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Chiroptical Detection During Liquid Chromatography, Part 5 [1]: On-Line Measurement of Circular Dichroism Spectra $\Delta\varepsilon(\lambda)$ During Stops of Chromatographic Flow**

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Summary. A procedure is described which serves to measure circular dichrograms $\Delta\varepsilon(\lambda)$ on line during stops of flow in liquid chromatography. Since the concentration of substrate in the spectrometer cell during the stop is not known, the differential absorption coefficients $\Delta\varepsilon$ are calculated from the experimental differential absorbances ΔA by means of UV absorption (i. e. photomultiplier voltage) data. Verifications of the procedure are obtained by its application to three substrates (Table 1), the $\Delta\varepsilon(\lambda)$ spectra of which were known. The present on-line technique is compared with a corresponding off-line method.

The N,N-dimethylthiobenzamides **1** and **2** as well as the 9,10-phenanthrenequinone **7** consist of interconvertible enantiomers because their planar states are destabilized by steric overcrowding of groups. The unknown dichrograms $\Delta\varepsilon(\lambda)$ of **1**, **2** and **7** are obtained (Figs. 2 and 4) and discussed with reference to the helicities of these molecules.

Keywords. Circular dichroism spectra; Liquid chromatography on optically active sorbents; 9,10-Phenanthrenequinones, enantiomers of ; Thiobenzamides, enantiomers of.

Chiroptische Detektion während der Flüssigkeits-Chromatographie, Teil 5 [1]: On-line-Messung von Circulardichroismus-Spektren $\Delta\varepsilon(\lambda)$ während des Anhaltens des chromatographischen Flusses

Zusammenfassung. Ein Verfahren zur on-line-Messung von Circulardichroismus-Spektren $\Delta\varepsilon(\lambda)$ während des Anhaltens des chromatographischen Flusses wird beschrieben. Die Konzentration des Substrats in der Spektrometer-Küvette während des Anhaltens ist nicht bekannt, weshalb die differentiellen Absorptionskoeffizienten $\Delta\varepsilon$ aus den experimentellen differentiellen Absorbanzen ΔA mit Hilfe von UV-Absorptions-Daten (d. h. Photomultiplier-Spannungen) berechnet werden. Die erfolgreiche Überprüfung des Verfahrens gelingt durch seine Anwendung auf drei Substrate (Tabelle 1), deren $\Delta\varepsilon(\lambda)$-Spektren bekannt waren. Die vorgestellte on-line-Technik wird mit einer entsprechenden off-line-Methode verglichen.

Die N,N-Dimethylthiobenzamide **1** und **2** sowie das 9,10-Phenanthrenchinon **7** bestehen aus interkonvertierenden Enantiomeren, weil ihre ebenen Zustände durch räumliche Gruppenhäufung destabilisiert sind. Die unbekannten Dichrogramme $\Delta\varepsilon(\lambda)$ von **1**, **2** und **7** werden ermittelt (Fig. 2 und 4) und im Hinblick auf die Helizitäten dieser Moleküle diskutiert.

** In memory of the late Professor Dr. Dr. h. c. Günther Snatzke.

Introduction

Circular dichroism (CD) spectroscopy is probably the most significant method for the characterization and structural investigation of chiral molecules. The study of enantiomers, on the other hand, is of growing interest to chemistry, biology and pharmacy, one of the reasons being the rapid development of liquid chromatography (LC) techniques. The latter can be performed on optically inactive or on optically active sorbents.

Therefore, the joint use of these two methods has been accomplished by several groups during the last years, the literature being mentioned in two articles [1, 3]. This combination means that a CD spectrum is recorded on line during a stop of the LC flow. The spectrum obtained is of the type $\Delta A(\lambda)$, i.e. the differential absorbance ΔA is measured, not the desired differential absorption coefficient $\Delta\varepsilon$ [4, 5], referred to an enantiomeric purity of unity. $\Delta\varepsilon(\lambda)$ can be calculated if the molar concentration of the substrate, trapped in the spectrometer cell during the stop, is known. Takakuwa and his coworkers [4] have determined such concentrations in the case of a protein mixture via off-line CD spectra at known concentrations. To our knowledge, there is no publication of a further attempt to obtain on-line dichrograms $\Delta\varepsilon(\lambda)$ during LC stops.

