Journal of Chromatography, 396 (1987) 177–182
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 19 455

CHIRAL HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC STUDIES OF 2-(4-CHLORO-2-METHYLPHENOXY)PROPANOIC ACID

B. BLESSINGTON*
Pharmaceutical Chemistry Department, Bradford University, Bradford BD7 1DP (U.K.)
and
N. CRABB and J. O'SULLIVAN
Pharmacy Department, Barnsley District General Hospital, Gawber Road, Barnsley (U.K.)
(First received November 19th, 1986; revised manuscript received January 21st, 1987)

SUMMARY

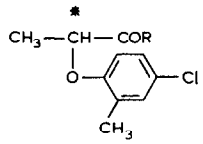
The direct enantiomeric resolution of the racemic herbicide 2-(4-chloro-2-methylphenoxy)propanoic acid (CMPP) was demonstrated on an Enantiopac (α_1-acid glycoprotein) chiral high-performance liquid chromatographic (HPLC) column. The HPLC separation of various amide derivatives of CMPP on a chiral "Ionic Pirkle" column comprising of N-(3,5-dinitrobenzoyl) (*R*)-(−)phenylglycine as chiral ligand, was also accomplished. These amides and racemic ibuprofen, however could not be separated on the Enantiopac system. The performance, stability and cost of the two systems were compared. Using optically pure CMPP enantiomers the elution order was determined and shown to reverse between the two systems. It was also shown that negligible racemisation occurred during derivatization.

INTRODUCTION

2-(4-Chloro-2-methylphenoxy)propanoic acid (CMPP) (Chemical Abstracts Registry No. 7085-19-0) (A) is a widely used herbicide supplied in the racemic form. Only the (+) enantiomer, however, has herbicidal activity[1]. Moreover, toxicity differences are known to exist between the (+) (Chemical Abstracts Registry No. 16484-77-8) and (−) (Chemical Abstracts Registry No. 25333-13-5) enantiomers[2]. There is therefore considerable interest for ecological reasons in producing optically pure CMPP on an industrial scale[3].

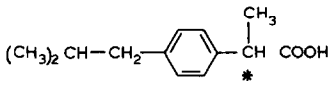
A range of analytical methods have been reported which can be used to study the production and occurrence of these enantiomers. These include direct polarimetry and indirect studies [gas chromatography (GC), high-performance liquid chromatography (HPLC)[4] and NMR[5]] of diastereoisomers produced from the enantiomers. We now wish to report the direct separation of CMPP enantiomers by chiral HPLC using Pirkle Ionic[6] and Enantiopac[7,8] chiral stationary phases (CSPs). This approach should facilitate studies of CMPP in aqueous biological systems and in industrial production process control. We can also compare the relative performance of these two important CSPs.

0021-9673/87/$03.50



A: R = –OH; B: R = $-NH_2$

C: R = $-NHCH_2C_6H_5$; D: R = $-N(C_6H_5)_2$



E

EXPERIMENTAL

Materials

All CMPP samples were provided by A. H. Marks Co. (Bradford, U.K.). γ-Aminopropyl silica (5 μm) was purchased as Spherisorb NH_2 from Jones Chromatography and packed into a 30 cm × 4.6 mm I.D. column. N-(3,5-dinitrobenzoyl)-(*R*)-(−)-phenylglycine was purchased from Sigma. The 10-cm Enantiopac column was purchased from LKB (Bromma, Sweden) at a cost of £ 400. All solvents used were HPLC-grade, other materials were of laboratory grade and used as purchased.

Equipment

HPLC. The system used consisted of a Pye Unicam PU 4010 pump, PU 4020 UV detector, PU 4047 column module and a Pye Unicam DP 88 computing integrator as required.

IR. Samples were examined as Nujol mulls on a Perkin-Elmer 157 G Grating infrared spectrophotometer.

Mass spectrometry (MS). Mass spectra were run, using a direct insertion probe, on an AE1 MS-902 instrument (source 250°C; 70 eV ionising energy; 100 μA emission) equipped with a Mass Spectrometry Services data system.

Storage and testing of Enantiopac column

The Enantiopac column was stored in propan-2-ol–water (50:50, v/v) at a temperature of 10°C. Prior to each use the column was equilibrated in a mobile phase of propan-2-ol–0.1 *M* sodium chloride in phosphate buffer (10 m*M*, pH 6) (8:92, v/v) and tested by the injection of 20 μl disopyramide (0.1 mg/ml) in mobile phase. Baseline resolution for this reference racemate was always obtained during our study.

