*Journal of Chromatography*, 623 (1992) 159–162
Elsevier Science Publishers B V , Amsterdam

CHROM 24 482

# Short Communication

# Direct liquid chromatographic separation of the enantiomers of a 1,3-dihydrophenylindol-2-one and of a 2,3-dihydro-2-oxo-benzofuran derivative

Salvatore Caccamese

*Dipartimento di Scienze Chimiche, Universita di Catania, Viale A Doria 6, 95125 Catania (Italy)*

Tiziana Benincori

*Dipartimento di Chimica Organica e Industriale, Universita di Milano, Via Golgi 19, 20133 Milan (Italy)*

Grazia Principato

*Dipartimento di Scienze Chimiche, Universita di Catania, Viale A Doria 6, 95125 Catania (Italy)*

(First received April 28th, 1992, revised manuscript received June 22nd, 1992)

ABSTRACT

The enantiomers of a 1,3-dihydrophenylindol-2-one derivative were separated on a Pirkle chiral phase This phase was unable to separate the enantiomers of a structurally similar 2,3-dihydro-2-oxo-benzofuran derivative, where an oxygen atom replaces the N-$CH_3$ in the hetero-ring The enantiomers of this compound were instead separated using a Chiralpak OP(+) column

## INTRODUCTION

1,3-Dihydro-1,3,4,6-tetramethyl-3-(2-amino)phenylindol-2-one (**1**), 3-(2-amino)phenyl-2,3-dihydro-2-oxo-3,4,6-trimethylbenzofuran (**2**) and 3-(4-amino)-phenyl-2,3-dihydro-2-imino-3,4,6-trimethylthianaphthene (**3**) (Fig 1) were the main racemic products of the rearrangements of 2-phenylhydrazo-1,3,4,6-tetramethylindole, 2-phenylhydrazo-3,4,6-trimethylbenzofuran and 2-phenylhydrazo-3,4,6-trimethylthianaphthene, respectively, in acidic medium [1], these new compounds are expected to show some pharmacological activity as they possess a skeleton which is known to be biologically active [2–5]

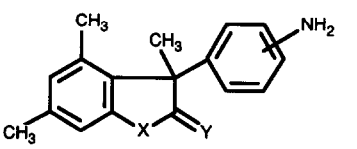


Fig 1 Structures of compounds **1–3** **1** X = N($CH_3$), Y = O, *ortho* $NH_2$, **2** X = O, Y = O, *ortho* $NH_2$, **3** X = S, Y = NH, *para* $NH_2$

*Correspondence to* Dr S Caccamese, Dipartimento di Scienze Chimiche, Universita di Catania, Viale A Doria 6, 95125 Catania, Italy

0021-9673/92/$05 00 

This paper reports the direct enantiomeric resolution of compound **1** by high-performance liquid chromatography (HPLC) on a donor–acceptor chiral stationary phase (CSP) (Pirkle column) and of compound **2** on a helical column [Chiralpak OP(+)] The resolution factor of compound **1** afforded milligram separation and circular dichroism (CD) measurement of the enantiomeric pair

## EXPERIMENTAL

### *Apparatus*

The HPLC system consisted of a Varian 5060 liquid chromatograph with a 10-$\mu$l Valco sample loop, a Jasco Uvidec 100-III UV spectrophotometric detector operating at 240 nm and a Varian CDS 401 data system For semi-preparative purpose repetitive injections on a 50-$\mu$l loop were performed CD spectra were recorded on a Jasco 600 spectropolarimeter The columns (25 cm × 4 6 mm I D ) were packed with (*R*)-N-3,5 dinitrobenzoylphenylglycine covalently bonded to $\gamma$-aminopropylsilanized silica (DNPBG) from Regis (Morton Grove, IL, USA) and with (+)-polydiphenyl-2-pyridylmethylmethacrylate [6] coated on silica gel [Chiralpak OP(+)] from Daicel (Tokyo) The void volume ($t_0$) was determined by injection of 1,3,5-tri-*tert* -butylbenzene as a non-retained sample

### *Chemicals*

Compounds **1–3** were prepared by treatment with acetic acid and concentrated sulphuric acid of 2-phenylhydrazo-1,3,4,6-tetramethylindole, 2-phenylhydrazo-3,4,6-trimethylbenzofuran and 2-phenylhydrazo-3,4,6-trimethylthianaphthene, respectively

