then for α, and again for A (cf. Experimental Part); the earlier[1] as well as the present work used this technique, reproducing several known P-values satisfactorily. (By the way, our α and A detections can be carried out at *unequal* wavelengths). For the P determination of a mixture according to *Mason* et al.[15], a mean $\Delta A/A$-ratio over the chromatogram is needed, but the authors did not describe how this ratio can be obtained nor did they give examples for the measurement of the overall P of mixtures.

It should be noted that the applications given in this paper do not correspond to optimized experimental conditions concerning eluents, sorbents, particle sizes, column diameters[16], and detections. In our opinion, future improvements will originate mainly from the accuracy of polarimeters and from the dimensions of polarimetric cells.

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Experimental Part

Mixture of (+)- and (−)-2-chloro-N,N-dimethylthiobenzamides (**1**): Obtained by semipreparative liquid chromatography[10] of (±)-**1**[17] on triacetylcellulose: $[\alpha_m]_{436}^{22}$ = +306 ± 20° ml g^{-1} dm^{-1} (0.76 g/l ethanol/water, 96:4), m.p. 110–113 °C, P = 0.47, determined (Fig. 1) according to the novel procedure.

Mixture of (+)- and (−)-N,N,N',N'-tetramethylethanedithioamides (**2**): Obtained by semipreparative liquid chromatography[10] of (±)-**2**[18] on triacetylcellulose: $[\alpha_m]_{365}^{34.5}$ = +2309 ± 346° ml g^{-1} dm^{-1} (0.08 g/l $CHCl_3$), m.p. 137–138 °C, P = 0.51, determined according to the novel procedure.

(±)-2-Methyl-3-(2-methylphenyl)-4(3H)-quinazolinone (**3**)[19]: M.p. 116–117 °C (lit.[19] 113–115 °C).

Mixture of (+)- and (−)-2-methyl-3-(2-methylphenyl)-4(3H)-quinazolinones (**3**): Obtained by semipreparative liquid chromatography[10] of (±)-**3** on triacetylcellulose: $[\alpha_m]_{365}^{21}$ = +455 ± 15° ml g^{-1} dm^{-1} (3.8 g/l ethanol/water, 95:5), m.p. 91 °C, P = 0.64, determined according to the novel procedure (Table 1).

(±)-(2,2-Dimethyl-2-phenylethyl)methylphenyl(triphenylmethyl)tin (**4**)[5] *and mixtures*[5] *of* (+)- *and* (−)-**4** in Table 2.

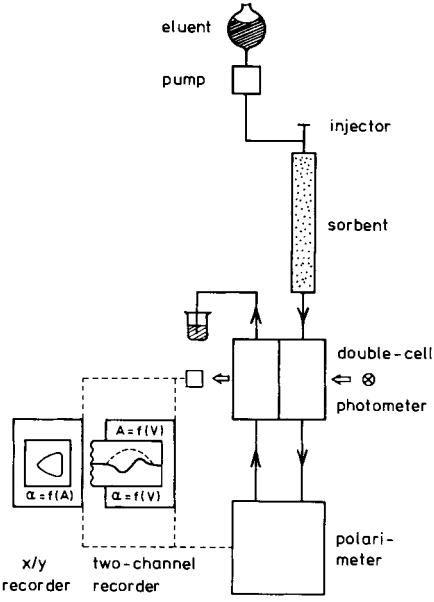

Fig. 2. Scheme of chromatography and detection equipment used for determination of enantiomeric purity

Chromatography equipment: Microcrystalline triacetylcellulose, prepared, milled, and pre-separated as described[11], was air-separated (Zickzacksichter A 100 MZR Alpine AG, Augsburg). Fractions with particles sizes of 20 to 30 µm or 32 to 60 µm were swollen and slurry-packed as described[11] into glass columns (30 × 2.5 cm, Serva GmbH, Heidelberg) under 7 to 8 bar. Silica (63 to 200 µm; E. Merck, Darmstadt) was packed into glass columns (25 × 2.5 cm) or used as a Fertigsäule (40 to 63 µm; E. Merck, Darmstadt) of the same size. The temperatures of the columns were 22 °C. Ethanol/water (96:4) served as the eluent. The membrane pumps ProMinent Electronic B2505 or SI (Chemie und Filter GmbH, Heidelberg) generated pressures of 1.5 to 4 bar and flows of 4 to 7 ml/min.

Detections: The eluate was passed through one compartment (path length 2.0 mm, volume 0.08 ml) of a photometer double-cell (Hellma GmbH & Co., Müllheim), then through the polarimeter cell (path length 100 mm, volume 1 ml) and back through the photometer (Fig. 2). Photometer: LKB Uvicord S. Polarimeter: Perkin-Elmer 141 or 241. The two-channel recorder RE 571 Servogor 2S (Goerz Electro GmbH, Wien, Austria) was used for the $A(v)$ and $\alpha(v)$ chromatograms, the x/y recorder Servogor XY 733 (same origin) for the $\alpha(A)$ diagrams.

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[6] *A. Mannschreck, G. Stühler,* and *H. Koller,* unpublished results.
[7] Conventions urge for Beer's law concentrations in mol/l and path lengths in cm, however for Biot's law concentrations in g/ml and path lengths in dm. No confusion can arise since concentrations and path lengths are eliminated from the final eq. (7).
[8] Cf. *R. L. Pecsok, L. D. Shields, T. Cairns,* and *I. G. McWilliam,* Modern Methods of Chemical Analysis, 2nd ed., p. 102, Wiley, New York 1976.
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