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Molecular modelling studies and the chromatographic behaviour of oxiracetam and some closely related molecules

Patrick Camilleri, Jose A. Murphy, Martin R. Saunders and Christopher J. Thorpe

SmithKline Beecham Ltd., The Frythe, Welwyn, Herts. AL6 9AR, U.K.

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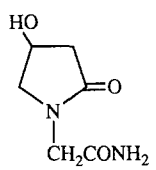
SUMMARY

Modelling studies have been carried out on the cellulose-based chiral stationary phase used to separate the enantiomers of three simple lactams. These studies have helped in understanding differences in the chromatographic behaviour of these molecules.

INTRODUCTION

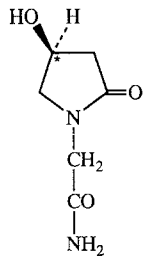
Oxiracetam (4-hydroxy-2-oxo-1-pyrolidine acetamide) (**1**) is a neurochemically active drug that shows inotropic properties, especially in elderly patients [1]. The molecular structure of oxiracetam shows that it is a simple polar lactam of low molecular mass. It also contains one chiral centre in the 4-position of the ring so that it can exist in two forms, **1a** and **1b**, which share an enantiomeric relationship.



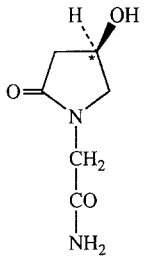
(1)

The configuration of these two enantiomers is, by convention [2,3], designated 'R' and 'S' respectively.

Recently we reported [4] the chiral resolution of the 'R' and 'S' optical isomers of **1** using a CHIRALCEL OC chromatographic column. The chiral stationary phase (CSP) in this column consisted of low-molecular-weight cellulose appropriately derivatised to give the corresponding



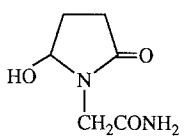
(1a)-S



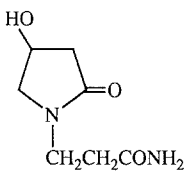
(1b)-R

triphenylcarbamate. This material is then coated on microporous silica gel [5,6], as shown schematically in Fig. 1.

To assist our understanding of the stereoselective mechanisms that are involved in the chiral resolution of **1a** and **1b**, we also reported the chromatographic behaviour of two closely related compounds **2** and **3**, both of which contain one chiral centre.



(2)



(3)

Compound **2** is an isomer of **1** where the hydroxy group has been moved closer to the nitrogen of the lactam ring. Compound **3** has the same substitution pattern in the lactam moiety as **1**, but the primary amide group is now separated by two methylene groups from the ring nitrogen. As reported by Camilleri et al. [4], using the same chromatographic conditions for the resolution of the enantiomers of **1**, it was found that the corresponding antipodes of **2** were not resolved and those of **3** were only partially resolved. We report here molecular-modelling studies on **1**, **2** and **3** in an attempt to relate stereoselectivity to the structural characteristics of these molecules and that of the chiral support.

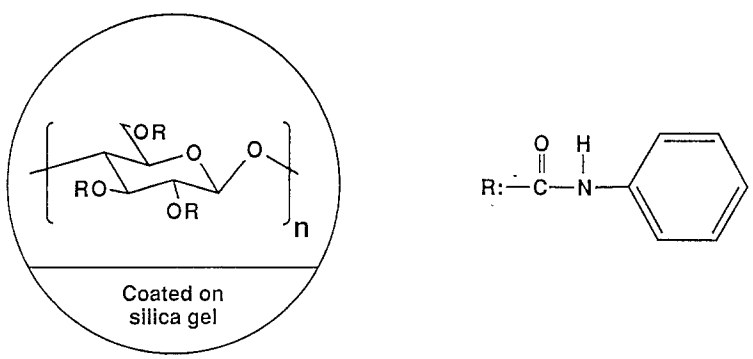


Fig. 1. Schematic representation of the chiral stationary phase.