1,3,4,6-Tetramethyl-3-(2-amino)phenylindolinone (**1**) was obtained in 30% yield, m p 133°C, $^1$H NMR ($C^2HCl_3$) $\delta$ 7 35 (1H, dd, aromatic), 7 08 (1H, m, aromatic), 6 98 (1H, m, aromatic), 6 68–6 40 (3H, m, aromatic), 3 25 (3H, s, $CH_3$ in position 1), 3 10 (2H, broad s exchangeable with $^2H_2O$, $NH_2$), 2 30, 1 95 and 1 80 (3 × 3H, 3s, 3 $CH_3$)

3-(2-Amino)phenyl-2,3-dihydro-2-oxo-3,4,6-trimethylbenzofuran (**2**) was obtained in 58% yield, m p 195°C, $^1$H NMR ($C^2HCl_3$) $\delta$ 7 25–6 20 (6H, m, aromatic), 4 2 (2H, broad s exchangeable with $^2H_2O$, $NH_2$, 2 35 (2 × 3H, s, 2 $CH_3$ in position 4 and 6), 1 80 (3H, s, $CH_3$ in position 1)

3-(4-Amino)phenyl-2,3-dihydro-2-imino-3,4,6-trimethylthianaphthene (**3**) was obtained in 47% yield, m p 152°C, $^1$H NMR ($C^2HCl_3$) $\delta$ 7 10–6 90 (3H, m, aromatic), 6 50–6 75 (3H, m, aromatic), 5 3 (3H, broad s exchangeable with $^2H_2O$, NH and $NH_2$), 2 30 and 1 80 (9H, 2s, 3 $CH_3$)

## RESULTS AND DISCUSSION

The chromatographic results are presented in Table I Racemic compound **1** was completely resolved using the Pirkle-type DNBPG column, obtaining a good separation factor ($\alpha$) which was slightly affected by an increase in the percentage of propan-2-ol in the mobile phase, and excellent resolution factors ($R$) as shown in Table I and Fig 2a This gave a quantitative separation of the enantiomers by repeated 50-$\mu$l injections of racemic

TABLE I

CSP-HPLC RESOLUTION OF ENANTIOMERIC COMPOUNDS 1–3

| Compound | CSP[a] | A[b] (%) | $k'$[c] | $\alpha$ | $R$ |
|---|---|---|---|---|---|
| **1** | DNBPG | 3 | 5 25 | 1 44 | 2 5 |
| | DNBPG | 5 | 3 70 | 1 42 | 2 8 |
| | DNBPG | 10 | 2 41[d] | 1 39 | 2 5 |
| | DNBPG | 15 | 1 74 | 1 33 | 2 5 |
| | Chiralpak OP(+) | 5[e] | 4 32[f] | NS[g] | |
| **2** | DNBPG | 0 | 1 74[f] | 1 02 | |
| | Chiralpak OP(+) | 0[e] | 4 79 | 1 19 | 0 98 |
| | Chiralpak OP(+) | 5[e] | 2 61 | 1 16 | 0 69 |
| | Chiralpak OP(+) | 10[e] | 2 04 | 1 15 | 0 57 |
| | Chiralpak OP(+) | 10[h] | 2 12 | 1 16 | 0 68 |
| **3** | DNBPG | 10 | 6 19 | NS | |
| | DNBPG | 10[i] | 4 07[f] | NS | |
| | DNBPG | 10[i,j] | 4 49[f] | 1 02 | |
| | Chiralpak OP(+) | 10 | 6 64[f] | NS | |

[a] CSPs are described under Experimental
[b] Percentage of propan-2-ol in *n*-hexane at a flow-rate of 1 5 ml/min unless otherwise specified, $t_0$ = 2 37 min
[c] Capacity factor of the first eluted enantiomer
[d] CD-positive enantiomer, see text
[e] Flow-rate of 1 ml/min, $t_0$ = 3 21 min
[f] Shoulder in the rising edge of the peak
[g] Not separated
[h] Flow-rate of 0 7 ml/min
[i] Propan-2-ol doped with 0 5% diethylamine
[j] Flow-rate of 0 7 ml/min, $t_0$ = 5 50 min

