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SEPARATION AND IDENTIFICATION OF TOFISOPAM STEREOISOMERS BY HYPHENATED HPLC-CD TECHNIQUE

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ABSTRACT

The published method allows the separation of the four isomers of the anxiolytic 2,3-diazepine derivative, tofisopam. Assignment for the peaks of the two central and two helical isomers is performed by using the chromatogram of the pure tofisopam enantiomers and also considering the conformer-equilibrium was suggested by previous authors. The detection has been made by CD. The in situ CD spectra of the peaks, obtained by stop-flow method, definitely show different chiral character.

INTRODUCTION

The stereochemistry of the 2,3-benzodiazepine derivative anxiolytic, tofisopam (Grandaxin^R, Fig. 1) has been the subject of NMR¹ CD² and X-ray³ investigations. As C(5)-atom generates a central chirality in the molecule, the diazepine-ring effects the existence of two helical conformers.⁴ It was suggested, that in solid state the C(5)-C₂H₅ should exist in the preferred pseudo-equatorial position. While the dissolution of tofisopam results in the formation of an equilibrial mixture of both conformers.⁵⁻⁸

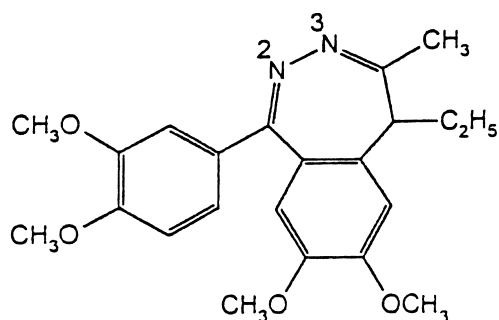


Figure 1. Tofisopam [1-(3,4-dimethoxyphenyl)-5-ethyl-7,8-dimethoxy-methyl-5H-2,3-benzodiazepine].

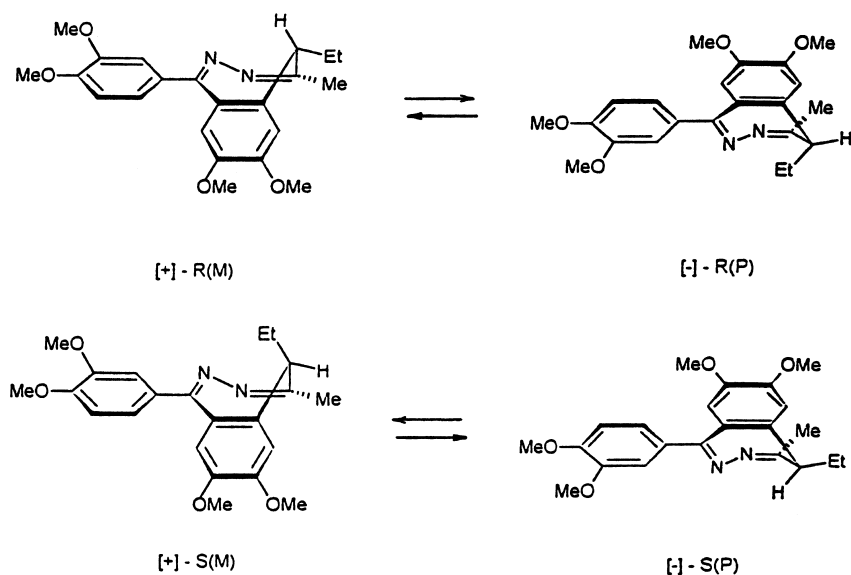


Figure 2. Stereoisomeric equilibrium in (±)-tofisopam solution [sign of optical rotation].

Since the enantiomer-conformer pairs (Fig. 2) R(M), R(P), and S(M), S(P) are in diastereomer relation, they could be separated even in achiral chromatographic system by TLC⁴ and HPLC^{7,9} as well. Conformation P defined by the positive sign of C(1)-N(2)-N(3)-C(4) torsion angle. However, the overall separation of the four isomers evidently needs the application of chiral analysis.