A procedure of this type will be described in detail in the present paper. However, for calibration it does not use the off-line CD spectrum at known concentration (which is convenient if the non-racemic substrate is available), but it uses *UV absorption* (i.e. photomultiplier voltage) data of a *racemate* at known concentration (which is convenient if the non-racemic substrate is not available).

Subsequently, this procedure will be applied to two types of molecules exhibiting interconvertible enantiomers: thiobenzamides and phenanthrenequinones.

Method and Results

Description of the Procedure; Relative Helicities of Thiobenzamides

In order to obtain an on-line dichrogram $\Delta\varepsilon(\lambda)$, the first step is the acquisition [1, 3] of the corresponding spectrum $\Delta A(\lambda)$ during a stop of the LC flow on the peak of an enantiomer (or an enriched enantiomer). For this purpose, the spectrometer must be equipped with a flow-through cell and an optical beam condensor. The principle of the procedure is given schematically in Ref. [1] together with the example of a curve $\Delta A(\lambda)$.

A corresponding dichrogram $\Delta\varepsilon(\lambda)$ is obtained by a second step, a calibration using the same solution, trapped by stopped flow, the same cell and the same spectrometer, but in its "absorption", not its CD mode. We have made sure by additional measurements that the UV "absorption" mode of an instrument like the Jasco J-40 A, including an additional beam condenser, can be used successfully even if its output is the photomultiplier voltage E which differs from the absorbance A [6]. It turned out that the E-values of our J-40 A were linear with respect to the substrate concentration c up to $E = 500\,\text{mV}$. If the solvent contributes to E, the *difference* ΔE between the values of the solvent and the substrate has to be used as a measure of absorption, i.e. ΔE and c are proportional.

The technique of calibration will described for (*MP*)-2-dimethylamino-N,N-dimethylthiobenzamide (**1**) which owes its chirality to hindered rotation of a thio-

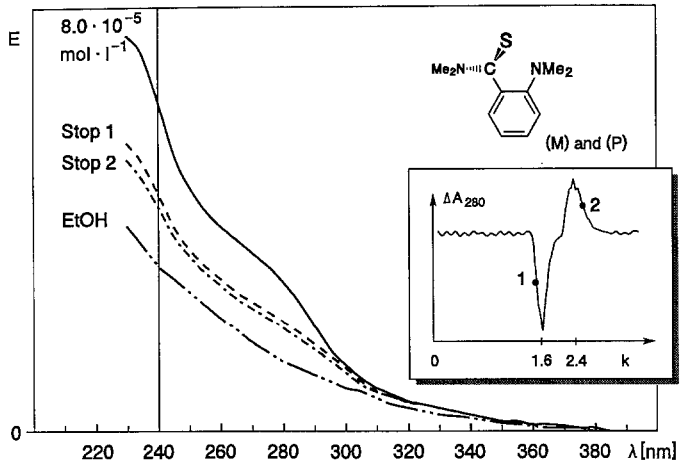


Fig. 1. (*M*)- and (*P*)-2-dimethylamino-N,N-dimethylthiobenzamides (**1**) in *Et*OH/H_2O (96 : 4) at 15 °C. Insert: Chromatogram of 120 µg of (*MP*)-**1** on triacetylcellulose at ~ 92 bar; ΔA_{280}, differential absorbance at 280 nm; *k*, capacity factor; stops of flow no. 1 and 2 for recording of $E(\lambda)$ spectra are marked. Main diagram: $E(\lambda)$ spectra, *E* being the photomultiplier voltage [6]. ----- On-line spectra of (*M*)- and (*P*)-**1** at stops no. 1 and 2; ——— off-line spectrum of (*MP*)-**1**, $c_k = 8.0 \cdot 10^{-5}$ mol l^{-1}; —··—·· off-line spectrum of the solvent *Et*OH/H_2O (96 : 4)

carbamoyl group about a C(sp^2)-C(sp^2) bond [7 – 10]. Its dichrograms $\Delta\varepsilon(\lambda)$ are not accessible in the usual way because the low barrier to enantiomerization (98.5 kJ/mol, 30.7 °C, *Et*OH [11]) renders the quantitative investigation of the enantiomers difficult at room temperature. However, they can be trapped at 15 °C in the cell of the spectrometer by LC (Fig. 1, insert). During a first stop of flow, a CD curve $\Delta A(\lambda)$ (not shown) and an absorption spectrum $E(\lambda)$ (Fig. 1, main diagram, Stop 1) are recorded, followed by the off-line spectra $E(\lambda)$ of (*MP*)-**1** in known concentration and the pure solvent used for LC (Fig. 1, main diagram, highest and lowest traces).

