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SIMULTANEOUS MONITORING OF LIGHT-ABSORPTION AND OPTICAL ACTIVITY IN THE LIQUID CHROMATOGRAPHY OF CHIRAL SUBSTANCES

ALEX F. DRAKE, JOHN M. GOULD and STEPHEN F. MASON*
Chemistry Department, King's College, London WC2R 2LS (Great Britain)
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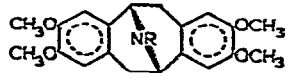
SUMMARY

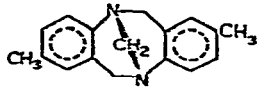
A detection system for the simultaneous monitoring of the light-absorption (absorbance, A) and the optical activity as circular dichroism (differential absorbance for left- and right-circularly polarized light, $\Delta A = A_L - A_R$) of the eluent from a liquid-chromatography column is described. The optical resolution of pavine by liquid chromatography on a triacetyl cellulose column is reported.

INTRODUCTION

The optical resolution of a synthetic racemate by the traditional method of the fractional crystallisation of the two diastereomeric derivatives afforded by an enantiomerically-pure reagent is laborious and the outcome is not always certain or reproducible. The method has been described as a matter of trial and error[1] and as an art[2]. These descriptions are apt, in our experience, for the literature reports[3,4] of the optical resolution of synthetic pavine (I) through the (+)-bromocamphorsulphonic acid salts.



(I) R = H
(III) R = CH_3



(II)

In view of the versatility of column liquid chromatography for the optical resolution of both inorganic[5] and organic[6] racemates, a chromatographic alternative to the literature methods[3,4] was sought for the separation of the pavine enantiomers.

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Pavine and Tröger's base (II) have similar molecular morphologies, and the complete optical resolution of II on a column of microcrystalline triacetylcellulose (MCTC) has been reported[7], and extended to a range of organic racemates[6,8]. In reproducing the optical resolution[7] of II, and extending the method to pavine, the problem arose of monitoring the solute in the eluent from the MCTC column by light-absorption at one wavelength and the optical activity of any enantiomeric solutes with a separate instrument at a different wavelength, typically in a transparent frequency region. Inevitably, the two detection systems did not record, at a given time, the light-absorption and the optical activity of the same solute fraction.

Accordingly, an attachment has been constructed, to replace the sample and detector compartments of a standard spectrophotometer, to record simultaneously at a given monitoring wavelength the light-absorption as optical-density or absorbance (A) and the circular dichroism, *i.e.* the differential absorbance of left- (LCP) and right-circularly polarised (RCP) light, ($\Delta A = A_L - A_R$), which is the absorption counterpart of optical rotation in transparent wavelength regions. The design of the attachment is illustrated in Fig. 1, and chromatograms of I and II optically resolved on a MCTC column are recorded in Fig. 2.

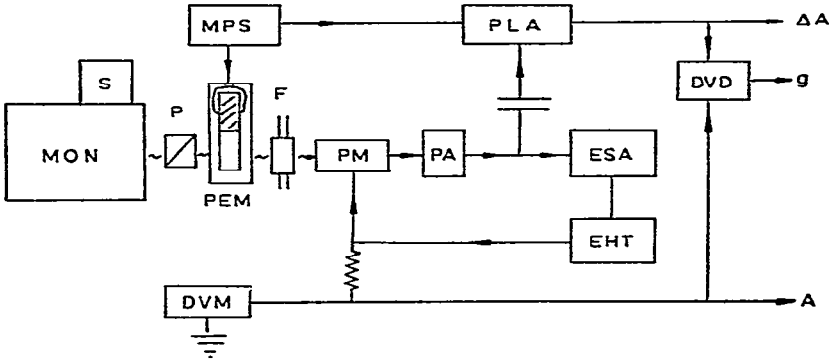

Fig. 1. The spectrophotometer modification for the simultaneous monitoring of absorbance (A) and circular dichroism ($\triangle A$) of chiral solutes in the eluent from a liquid chromatography column. The source (S) and monochromator (MON) are those of the original instrument. Radiation from the monochromator exit slit passes through a polarizing prism (P) and a photoelastic modulator (PEM) (Hinds Internat. Portland, OR, U.S.A.) to the flow-cell (F) and photomultiplier detector (PM). After the preamplifier (PA) the PM signal is fed to the error-signal servo-amplifier (ESA) which governs the extra high-tension voltage supply (EHT) to the PM registered by the digital voltmeter (DVM) in order to maintain the $V_{d.c.}$ constant at a pre-set level. A signal proportional to the change in the EHT supply is fed to one channel of a multipen recorder to measure the absorbance, A. The $V_{a.c.}$ signal from the PM after the PA is fed to the phase-lock amplifier (PLA) which receives a reference signal from the PEM power supply (MPS). The output from the PLA is fed to a second channel of the multipen recorder to measure the circular dichroism, $\triangle A = (A_L - A_R)$, and to one input of a ratiometer, the divider (DVD). The second input to the DVD is the absorbance signal, so that the DVD output measures the g ratio, $\triangle A/A$, which is also recorded as a function of the elution volume.