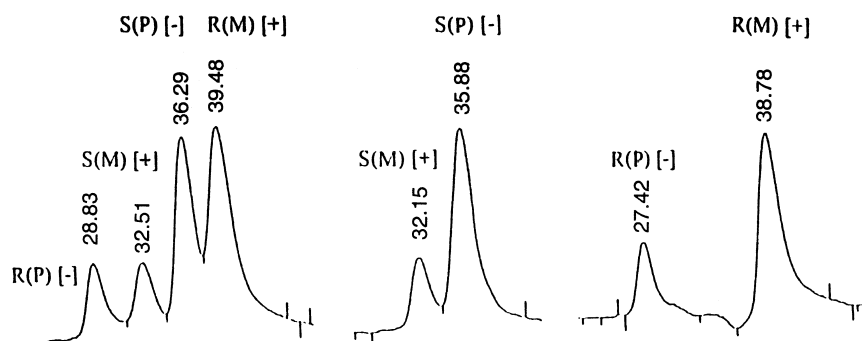


Figure 3. Separation of tofisopam stereoisomers. From the left: (±)-tofisopam, S-(-)-tofisopam, R-(+)-tofisopam, 0.1 % methanolic solutions. Other conditions, see in Experimental.

Visy and Simonyi⁴ could assign four peaks analyzing the ethanolic solution of tofisopam by affinity chromatography on human serum albumin stationary phase. The present paper reports about the complete and sharp separation of the four tofisopam stereoisomers, using a normal phase chromatographic system. The assignment of the peaks was made utilizing the advantage of the CD method^{10,11} by the analysis of in situ CD spectra of the peaks.

EXPERIMENTAL

Instrumental

The HPLC apparatus comprised a JASCO intelligent pump type PU-980, combined with a Rheodyne 7725 injector with 20 μ L loop. JASCO spectropolarimeter type J-720 equipped with flow through cell, length 0.5 cm, suitable for the simultaneous detection of CD-ORD and UV was used. Detector operated at 250.2 nm. The sorbent Chiralcel OJ (Daicel) was purchased (Baker, Deventer, Netherland) in a stainless column, 5 μ m particle size, 250 x 4.6 mm I.D.

The samples were injected from 0.1 % (for UV detection) or 0.4 % (for CD detection) methanolic solutions. The equipment units subsequent to the pump, were thermostatted at 25 (\pm 0.1) $^{\circ}$ C (Column chiller Model 7955, Jones Chromatography, Wales) As mobile phase sonically degassed and filtered mixture of n-hexane, 2-propanol and methanol (72 : 1.5 : 3) was applied. Flow rate: 0.8 mL/min.

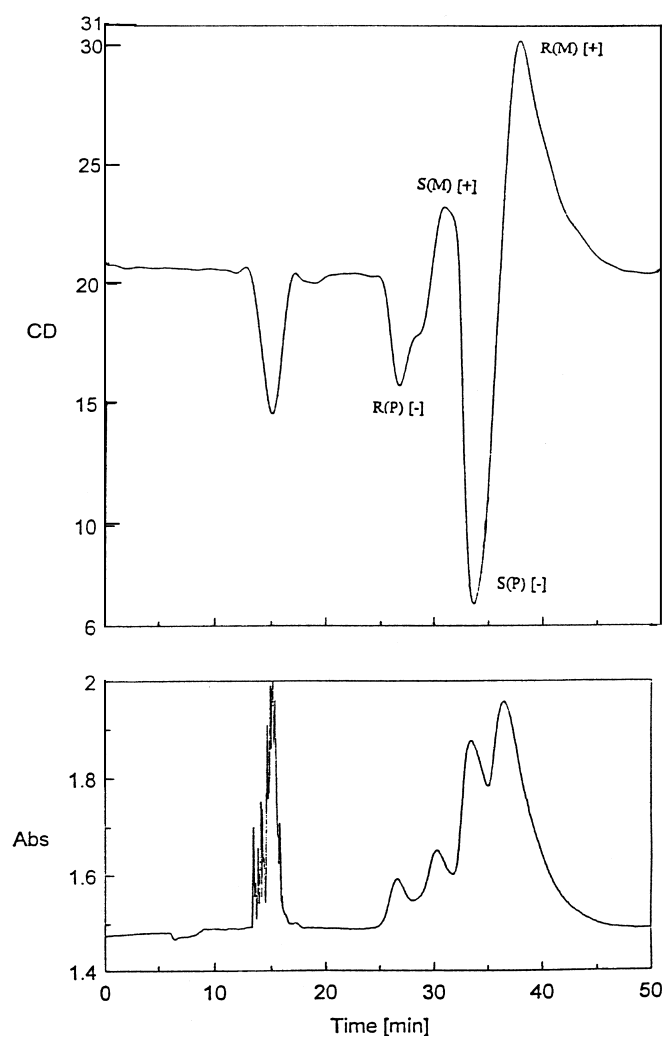


Figure 4. Separation of tofisopam stereoisomers, CD detection, 0.4 % methanolic solutions. Below: the chromatogram; above: CD spectra of the peaks. Other conditions, see in Fig. 3.