According to experience, based upon deconvolutions of overlapped chromatographic peaks [1], we can safely assume that the enantiomeric purity at stop no. 1 in Fig. 1, insert, is unity. Therefore, the $\Delta A(\lambda)$ curve measured above can be transformed into the desired dichrogram $\Delta\varepsilon(\lambda)$ by

$$\Delta\varepsilon = \frac{\Delta A}{b \cdot c} \tag{1}$$

b = path length of cell
c = unknown molar concentration of substrate, trapped in the cell during stop of flow

We have stated above the proportionality between c and ΔE, e. g.,

$$c/\Delta E = c_k/\Delta E_k \tag{2}$$

ΔE = experimental difference between the *E*-values of the solvent and the substrate trapped in the cell
C_k = known molar concentration of substrate, injected off line into the cell

ΔE_k = experimental difference between the E-values of the solvent and the substrate, injected off line into the cell
Eqs. (1) and (2) result in

$$\Delta\varepsilon = \frac{\Delta A \cdot \Delta E_k}{b \cdot c_k \cdot \Delta E} \tag{3}$$

the equation transforming the measured $\Delta A\,(\lambda)$ curve into the desired dichrogram $\Delta\varepsilon\,(\lambda)$ by means of the known values of b, c_k, ΔE_k and ΔE. In Fig. 1 (main diagram), ΔE_k can be taken, e. g. at 240 nm, as the difference between the highest and the lowest trace, ΔE as the difference between the traces marked "Stop 1" and "*Et*OH". Fig. 2 contains the dichrogram, resulting from Eq. (3), for one of the enantiomers of **2**.

We measured exactly the opposite dichrogram, when LC was continued after stop no. 1 in Fig. 1 and CD was recorded during stop no. 2. For this spectrum, an enantiomeric purity of less than unity, resulting from some overlap of the peaks, had to be taken into account.

In the same way, the on-line dichrogram (Fig. 2) of an enantiomer of **2** was obtained [11]. The barrier to enantiomerization for the thiobenzamide **3** (103.1 kJ/mol, 31.9 °C, $CHCl_3$ [19]) is higher than for **1** and **2**. Therefore, its CD spectrum (Fig. 2) could be measured off line after preparative separation of the antipodes, i. e. in the usual way. On the basis of the curves in Fig. 2, we propose identical relative helicities for these enantiomers of thiobenzamides **1**, **2** and **3**.

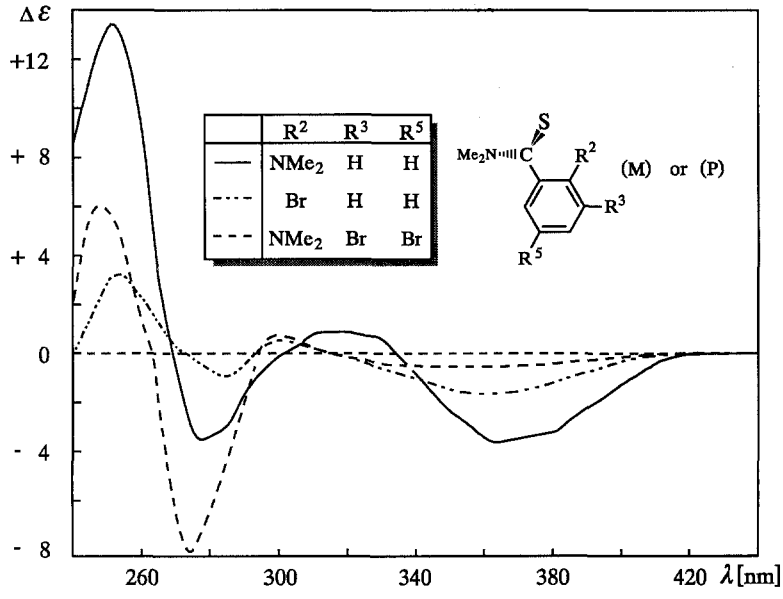


Fig. 2. Circular dichrograms on the basis of which identical relative helicities of $(-)_{436}$-$[-]_{360}$-thiobenzamides are proposed. The designations (−) and [−] refer to polarimetry and CD, respectively. ——— On-line CD of $(-)_{436}$-**1**, enantiomeric purity 1.0, in *Et*OH/H_2O (96 : 4) at 15 °C at stop no. 1 of Fig. 1, calibrated for $\Delta\varepsilon$ (see text). —··—·· Off-line CD of $(-)_{436}$-**3** [8], enantiomeric purity 1.0, in *Me*OH at 23 °C. ----- On-line CD of $(-)_{436}$-**2**, enantiomeric purity 1.0, in *Me*OH at 22 °C according to Ref. [1], calibrated for $\Delta\varepsilon$ (see text)