Preparation, testing and storage of Pirkle column

Preparation of the Pirkle CSP, from a prepacked 30 cm γ-aminopropyl silica column and a solution of N-(3,5-dinitrobenzoyl)-(*R*)-(−)-phenylglycine, was in accordance with the *"in situ"* method described by Pirkle and Myung Ho Hymn[6]. The final stage of preparation involved equilibration in a mobile phase of propan-2-ol–hexane (10:90, v/v). On a achieving a stable baseline the column was tested using phensuximide as the test racemate. On injection of 20 μl of a 1 mg/ml solution, clear separation (but not to baseline) of the enantiomers was considered satisfactory. When not in use, the column was stored in propan-2-ol–hexane (10:90) at 10°C. The cost of column preparation was about £ 40.

Preparation and characterisation of CMPP derivatives

The primary (B), benzyl (C) and diphenyl (D) amides of (±)CMPP (A) were prepared in the following manner: 500 mg (2.4 mmol) of (±)CMPP was weighed into a 5-ml reactivial along with 300 mg (slight excess) of thionyl chloride. The contents were heated on a steam bath for 1 h before evaporating to dryness under reduced pressure. The solid obtained was dissolved in chloroform and 2.4 mmol of the appropriate amine was added (dissolved in 0.5 ml chloroform). The reactivial was shaken regularly over a period of 1 h and a crude product was obtained after evaporation. The crude material was dissolved in 99.8% ethanol (5 ml) and filtered. The filtrate was reduced to a volume of 1 ml before adding a single drop of water and placing in a refrigerator. On crystallisation, the purified material was obtained by centrifugation and dried. The derivatives so obtained were characterised by IR and MS.

Optically pure derivatives were prepared on a smaller scale but isolation and characterisation was not carried out. Quantities of 50 mg of (+)- or (−)CMPP were used and the only purification process used was washing the crude derivative in hexane prior to HPLC analysis.

Characterisation of derivatives

(±)CMPP (A). MS: molecular peak (^{35}Cl) m/z = 214, relative intensity = 50%. IR: 3300–2500 cm^{-1} (OH), 1750–1650 cm^{-1} (C=O).

Primary amide (B). MS: molecular peak (^{35}Cl) m/z = 213, relative intensity = 51%. IR: 3220–3180 cm^{-1} and 3410–3370 cm^{-1} (primary amide NH), 1670–1650 cm^{-1} (primary amide C=O).

Benzyl amide (C). MS: molecular peak (^{35}Cl) m/z = 303, relative intensity = 41%. IR: 3300–3200 cm^{-1} (secondary NH), 1680–1630 cm^{-1} (secondary amide C=O).

Diphenyl amide (D). MS: molecular peak (^{35}Cl) m/z = 365, relative intensity = 20%. IR: 1700–1650 cm^{-1} (tertiary amide C=O), no NH peaks observed.

RESULTS AND DISCUSSION

Enantiopac column

The Enantiopac column used was still operational after about six months, during which time several hundred samples had been analysed. It is interesting to note that the resolving power of the column for (±)CMPP increased after a period of storage (see Fig. 1) whilst remaining largely unaltered for disopyramide. This selective change was difficult to explain but may have involved a change in configuration of the acid glycoprotein. During the course of this work the elution time for CMPP increased by about 15%. These changes may have been due to settlement of the packing.

Previously reported resolutions of chiral acids on this CSP have involved the use of N,N-dimethyloctylamine (DMOA) as a organic modifier[7]; DMOA however, may reduce column life[8]. In this investigation, through systematic alteration of variables, (±)CMPP was resolved to baseline without employing DMOA (see Fig. 2). It was noted that no apparent resolution of the racemic anti-inflammatory drug ibuprofen (E) was observed under these conditions.