EXPERIMENTAL

Apparatus and method

The sample and detector compartments of a Unicam SP 600 were modified by the insertion of a Glan polarizing prism of calcite (P) and a photo-elastic

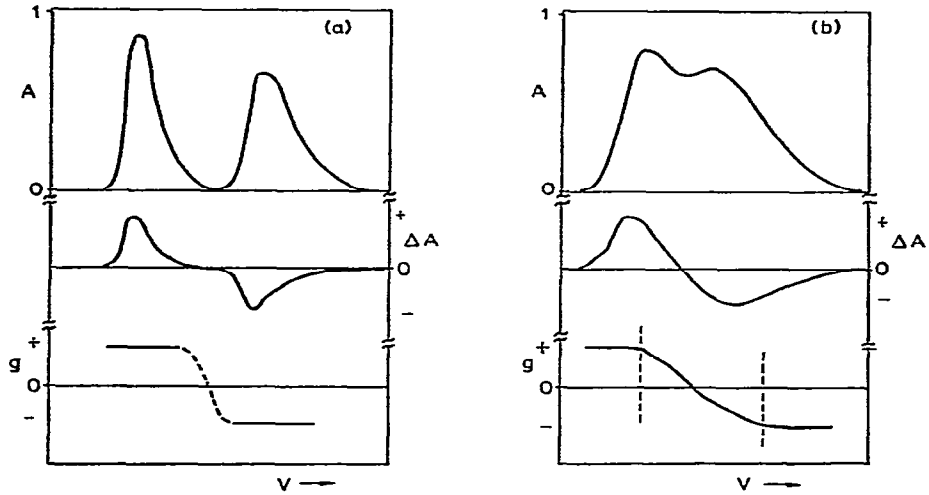

Fig. 2. Chromatograms of the optical resolution on a microcrystalline triacetylcellulose column (400 × 15 mm I.D.) of (a) Tröger's base (II), and (b) pavine (I), with a mobile phase of ethanol–water (9:1). The chromatograms record the absorbance (A), differential absorbance of LCP and RCP radiation, ($\triangle A$), and the g ratio, ($\triangle A/A$), as a function of the elution volume, (V). In (b) the fractions of pavine eluted in the volume between the vertical dashed lines are incompletely resolved, the volume being recycled to achieve a further separation.

modulator (PEM) between the monochromator and the sample chamber, and by replacing the phototube with a photomultiplier (PM) detector (Fig. 1). The photoelastic modulator consists of a transparent isotropic optical element, vitreous silica or calcium fluoride, which is periodically stressed by a single-crystal quartz transducer, to which the optical element is cemented[9,10]. The quartz crystal, some 10 × 20 × 50 mm, with electrodes on the two largest faces, has a piezoelectric resonance at ≈ 50 kHz in a uniaxial mode in the direction of the long axis, and the optical element, with dimensions ensuring acoustic resonance at the same frequency, is attached to one of the small end faces of the crystal[9,10]. The application of a sine-wave alternating voltage to the quartz crystal produces a periodic birefringence in the otherwise-isotropic optical element, and the power-level applied to the quartz transducer is adjusted so that the periodic birefringence maxima conform to the quarter-wavelength retardation condition, $d(n_x - n_y) = \lambda/4$, where d is the plate thickness, at the wavelength of interest, λ. The plane-polarized radiation emerging from the polarizing prism at the exit slit of the monochromator is thence transformed into LCP and RCP radiation alternating sinusoidally at ≈ 50 kHz.