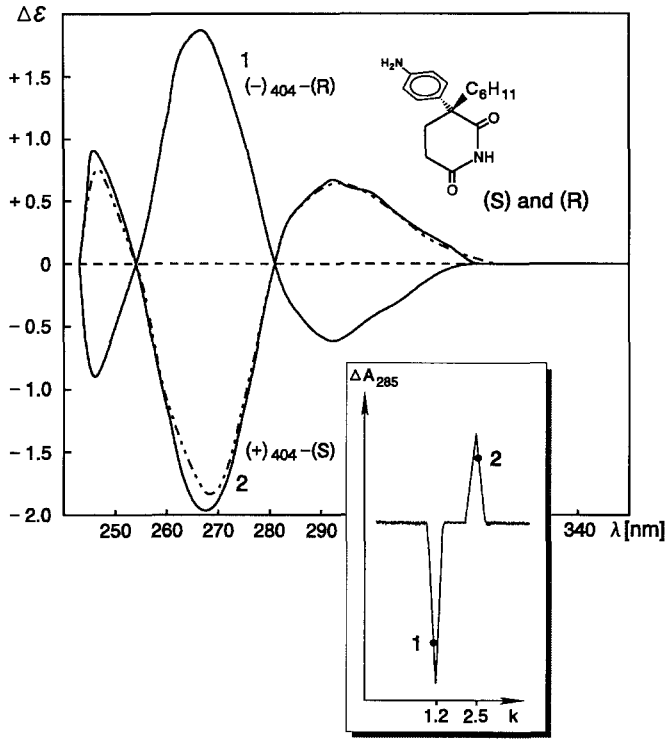


Fig. 3. (*R*)- and (*S*)-3-(4-aminophenyl)-3-cyclohexyl-2,6-piperidinediones (**4**) [12, 13] in *Et*OH/H_2O (96 : 4) at 23 °C. Insert: Chromatogram of 40 µg of (*RS*)-**4** on tris(phenylcarbamoyl)cellulose/SiO_2 at ~ 130 bar; ΔA_{285}, differential absorbance at 285 nm; *k*, capacity factor; stops of flow no. 1 and 2 for recording of dichrograms are marked. Main diagram: ——— On-line circular dichrograms at stops no. 1 and 2, calibrated for $\Delta\varepsilon$ (see text), enantiomeric purities being 1.0 each; ----- off-line circular dichrogram of 0.5 µg of $(+)_{404}$-(*S*)-**4** [12, 13], enantiomeric purity of 1.0, injected into cell

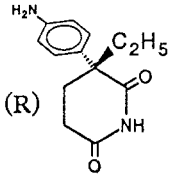
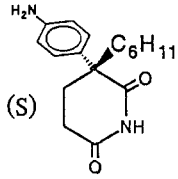
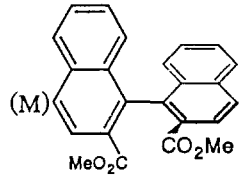
Experimental Verification of the Procedure

The on-line dichrograms (Fig. 3) of (*R*)- and (*S*)-3-(4-aminophenyl)-3-cyclohexyl-2,6-piperidinediones (**4**) were obtained during two stops of flow on base-line separated peaks. Therefore, both spectra correspond to enantiomeric purities of unity and are related to one another by precise mirror symmetry. (*S*)-**4** has turned out to be one of the most potent aromatase inhibitors and is therefore of great interest for the treatment of certain types of cancer [12, 13]. Its off-line CD spectrum (Fig. 3) fits well to the on-line dichrogram, thus providing an experimental verification of our stopped-flow technique.

A similar verification (Table 1) was performed using the ethyl analogue **5** (aminoglutethimide) [12 – 14]. The $\Delta\varepsilon$-scales given previously [12] for (*S*)-**4** and (*S*)-**5** show values which are low because of erroneous calibrations of the dichrograph.