Having achieved baseline resolution, the method was used to analyse a number

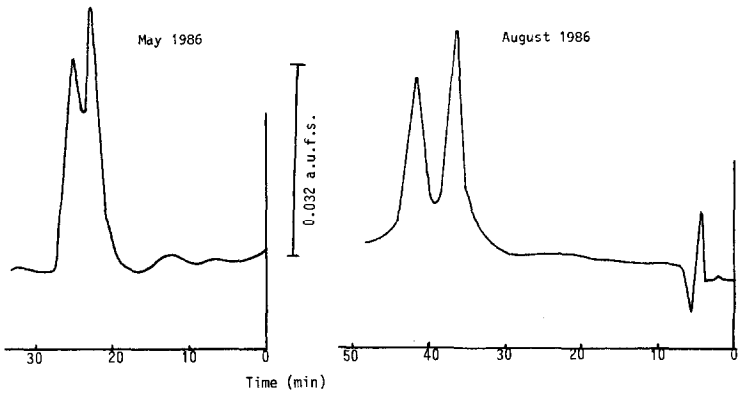


Fig. 1. Enantiopac CSP: change in resolving power and retention time for (±)CMPP. Mobile phase: propan-2-ol–0.1 *M* sodium chloride in phosphate buffer (10 m*M*, pH 6) (8:92, v/v). Flow-rate: 0.2 ml/min. Detection: 240 nm. Samples: 1 mg/ml (±)CMPP in mobile phase.

of pilot plant production samples. An integrator response of 1:1 for the enantiomers of CMPP was established prior to the analysis of other samples. Some of the results obtained are shown in Fig. 2. This method represents a valuable advance in the determination of the optical constitution of CMPP. Being a direct separation, procedures are simple, total analysis time is relatively short and the method can be applied directly to aqueous solutions. Apart from polarimetry other methods of chiral investigation used for CMPP involve diasteriomeric conversion which is limited by the optical purity of derivatising agents and the possible occurrence of racemisation during derivatisation. Polarimetry is limited through poor sensitivity and chemical impurities will influence the optical rotation.

Pirkle column

The Pirkle Ionic CSP consisted of N-(3,5-dinitrobenzoyl)-(*R*)-(−)-phenylgly-

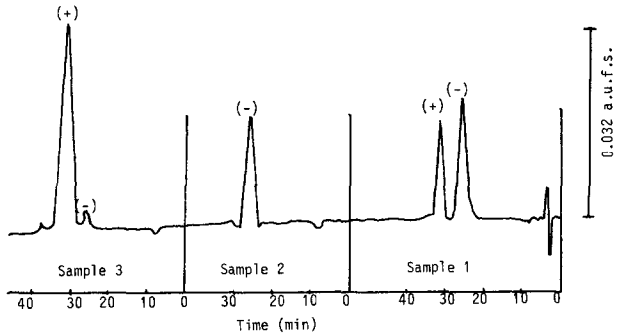


Fig. 2. Baseline resolution of (±)CMPP and analysis of pilot plant production samples on Enantiopac column. Sample 1: (±)CMPP, 0.08 mg/ml in mobile phase, 49.89% (+)- and 50.11% (−)enantiomer. Sample 2: (−)CMPP 0.06 mg/ml in mobile phase, 100.00% (−)enantiomer. Sample 3: (+)CMPP, 0.10 mg/ml in mobile phase, 90.70% (+)- and 9.30% (−)enantiomer. Mobile phase: propan-2-ol–0.1 *M* sodium chloride in phosphate buffer (10 m*M*, pH 6) (4:96, v/v). Flow-rate: 0.3 ml/min. Detection: 230 nm.

