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Chiroptical detection during liquid chromatography

III☆. Non-stop acquisition of circular dichroism spectra during liquid chromatography☆☆

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ABSTRACT

A new type of spectrometer, built in-house, served as a circular dichroism detector for liquid chromatography. It acquired differential absorbances $\Delta A = f(\lambda)$ at all wavelengths, λ, between 208 and 268 nm simultaneously within a time interval of 9 s. Non-stop acquisition of circular dichroism spectra during liquid chromatography is described for the first time. The novel set-up gives automatic access to dichrograms without preparative enrichment of enantiomers, if some analytical separation on an optically active sorbent can be achieved. Like photodiode-array detection, the new technique collects ultraviolet spectra $A = f(\lambda)$, but furnishes positive/negative information $\Delta A = f(\lambda)$ in addition. It was applied to (±)-2,2′-spirobi[2*H*-chromene] as a test sample and microcrystalline tribenzoylcellulose as a sorbent. Future applications of non-stop liquid chromatographic circular dichroic–ultraviolet data are discussed.

INTRODUCTION

Circular dichroism (CD) detection during liquid chromatography (LC) at a fixed wavelength has been performed successfully in the past by several groups [1–6], its advantages [7] compared with polarimetry being the increased selectivity and the possibility of determining absolute molecular chirality. Recording of CD spectra during stoppages [1,8–10] of chromatographic flow represented a considerable progresss, since this procedure gives access to dichrograms without preparative enrichment of enantiomers. On the other hand, CD spectra provide the most important information for the charac-

☆ For Part II see ref. 23.

☆☆ Dedicated to Professor Heinz A. Staab on the occasion of his 65th birthday.

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terization and the structural investigation of chiral molecules.

However, stoppages of eluent flow, besides requiring the attention and activity of the experimenter, generate errors in the apparent retentions of the substrates because the LC pump has to be stopped and restarted. Therefore, an automatic procedure avoiding any stoppage would be very useful for the analysis of LC peaks and for the acquisition of unknown CD spectra. Such a procedure is well known for UV detection: the use of photodiode-array technology [11]. To our knowledge, no technique for non-stop recording of CD spectra during LC has yet been described.

This technique was made possible by a novel spectrometer [12–14] which allows the simultaneous measurement of CD and UV absorptions at all wavelengths within a limited spectral range. In this apparatus, a polychromator and a charge-coupled device serving as a multichannel sensor are arranged behind the flow cell, in contrast to commercial circular dichrographs in which a monochromator is located in front of the cell and a photomultiplier behind it.

EXPERIMENTAL

Microcrystalline tribenzoylcellulose [15,16] (particle sizes 10–15 μm) from Riedel-de Haën (Seelze, Germany) was packed into a Knauer steel column (250 × 8 mm I.D.) using methanol as an eluent at *ca.* 170 bar and a flow-rate of 6 ml/min according to ref. 15. For LC work, the IRICA Σ - 871 pump (Irica Instruments, Kyoto, Japan) produced a pressure of 17 bar at a flow-rate of 0.7 ml/min. Rheodyne 7125 served as an injector and ERC 7210 (ERMA Optical Works, Tokyo, Japan) as a photometric detector at 254 nm. The latter allowed the quality of the separation to be checked and the capacity factors, k', to be determined. The k' values are given relative to 1,3,5-trihydroxybenzene [16] ($k' = 0$).

Following the photometer, a novel spectrometer [12–14] built in-house was connected as a CD and UV detector. A polychromator (holographic concave grating) and a charge-coupled device (L172D; VEB Werk für Fernsehelektronik, Berlin, Germany) were located behind the flow cell of this spectrometer. A schematic set-up is presented in Fig. 1. The home-made cell had a cylindrical form of 1.5