The monitoring wavelength, λ, for the chiral solute passing through the flow-cell (F) in the eluent from the chromatographic column is chosen by reference to the absorption and circular dichroism (CD) spectrum of an enantiomer of the solute or of an analogous compound. In general the wavelength of the lowest frequency CD band maximum is adopted, or that of the major CD band where more than one CD band is associated with the lowest-energy absorption band. The CD spectrum of (—)-Tröger's base (II) has been reported[11], and that[12] of (—)-argemonine (III) which is a close analogue of (—)-pavine (I). The CD spectra of II and III (Fig. 3)

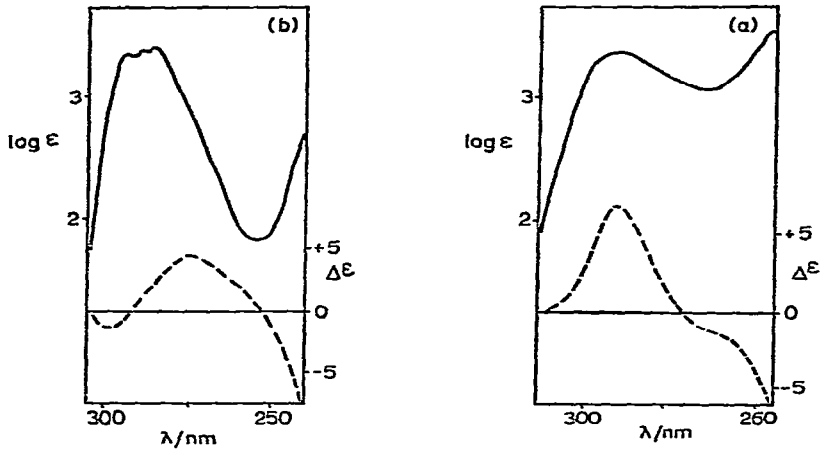

Fig. 3. The absorption spectra (upper curves) and circular dichroism spectra (lower curves) of (a), (−)-Tröger's base (II), and (b) (−)-argemonine (III) in ethanol. The corresponding spectra of (−)-pavine (I) are virtually identical to those of (III), with $g = 1.8 \cdot 10^{-3}$ at 275 nm. In the case of (II), $g = 4.5 \cdot 10^{-3}$ at 289 nm.

suggest, from the observed CD band maxima, the adoption of 280 nm for II and 275 nm for I.

With the monitoring wavelength appropriately set, the modulated LCP and RCP radiation from the PEM passes through the flow-cell without absorption or differential absorption during the initial chromatographic forerun, so that the photomultiplier registers only a direct current (d.c.) signal which provides a base-line for both the subsequent absorption (A) and differential absorption of LCP and RCP radiation ($\Delta A = A_L - A_R$). After the solute break-through from the chromatographic column, the modulated radiation transmitted through the flow-cell undergoes an overall absorption (A) and a differential absorption (ΔA), the latter being registered by the photomultiplier as a small 50-kHz a.c. signal superimposed on the now-reduced d.c. signals. Strictly the differential absorption is measured by the ratio of the two signal voltages[13], ($V_{a.c.}/V_{d.c.}$).

The ratio giving ΔA is conveniently measured, together with the overall absorption A, by maintaining the denominator ($V_{d.c.}$) at a constant level, that of the original zero-absorption d.c. signal during the forerun period, using an error-signal servo-amplifier[14] (ESA) (Fig. 1). During the absorption of radiation, the fall in the d.c. signal from the photo-multiplier is registered by the ESA, which governs the extra high-tension voltage supply (EHT) to the PM, increasing the voltage to restore the $V_{d.c.}$ from the PM to its original forerun base-line level. As the response of the PM to the applied EHT voltage is nearly logarithmic, the increase in that voltage, governed by the ESA, measures in good approximation the optical density or absorbance A of the solute passing through the flow-cell. A signal proportional to the EHT voltage-change is fed to one channel of a two-pen recorder in order to monitor the corresponding changes in absorbance A. Since the denominator of the ($V_{a.c.}/V_{d.c.}$) ratio is constant, the small 50 kHz a.c. signal now measures directly the differential absorbance ΔA. The a.c. component from the preamplifier (PA) following the photo-multiplier is fed to a phase-sensitive detector and amplifier, the phase-lock

amplifier (PLA) which receives a reference signal from the PEM (Fig. 1). The PLA discriminates the 50 kKz from other signals, and distinguishes between positive and negative differential absorbance ΔA, which is registered by the second channel of the two-pen recorder.

