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Chiral high-performance liquid chromatography of some related bicyclic lactams

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ABSTRACT

Chromatographic methods utilising a Chiralcel OC cellulose-based column were developed for the chiral resolution of optical isomers of the cognition-enhancing ISF 4185 and related bicyclic lactams. These methods were scaled up for the preparation of purified samples of enantiomers, one pair of which was submitted to X-ray analysis. The resolution of the enantiomers derived from these compounds appears to be mainly dependent on their ability to hydrogen bond to the chiral stationary phase.

INTRODUCTION

2,5-Dioxohexahydro-1*H*-pyrrolo[1,2-*a*] imidazole (ISF 4185) (**1**) is currently being developed as Dimiracetam by ISF (Milan, Italy) because of its potency as a cognition enhancer [1]. The therapeutic efficacy of compounds of this class has been demonstrated [2] in the case of oxiracetam (**2**), a derivative of γ-amino-β-hydroxybutyric acid (GABOB). Both **1** and **2** contain an asymmetric centre (denoted by an asterisk) so that each molecule can exist in two enantiomeric forms.

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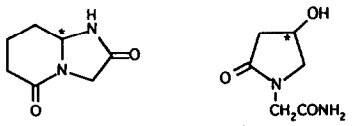

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We reported previously the separation of the enantiomers of **2** using a Chiralcel OC column [3]. In this study, we analysed the chromatographic behaviour of **1** and a number of structurally related molecules. Pure samples of the enantiomers of one of the lactams were also chromatographically prepared for the determination of its absolute configuration by X-ray analysis.

EXPERIMENTAL

Materials and reagents

All compounds used were greater than 99% pure and were used without further purification. *n*-Hexane (Rathburn Chemicals), 2-propanol (BDH) and chloroform (May and Baker) were degassed with helium before use.

High-performance liquid chromatography

The HPLC pump system used was either a Gilson Model 303 or a Perkin-Elmer Series 3B or Series 4. Samples were injected with a Perkin-Elmer ISS-100 autoinjector or a Rheodyne Model 7125 manual injector and detected using a Gilson HM/Holochrome or a Perkin-Elmer LC90 variable-wavelength detector. Chromatographic peaks were injected using either the Perkin-Elmer LIMS 2000/CLAS or the Nelson 2600 data systems.

For the analytical separations a Chiralcel OC column (250 mm × 4.6 mm I.D.), supplied by Daicel, was used. Preparative separations were carried out on a Chiralcel OC column (250 mm × 10 mm I.D.). The mobile phase for chiral resolution consisted of various amounts of hexane and 2-propanol at flow-rates between 1 and 4 ml min^{-1}. Compounds were injected on to the column at a concentration of about 1 mg ml^{-1} in the mobile phase. The temperature for these chiral separations was ambient. UV detection was at 205 or 210 nm.

Polarimetry

In order to determine the enantiomeric composition of the separated enantiomers, their specific rotation, $[\alpha]_D^{25}$, was measured using a Perkin-Elmer Model 241 polarimeter set at a wavelength of 589 nm (sodium D-line). Optical rotation was determined in a cell of 100 mm path length. Compounds were dissolved in methanol at a concentration of 1 mg ml^{-1}.

X-Ray diffraction

Three-dimensional X-ray diffraction data were collected for each compound on an Enraf-Nonius CAD-4 diffractor equipped with incident beam graphite monochromated copper radiation. Preliminary crystal examination and the data collection set-up were the same for each sample. The crystal was mounted with epoxy on glass-fibre and centred optically on the goniostat of the diffractometer. Preliminary lattice parameters were obtained either through a random search of reciprocal space or by use of a rotation photograph from which diffraction spots were measured.

After attaining a preliminary cell the diffraction symmetry was checked and the crystal quality was assessed using a plot of intensities for several axial reflections in the plane of the scanning monitors θ and ω. A small shell of higher order data was then collected rapidly in order to assess the diffraction range and to select 25 reflections well distributed in reciprocal space which were accurately centred to provide the final lattice parameters. Intensity data were then collected using variable-speed $\omega - 2\theta$ scans with the final scan speed selected according to the diffraction of any given reflection based on a prescan. Final scans were extended in width 25% on both sides to allow for estimation of background intensity. Three intensity standards were monitored every 3 h of X-ray exposure time in order to account for any crystal decay or significant fluctuations in the cold stream temperature for those data sets in which cooling was employed. The crystal orientation was also monitored every 250 reflections and adjustments to the orientation matrix were made automatically whenever the scattering vectors of the monitor

reflections had moved by a preset amount from the angle calculated based on the orientation matrix. A unique octant of data along with the Friedel mate of each reflection was collected for each crystal data set.