METHODS

Molecular-modelling studies were performed using the COSMIC and ASTRAL [7] software frameworks. Energy minimisation of the modelled compounds was carried out by gradient torsional minimisation and molecular orbital calculations by MOPAC within the COSMIC software framework. 'Guest' molecules were docked manually on to the 'host' molecule using ASTRAL, and were displayed on an Evans and Sutherland PS330 terminal, running on a Micro Vax 2II. 'Host' and 'guest' molecules were initially brought together so that all hydrogen bonding contacts were within 1.70 Å. These interaction complexes were then run into a single file and transferred back into COSMIC for Van der Waal's contact minimisation. The data file was minimised using 100 interactions of a SIMPLEX minimiser and converted to a PDB file format. This data file was displayed by the INSIGHT (II) package (Biosym) on a Silicon Graphics Personal Iris workstation. Partial CPK models were constructed by creating a CPK surface for the whole molecule at 720 polygons per sphere, and then displaying only those atoms actively involved in the three-point interactions on the 'host' and 'guest' molecules.

RESULTS AND DISCUSSION

The recognition process and resolution of a pair of optical isomers on a chiral stationary phase (CSP) is critically dependent on the interactions between the 'host' and 'guest' systems. To understand the mechanism of chiral recognition between the cellulose-derived CSP and oxiracetam (**1**) and the closely related molecules **2** and **3**, it is essential to model the cellulose phase itself.

Cellulose is a polysaccharide that is very abundant in nature. It is a polymer of D-glucose residues joined by β-1,4-linkages. Each glucose molecule is related to the next by a rotation of 180° and the hydroxy group in the 3-position of one glucose moiety is hydrogen-bonded to the ring sugar atom of a neighbouring glucose molecule (Fig. 2). For molecular modelling purposes, the trisaccharide in Fig. 2 was capped by two methyl groups, one at each end of the molecule shown. Energy minimisation gave the molecule in Fig. 3 where the colour coding is as follows: carbon, white; oxygen, red; hydrogen, blue; end methyl groups, purple.

Figure 4 shows the semi-CPK model of the trisaccharide where the two intramolecular hydrogen bonds have been highlighted. The β-configuration of cellulose allows it to form rigid long straight chains. This is in contrast to other polysaccharides such as starch and glycogen, where the α-1,4 linkages in these polymers do not allow them to adopt an extended conformation and which therefore undergo helical coiling. The cellulose triphenyl carbamate CSP shown in Fig. 1, where the hydroxy groups have been replaced by phenylcarbamate residues, was used to study the chiral behaviour of molecules **1**, **2** and **3**. Energy minimisation of a trisaccharide containing the

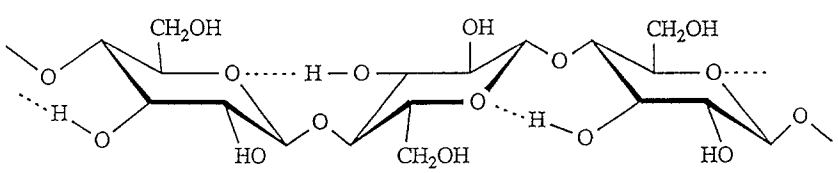


Fig. 2. Structure of cellulose.

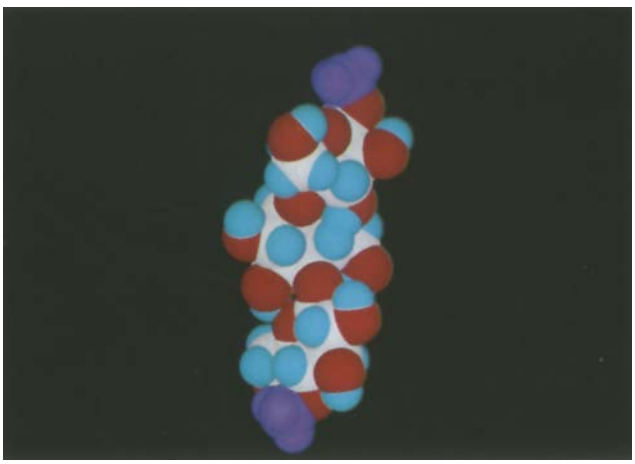


Fig. 3. CPK model of a trisaccharide used to model cellulose (for colour coding, see text).

carbamate substituents gave the configuration shown in Fig. 5(a). It appears from this molecular model that the introduction of the phenylcarbamate substituents imparts a helical conformation to the substituted cellulose, and this is mainly due to steric interaction between the phenyl moieties. Figure 5(b) shows the oxygen and hydrogen bonds that are most likely to be involved in chiral selectivity by the substituted cellulose support.

