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Note

High-performance liquid chromatographic resolution of the enantiomers of thioridazine, its metabolites and related compounds

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The neuroleptic thioridazine is chiral (Fig. 1.). The enantiomers have been resolved by fractional crystallization as the di(*p*-toluoyl) tartrate salts [1] but this is impractical for thioridazine extracted from biological material. The compound is a tertiary amine and has no functional groups suitable for derivatization to diastereoisomers. We report the use of a chiral high-performance liquid chromatographic (HPLC) stationary phase to resolve thioridazine and some of its metabolites. The resolutions of related compounds were investigated in an attempt to understand the structural requirements for optical recognition on this chiral stationary phase.

EXPERIMENTAL

Reagents and reference materials

HPLC-grade methanol, acetonitrile and dichloromethane (stabilized with pentene) were purchased from Fisons Scientific Apparatus (Loughborough, U.K.). Thioridazine, thioridazine-5-sulphoxide, thioridazine-2-sulphoxide (mezoridazine), thioridazine-2-sulphone (sulforidazine) and N-desmethylthioridazine were gifts from Sandoz (Feltham, Middlesex, U.K.). Dr. Maurer (Sandoz, Basle, Swizerland) provided (+)- and (−)-thioridazine. Diastereoisomeric 5-sulphoxides were obtained by oxidizing the appropriate sulphide with nitrous acid [2] and separated by thin-layer chromatography (silica; chloro-

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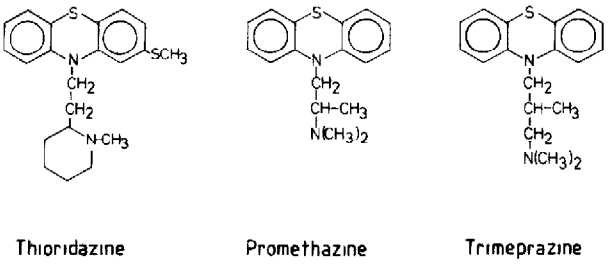

Fig. 1. Structures of thioridazine, promethazine and trimeprazine.

form–methanol–18 *M* ammonium hydroxide, 80:10:1, v/v). Promethazine, ethopropazine, trimeprazine, methotrimeprazine and trimiprazine were gifts from May and Baker (Dagenham, U.K.).

High-performance liquid chromatography

An Altex Model 330 isocratic system with fixed-wavelength (254 nm) UV absorbance detection was used. The column (250 mm×4.6 mm I.D.) which contained *R*-N-(α)-phenethyl-N-propylurea covalently bonded to Spherisorb 5-μm silica (Spherisorb S5 Chiral 1) was a generous gift from Phase Separations (Queensferry, U.K.). Several eluents were evaluated, including methanol [3], methanol–water mixtures, methanol–acetonitrile mixtures, dichloromethane–methanol mixtures and mixtures of hexane–dichloromethane–methanol.

Biological material

Rats received thioridazine hydrochloride in their drinking water for up to twelve months. Drug solutions were made up daily and stabilized with ascorbic acid (10% of drug weight). Black water bottles were used to prevent photo-oxidation. Body weight and water intake were monitored regularly and thioridazine concentrations adjusted to maintain a daily intake of ca. 50 mg/kg.

Rat livers were homogenized in 1.15% (w/v) potassium chloride in phosphate buffer, pH 7.6. Tissue homogenate (equivalent to 1 g tissue) was made alkaline with 2 *M* sodium hydroxide (1 ml) and extracted with heptane containing 1.5% (v/v) 1-pentanol (5 ml). The organic phase (4 ml) was evaporated to dryness at 50°C under nitrogen and reconstituted in methanol prior to HPLC.

RESULTS AND DISCUSSION

Using methanol as eluent, desmethylthioridazine was fully resolved but thioridazine, thioridazine-2-sulphone and thioridazine-2-sulphoxide were only partially resolved [3]. The addition of acetonitrile or water to methanol, in various proportions, did not improve the resolution. The addition of up to 50% (v/v) acetonitrile to methanol had little effect on the chromatography. The compounds did not elute with 100% acetonitrile. Addition of dichloromethane to methanol had a marked effect on the retention of thioridazine. In methanol–dichloromethane (50:50) the capacity factor (k') was ca. 1.4 compared with

TABLE I

SUMMARY OF CHROMATOGRAPHIC PROPERTIES

k_1' = Capacity factor of the first eluting isomer; α = separation factor (k_2'/k_1'); R_s = resolution.