Structure analysis, refinement and configuration assignment

The first structure was solved by direct methods using the MULTAN [4] program series. Subsequently, coordinates of this solution were used as a starting point for refinement of the others. After refinement with isotropic temperature factors the non-hydrogen atoms were assigned anisotropic parameters and refined to convergence. Hydrogen positions were suggested from difference Fourier maps in all instances. Hydrogen atoms were included as fixed contributions to the models at calculated positions based on geometric considerations and assuming a C–H or N–H bond length of 1.03 Å. Fixed isotropic temperature factors equal to $1.1 \times B_{iso}$ of the attached non-hydrogen atom were assigned to each hydrogen. Values of the neutral atom scattering factors and for anomalous dispersion corrections were taken from ref. 5.

Absolute configuration assignment samples were based both on an analysis of the signs of the intensity ratios of Friedel mates and on the purely statistical basis of Hamilton's *R*-factor ratio test [6]. Results from both methods were in agreement for both the data sets. The signs of the differences in F_0 and F_c for all Friedel mates significantly affected by anomalous dispersion (6% or greater) were in agreement for the correct enantiomer in each case. For (R) ISF 4393 (determined from sample code LCA/1/NC2-3/1) the *R*-factor ratio was 1.259 and is significant at the 99.995% confidence level compared with the theoretical value of 1.003 for the refinement. Similarly, for (S)-ISF-4393 (determined from sample code UP-23-48) the *R*-factor ratio of 1.067 is statistically significant at the 99.995% confidence level as compared with the calculated value of 1.008. Our findings for this latter sample are in agreement with its preparation from (*S*)-pyroglutamic acid under non-racemizing conditions.

RESULTS AND DISCUSSION

Compound **1** is a relatively hydrophilic molecule containing few aliphatic and no aromatic residues, essential for hydrophobic interactions and important for the resolution of enantiomers on most commercial chiral stationary phases (CSPs). The absence of aromatic residues also precludes the occurrence of $\pi-\pi$ interactions, important for the formation of charge-transfer transient complexes. However, **1** contains two amide moieties which can be involved in hydrogen-bonding interactions between a CSP and the enantiomers of this molecule. Hydrogen bonding will be enhanced in non-polar solvents.

Because of the above limitations, the choice of the most suitable CSP for the resolution of the enantiomers of **1** was limited. The use of either a Pirkle-type [7] or a cyclodextrin [8] column was very doubtful as the chemical structure of **1** does not give any indication that it can be associated with either charge transfer or inclusion in the hydrophobic cavity of cyclodextrin. The use of a protein column [9] was thought not to be practical as such a CSP can be used only for analytical and not preparative purposes. Moreover, most of the analytes resolved on such a column contain aromatic groups [10].

From our experience on the chiral separation of the enantiomers of oxiracetam and closely related molecules [11], a Chiralcel OC column was chosen for the resolution of the optical isomers of **1** and other lactams. This CSP is made up of cellulose with the three hydroxy groups on each D-glucose unit replaced by phenylcarbamate residues. The Chiralcel OC column is especially effective for the chiral resolution of polar racemates as the carbamate residues can be involved in stereoselective hydrogen bonding with a substrate [11]. The chiral resolution of the enantiomers of **1** using a semi-preparative column of this type is shown in Fig. 1. About 20 mg of each enantiomer of **1** were obtained using this column with hexane–2-propanol (50:50) as the mobile phase at a flow-rate of 4 ml min^{-1}.

