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Resolution of the enantiomers of tetrahydrozoline by chiral HPLC. The racemization of the enantiomers via an imine–enamine tautomerism

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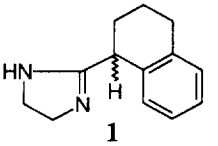
Abstract

Resolution of the enantiomers of the sympathomimetic drug tetrahydrozoline was obtained by chiral HPLC. The isolated enantiomers racemize easily and chiral HPLC experiments allowed the determination of the racemization rate constant. This process occurs via an imine–enamine tautomerism which was studied by UV and ^{1}H NMR spectroscopies.

1. Introduction

Tetrahydrozoline **1** [2-(1,2,3,4-tetrahydro-1-naphthyl)-2-imidazoline] is a sympathomimetic agent with marked α-adrenergic activity, used as a racemic mixture of the hydrochloride in pharmaceutical preparations (mainly ophthalmic solutions) as a vasoconstrictor and decongestant.[1] Its activity resides predominantly in the (−)-enantiomer.[2] Indeed, recent guidelines have been given for pharmacological evaluation of chiral drugs[3,4] because of the importance, previously underestimated,[5] of the enantioselectivity of chiral drugs with respect to the receptor sites. This prompted us to look for a direct HPLC method for the enantiomeric separation of **1**.



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During this study we isolated the individual enantiomers but soon discovered an easy racemization of them which can be followed up by chiral HPLC to determine the rate constant. This process proceeds via

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Table 1
HPLC behaviour of tetrahydrozoline on Chiralpak AD chiral stationary phase

A (%)[a]	FR[b]	K_1[c]	α	R_s
5[d]	0.7	2.684	—	NS[e]
5	0.7	1.687	1.20	1.60
5	1.0	2.108	1.15	1.17
10	1.0	0.783	1.24	1.08

[a] Percentage of ethanol doped with 2% of diethylamine in *n*-hexane.
[b] Flow rate (ml/min.); FR=0.7, t_0=5.9 min., FR=1.0, t_0=3.7 min..
[c] Capacity Factor for the the first eluted enantiomer.
[d] Percentage of ethanol in *n*-hexane.
[e] Not separated.

a tautomeric achiral enamine due to the lability of the acidic hydrogen on the stereogenic carbon of the imine form and spectroscopic evidence (UV, NMR) for the enamine form is reported.

2. Results and discussion

2.1. Chromatographic enantioseparation

Racemic tetrahydrozoline was resolved into its enantiomers on a chiral stationary phase (CSP), as shown in Table 1. The compound was injected directly as the hydrochloride in ethanolic solution. As expected, the hydrochloride eluted poorly from the HPLC column without enantioseparation. The addition of a small amount of diethylamine (DEA) to the mobile phase was crucial for good enantiomeric separation, as shown by the α values, and good resolution factor, as shown by the R_s values. DEA transforms the hydrochloride into the free base that can interact better with the active sites of the polysaccharide CSP. The beneficial effect of DEA on the enantioselectivity of basic drugs is well known and it was previously reported by us for chiral pyrroloimidazopyridine derivatives.[6] An increase in the polarity of the mobile phase has a detrimental effect on R_s, whereas a decrease in the flow rate of the mobile phase has a beneficial effect on α and R_s. In the absence of a diode array detector, the enantiomeric nature of the HPLC peaks was established by measuring the area ratio of the peaks (in a number of different experiments) with the UV detector set at various wavelengths (235, 260 and 272 nm). As expected for an enantiomeric pair, these area ratios were equal.[7] Also, stop flow scanning at many wavelengths while the maximum of the HPLC peaks was at rest in the flow cell[8] afforded the reconstruction of the UV spectra of the two eluting peaks which were equal as expected.

Only two reports concerning the enantioseparation of tetrahydrozoline were found in the literature. One method used various urea-linked Pirkle type CSPs[9] and the other one used an α_1-acid glycoprotein column.[10] Both papers were concerned with a survey of many basic drugs and details on the choice of chromatographic parameters were not given.

