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USE OF CIRCULAR DICHROISM AS A HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY DETECTOR

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SUMMARY

A circular dichroism (CD) spectrophotometer has been coupled to a high-performance liquid chromatograph. Natural pyrethrins, tryptophan, rotenoids and Amaryllidaceae alkaloids have been used to evaluate the system. Monitoring at selected wavelengths has enabled detection levels in the low μg range to be obtained. By using stopped-flow techniques, full CD spectra can be obtained, and the system has been used in conjunction with combined high-performance liquid chromatography–mass spectrometry to study an extract of timbo powder.

INTRODUCTION

The combination of high-performance liquid chromatography (HPLC) with some form of detector that is selective for optically active compounds has considerable potential in a wide variety of research areas. Studies aimed at the identification of known natural products or seeking new structural types are examples. A potentially more important area of application is metabolic studies of drugs and pesticides, where detectors of this type could assist in unravelling some of the stereochemistry involved in the metabolic process and provide increased specificity for quantitative and qualitative studies.

We report here the results we have obtained by coupling a circular dichrograph with a high-performance liquid chromatograph. An advantage of using a circular dichrograph is that it not only enables selective detection of optically active compounds with suitable chromophores, but that it enables wavelength selectivity to be utilised in a similar way to that available with a variable-wavelength UV detector. The high degree of selectivity thus obtained enables circular dichroism (CD)-active compounds to be detected in a complex matrix of other compounds such as is encountered in natural-product extracts, or biological fluids.

EXPERIMENTAL

A HPLC system consisting of a Waters M6000 pump connected to columns set up for on-column injection was connected to a Jobin-Yvon circular dichrograph III. Micro HPLC flow-cells of 1 mm and 10 mm path length (Cecil Instruments,

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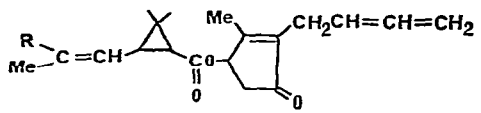
Cambridge, Great Britain) were initially used for fixed-wavelength studies. On-line CD spectra were obtained by using a cell machined from a block of PTFE. The flow channel was 1 mm^2 in area and 10 mm in length, and stress-free silica end windows were used. Solvents were distilled from an all-glass apparatus and were degassed before use.

Combined liquid chromatography–mass spectrometry (LC–MS) was performed on a Finnigan 4000 mass spectrometer equipped with a standard moving-belt interface[1] and coupled to an Incos data system. A source temperature of 240°C and source pressure of 0.2 Torr were used for the methane chemical-ionisation (CI) studies. A Cecil CE272 was used for UV detection in the HPLC studies. The HPLC conditions used are given in the text.

RESULTS AND DISCUSSION

Initial studies to evaluate the system utilised the amino acid L-tryptophan. Standard solutions were injected into the HPLC–CD system A Hypersil ODS (5 μm) column, (100 × 5 mm) was used, with methanol–water (1:1) at a flow-rate of 1 cm^3 min^{-1} as mobile phase, and the CD was monitored at 270 nm. Injection of a solution containing 3 μg of L-tryptophan could be detected with a signal-to-noise ratio greater than 2:1.

In order to assess the possibility of selective detection of CD-active compounds in crude mixtures, an extract of pale pyrethrum was examined. The extract consisted predominantly of pyrethrins I (1a) and II (1b). A column (100 × 5 mm) packed with Spherisorb ODS (5 μm), with methanol–water (4:1) at a flow-rate of 1 cm^3 min^{-1} was used as eluent in this study. By monitoring at 250 nm, both compounds are detected, with pyrethrin II giving the larger signal. None of the other components detected in the UV trace is detected. At 235 nm, the intensity of the signals is reversed, while at 220 nm only pyrethrin I can be detected.



1a R=Me
1b R=CO_2Me

Fig. 1 shows a comparison of the traces obtained from HPLC with UV detection, HPLC–CD and the reconstructed total-ion-current trace obtained during methane CI LC–MS of a chloroform extract of timbo powder. The features of the methane CI spectra are given in Table I. The extract consists of four main components. As can be seen from Table I, components A and B gave virtually identical methane CI spectra, indicating molecules of MW 410; components C and D also gave very similar spectra and had MW 394. The HPLC–CD trace enabled some differentiation to be made of the pairs of isomeric compounds, and HPLC–CD and LC–MS examination of a commercially available sample of rotenone (3) (BDH, Poole, Great Britain) showed that component C is rotenone. Although it might be expected from the HPLC–CD trace that component D might have the opposite

