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Short Communication

Chiral stationary phases and circular dichroism detection in high-performance liquid chromatography: determination of stereochemical purity of drugs*

CARLO BERTUCCI, CARLO ROSINI, DARIO PINI and PIERO SALVADORI†

Centro di Studio del C.N.R. per le Macromolecole Stereordinate ed Otticamente Attive, Dipartimento di Chimica e Chimica Industriale, via Risorgimento, 35, 56100 Pisa, Italy

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Introduction

About one-half of the 2050 pharmaceutical substances reported in the U.S. Pharmacopoeial Dictionary of Drug Names [1] are chiral compounds from both natural or synthetic origin. Chirality gives rise to the problem of stereoisomerism [2]: for instance, a widely used drug such as propranolol, the second most prescribed drug in the USA [3], exists as a pair of enantiomers. The main consequence of chirality for substances with biological activity is that the effects of the (+) enantiomer may be completely different from the effects of the (−) enantiomer, as demonstrated by several examples in the field of pharmacology [4]. This difference may be, in certain instances, particularly relevant because one of the antipodes can be toxic to the host, whilst the other shows its expected pharmaceutical properties. The tragic case of thalidomide [4] is perhaps the most striking example of the influence of chirality on the biological activity of organic molecules. The dimension of the problem has to be stressed: in the USA, 12 of the 20 most prescribed drugs and 104 of the top 200 contain one asymmetric centre [3]. For these reasons chiral pharmaceutical substances require a complete stereochemical characterization (i.e. determination of absolute configuration and enantiomeric purity) before marketing the product.

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†To whom correspondence should be addressed.

The problem of determining the stereochemical purity of a drug, as well as its absolute configuration, can be approached, at least in principle, by making use of liquid chromatography upon a chiral stationary phase coupled with a circular dichroism (c.d.) detector: from the area of the chromatographic peaks corresponding to the two enantiomers it is possible to evaluate the enantiomeric composition, whilst from the sign of the two peaks it is possible, in many cases, to determine the absolute configuration of each antipode eluted [5]. The rapidity, accuracy and sensitivity of chiral HPLC in determining the enantiomeric composition have to be pointed out: the enantiomeric purity of D-amphetamine has been determined [6] with the Pirkle chiral stationary phase [7] in 1 h using just one 10-mg tablet. As little as 0.5% of L-amphetamine can be detected in the presence of 99.5% of D-amphetamine.

Several chiral stationary phases are known in the literature [7–10] which are efficient in separating organic racemates having pharmacological interest. We have recently described [11, 12] the preparation and the use of a new chiral phase, SiSQuin, obtained by reacting γ-mercaptopropyl silanized silica with quinine. In this paper we present the performances of another chiral phase of this family, SiSQuinmei I [13], in separating some compounds having pharmaceutical interest and their analogues.

Experimental

Chromatographic resolution

The separations were carried out using a Jasco Twinkle apparatus. The absorption was obtained by means of a Uvidec-100V variable wavelength detector, whilst the c.d. detector was a Jasco J500C spectrometer, equipped with a micro-HPLC cell and a doublet of lenses to focus the light beam in the sample compartment. The two detectors were connected in series.

Preparation of the chiral stationary phase (I)

The c.s.p. I has been obtained by reacting N-methylquininium iodide with γ-mercaptopropylsilanized silica, prepared starting from LiChrosorb Si 60 (5μ, Merck, Darmstadt, FRG) and (3-mercaptopropyl)trimethoxysilane, as described elsewhere [13]. A 125 × 4 mm i.d. column was slurry packed (MeOH) by conventional techniques. The column was tested with a mixture of aromatic compounds (naphthalene, anthracene, fluoranthene, 3,4-benzopyrene), using methanol as the eluent (1 ml min^{-1}, U.V. detection at 254 nm), giving 21579 plates per metre. The chiral column remained very stable: after four months of continuous use it showed the same capacity and resolution factors for the test compounds. Mixtures of hexane, 2-propanol and methylene chloride were used as mobile phases. The solvents were HPLC grade chemicals by Merck and were filtered and degassed before use.