Fig. 2 shows the purity of the prepared samples. The peak areas of the (+)- and (−)-en-

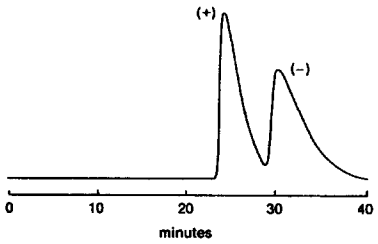


Fig. 1. Resolution of the enantiomers of **1** on a semi-preparative Chiralcel OC column. Column, Chiral OC (250 mm × 10 mm I.D.; 10 μm); mobile phase, hexane–2-propanol (50:50); flow-rate, 4 ml min^{-1}; temperature, ambient; detection at 205 nm.

antiomers integrated to 97% and 95%, respectively. The enantiomeric excess (ee) was calculated as 95 and 91% and the optical rotations, $[\alpha]_D^{25}$, were measured in methanol as +38.5 and −35.2. These values are in agreement with purity values for the individual enantiomers. The absolute configuration of the (+)- and (−)-enantiomers was *S* and *R*, respectively. The identity of the enantiomers was obtained by comparison of retention times with those of authentic samples of the separate enantiomers.

In order to evaluate the usefulness of the Chiralcel OC column for the chromatographic resolution of racemates closely related to **1**, several molecules were analysed using similar chromatographic conditions. Expanding one of the lactam rings of **1** to give the lactams **3** and **4** does not have much effect on either the resolution or the retention time of the enantiomers. Table I compares some of the properties of the three compounds. The separation factors for **3** and **4** are lower than that measure for **1**. Unlike

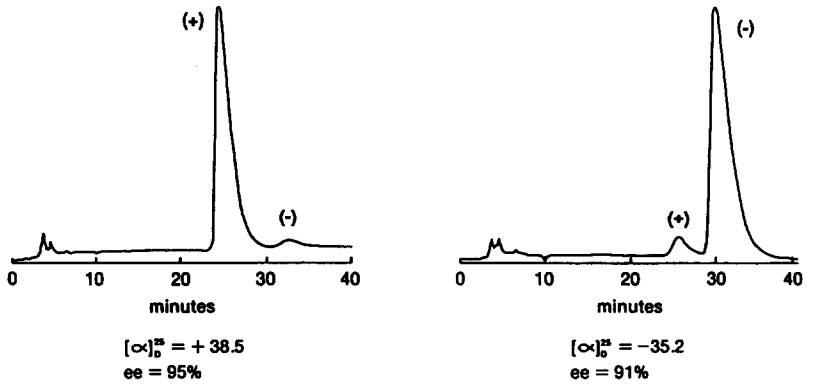
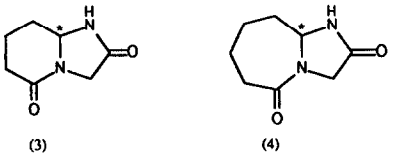


Fig. 2. Analysis of the separate enantiomers of **1**.

TABLE I

COMPARISON OF SOME PROPERTIES OF **1** AND CLOSELY RELATED COMPOUNDS

Compound	Separation factor	Mobile phase hexane–2-propanol	Specific rotation, $[\alpha]_D^{25}$ (°)	
			First-eluting enantiomer	Second-eluting enantiomer
1	1.38	50:50	+38.5	−35.2
3	1.28	50:50	−5.0	+4.0
4	1.35	50:50	+9.6	−9.3
5	1.26	60:40	–	–
6	1.29	60:40	–	–
7	1.17	80:20	+16.2	−15.7

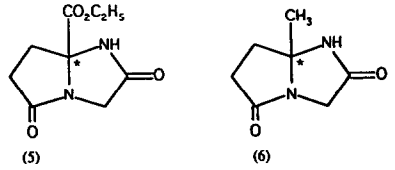


(3) (4)

1 and **4**, the (−)-enantiomer of **3** elutes before the (+)-isomer.

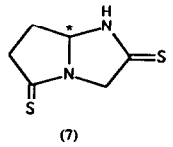
Expansion of one of the lactam rings by the addition of one or two methylene groups would have been expected to increase the hydrophobicity of the resulting molecules so that under the conditions of elution described retention of the three molecules would normally be predicted to be in the order **1** > **3** > **4**. The apparent lack of sensitivity of retention to the size of one of the lactam rings is remarkable and may emphasize that the hydrogen bonding character of these molecules is the dominant mechanism both for their retention and for their stereoselective interaction with the CSP.