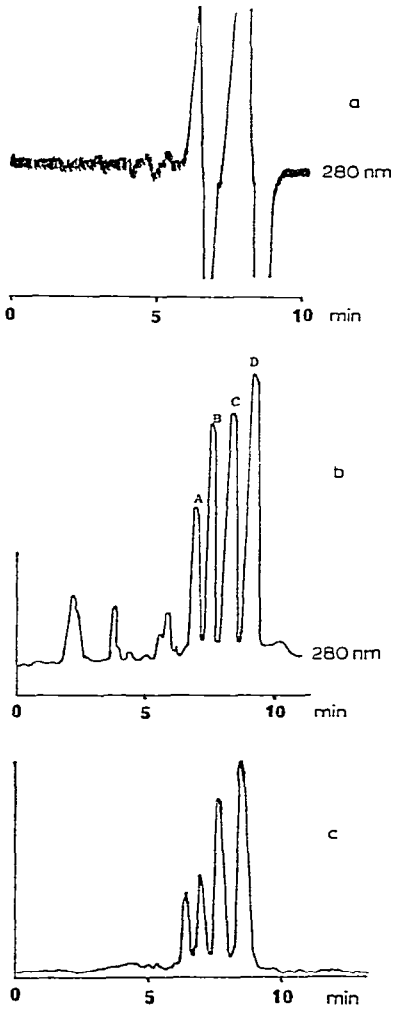
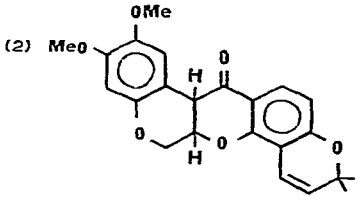


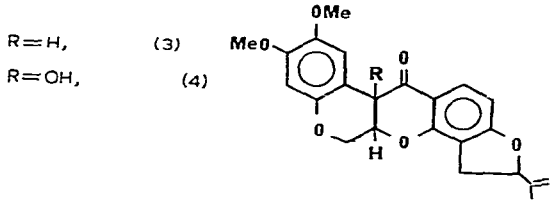
Fig. 1. Chomatograms of timbo powder extract. Column: 250 × 4.6 mm; Hypersil C_{22} (5 μm). Mobile phase: acetonitrile–water (4:1); flow-rate, 1 $cm^3\ min^{-1}$. (a) HPLC–CD; (b) HPLC–UV; (c) LC–MS.

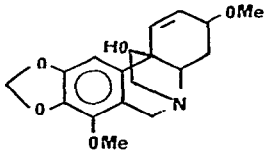
TABLE I

METHANE CI SPECTRA OBTAINED DURING LC–MS OF TIMBO POWDER EXTRACT

Peak	*m/z (% relative intensity)*
A	411(23), 410(16), 393(100), 323(10), 208(13)
B	411(30), 410(19), 393(100), 208(15)
C	395(100), 192(10)
D	395(100), 192(8)







(5)

configuration to rotenone, an alternative explanation could be that the compound is isomeric, with a different chromophore. This could be resolved by obtaining an on-line CD spectrum. Comparison of mass spectra, HPLC–CD and HPLC retention times in fact showed the latter explanation to be correct, and component D is deguelin (2). The identity of components A and B has not yet been satisfactorily resolved, but rotenalone (4) has a similar retention to component A.

In order to ascertain the possibility of obtaining on-line CD spectra, the base extract of bulbs of *Crinum glaucum* was examined. Preliminary examination of this extract showed the presence of a number of known Amaryllidaceae alkaloids, together with other alkaloids of unknown structure[2]. We have reported LC–MS studies on this extract elsewhere[3]. The extract is a complex mixture of alkaloids, and one of the major components is the alkaloid ambelline (5), the CD spectrum of which has been reported[4]. Fig. 2a shows the background-subtracted on-line CD spectrum of ambelline obtained during HPLC–CD of the extract, together with the CD spectrum obtained under standard conditions (Fig. 2b); as can be seen, the CD spectra are in good agreement.

CONCLUSIONS

HPLC–CD shows promise as a selective detection system for compounds that are CD-active. At present, detection is in the low μg range. An ability to obtain on-line CD spectra during HPLC–CD of complex mixtures has additional utility, particularly when used in combination with LC–MS in ascertaining the structures of components of natural extracts.

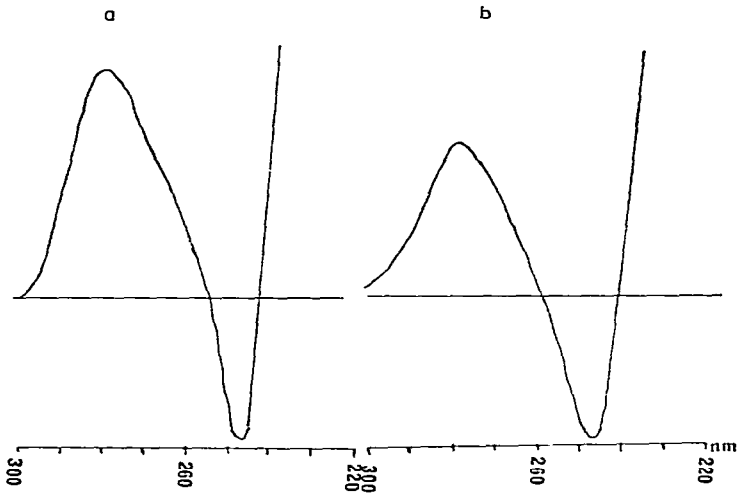


Fig. 2. (a) On-line CD spectrum: time constant, 10 sec; sensitivity, 1×10^{-6}. Separation was carried out on a column (150 × 5 mm) of C_8 bonded phase, with methanol–water (3:2) containing a trace of ammonia as eluent (1 cm^3 min^{-1}). (b), CD spectrum of ambelline.

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