Preparation of compounds **1–9**

Compounds **1**, **2** and **3** were commercial samples from Aldrich (USA) and were used without further purification. Compounds **4**, **5** and **8** were obtained by Soxhlet extraction (acetone) of commercial pharmaceuticals. Compounds **6** and **7** were kindly provided by Professor W.H. Pirkle, School of Chemical Sciences, University of Illinois at Urbana-Champaign. Compound **9** was prepared as follows: to a solution of 5 g (0.024 mol) 2-

(*para*-2-methylpropyl)-phenylpropionic acid (Ibuprofen) in 50 ml anhydrous ethylether, was added 10 ml thionyl chloride at 0°C under magnetic stirring, over 30 min. After the addition was completed the solution was refluxed for 5 h. The excess thionyl chloride was removed under reduced pressure. The resulting solid was dissolved in ether and added to 5.6 g (0.06 mol) aniline in 100 ml ether. This mixture was refluxed for 3 h. After treatment with 1 M HCl to weak acidity, the ether phase was washed with a 5% Na_2CO_3 solution and dried over sodium sulphate. Evaporation of the solvent yielded a solid, which was recrystallized from methanol/water to produce a white crystalline material (70% yield), having m.p. 137–139°C.

Results and Discussion

The chiral phase I (Chart 1) has been employed to separate the compounds reported in Chart 2. Compound **1** is a structural analogue of the anti-convulsant drug mesantoin, for which different activities have been observed for the two enantiomers [14]. Compound **2** is the major metabolite of another anti-convulsant drug, methsuximide [15], and a potent activity of this type has been shown [16] also for compound **3**. Compound **4** is the diuretic drug etozoline which is metabolized to the active ozolinone. The (−) isomer shows diuretic properties, while the (+) isomer does not and actually inhibits the diuretic properties of the active enantiomer [4]. Compounds **5** and **8** are the tranquilisers oxazepam and lorazepam, while **6** and **7** are structural analogues of oxazepam. The interest in the stereochemical characterization of benzodiazepinones is related not only to the study of the relation between activity and stereochemistry, but also to the study of the mechanism of racemization of some of these molecules and of their metabolites [17]. Finally compound **9** is the anilide of the very popular anti-inflammatory drug ibuprofen. The resolution of these compounds on the chiral stationary phase I, using different mixtures of hexane, 2-propanol and methylene chloride, are reported in Table 1, whilst in Fig. 1, the resolution of **8** is shown as a representative example. The data in the table show that compounds **1**, **2**, **4**, **5** and **8** are directly and satisfactorily resolved on the present chiral phase: the resolution is good also in the case of ibuprofen, even if a simple derivatization of such an acid is required.

The availability of four different benzodiazepinone derivatives allows one examination of the dependence of the chromatographic resolution on the structure of the substrate: it is noteworthy that compounds **5** and **8**, where a free hydroxyl group is present at C(3), are efficiently separated on **I** (α = 1.17 and 1.59 for **5** and **8**, respectively). Blocking of this functional group with an acetyl group (compound **6**) or its substitution with an alkyl residue (compound **7**), strongly decreases the retention (K_1 values being 4.5 for **5**, 3.1 for **6** and 1.7 for **7**), causing also a marked reduction of the resolution. Indeed only a single peak is observable with the absorption detector for **6** and **7**, the separation of the enantiomers being indicated only by the c.d. detector. This observation points out the usefulness of the c.d. detector coupled with the standard absorption detector. First of all, it is possible to see from chiral detection that a partial resolution has been obtained for compounds **3**, **6** and **7**. The enantiomer showing negative c.d. at 254 nm is eluted first in the case of **3** and **6**, whilst for **7** the positive enantiomer is retained less. Secondly, it is possible to establish the absolute configuration of the enantiomer corresponding to each peak observed, i.e. the determination of the elution order [5]. This order can be easily established for compound **1** considering that the c.d. data on some phenylalkyl substituted hydantoins have been reported [18], e.g. (+)-(S)-5-cyclohexyl-5-phenyl-

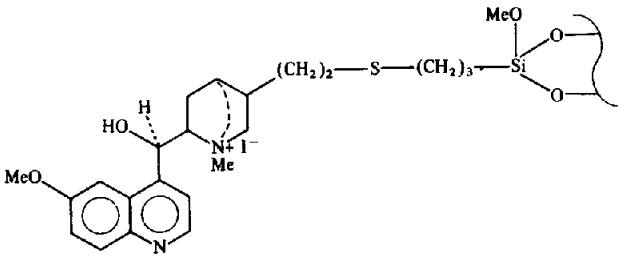
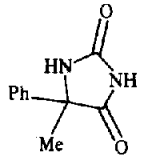
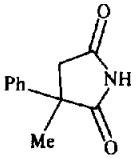


Chart 1
Chiral phase I



1



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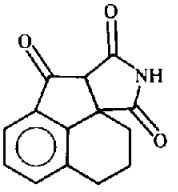