Finally, the corresponding spectra $\Delta\varepsilon(\lambda)$ of the binaphthyl derivative **6** [15, 16] agree equally well (Table 1). The deviations between the on-line and the usual off-line data in the whole Table 1 amount to roughly 10% of the latter.

Table 1. Comparison of CD off-line results with data which were acquired on line and calibrated for $\Delta\varepsilon$ (see text). Ethylpiperidinedione derivative **5** [12–14] and its cyclohexylpiperidinedione analogue **4** [12, 13] (cf. Fig. 3) in *Et*OH/H_2O (96:4); binaphthyl derivative **6** [15, 16] in *Me*OH

$(+)_{404}$-Enantiomer	On-Line Acquisition, Calibrated $\Delta\varepsilon_{max}$ [l mol^{-1} cm^{-1}]	Off-Line Acquisition $\Delta\varepsilon_{max}$ [l mol^{-1} cm^{-1}]	λ_{max} [nm]
 (R)	+ 0.7	+ 0.8	290
	− 2.3	− 2.4	263
 (S)	+ 0.7	+ 0.6	293
	− 2.0	− 1.8	267
	+ 0.8	+ 0.7	245
 (M)	+ 3.4	+ 3.3	323
	+17.1	+16.5	283

(MP)-4,5-Dimethyl-9,10-phenanthrenequinone (**7**)

Quinone **7** owes its chirality to overcrowding of the methyl groups and, probably, the oxygen atoms in its planar state [17, 18]. The CD spectra of **7** are not easily accessible in the usual way for the following reasons: a) The barrier to enantiomerization (90.2 kJ/mol, 25.0 °C, *Et*OH/H_2O, 96:4 [18]) is low.

b) A semipreparative enrichment would have been somewhat time-consuming if we applied the 4 × 250 mm LC column which resulted in the mediocre resolution shown in Fig. 4 (insert); on the other hand, larger amounts of that sorbent are very expensive.

c) Triacetylcellulose cannot be used preparatively because its enantioselectivity is low in this case [18].

According to an empirical rule [20], an enantiomer which exhibits a C_2 axis and is more retained upon LC on (+)-poly(trityl methacrylate) has (*P*) helicity. This applies to the enantiomer of stop no. 2 in Fig. 4. Upon polarimetric instead of dichroic detection of LC on the sorbent mentioned above, the more retained enantiomer was dextrorotatory [18] at 436 nm, i.e. $(+)_{436}$ – $[+]_{290}$ – (*P*)-**7**, where the designations (+) and [+] refer to polarimetry and CD, respectively. The CD spectra $\Delta\varepsilon(\lambda)$ of (*MP*)-**7** (Fig. 4) will serve to assign relative helicities to a series of 9,10-phenanthrenequinones [17, 21] in a way similar to the one depicted in Fig. 2 for some thiobenzamides.