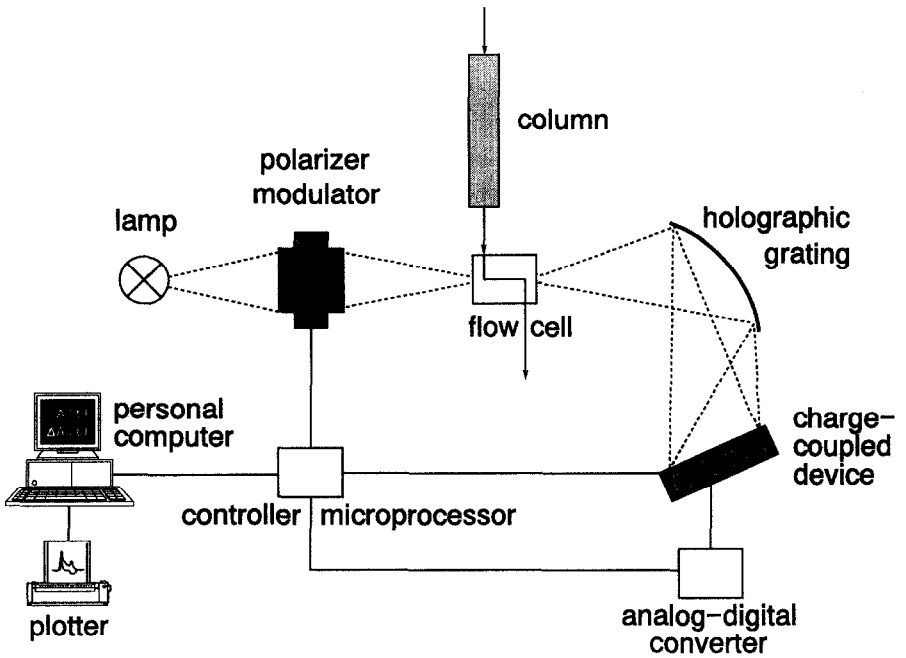

Fig. 1. Schematic set-up for non-stop aquisition of LC–CD–UV data.

mm diameter and 5.8 mm length (light path), resulting in a volume of 10 μl. It was located closely behind the entrance slit of the polychromator. The flow within the cell was in the direction of the light beam. A more detailed description of the whole spectrometer and its operation is given elsewhere [14]. The acquisition time for one spectrum was 9 s, the spectral bandwith being approximately 3 nm. The acquisition time was a consequence of using the available charge-coupled device (see above) and computer (see below). We assume that replacing these items with more efficient ones will reduce the acquisition time to *1 s or less* without significant loss of sensitivity. These modifications should allow application of the detector to more rapid separations. Systematic errors were avoided because there was no time shift between the measurement of CD and UV absorptions and the measurements at different wavelengths within a limited spectral range. The calibration of differential absorbance, ΔA, was performed using the Cotton effect [14] at 220 nm of a solution of D-pantolactone in water (0.165 g/l) in a static cell with a 1.0-mm light path. CAMAC hardware and a Robotron A 7100 personal computer served for instrumental control and data processing, respectively, using specialized programs.

(±)-2,2′-Spirobi[2*H*-chromene] (**1**) was prepared according to ref. 17. Its chromatograms, $A = f(t)$ and $\Delta A = f(t)$ (for example, see Fig. 3), correspond to our earlier results [18]. The UV spectrum $A = f(\lambda)$ (for example, see Fig. 4) is in agreement with the literature [19]. The circular dichrogram $\Delta A = f(\lambda)$ (*e.g.* Fig. 4) corresponds to our spectrum $\Delta\varepsilon = f(\lambda)$, obtained [20] for (+)−**1** [18] (enantiomeric purity $P = 94\%$) at 20°C in methanol and calculated for $P = 100\%$: + 69 (212), −44 (225), +52 (258), +11 l mol^{-1} cm^{-1} (300 nm).

The amount of 0.05 mg of (±) −**1** in 0.05 ml of methanol was chosen to give an absorbance of less than $A = 0.8$ for the peak maximum at $k' = 3.4$ in the measured spectral range. This limit was a consequence of the dataprocessing program mentioned above.

Although some information about signal-to-noise ratios achieved with this detector in its present state is available [14], final experiments concerning the noise level and the limit of detection of the new dichrograph have not yet been performed. Therefore, the experiment reported in Figs. 2–5 does not indicate a limitation of the utility. A preliminary measurement indicates that values of $\Delta A \approx 10^{-4}$ can still be detected above noise.