Chiral resolution on a chromatographic support such as substituted cellulose can, in principle, be due either to the helical nature of the support itself or to the D-configuration of the glucose units. However, in the present study, as the substrates **1**, **2** and **3** are small in size, chiral selectivity is thought to be mainly caused by specific interactions, such as hydrogen bonding between these molecules and each of the substituted D-glucose units. The phenylcarbamate cellulose is a normal (polar) phase chromatographic support, whereas the mobile phase used in conjunction with this

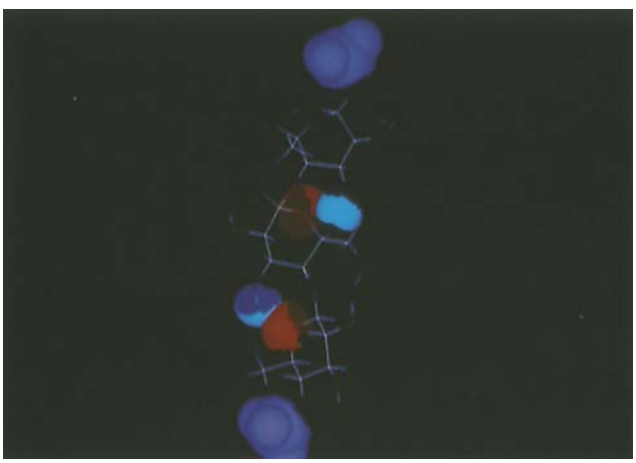


Fig. 4. Semi-CPK model of the trisaccharide in Fig. 3, showing intramolecular hydrogen bonds characteristic of the cellulose structure.

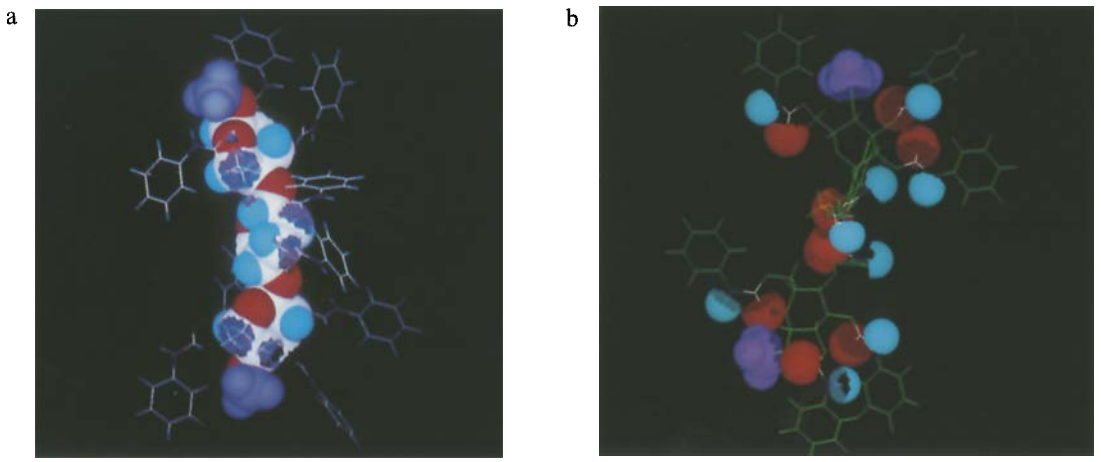
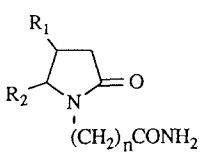


Fig. 5. (a) Model of the phenylcarbamate-substituted trisaccharide showing the helical conformation; (b) Hydrogen bonds that can be involved in chiral selectivity.

CSP is usually a mixture of miscible organic solvents such as hexane and isopropanol. Differences in the migration speeds of the components of a mixture are caused by differences in polar interactions between these components and the two phases. In chiral chromatography, other interactions are also operative. In fact, a prerequisite for chiral resolution is that a transient diastereoisomeric association must occur between the CSP and at least one of the analyte enantiomers. These transient species differ geometrically, and can therefore differ in their free energy of formation, leading to different speeds of migration. Types of interaction between the enantiomeric solute and the CSP are hydrogen bonding, dipole stacking, steric repulsion and π-π interactions. In the case of