A refinement allows the enantiomeric purity of the solute passing through the flow-cell to be recorded. The dissymmetry ratio, $g = \Delta A/A$, introduced by Kuhn[15], is a constant at a given wavelength with an optimum value for an optically-pure enantiomer. The individual absorbance A and differential absorbance ΔA signals fed to a divider (DVD) give an output measuring the g ratio. For a racemate which is completely resolved into its enantiomers chromatographically, as in the case of Tröger's base (II) on a MCTC column[7], the g ratio has its constant optimum value throughout the elution band of each enantiomer, the g ratio at 289 nm being positive for the first-eluted (−)-isomer of II and negative for the second, the (+)-isomer with $|g| = 4.5 \cdot 10^{-3}$ (Fig. 2). For a racemate which is incompletely resolved chromatographically, giving a single absorbance elution band A, but a bisignate double differential-absorbance band ΔA, as in the case of pavine on a MCTC column, the g ratio *versus* elution-volume record has positive and negative stationary sections at the beginning and at the end of the elution band, joined by an intermediate section in which the value of $|g|$ progressively diminishes from the first optimum and increases to the second (Fig. 2). The g ratio trace identifies the enantiomerically-pure fractions, and provides a measure of the optical purity of the intermediate fractions. The lower limit of the enantiomeric purity which may be determined by the method is 1% or less, as the instrumental limitation is $|g| \approx 10^{-5}$. and the optimum g ratio at a wavelength within the range of the lowest-energy absorption band is generally $\geqslant 10^{-3}$ for a wide range of chiral molecules[16].

Materials

Pavine was prepared from papaverine (BDH, Poole, Great Britain) by the method of Pyman[17], and the Tröger's base from Aldrich (Milwaukee, WI, U.S.A.) was recrystallised from ethanol. MCTC was prepared by the heterogeneous acetylation procedure of Hesse and Hagel[7,8]. As the optical isomers of Tröger's base racemise under even weakly acidic conditions[18], the washed MCTC product (80 g) was suspended in a mixture of ethanol (250 ml), water (30 ml) and 0.88 sp. gr. ammonia (10 ml), and stirred under reflux for 1 h. The treatment not only removes acidic residues, but also swells the MCTC. The slurry obtained was used to pack a glass chromatography column, 400 × 15 mm I.D. The mobile phase employed was ethanol–water (9:1) under a positive pressure of 1.7 atmosphere of nitrogen, which gave a flow-rate of 25 ml/h. Samples of up to 50 mg of racemic I or II were separated into their respective enantiomers on the column. In the case of I the intermediate fraction, where the g ratio declines from the positive optimum and subsequently increases to the negative optimum, gave further enantiomerically-pure fractions on recycling through the column.

CONCLUSION

The system described has the advantage, over the sequential monitoring of the absorbance and the optical rotation of enantiomeric solutes in the eluent from a

chromatographic column for the optical resolution of a racemate, not only of recording simultaneously the concentration, as the absorbance A, and the optical activity, as ΔA, of a given fraction with a single detection system, but also of measuring the enantiomeric purity of the solute in that fraction. In case where the g ratio, $\Delta A/A$, of the enantiomers at the analytical wavelength employed is not previously determined, the observation of two segments, in the g ratio *versus* elution volume relation, where the g ratio is invariant for a limited range, and equal in magnitude but opposite in sign for the two segments, affords an indication that the optical resolution is complete over those limited ranges, and that the corresponding fractions are enantiomerically pure.

The optical purity of products given by asymmetric syntheses, which generally provide less than 100% of a single enantiomer, is estimated chromatographically with the detection system described more readily than by the corresponding optical-rotation procedure[19]. The chromatographic estimation of the optical purity of an enantiomeric mixture is problematic only when the elution peaks of the two enantiomers overlap or are fused[19]. Provided that the elution fractions of the more abundant enantiomer in the mixture give a segment in the g ratio *versus* elution volume relation which is invariant over a limited range, and thus is optimum, $|g|_{opt}$, the optical purity of the mixture is given by the ratio of the mean g-ratio over the chromatogram of the enantiomer mixture to its optimum value, $|\bar{g}|/|g|_{opt}$.

The principal limitation of the simultaneous absorbance, A, and circular dichroism, ΔA, detection system in its present form is the use of a calcite polarizing prism, which has a shorter-wavelength transmission limit of ≈ 240 nm. The limit is reducible to ≈ 185 nm by replacing the calcite prism with a Rochon quartz polarizing prism. The replacement involves the introduction of a fused silica lens or, better, a silica–fluorite achroamt, to bring to a focus the images of the exit slit of the monochromator given by the ordinary and the extraordinary polarized rays transmitted by the Rochon prism, so that the unwanted extraordinary beam may be masked off. Even with the replacement envisaged, the monitoring of chiral hydrocarbons in the eluent from a chromatography column would not be practicable by the simultaneous absorption and circular dichroism procedure, which was designed essentially for the detection of chiral benzenoid and larger aromatic derivatives absorbing at wavelengths longer than ≈ 250 nm.

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* *Editor's Note:* See also S. A. Westwood, D. E. Games and L. Sheen, *J. Chromatogr.*, 204 (1981) 103, which had not been published when this paper was received.

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