The experimental data were externally processed and plotted by means of an Acer 910 personal computer and specialized programs [21].

RESULTS

Stopped-flow measurements were performed with several racemates, including (±)-2,2′-bis(methoxycarbonyl)-1,1′-binaphthyl, using the set-up described above. Of these, (±)-spirobi[2*H*-chromene] (**1**) (for formula, see Fig. 2) was the most successful because of its high $\Delta\varepsilon$ values [20] (see Experimental section) and the excellent separation [18] of its enantiomers on microcrystalline tribenzoylcellulose [15,16] as an optically active sorbent. Therefore, (±)-**1** was chosen for non-stop acquisition of spectra during LC. This spirochromene and its enantiomers [18] are of interest in connection with thermal racemization [18] and photochromism [17].

On the other hand, under these conditions the peaks of (+)- and (−)-**1** are relatively broad because of partial racemization [18] during chromatography and because of the particular kinetic properties of the sorbent. Further investigations must include examples with sharper peaks.

The LC–CD data for (±)-**1** (Fig. 2) were obtained by recording dichrograms $\Delta A = f(\lambda)$ at certain retention times t ($t = 0$ upon injection). During the acquisition time of 9 s, the differential absorbance ΔA was recorded at all wavelengths between 208 and 268 nm simultaneously. The dichrograph used measured ΔA values within a λ-window of 76 nm, but the region of low wavelength was lost in the present case because of the UV absorption of methanol serving as an eluent. A visual overview and quantitative evaluation of three-dimensional plots such as in Fig. 2 is not easy. Therefore, we have prepared cross-sections of these data, two of which are presented as a CD-detected chromatogram $\Delta A = f(t)$ of (±)-**1** (Fig. 3, solid line) and as a circular dichrogram $\Delta A = f(\lambda)$ of (±)-**1** (Fig. 4, solid line).

The solid line in Fig. 4 corresponds to (+)-**1**, *i.e.* the enantiomer with positive polarimetric rotation angles [18,20] between 365 and 578 nm. This assignment follows from the CD spectrum $\Delta\varepsilon = f(\lambda)$ given

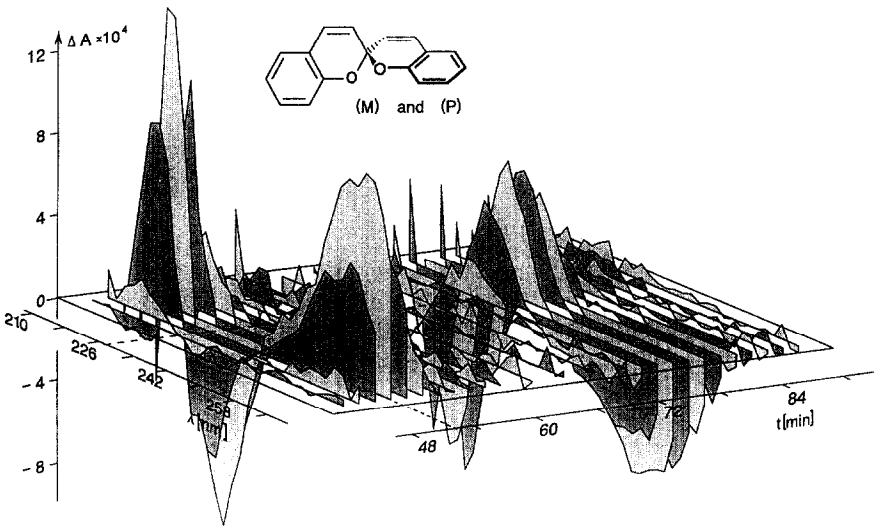

Fig. 2. Non-stop acquisition of circular dichroism (CD) spectra during LC of 0.05 mg of (±)-**1** in methanol on microcrystalline tribenzoylcellulose. Flow-rate 0.7 ml/min, linear velocity 0.35 mm/s, retention time t (t = 0 upon injection), wavelength λ, differential absorbance ΔA. The peaks of the enantiomers at t = 52 and 80 min correspond to the capacity factors k' = 3.4 and 5.7, respectively. The absorptions at λ = 208 nm are due to a Cotton effect around λ = 200 nm. The CD spectra shown for certain times (differing by 1.5 or by 3.0 min) were acquired during time intervals of 9 s each. ----- = Cross-sections used for Figs. 3 and 4.