2.2. Racemization

The good resolution factor (R_s=1.6) obtained afforded a semipreparative separation of the enantiomers of tetrahydrozoline by repeated injections, according to the conditions in Table 1. However, an analytical rerun of the eluates from the peaks to check their enantiomeric purity indicated a gradual increase of

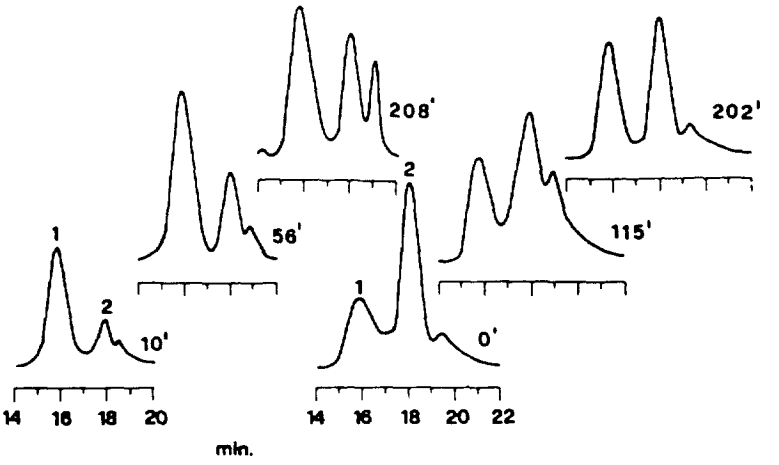


Fig. 1. HPLC profiles of the racemization course of enantiomer **1** (left) and enantiomer **2** (right). Conditions as in the second line of Table 1

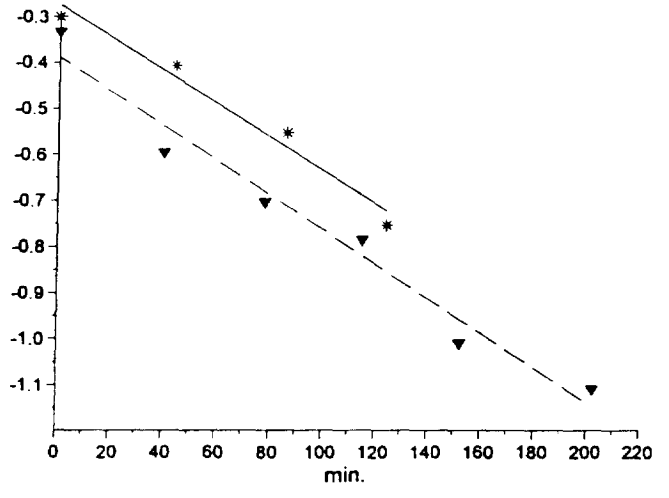


Fig. 2. Plot of $\log[(h_1-h_2)/(h_1+h_2)]$ for peak 1 (*) and peak 2 (▼) versus time, to obtain racemization constant

the second enantiomer in the peak of the first enantiomer and *vice versa*, as shown in Fig. 1. This fact prompted us to run a time-controlled experiment to determine the rate constant for racemization. The eluates of peak 1 from three consecutive injections were collected at −20°C and kept at this temperature for about 2 h. The temperature was then raised to 19°C and this was assumed as t=0 min, although racemization was in fact already in process. HPLC injections at known times t gave the change into enantiomer 2 as a function of time. Analogous experiments were performed with the eluates from peak 2.

The racemization constant was calculated from the slope of a semi-log plot of the data by means of the equation $-2kt/2.3=\log[(h_1-h_2)/(h_1+h_2)]$ where k is the interconversion constant and 2k is the racemization constant.[11] This equation is an adaptation of the usual expression[12] for a reversible, first-order reaction where the equilibrium constant K is 1. The use of heights instead of the areas of the peaks avoids the introduction of errors due to baseline drift or partial overlapping of the peaks.[13]

The obtained plots are shown in Fig. 2 giving the straight lines (R^2=0.971 and 0.970) for enantiomers 1 and 2, respectively. The slope of the lines established the value of the rate constant for racemization (2k) which resulted in 1.38×10^{-4} and 1.43×10^{-4} s^{-1} for the first and second eluted enantiomers, respectively.

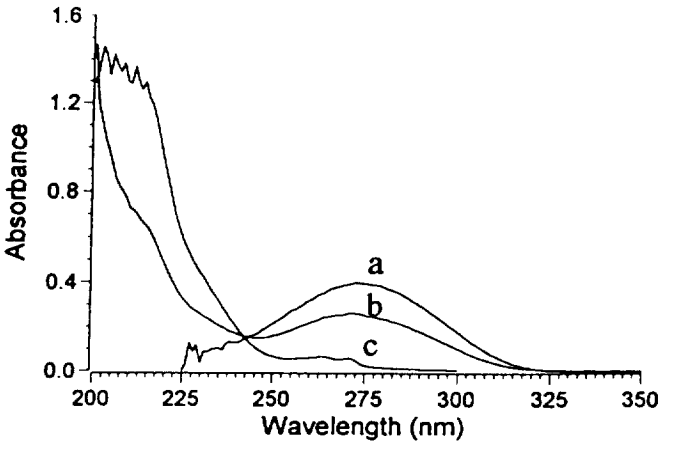
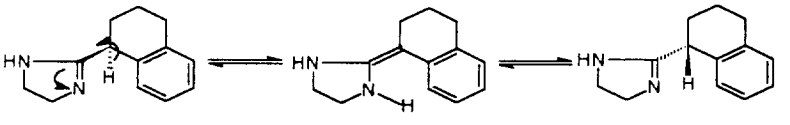