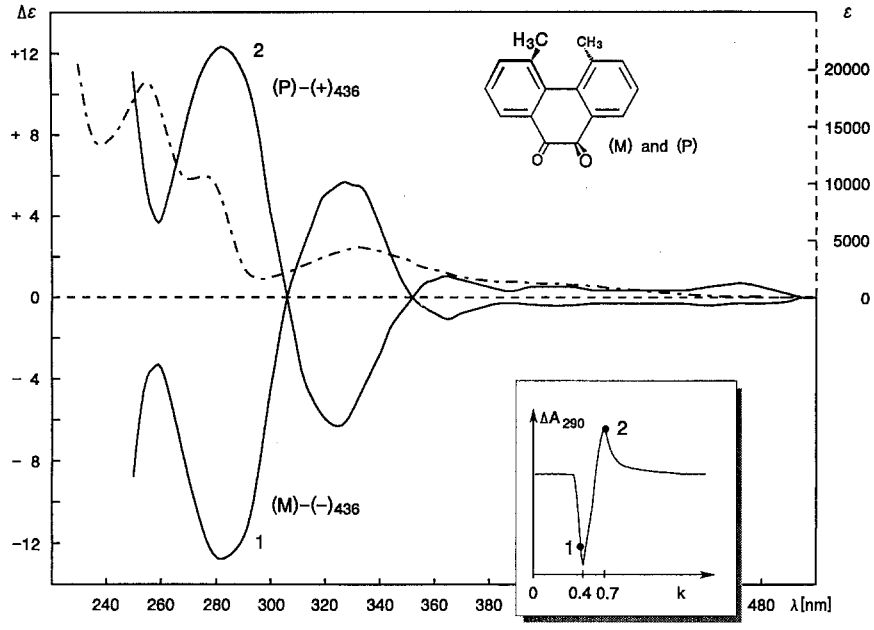


Fig. 4. (*M*)- and (*P*)-4,5-dimethyl-9,10-phenanthrenequinones (**7**) [18, 19] in *Me*OH at −20 °C. Insert: Chromatogram of 17 µg of (*MP*)-**7** on (+)-poly(trityl methacrylate)/SiO_2 at ~ 103 bar; ΔA_{290}, differential absorbance at 290 nm; *k*, capacity factor; stops of flow no. 1 and 2 for recording of dichrograms are marked. Main diagram: ——— On-line circular dichrograms at stops no. 1 and 2, calibrated for Δε, enantiomeric purities being 1.0 and 0.8, respectively (see text); ----- UV spectrum of (*MP*)-**7** in *Me*OH

Discussion

The on-line method described here for the first time furnishes CD spectra $\Delta\varepsilon(\lambda)$ by using *racemates* instead of enantiomers, provided an analytical LC separation of the latter is accomplished. As mentioned above for (*MP*)-**7**, time-consuming or expensive semipreparative enrichments can be avoided in this way. This technique may serve to determine relative configurations of interconverting antipodes, by thermostatting the cell, if necessary. Both the CD and the UV measurements can be performed in the same spectrometer.

Schlögl and co-workers [22] manually transferred LC fractions in solution from the column to the cell of a dichrograph and to the cell of a UV instrument. This means that they obtain $\Delta\varepsilon(\lambda)$ spectra as well, but they do not need a flow-through cell and a beam condenser [22]. This off-line approach, in our opinion, requires a certain expenditure concerning sample handling, whereas the on-line approach admittedly requires a certain expenditure concerning the above equipment. A choice between the two possibilities would mainly depend upon an estimation, how often flow-through CD measurements of all types [1] will be required in a given laboratory.

The necessity to stop the eluent flow is a serious drawback of the present method. Besides requiring the attention and activity of the experimenter, stops of flow may generate errors of the retentions of the substrates because the LC pump has to be switched off and on. We have recently described [2] *non-stop* acquisition of di-

chrograms $\Delta A(\lambda)$ and UV spectra $A(\lambda)$ during LC. Preliminary experiments [23] have shown that on-line CD spectra $\Delta\varepsilon(\lambda)$ are accessible as well, if a UV spectrum $A(\lambda)$ with known concentration is recorded before or after such a non-stop acquisition [2] of LC-CD-UV data.

Experimental Part

High-Pressure Liquid Chromatography on Optically Active Sorbents

Microcrystalline triacetylcellulose, particle 10–20 µm, was prepared in a way similar to Ref. [24], but starting from Avicel 2330 (E. Merck), wind-sieved, swollen and slurry-packed at 214 bar in a 8 × 250 mm Knauer column. The capacity factors *k* refer to 1,3,5-tri-*tert*-butylbenzene. (+)-Poly(trityl methacrylate)/SiO_2 [25], was filled into a Knauer column (4 × 250 mm). The capacity factors refer to water. Tris(phenylcarbamoyl)cellulose/SiO_2 [26] was used in the column Chiracel OC, commercially availiable from Daicel, Himeji, Japan. The capacity factors refer to 1,3,5-tri-*tert*-butylbenzene. LC was performed with the injector Rheodyne 7125 and the pump Irica Σ-871 (ERMA Optical Works, Tokyo, Japan) at pressures of 103 to 168 bar. Additional cooling of the columns was achieved by using the thermostates of Colora Meßtechnik und Haake Mess-Technik as well as homemade column jackets.

Further Methods

Melting points were determined on a Büchi 530 and are uncorrected. ^{1}H-NMR spectra were measured on a Bruker WM 250, UV spectra on a Hitachi U-2000 spectrometer. Mass spectra were obtained on a Varian CH5 at low resolution. Specific rotations were recorded on a Polarimeter Perkin Elmer 241; its cell temperature was kept constant by a thermostat. Detection of the CD spectra was performed on a Jasco J-40 A with a flow-through cell by Hellma GmbH (10 mm light path with 1 mm diameter; volume 8 µl) and an additional homemade beam condenser [5]. Evaluations and plots of UV and CD data were performed with the computer program CD/UV [11], which takes the experimental baseline into account and achieves a smoothing of the spectra.