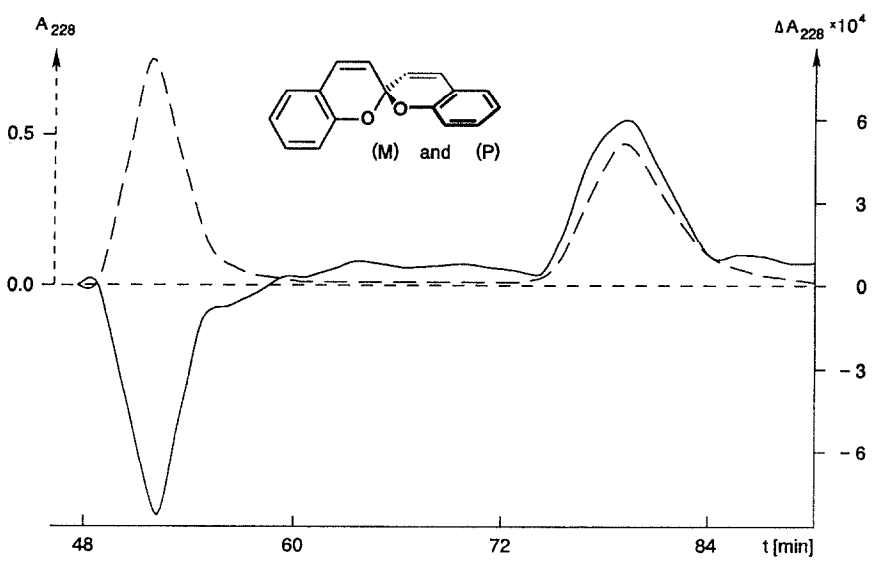

Fig. 3. LC of (±)-**1** on microcrystalline tribenzoylcellulose. The peaks of the enantiomers correspond to the capacity factors k' = 3.4 and 5.7. See Fig. 2 for further experimental details. ———— = Circular dichroism detection ΔA = f(t), obtained as a cross-section at λ = 228 nm of the data ΔA = f(λ,t) in Fig. 2. ----- = UV detection A = f(t), obtained as a cross-section at λ = 228 nm of the data A = f(λ,t) in Fig. 5.

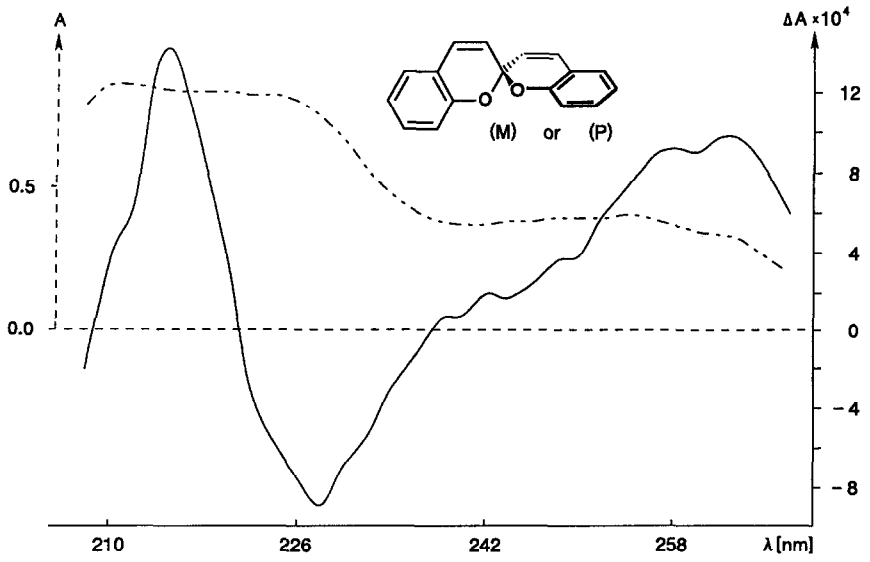

Fig 4. Circular dichroism (CD) and UV spectra of (+)-**1**, recorded during LC of (±)-**1** on microcrystalline tribenzoylcellulose. See text for notation of the enantiomer (+)-**1**; see Fig. 2 for further experimental details. ——— = CD spectrum $\Delta A = f(\lambda)$, obtained as a cross-section at $t = 52$ min of the data $\Delta A = f(\lambda,t)$ in Fig. 2. ----- = UV spectrum $A = f(\lambda)$, obtained as a cross-section at $t = 52$ min of the data $A = f(\lambda,t)$ in Fig. 5.