Fig. 3. UV spectra of tetrahydrozoline (1×10^{-4} M) in: (a) dioxane; (b) acetonitrile; and (c) water

2.3. *Imine–enamine tautomerism*

The existence of a tautomeric achiral enamine makes possible the interconversion between the two enantiomers of tetrahydrozoline, according to the following scheme.



(S) - Tetrahydrozoline Enamine (R) - Tetrahydrozoline

The acidic hydrogen attached to the carbon β to the nitrogen, in the presence of the DEA added to the mobile phase which acts as a basic catalyst, can migrate to the imino nitrogen and a tautomeric equilibrium is established between the secondary enamine and both enantiomeric imines. The existence of a tautomeric mixture is favoured by an α,β-unsaturation conjugated with the aromatic ring. A similar case was reported in the literature for 2-benzylpyrroline.[14] Evidence for this tautomerism came from UV and NMR spectroscopies. An α,β-unsaturated secondary amine exhibits a bathochromic and hyperchromic shift[15] in the UV spectrum which is particularly strong if the auxochromic lone pair on nitrogen is conjugated with the aromatic ring. A similar shift (at λ=278 nm) is reported when a lone pair of the nitrogen interacts with a diene conjugated system in A and B steroid rings.[16] This B band, shifted to longer wavelength (about 272 nm), has an absorbance very sensitive to the nature of the solvent since the proportion of the enamine form is much greater in non-polar solvents such as 1,4-dioxane than in polar solvent such as water and in proton-accepting solvents such as acetonitrile. This is shown in Fig. 3 where the UV spectra of tetrahydrozoline, as the free base, taken in various solvents at the same molarity, are reported. The absorbance at 270–272 nm in acetonitrile and dioxane is much higher than in water while intermediate absorbances (unreported) in the same region were obtained in 1:1 mixtures of dioxane:water and acetonitrile:water. Similar solvent effects were reported on several keto–enol equilibria.[17]

The stereochemical lability of the methine proton β to the NH group responsible for the imine–enamine tautomerism, was proven via a deuterium exchange experiment in D_2O catalyzed by NaOD. The ^1H NMR spectra of tetrahydrozoline in D_2O and NaOD are shown in Fig. 4. In the spectrum in D_2O (bottom) the methine proton appears as a triplet at δ 3.79 ppm. After addition of few drops of 0.5 N NaOD in D_2O into the NMR tube and filtration of the most part of the precipitated tetrahydrozoline, the dilute solution shows a spectrum where the methine proton signal has totally disappeared.