2-Dimethylamino-N,N-dimethylbenzamide (**8**)

4.28 g (36 mmol) of thionylchloride were dropped during 30 min at room temperature to the solution of 4.96 g (30 mmol) of 2-dimethylaminobenzoic acid [27] and 3.32 g (42 mmol) of pyridine. After additional stirring of 30 min colourless pyridiniumchloride precipitated. Dimethylamine was added until no further precipitation occurred. The filtrate was evaporated, dissolved in 100 ml of $CHCl_3$, and washed three times with 50 ml of H_2O. Drying over $MgSO_4$, removing the solvent, and purifying by column chromatography on SiO_2 (*Et*OH) resulted in a yellow oil which was freezed by liquid N_2 to afford 5.48 g (95%) of colourless crystals of **8**. M.p. 32–34 °C. ^{1}H NMR ($CDCl_3$): δ = 2.86 ppm (s, 6 H, *Ar*-N(CH_3)$_2$), 2.90 (s, 3 H, CO-N-CH_3^E), 3.18 (s, 3 H, CO-N-CH_3^Z), 6.70–7.45 (m, 4 H, *Ar*-H). MS (70 eV): *m/z* (%) = 192 (39) [M^+], 191 (12) [M^+-H], 148 (100) [M^+-N*Me*$_2$], 147 (63) [M^+-HN*Me*$_2$], 120 (18) [M^+-N*Me*$_2$-CO]. $C_{11}H_{16}N_2O$ (192.3). Calcd. C 68.27, H 8.39, N 14.57. Found C 68.14, H 8.23, N 14.37.

(±)-2-Dimethylamino-N,N-dimethylthiobenzamide (**1**)

1.00 g (5.20 mmol) of **8** in 30 ml abs. toluene were treated under reflux with an equimolar amount of Lawesson's reagent. Monitoring of the reaction via thin-layer chromatography showed the completeness of the reaction. After cooling the mixture to room temperature and 10 h of additional stirring a yellow oil precipitated. It was removed and the solution evaporated. Repeated purifications of the reaction product were achieved by column chromatography on SiO_2 (ethyl acetate and petroleum

ether/ethyl acetate, 3 : 1) and afforded 0.45 g (42%) of slightly yellow crystals of **1**. M.p. 66 – 68 °C. ^{1}H NMR ($CDCl_3$): δ = 2.78 ppm (s, 6 H, *Ar*-N(CH_3)$_2$), 3.07 (s; 3 H, CS-N-CH_3^E), 3.60 (s, 3 H, CS-N-CH_3^Z), 6.7 – 7.5 (m, 4 H, *Ar*-H). UV (*Et*OH): λ_{max} (lg ε) = 232 nm (3.98), 278 (3.84), 364 sh (2.75). $C_{11}H_{16}N_2S$ (208.9). Calcd. C 63.43, H 7.74, N 13.45. Found C 63.30, H 7.54, N 13.36.

Methyl 3,5-dibromo-2-dimethylaminobenzoate (**9**)

With vigorous stirring 10.58 g (84.0 mmol) of dimethylsulfate were carefully dropped to the solution of 9.91 g (33.6 mmol) 3,5-dibromoanthranilic acid [28] in 200 ml of H_2O which contained enough NaOH (40%) to keep the *pH*-value between 7 and 8. The mixture was slowly warmed to 40 – 50 °C and another 10.58 g (84.0 mmol) of dimethylsulfate were added (exothermic reaction!). During additional stirring for 2 h, addition of NaOH (40%) was necessary to keep the *pH*-value between 7 and 8. The solution was extracted with $CHCl_3$ and the organic solvent removed. After purification of the brown oil by column chromatography on SiO_2 (CCl_4), the resulting product was dissolved in petroleum ether. The precipitating colourless crystals of methyl 2-amino-3,5-dibromobenzoate were removed and the petroleum ether was evaporated. Further column chromatography on SiO_2 (petroleum ether/ethyl acetate, 20 : 1) afforded 4.26 g (41%) of **9** as a yellow oil. ^{1}H NMR ($CDCl_3$): δ = 2.77 ppm (s, 6 H, N(CH_3)$_2$), 3.83 (s, 3 H, O*Me*), 7.55 (d, 1 H, *Ar*-H), 7.73 (d, 1 H, *Ar*-H), $^4J_{meta}$ = 2.4 Hz.