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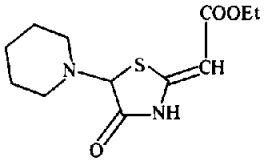

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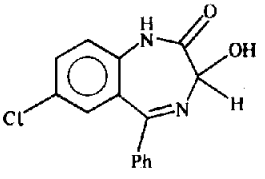

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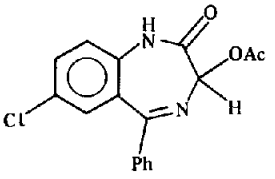

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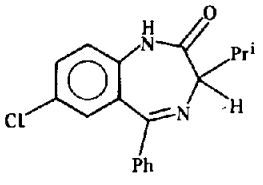

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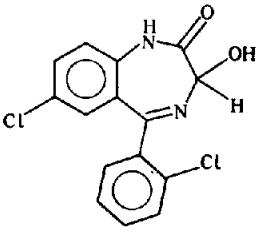

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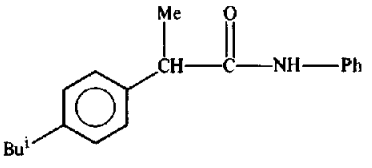

9

Chart 2

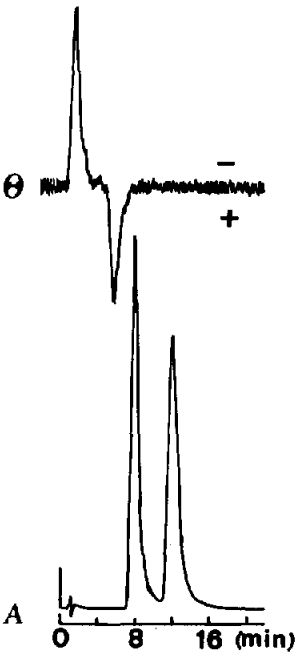

Figure 1
The resolution of lorazepam, **8**, on the chiral stationary phase I. Eluent: hexane–2-propanol–CH_2Cl_2 (100: 28:24; v/v/v). Absorption (A) and c.d. (Θ) detection at 254 nm.

Table 1
Resolution of some pharmaceutical compounds and analogues

Compounds	Eluent composition (ml) n-C_6H_{14}	Pr^iOH	CH_2Cl_2	K_1	α	CD sign*	Absolute configuration
1	80	20	0	5.0	1.14	−	R
2	95	5	5	8.0	1.09	−	—
3	100	28	24	7.0	1.00	−	—
4	90	2	8	3.7	1.13	−	—
5	100	28	24	4.7	1.17	−	R
6	100	28	24	3.1	1.00	−	R
7	100	28	24	1.7	1.00	+	S
8	100	28	24	5.7	1.59	−	R
9	90	10	0	6.6	1.09	+	—

*First eluted enantiomer, λ 254 nm.

hydantoin shows positive c.d. at 254 nm indicating then that for **1** the first eluted peak (negative c.d. at 254 nm, Table 1) is due to the (R) enantiomer. However similar correlations cannot be made in the case of **2**, **3** and **4** for which, to the best of our knowledge, no reference compounds are available in the literature. As far as the group of benzodiazepinone derivatives is concerned, the elution order can be established considering that it has been reported [19] that (S)-**7** shows positive c.d. at 260 nm. Taking into account that the structures of compounds **5–8** are very similar, from a spectroscopic point of view it can be reasonably assumed that a positive c.d. in the range 250–260 nm can be then correlated to the (S) stereochemistry. The negative c.d.s observed corresponding to the first eluted enantiomer for compounds **5**, **6** and **8** indicate that in

these cases the (R) enantiomer is the less retained one. On the contrary, the same enantiomer is the most retained for compound **7**.

The results discussed in the present paper strongly indicate the usefulness of Cinchona alkaloids and/or their derivatives in preparing efficient chiral phases which can find interesting applications in the field of quality control of optically active drugs. Actually, since the different behaviour of enantiomeric drugs is nowadays quite well documented, the demand for optically pure compounds is increasing in the field of pharmacology. It is noteworthy that the chromatographic resolution of the racemates allows separation of small amounts of the two enantiomers of a new compound whose biological properties have to be tested. The combined use of the chiral stationary phase I and of the c.d. detector looks a very promising technique considering that in many cases the absolute configuration can be directly assigned to the eluates [5], which is of importance in the study of the relationship between stereochemistry and the pharmacological properties of a drug.

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