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Note

Direct chromatographic separation of the enantiomers of oxiracetam

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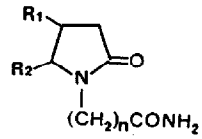
Oxiracetam (4-hydroxy-2-oxo-1-pyrolidineacetamide) (**1**) is a new drug that has neurochemical properties mostly expressed in the improvement of both learning and memory[1,2].



(**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

As oxiracetam has a chiral centre, a detailed investigation of both its pharmacokinetic and pharmacodynamic properties could require a knowledge of the individual behaviour of each of the enantiomers under a variety of biological conditions. As a first step of achieving this a method has to be developed that would allow the chromatographic separation of these optical isomers, preferably without derivatisation. In this paper we describe the direct separation and characterisation of the enantiomers of **1** on a chiral stationary phase. We also compare this chromatographic separation with that of two of its closely related molecules, namely, 5-hydroxy-2-oxo-1-pyrolidineacetamide (**2**) and 4-hydroxy-2-oxo-1-propionemide pyrolidine (**3**) in order to collect information about the possible mechanism of retention and chiral recognition.

EXPERIMENTAL

n-Hexane (Rathburn) and absolute ethanol (James Burrough) were degassed with helium before use. Oxiracetam (**1**) and its derivatives **2** and **3** were supplied by Dr. M. Pinza (ISF, Trezzano sul Naviglio, Italy).

High-performance liquid chromatography (HPLC)

HPLC analysis of compounds **1–3** was carried out on a Perkin-Elmer Series 4 liquid chromatograph. A Chiralcel OC (cellulose triphenylcarbamate) column (250 mm × 4.6 mm I.D.) gave the best resolution of the optical isomers of **1** and **3**, using hexane-ethanol at ratios 75:25, 80:20 and 85:15 (as appropriate, see Table I) flowing

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TABLE I

VARIATION IN RETENTION TIMES OF **1**, **2** AND **3** WITH VARIATION IN THE RATIO OF HEXANE TO ETHANOL AT 1 ml min^{-1}

Compound	*Hexane–ethanol*	*Retention times (min)*	*Separation factor*
1	75:25	39.4, 44.1	1.15
2		32.7	
3		34.1, 35.1	1.03
1	80:20	60.2, 67.2	1.13
2		50.1	
3		48.3, 50.1	1.04
1	85:15	113.3, 126.4	1.12
2		88.1	
3		92.6, 96.2	1.04

at a rate of 1 ml min^{-1}. The chromatography column was operated at ambient temperature and compounds were detected at 205 nm.

RESULTS AND DISCUSSION

The chromatographic resolution of the optical isomers of oxiracetam (**1**) is shown in Fig 1a, using a Chiralcel OC column and hexane–ethanol (75:25) as eluent. This column was the only one that gave excellent separation of the enantiomers. No resolution was obtained using a variety of other columns, such a Chiralcel OJ or OF, β-cyclodextrin and α_1-acid glycoprotein.

The separation factor α from the data in Fig. 1a was calculated as 1.15. The first and second peaks were assigned as $S(-)$ and $R(+)$ by comparison to authentic samples of the respective enantiomers (kindly supplied by Dr. M. Pinza, ISF).

Oxiracetam (**1**) is a relatively simple molecule. The absence of one or more aromatic groups in this molecule precludes the occurence of any π–π interactions with the phenyl carbamate residues on the cellulose adsorbed on the column silica gel support. However, the abundance of polar groups, the pyrolidone ring itself and the polar substituents –OH and $-CH_2\,CONH_2$, increases the possibility of chiral discrimination taking place due to "three-point" interaction(s)[3] solely involving hydrogen bonding and/or dipole interactions between **1** and the ciral stationary phase. Such interactions are expected to be stronger when the eluting solvent is relatively non-polar.

Fig. 1b shows the chromatogram of **2**, and isomer of oxiracetam, where the hydroxy substituent on the 2-pyrolidone ring (and hence the chiral centre) is closer to the nitrogen of the lactam moiety. No chiral resolution of the optical isomers of **2** could be obtained as shown in Table I. It is also clear from this table that the retention time of **2** is considerably shorter than that of either of the optical isomers of **1** under the same chromatographic conditions. These differences in retention behaviour are almost certainly due to differences in the hydrophobic nature of the two positional isomers. It is only in the case of molecule **2** that hydrogen bonding is possible between the hydroxy group and the carbonyl moiety in the substituent attached to the lactam nitrogen (Fig. 2).