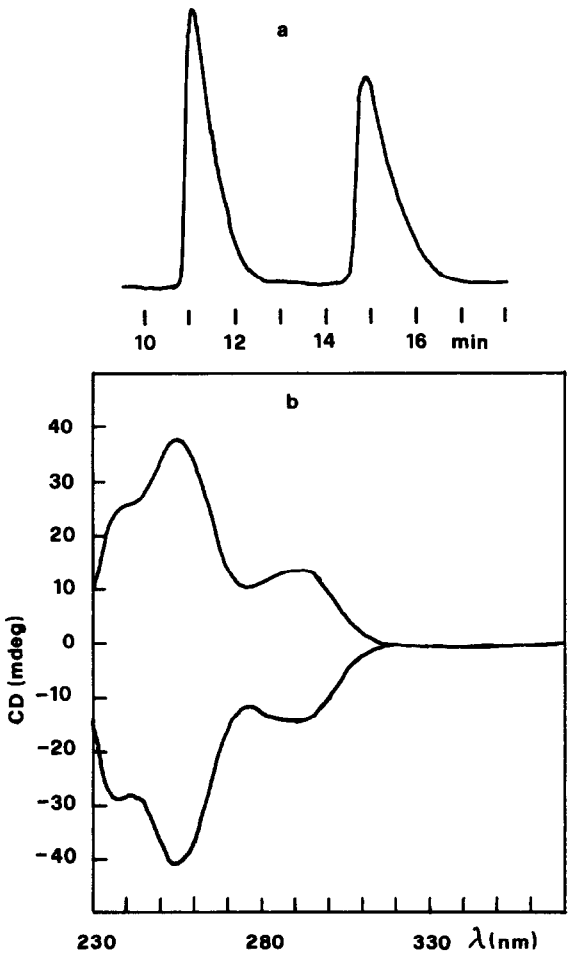


Fig 2 (a) HPLC separation of the enantiomeric pair of compound **1** on DNBPG, mobile phase *n*-hexane–propan-2-ol (95 5, v/v) at 1 5 ml/min (b) CD spectra of the enantiomeric pair of compound **1** obtained from the first eluted peak (positive) and from the second peak (negative) in ethanol at 22°C

**1** and collection of the eluates from the two chromatographic peaks The CD spectra of both eluates were measured and these were mirror images of each other as shown in Fig 2b, indicating that the two eluates are optical isomers Analytical HPLC reruns of the eluates indicated a 100% enantiomeric purity of each peak Their UV spectra are also identical No separation of the enantiomers of compound **1** was achieved using the Chiralpak OP(+) column, which owes its chirality to the macromolecular helicity, as shown in Table I and from other trials with various percentages of propan-2-ol in hexane

Compound **2** was well resolved using the Chiralpak OP(+) column, as shown in Table I The best results were achieved by eliminating the propan-2-ol in the eluent and by decreasing the flow-rate of the mobile phase, thus obtaining a beneficial effect on the resolution factor The Pirkle-type CSP was ineffective in the resolution of compound **2** A single sharp peak was observed using hexane–propan-2-ol mobile phases and barely resolved peaks were observed using only *n*-hexane as the eluent, as shown in Table I

Compound **3** was only slightly resolved using the DNBPG column and the addition of a small amount of diethylamine, combined with a low flow-rate (0 7 ml/min) was crucial to observe this poor resolution, as shown in Table I No separation was achieved using the Chiralpak OP(+) column, as shown in Table I

The different behaviour of compounds **1** and **2**, structurally very similar, to the DNBPG chiral stationary phase is explained using the chiral recognition model proposed by Pirkle for its DNBPG phase [7] We envisage three simultaneous interactions for the relative configurations of compound **1** (a) a $\pi$–$\pi$ bonding interaction between the 3,5-dimethylphenyl ring of the analyte and the 3,5-dinitrobenzoyl group of the CSP, (b) a dipole-stacking [8] between the N-methyllactame moiety of compound **1** and the 3,5-DNB carboxamide group of the CSP, and (c) a stereochemically dependent steric interaction between the analyte and the CSP The dipolar interaction in (b) is strong if the dipole moments of the N-methylpyrrolidone (4 06 D [9]) and of the *trans*-carboxamide group of the CSP (3 9 D [10]) are considered as a model

The dipole stacking is instead much less effective between the lactone function of compound **2** and the carboxamide group of the DNBPG CSP and this results in a minor enantioselectivity with respect to compound **1** The estereal function of analyte **2** interacts in some way with the estereal function of the Chiralpak OP(+) column, resulting in the resolution of the enantiomers

Thus the use of the correct CSP is crucial to obtain enantioselectivity sufficient for the preparative resolution of compounds **1** and **2**

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