The chromatographic behaviour of two more derivatives of **1** was also studied to provide information on the influence of substituents on chiral resolution. The enantiomers of both **5** and **6** were resolved (see Table 1), showing that introduction of substituents that differ widely in their size and their electronic and hydrophobic characteristics [12] is tolerated at the chiral centre. No optical rotation studies were carried out on **5** and **6**.



(5) (6)

We finally analysed the chromatographic behaviour of the dithio derivative of **1**. The chromatographic resolution of the two optical isomers of this compound (**7**) on a semi-preparative Chiralcel column is shown in Fig. 3.



(7)

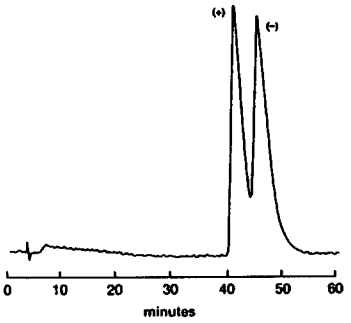


Fig. 3. Chiral resolution of the enantiomers of 7 on a semi-preparative column. Conditions as in Fig. 1 except hexane–2-propanol (80:20) and UV detection at 210 nm.

The enantiomers of **7** were not completely resolved over a wide range of hexane-to-2-propanol ratios. This may be due to the weaker hydrogen-bonding efficacy of sulphur compared with oxygen and to the larger atomic size of

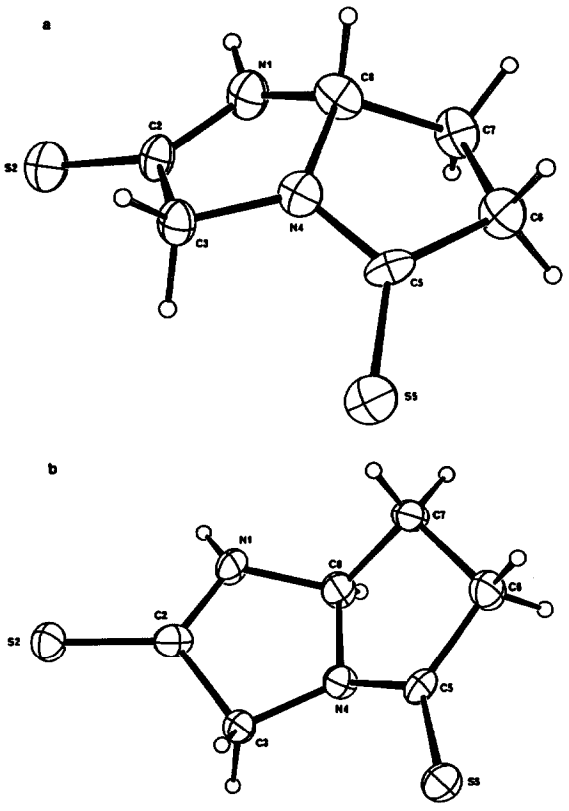


Fig. 4. X-Ray structures of (a) (*S*)-7 and (b) (*R*)-7.

sulphur. These two effects may both contribute to a smaller energy difference associated with the transient diastereoisomers between each enantiomer and the CSP, although different mechanisms for enantiorecognition may also be involved. The two enantiomers of **7** were prepared with ee values greater than 98% for the (+)-isomer and 93% for the (−)-isomer. The $[\alpha]_D^{25}$ values for the respective enantiomers were measured as +16.2 and −15.7.

The purified enantiomers were submitted to X-ray crystallography in order to determine absolute configuration. Results from these studies are given in Fig. 4 and Table II.

X-Ray studies on **7** were done to relate the absolute configuration of the enantiomers of this compound to those of the antipodes of **1**. These studies showed that although that although the (+)- and (−)-enantiomers of the two compounds eluted in the same order, the absolute configuration was the reverse, that is, the (+)- and (−)-enantiomers had the *R* and *S* absolute configuration, respectively, as confirmed by comparison of the retention times of the two antipodes with those of authentic samples [13,14]. This result was surprising and the reverse order of elution of the antipodes of **7** compared with those of **1** on a Chiralcel OC column appears to indicate that one or more of the three points of interaction (necessary for chiral recognition) between these