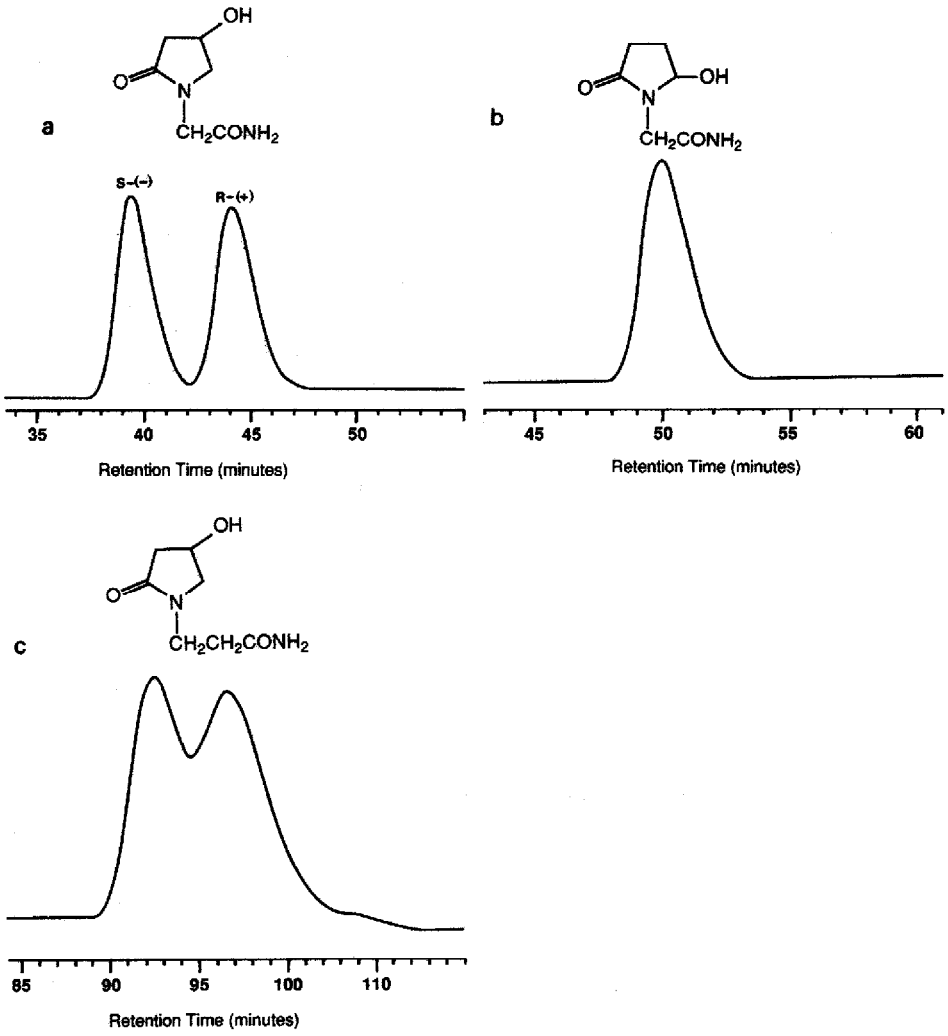

Fig. 1. (a) Chromatographic resolution of oxiracetam (**1**). Eluent, hexane–ethanol (75:25, v/v). (b) Chromatogram obtained for **2**. Eluent, hexane–ethanol (80:20, v/v). (c) Separation of the optical isomers of **3**. Eluent, hexane–ethanol (85:15, v/v). Other conditions given in the experimental section.

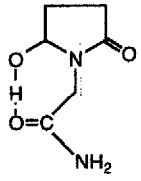

Fig. 2. Proposed hydrogen bonding that can occur in **2**, preventing the resolution of the optical isomers of this molecule.

The hydroxyl and carbonyl groups in **1** are too far apart for this type of intramolecular hydrogen bonding to occur. Moreover, the hydrogen bonding in **2** is expected to be favoured under non-polar conditions of chromatography. "Tying in" the hydroxyl group in intramolecular bonding hinders it and the amide carbonyl group from hydrogen bonding to the chiral support, thus allowing **2** to elute faster than oxiracetam (**1**).

The lack of separation of the optical isomers of **2** may also be an indication of the importance of the hydroxyl substituent (attached to the chiral centre) in the three-point interaction essential for chiral discrimination of the enantiomers of **1** by the Chiralcel OC column. We have carried out preliminary molecular modelling studies (results not shown) that confirm the occurrence of three hydrogen bonding interactions of **1** with the chiral stationary phase, stereoselective for interactions involving the hydroxyl substituent. It is only in the *R* configuration that chiral recognition is possible. We have also modelled a number of other non-stereoselective interactions between **1** and chiral stationary phase; such interactions contribute to the extent **1** is retained on the column.

To gain further insight into the mechanism of retention of oxiracetam (**1**) on the chiral support we studied the chromatographic behaviour of **3** which has two methylene groups separating the primary amide group from the lactam. As expected, **3** has a shorter retention time than **1** (Fig. 1c) because the "extra" methylene group in **3** makes it more hydrophobic than **1**. The substituted constant π is a good measure of hydrophobicity[4] and the π values for $-CH_2CONH_2$ and $CH_2CH_3CONH_2$ are -1.68 and -1.21, respectively. The separation factor for **3** is also smaller than for **1** (Table I) indicating smaller energy differences involved in the equilibrium processes of each of the optical isomers of **3** between the mobile phase and the chiral stationary phase. These smaller differences may arise due to weaker (longer) hydrogen bond interactions of this molecule with the chiral support, leading to a low separation factor for the optical isomers of **3**.

The excellent resolution of the optical isomers of oxiracetam (**1**) make the chromatographic method outlined most suitable for studies designed to probe the enantiomeric distribution of the antipodes of **1** in biological media.

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