Compound	k_1'	α	R_s
Thioridazine	6.7	1.12	1.1
Desmethylthioridazine	12.2	1.35	2.6
Thioridazine-2-sulphone	8.2	1.10	0.8
Thioridazine-2-sulphoxide	14.3	1.12	1.0
Thioridazine-5-sulphoxide (1)*	12.0	1.06	0.5
Thioridazine-5-sulphoxide (2)	12.7	1.06	0.5
Promethazine	3.8	1.13	0.8
Ethopropazine	5.2	1.06	0.4
Trimeprazine	2.1	1.00	–
Methotrimeprazine	1.7	1.00	–
Trimipramine	1.3	1.00	–

*Slower migrating diastereoisomer on TLC plate.

ca. 7 for 100% methanol. With 75% dichloromethane k' was less than 1, but then increased as the proportion of dichloromethane was increased to 0.9 ($k' \sim 1.3$) and 0.95 ($k' \sim 2.2$). Thioridazine did not elute with 100% dichloromethane. Hexane was added to the methanol–dichloromethane mixture in an attempt to increase the retention time and improve the resolution of the isomers. This was achieved with hexane–dichloromethane–methanol (45:45:10, v/v). The capacity factors increased with little change in relative retention α (Table I) of the enantiomers. The peak shapes were improved, despite the increased retention volume, so that almost baseline resolution of thioridazine enantiomers was obtained (Fig. 2).

Hexane–dichloromethane–methanol (45:45:10, v/v) was used as eluent for the separations presented in Figs. 2–4 and Table I. Desmethylthioridazine enantiomers were completely resolved (Fig. 2). (+)-Thioridazine eluted first. Fig. 3 shows the chromatographic behaviour of some of the oxide metabolties of thioridazine. Thioridazine-2-sulphone (sulforidazine) enantiomers were not as well resolved as those of the parent compound, although the retention volumes were greater. The resolution of the 2-sulphoxide (mezoridazine) was better, being similar to thioridazine (Table I). Oxidation of thioridazine with nitrous acid produces the 5-sulphoxide and as the sulphur is chiral there are four possible isomers [2,3]. The reaction mixture gave three peaks when chromatographed on the chiral column (Fig. 3, bottom). This was shown to be due to the retention volume of the second eluting enantiomer of one diastereoisomer being the same as the retention volume of the first eluting enantiomer of the other diastereoisomer pair. From the appearance of the chromatographic traces of the diastereoisomers injected individually the four isomers appeared to have been formed in equal quantities.

It is not possible to be precise about the mechanism of the chiral separation but some clues can be obtained by considering the behaviour of the different

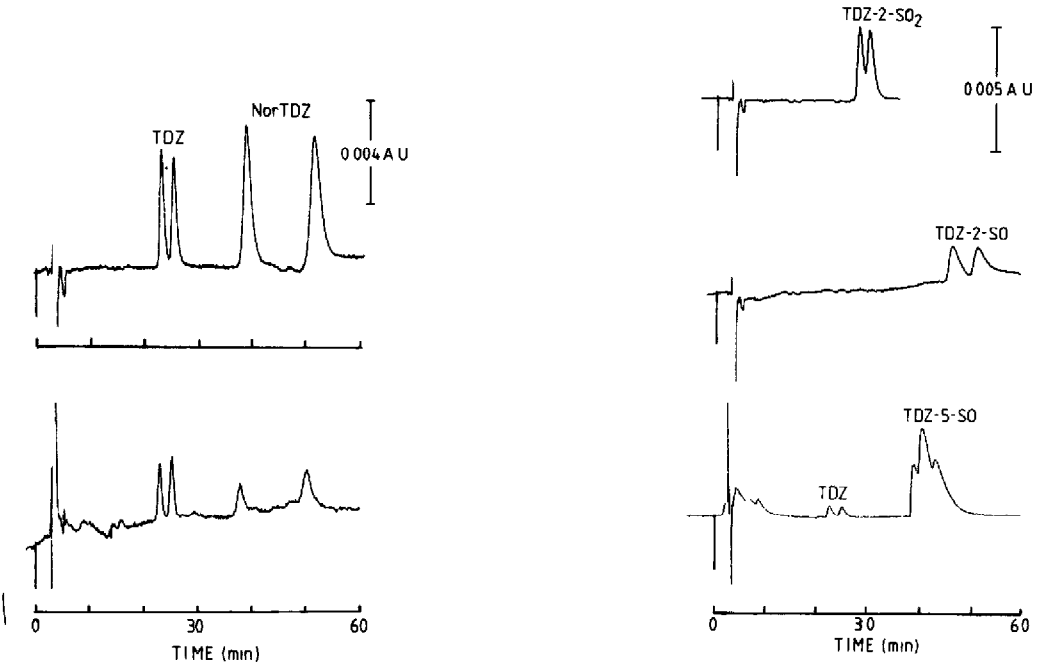

Fig. 2. (Top) Resolution of thioridazine (TDZ) and desmethylthioridazine (NorTDZ) reference compounds. (Bottom) Chromatogram of heptane-extractable material from homogenised liver from rat dosed with TDZ. Conditions: mobile phase, hexane–dichloromethane–methanol (45:45:10, v/v); flow-rate, 1.0 ml/min; UV detection, 254 nm.