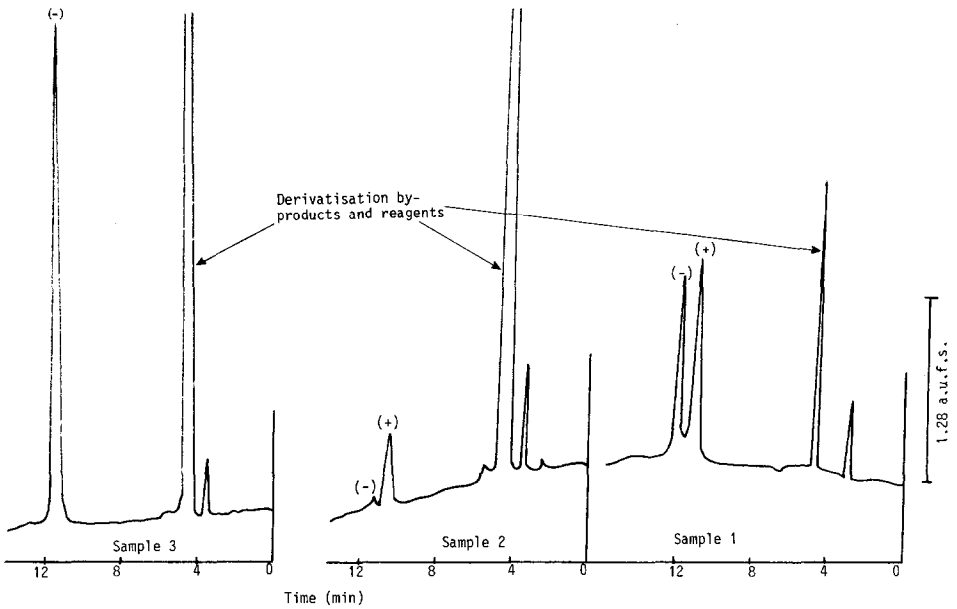


Fig. 3. Baseline resolution of CMPP as diphenyl amide derivative on Pirkle Ionic column. Mobile phase: propan-2-ol–hexane (10:90). Detection: 260 nm. Flow-rate: 1 ml/min. Samples: 1 = (±)CMPP 0.5 mg/ml in mobile phase, 2 = (+)CMPP (crude), 3 = (−)CMPP (crude).

cine bonded ionically to an achiral support of γ-aminopropyl silica. This CSP was unsuitable for the direct resolution of acidic racemates, amide derivatives were therefore prepared.

Primary, benzyl and diphenyl amides of (±)CMPP were prepared and characterised by IR and MS. Diphenyl amides were also prepared for (+)- and (−)CMPP but here the compounds were not isolated (due to smaller scale preparation) and spectroscopic characterisation was not carried out.

Of the racemic derivatives, on chromatographic examination no separation was observed for the primary amide, some separation was observed for the N-benzyl amide and baseline separation was observed for the diphenyl amide. The diphenyl amides of the optically pure CMPP enantiomers gave single peaks corresponding to one of the peaks observed for the racemate. There was no evidence of racemisation during the derivatisation (Fig. 3, sample 3). The small quantity of the (−)enantiomer evident on the analysis of the (+)derivative (Fig. 3, sample 2) can be accounted for by 9.3% optical impurity present in the CMPP as found during the Enantiopac study.

The amide derivates of CMPP were all examined on the Enantiopac column but in each case no separation was observed. This Pirkle CSP could be of value as an enantiospecific assay. It was clearly less costly than the Enantiopac but could not be used for aqueous samples and the derivatisation step was time consuming. We also found the usable life of this CSP to be limited to about two months. On comparing the two systems it was interesting to note that the elution orders were reversed. The (−)enantiomer of CMPP wass first eluted from the Enantiopac CSP while the (+)enantiomer of the diphenyl amide was first eluted from the Pirkle CSP. This may

be of use for the accurate quantification of the minor enantiomer in the presence of a large excess of the other.

ACKNOWLEDGEMENTS

Thanks are due to Barnsley District Health Authority for funding this work and to A. H. Marks & Co. Ltd. for the kind gifts of CMPP samples.

REFERENCES

1 R. Wegler and L. Eue, in R. Wegler (Editor), *Chemie der Pflanzenschutz- und Schadlingsbekämpfungsmittel*, Vol. 2, Springer, Berlin, 1970, p. 278.
2 R. Roll and G. Matthiaschk, *Arzneim.-Forsch.*, 33 (1983) 1479–83.
3 Rhone-Poulenc-Agrochimie, *Brit. Pat.*, 2,054,570 B (1983).
4 R. W. Souter (Editor), *Chromatographic Separations of Stereoisomers*, CRC Press, Boca Raton, FL, 1985.
5 Y. Tsuda, K. Kawai and S. Nakajima, *Chem. Pharm. Bull.*, 33 (1985) 1955–1960.
6 W. H. Pirkle and Myung Ho Hyum, *J. Org. Chem.*, 49 (1984) 3043–3046.
7 J. Hermansson and M. Eriksson, *J. Liq. Chromatogr.*, 9 (1986) 621–639.
8 *EnantioPac — The Chiral Separation Column*, LKB-Produkter, Bromma, No. 2134.