TABLE I
VARIATION IN RETENTION TIME WITH THE RATIO OF HEXANE TO ETHANOL, AT 1 ML MIN^{-1}



(1) R_1=OH, R_2=H, n=1
(2) R_1=H, R_2=OH, n=1
(3) R_1=OH, R_2=H, n=2

Compound	Hexane : Ethanol	$t_A$min		$t_B$min	α
1	75 : 25	39.4		44.1	1.15
2	75 : 25		32.7		
3	75 : 25	34.1		35.1	1.03
1	80 : 20	60.2		67.2	1.13
2	80 : 20		50.1		
3	80 : 20	48.3		50.1	1.04
1	85 : 15	113.3		126.4	1.12
2	85 : 15		88.1		
3	85 : 15	92.6		96.2	1.04

molecules **1** to **3**, the absence of aromatic and bulky groups precludes the occurrence of either π-π interactions or steric repulsion, respectively. Moreover, the presence of two amide residues and a hydroxyl group in these molecules increases the possibility of hydrogen bonding being involved in the stereoselective discrimination between the optical isomers.

As published in an earlier report [4] and as shown in Table 1 the triphenyl-substituted cellulose column gives excellent resolution of the enantiomers of **1** (Fig. 6), unlike the case of the positional isomer **2** where no chiral resolution is observed under a variety of chromatographic conditions. Again, the optical isomers of **3** are only partially resolved. With the aid of molecular modelling, we have attempted to identify possible interactions that can contribute to energy variations in the formation of transient diastereoisomeric complexes and hence to differences in chromatographic behaviour.

Figure 7 shows a model of the transient complexation of **1** with a D-glucose unit where the three hydroxy groups have been replaced by phenylcarbamate substituents. It is only in the case of the R-enantiomer that a three-point interaction (a prerequisite for chiral recognition) is possible. This interaction involves hydrogen bonding between the 'host' and 'guest' molecules. For the S-isomer, the hydroxy group is not suitably positioned to hydrogen-bond with the chiral support. This differentiation between the two antipodes is in agreement with their order of elution (Fig. 6).

As stated earlier, the chromatographic behaviour of the enantiomers of **2** is very different from that shown by the enantiomers of **1**, despite the close structural similarity of the molecules. The absence of chromatographic resolution of the enantiomers of **2** is undoubtedly due to an ortho-effect. The hydroxyl group in these molecules is involved in internal hydrogen bonding with the carbonyl group of the external amide (Fig. 8). This internal recognition process will be entro-

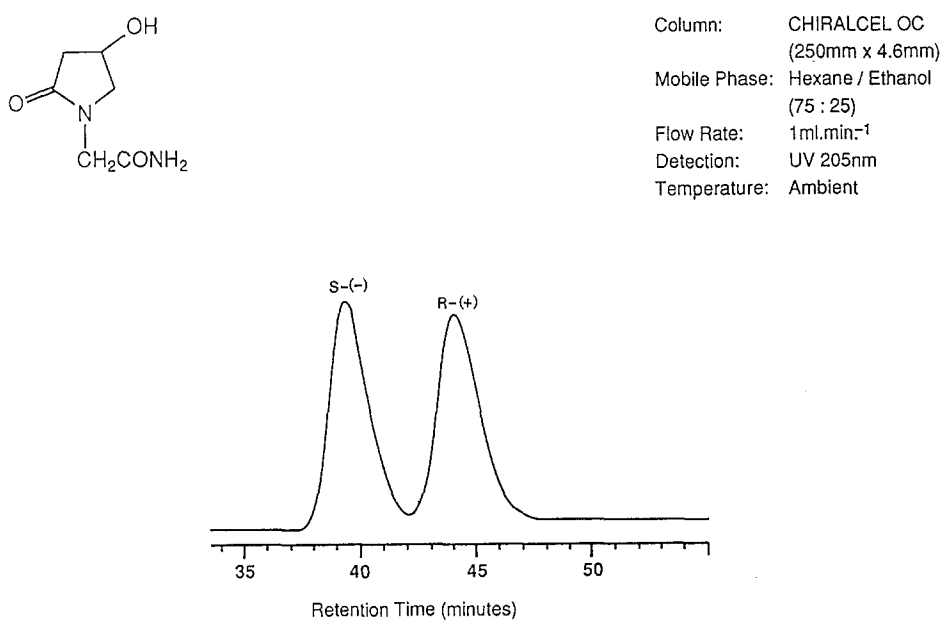


Fig. 6. Chromatogram of (1) resolved on a CHIRALCEL OC column (for experimental conditions see Ref. 4).