TABLE II

CRYSTALLOGRAPHIC DATA FOR THE ENANTIOMERS OF **7**

Parameter	(*S*)-**7**	(*R*)-**7**
Formula	$C_6H_8N_2S_2$	$C_6H_8N_2S_2$
M_r	172.27	172.27
Crystal shape and dimensions (mm)	Ellipsoidal, 0.40 × 0.30 × 0.30	Tabloid, 0.35 × 0.15 × 0.30
Space group	$P2_12_12_1$	$P2_12_12_1$
Temperature (K)	173	145
Lattice parameters (Å):		
a	6.667(2)	6.650(4)
b	7.689(10)	7.675(3)
c	14.984(5)	14.982(5)
Volume (Å^3)	768.1(1)	764.6(7)
Z	4	4
P_{calc} (g cm^{-1})	1.490	1.496
μ (cm^{-1})	55.700	55.955
F(000)	360	360
Range of data	$20 \leq 135°$	$20 \leq 135°$
	$h \leq 7$	$h \leq 7$
	$k \leq 9$	$k \leq 9$
	$l \leq 19$	$l \leq 19$
Total observed data including Friedel mates	1259	1305
Unique data	774	818
Variables	92	92
R [a]	0.0584 (0.0635)	0.0386 (0.0478)
R_w [a]	0.0696 (0.730)	0.0504 (0.0653)
Goodness of fit	1.432	2.358
Extinction correction (×10^{-6})	11.14	9.101
Absorbtion corrections:		
Max.	0.9986	0.9991
Min.	0.8399	0.7499
Decay corrections	–	–

[a] Values in parentheses are crystallographic residuals for the enantiomer of each data set.

substrates and the CSP are different; for example, a different extent of enolization would be expected for **7** than for **1**. This will be investigated by constructing chiral recognition models in the same way as has been done for oxiracetam [11].

In conclusion, we have shown that it is possible to isolate enough material chromatographically for structure determination by X-ray crystallography. Moreover, the influence of the structure of the bicyclic lactam **1** on the chiral resolution of its enantiomers has been investigated and it has been shown that resolution is not greatly dependent on hydrophobicity but on the ability of the molecule to hydrogen bond to the cellulose-based chiral stationary phase.

REFERENCES

1 M. Pinza, C. Farina, U. Pfeiffer, M.T. Riccaboni, R. Begetti, O. Pozzi and L. Dorigotti, presented at the *XIth International Symposium on Medicinal Chemistry, Jerusalem, 2–7 September, 1990.*

2 C. Villardita, J. Parini, S. Grioli, M. Quattropani, C. Lomeo and U. Scapagnini, *J. Neural Transm., Suppl.*, 24 (1987) 293.

3 P. Camilleri, J.A. Murphy and C.J. Thorpe, *J. Chromatogr.*, 508 (1990) 208.

4 P. Main, S.J. Fiske, S.E. Hull, L. Lessinger, G. Germain, J.P. Declercq and M.M. Woolfson, *MULTAN80 – a System of Programs for the Automatic Solution of Crystal Structures from X-Ray Diffraction Data*, Universities of York, UK, and Louvain, Belgium, 1980.

5 *International Tables for X-Ray Crystallography*, Vol. IV, Kynoch Press, Birmingham, 1974; present distributor, Reidel, Dordrecht.

6 W.C. Hamilton, *Acta Crystallogr.*, 18 (1965) 502.

7 W.H. Pirkle and C.J. Welch, *J. Org. Chem.*, 49 (1984) 148.

8 W.L. Hinze, T.E. Riehl, D.W. Armstrong, W. Demond, A. Alak and T. Ward, *Anal. Chem.*, 57 (1985) 237.

9 S. Allenmark, B. Bomgren and H. Boren, *J. Chromatogr.*, 316 (1984) 617.

10 S. Allenmark, in A.M. Krstulović (Editor), *Chiral Separations by HPLC*, Wiley, New York, 1989, p. 285.

11 P. Camilleri, J.A. Murphy, M.R. Saunders and C.J. Thorpe, *J. Comput.-Aided Mol. Des.*, 5 (1991) 277.

12 C. Hansch and A. Leo, *Substituent Constants for Correlation Analysis in Chemistry and Biology*, Wiley, New York, 1979.

13 SmithKline Beecham, *UK Pat. Appl.*, 9123641.4 (1991).

14 SmithKline Beecham, *UK Pat. Appl.*, 9206618.2 (1992).