Materials

(±)-Tofisopam;* S(-)-Tofisopam;** R(+)-Tofisopam;** n-Hexane for HPLC (Chemolab, Budapest); 2-Propanol for HPLC (Chemolab, Budapest); Methanol for HPLC (Chemolab, Budapest). Note: *As gift of EGIS Pharmaceutical Works, Budapest. **As a gift of Central Research Institute for Chemistry, Budapest.

RESULTS AND DISCUSSION

The sharp separation of the four tofisopam isomers on Chiralcel OJ surface was achieved after optimization of the development conditions (eluent flow rate and composition of the mobile phase).

Like earlier¹² the addition of methanol definitely increased the chiral stereoselectivity of the commonly used n-hexane-isopropanol mixture and this time resulted in the isocratic separation of tofisopam enantiomers and their conformers as well (Fig. 3). The identification of the two enantiomer peaks might be directly performed by parallel chromatography of the pure antipodes (Fig. 3).

For the assignment of the conformer peaks the data of former investigations were used.⁴ In accordance with the published scheme of conformer equilibrium of energetically preferred conformers (Fig. 2) the major peaks developed from the methanolic solution of the pure enantiomers evidently represent the isomers as R(M) and S(P) followed by the minor peaks of the two unpreferred conformers S(M) and R(P).

This suggested order of elution strongly depends on the stereochemistry of the chirality center C(5). Due to its pseudo-axial position C(5)-C₂H₅ group may decrease the strength of the interaction between the respective adsorption site of the cellulose surface and the diazepine ring.

Just to the opposite, the rotation inhibitory effect of the pseudo-equatorial C(5)-C₂H₅ in S(M) and R(P) should be weaker, resulting in the stronger retention of these conformers. The assumptions above are confirmed by the chromatogram obtained by CD detection (Fig. 4).

The CD spectra plotted directly from the peaks (stop-flow method) evidently proves the antipode character of the separated substances. It clearly seems, that the antipode pairs generate Cotton effect curves with quite the opposite signs.

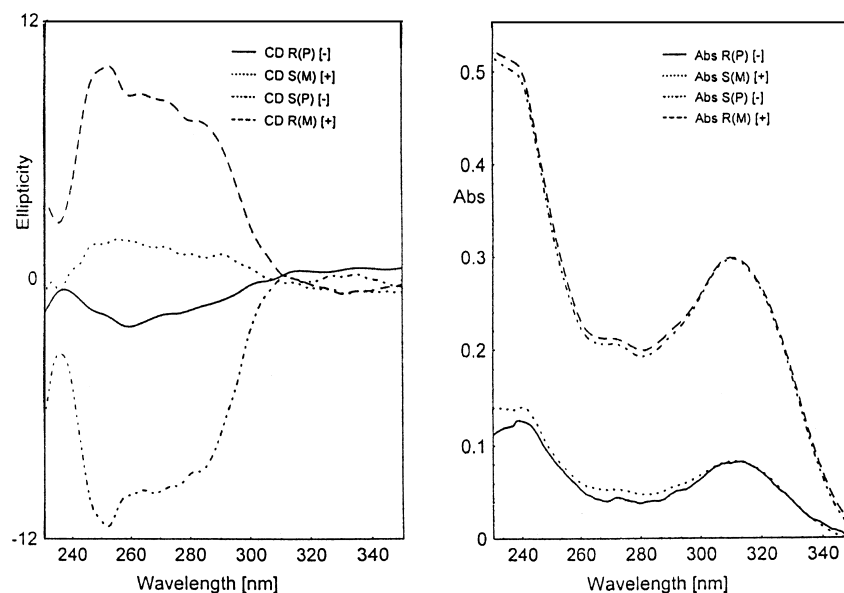


Figure 5. CD (left) and UV (right) spectra of the tofisopam stereoisomers. Other conditions see in Experimental.

Consequently, the positive or negative feature of the Cotton effect at tofisopam isomers primarily depends upon the helical chirality of the diazepine ring. The CD spectra in Fig. 5 say, that the M-conformer generates the formation of a positive, the P-conformer a negative Cotton effect curve.

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