Fig. 3. Chromatograms of thioridazine-2-sulphone (top), thioridazine-2-sulphoxide (middle) and thioridazine treated with nitrous acid to produce 5-sulphoxide isomers (bottom). Conditions as in Fig. 2.

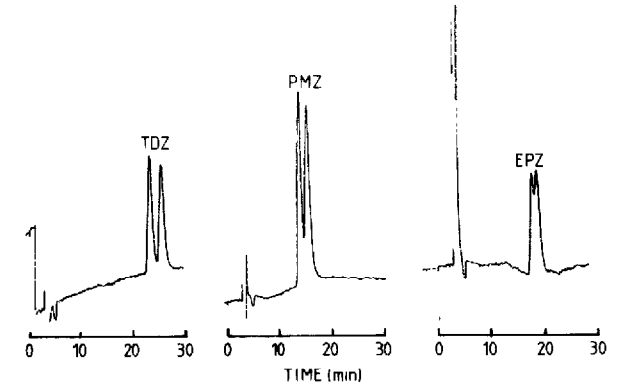

Fig. 4. Comparison of the resolution of thioridazine (TDZ), promethazine (PMZ) and ethopropazine (EPZ) isomers. Conditions as in Fig. 2.

structures. According to the Dalgliesh model [4] three sites of interaction between the analyte and the chiral stationary phase are necessary for chiral separation. The likely interactions on this type of column are π-bonding between aromatic rings, hydrogen bonding, dipole–dipole interactions and steric hindrance. The thioridazine compounds can be ranked in order of the resolution of the enantiomers (Table I). This order, desmethylthioridazine > thioridazine = thioridazine-2-sulphoxide > thioridazine-2-sulphone > thioridazine-5-sulphoxide cannot be related to the elution volumes. The reduced resolution of the 2-sulphoxide and 2-sulphone compounds relative to the parent compound may be due to the increased electron-withdrawing effects or steric effects influencing the π-interaction.

Compared to the tertiary amine compounds, the secondary amine desmethylthioridazine was well resolved in the majority of the eluents tested. This could be due to several factors: changed basicity of the nitrogen, hydrogen bonding to the N–H group or reduced steric hindrance. The fact that steric hindrance by nitrogen substituents may reduce chiral recognition can be seen by comparing the resolution of promethazine enantiomers and those of ethopropazine which differs from promethazine (Fig. 1) in having N,N-diethyl substituents rather than N,N-dimethyl (Fig. 4, Table I). Trimeprazine (which differs from promethazine by the insertion of a methylene group between the chiral carbon and the amine), methotrimeprazine (2-methoxytrimeprazine) and trimiprazine enantiomers were not resolved. These compounds had low k' values (Table I). It would appear that for chiral recognition on this stationary phase the analyte should contain an aromatic ring close to the chiral centre and an amine adjacent to it.

The column is suitable for samples extracted from biological materials as seen from Fig. 2 (bottom). The concentration of (−)-thioridazine was slightly greater than the (+)-isomer. It is not possible from this study to say whether this is due to enantiomer differences in metabolism or distribution. The limit of detection (three times the noise) for thioridazine was 10 ng (i.e. 5 ng of each enantiomer). Replicate injections ($n=5$) of 20 ng (±)-thioridazine gave coefficients of variation of 12.3 and 13.8% for the (−)- and (+)-isomers, respectively. For 100-ng injections the corresponding values were 1.9 and 2.2%.

The column appears to be reasonably robust. We have used it intermittently since March 1983 during which time 3-hydroxybenzodiazepines (oxazepam, temazepam and lorazepam), local anaesthetics (prilocaine, bupivacaine and mepivicaine) and the analgesic nefopam and its desmethyl metabolite have been partially resolved. The column is currently being used to evaluate the use of derivatives of chiral compounds. A guard column was not used for the work reported here but a Whatman Co-Pell guard column has been used without appreciable adverse effects.

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