for preparatively enriched (+)-**1** in the Experimental section. It should be noted that the absolute chiralities [22] (M) and (P) of the enantiomers of **1** are not known (see formula in Fig. 4).

The computer dedicated to the circular dichrograph is also capable [14] of calculating and storing the absorbance A and the ratios $\Delta A/A$ immediately after the measurement of ΔA. The LC–UV data for (±)-**1** (Fig. 5) were obtained in this way. They correspond to the results which would be obtained by a photodiode-array detector [11]. Two cross-sections of these data are presented as a UV-detected chromatogram $A = f(t)$ (Fig. 3, dotted line) and as a UV spectrum $A = f(\lambda)$ (Fig. 4, dotted line).

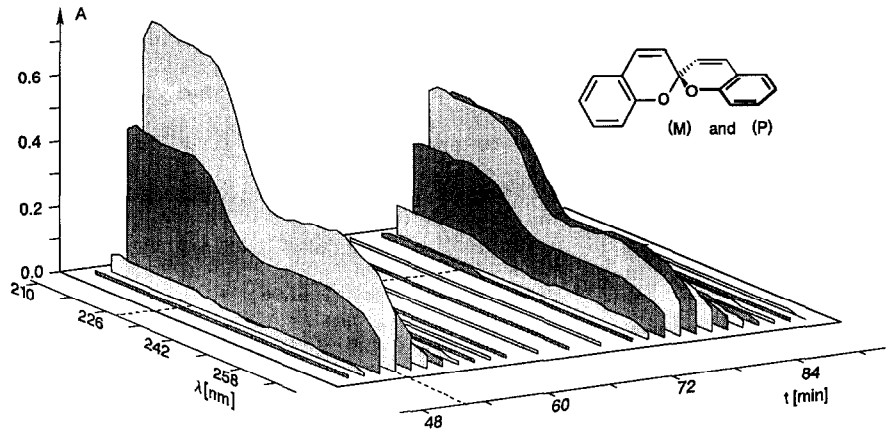

Fig. 5. Non-stop acquisition of UV spectra during LC of (±)-**1** on microcrystalline tribenzoylcellulose. The UV spectra shown for certain times (differing by 1.5 or by 3.0 min) were acquired during time intervals of 9 s each. See Fig. 2 for further experimental details. ----- = Cross-sections used for Figs. 3 and 4.

Similar measurements were successfully performed for (±)-2,2′-bis(methoxycarbonyl)-1,1′-binaphthyl.

DISCUSSION

Non-stop acquisition of LC–CD–UV data has been described above for the first time. It gives automatic access to dichrograms without preparative enrichment of the components of a mixture, provided an LC sorbent accomplishes some analytical separation.

As far as (±)-**1** is concerned, all the information in Figs. 2–5 was known in advance (see Experimental section), which was the reason for choosing it as a test sample. The sensitivity of the dichrograph remains to be demonstrated for compounds which are less ideal than (±)-**1** and for mixtures of compounds.

There are many examples for which the new technique will yield data and conclusions not easily obtainable by other methods. In chemistry, it will be useful for the stereodynamic investigation of enantiomers which cannot be separated because intramolecular processes [1] interconvert them. We have also encountered racemates whose components would have to be enriched on an analytical column by many time-consuming injections because the corresponding preparative column is too expensive. In these cases, the unknown dichrograms can now be measured without preparative enrichment of the enantiomers.

On the other hand, the above technique can also serve for the CD and UV analysis of LC peaks as a means of checking their identity and purity. This is a frequent task [11] in pharmacy, biology and medicine, where chiral drugs and their metabolites and chiral biomolecules or mixtures containing them have to be investigated.

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