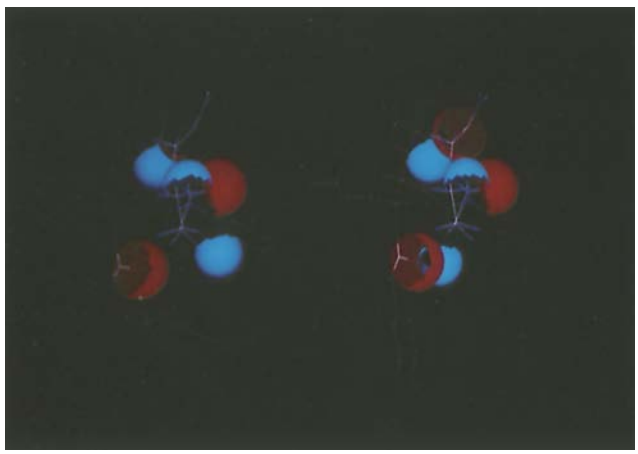


Fig. 7. Model of the possible transient complexation of (1) with a D-glucose unit in the phenylcarbamate-substituted cellulose.

pically preferred to external recognition. This effect is also seen in the mean retention times of the two pairs of enantiomers (Table 1). Molecules **2** elute faster than **1** as again substituents in the former are available to a lesser extent for noncovalent bonding with the stationary phase.

A model for the possible complexation of the enantiomers of **3** with the chiral support is shown in Fig. 9. As in the case of the enantiomers of **1**, chiral resolution is predicted. In practice, only partial resolution of the enantiomers is obtained. This may be due to the extra methylene group in the primary amide chain increasing the degrees of freedom of this group involved in the three-point interaction process and thus decreasing the difference in the free energy of formation of the two enantiomers.

The chromatographic behaviour of the enantiomeric forms of **1**, **2** and **3** on a cellulose-based column and the interpretation of these data using detailed chiral recognition models and molecular graphics have proved to be useful to identify some of the possible stereoselective interactions

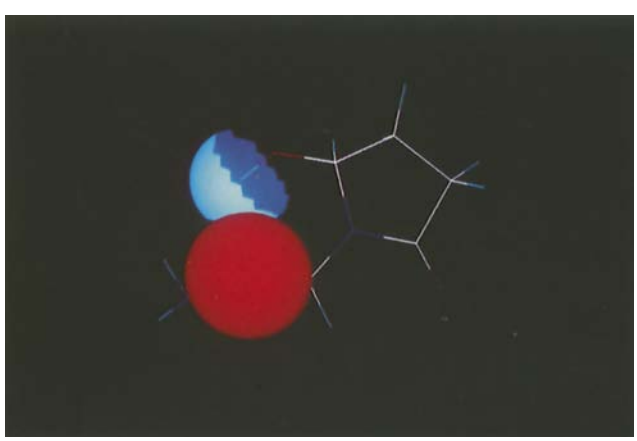


Fig. 8. Internal hydrogen bonding in molecule (2).

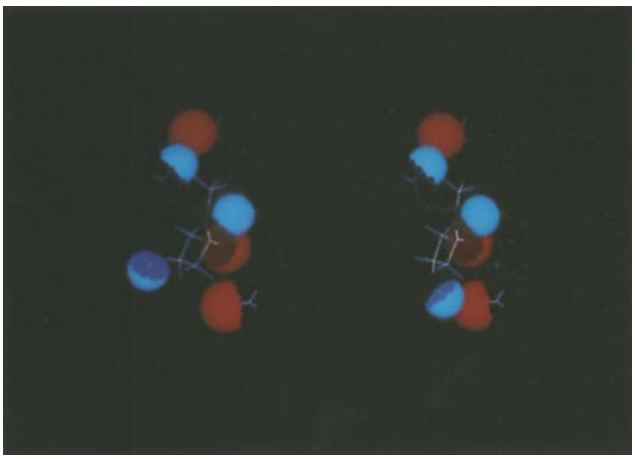


Fig. 9. Possible transient diastereoisomeric complexation of (3) with the chromatographic chiral stationary phase.

that can lead to the separation of enantiomers. Understanding separation mechanisms and chiral selectivity is especially of interest in the characterisation of chiral columns and in predicting with greater certainty the choice of a chiral column for a particular enantiomeric resolution.

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