3,5-Dibromo-2-dimethylaminobenzoic acid (**10**)

2.30 g (6.82 mmol) of **9** were refluxed for 24 h in 2 *N* NaOH. After neutralisation and evaporation of H_2O the residue was extracted three times with 100 ml of *THF*. Removal of the solvent and recrystallisation from *Et*OH afforded 1.32 g (60%) of colourless crystals of **10**. M.p. 151 – 154 °C. ^{1}HNMR ($CDCl_3$): δ = 3.05 ppm (s, 6 H, N(CH_3)$_2$), 7.68 (d, 1 H, *Ar*-H), 8.42 (d, 1 H, *Ar*-H), $^4J_{meta}$ = 2.4 Hz. $C_9H_9Br_2NO_2$ (323.0). Calcd. C 33.47, H 2.81, N 4.34. Found C 33.44, H 2.75, N 4.26.

3,5-Dibromo-2-dimethylamino-N,N-dimethylbenzamide (**11**)

The reaction of 9.69 g (30 mmol) of **10** was performed as described for **8**. Purification by column chromatography on SiO_2 (ethyl acetate) resulted in 8.82 g (84%) yellowish oily **11**. ^{1}H NMR (CCl_4): δ = 2.73 ppm (s, 6 H, N(CH_3)$_2$), 2.88 (s, 3 H, CO-N-CH_3^E), 3.00 (s, 3 H, CO-N-CH_3^Z), 7.11 (d, 1 H, *Ar*-H), 7.62 (d, 1 H, *Ar*-H), $^4J_{meta}$ = 2.4 Hz. $C_{11}H_{14}Br_2N_2O$ (350.0). Calcd. C 37.75, H 4.03, N 8.00. Found C 37.63, H 3.92, N 8.21.

(±)-3,5-Dibromo-2-dimethylamino-N,N-dimethylthiobenzamide (**2**)

The reaction of 1.82 g (5.20 mmol) of **11** was carried out as described for **1**. Repeated purification of the product by column chromatography on SiO_2 (ethyl acetate and petroleum ether/ethyl acetate, 3 : 1) and recrystallisation from *Et*OH afforded 1.16 g (61%) of yellow crystals of **2**. M.p. 105 – 108 °C. ^{1}H NMR ($CDCl_3$): δ = 2.83 ppm (s, 6 H, N(CH_3)$_2$), 3.12 (s, 3 H, CO-N-CH_3^E), 3.55 (s, 3 H, CO-N-CH_3^Z), 7.12 (d, 1 H, *Ar*-H), 7.57 (d, 1 H, *Ar*-H), $^4J_{meta}$ = 2.0 Hz. $C_{11}H_{14}Br_2N_2S$ (366.1). Calcd. C 36.09, H 3.86, N 7.65. Found C 36.29, H 3.85, N 7.60.

(–)$_{436}$-2-Bromo-N,N-dimethylthiobenzamide (**3**)

Prepared by LC [29] of (±)-**3** [30] in *Et*OH/H_2O (96 : 4) on microcrystalline triacetylcellulose. Yellow crystals. M.p. 128 – 130 °C. $[\alpha]_{436}^{22}$ = −370 ± 20° ml g^{-1} dm^{-1} (*Et*OH/H_2O, 96 : 4). Enantiomeric purity 0.8, determined by LC [29].

(±)-3-(4-Aminophenyl)-3-cyclohexyl-2,6-piperidinedione (**4**) [12, 13].
$(+)_{404}$-3-(4-Aminophenyl)-3-cyclohexyl-2,6-piperidinedione (**4**) [12, 13].

(±)-3-(4-Aminophenyl)-3-ethyl-2,6-piperidinedione (**5**) [12–14].
$(+)_{404}$-3-(4-Aminophenyl)-3-ethyl-2,6-piperidinedione (**5**) [12–14].

(±)-2,2'-Bis(methoxycarbonyl)-1,1'binaphthyl (**6**) [16].
$(+)_{404}$-2,2'-Bis(methoxycarbonyl)-1,1'binaphthyl (**6**) [16].

(±)-4,5-Dimethyl-9,10-phenanthrenequinone (**7**) [18, 19].

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