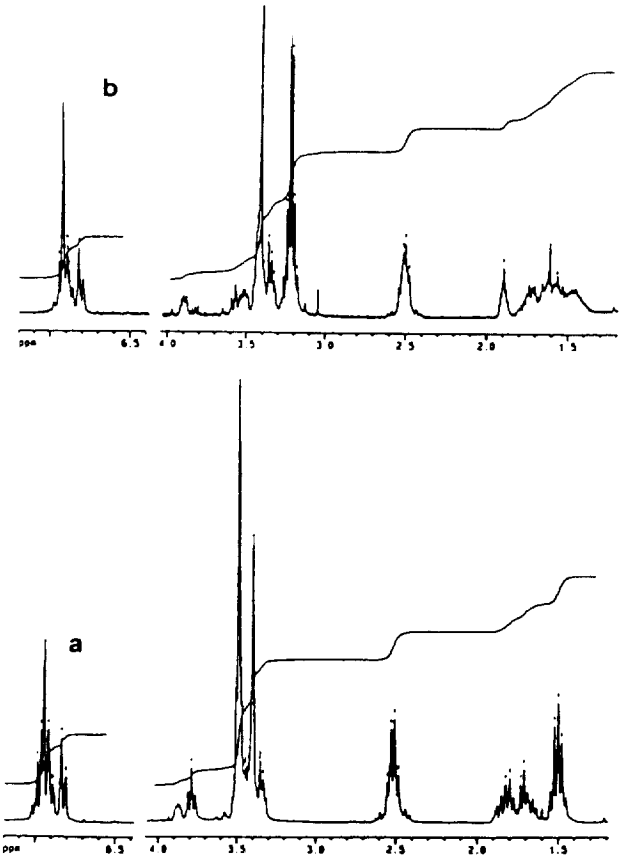


Fig. 4. ^{1}H NMR spectra of tetrahydrozoline in: (a) D_2O; and (b) D_2O+NaOD

In addition, significant differences are present in the ^{1}H NMR spectrum taken in C_6D_6 and in the spectrum in C_6D_6 after addition of three drops of NaOD (Fig. 5). Indeed, in the latter spectrum (top) a broad reproducible signal of the NH group in the enamine appears in the region between 5.6 and 6.1 ppm, similar to the NH signal in the enamine $C_6H_5C(CH_3)$=CH–NH–CH_3,[18] while the NH signal of the imine form exchanges with D_2O. The NH enamine signal becomes more intense after successive additions of one, two or three drops of NaOD, with the area ratio between this signal and that of the methine of the imine form at δ 3.86 ppm (same value as in C_6D_6) becoming larger. Further, the methylene signal of the imidazoline ring observed as a multiplet at δ 3.17 ppm in C_6D_6 splits into *two* multiplets centered at δ 3.65 and 2.66 ppm after addition of NaOD, suggesting a mixture of the imine and enamine forms. The methylene signal ortho to the phenyl ring remains unchanged at δ 2.47 ppm, as well as the other signals.

3. Experimental

rac-Tetrahydrozoline hydrochloride was purchased from Sigma. After dissolution in water, the free base was obtained by addition of a slight stoichiometric excess of NaOH (0.5 N), extraction of the base with $CHCl_3$ and separation of the phases. The combined organic layers, after drying over anydrous Na_2SO_4 and removal of the solvent on a rotary evaporator yielded solid **1**.

The HPLC system consisted of a Varian 5060 liquid chromatograph with Valco sample loops, a

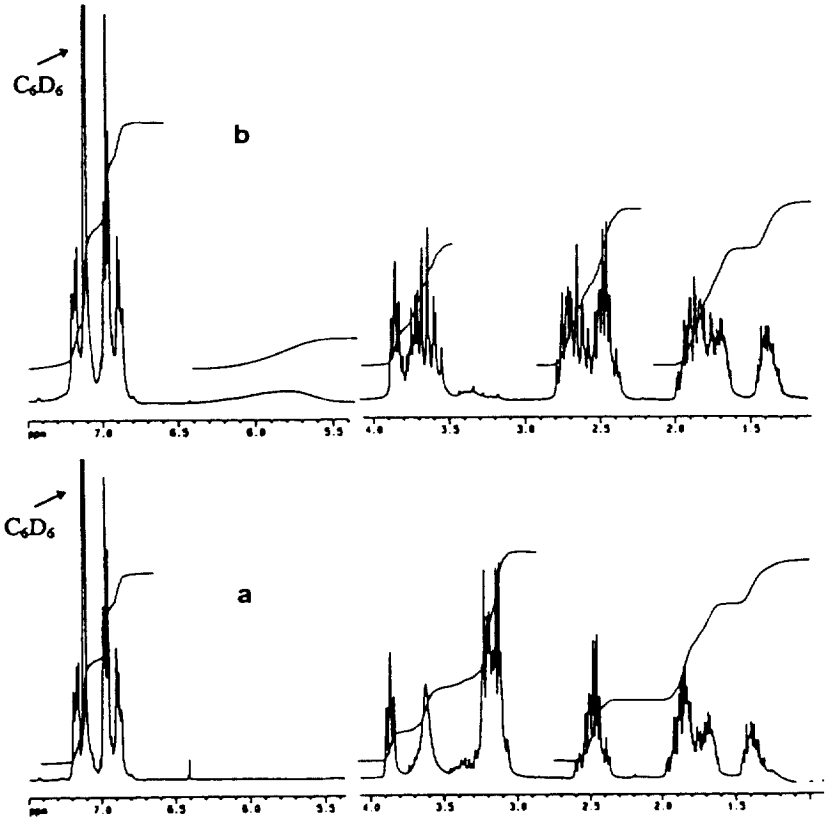


Fig. 5. ^{1}H NMR spectra of tetrahydrozoline in: (a) C_6D_6; and (b) C_6D_6+NaOD

Jasco Uvidec III UV spectrophotometer operating at 245 nm and a Varian Data System or Omniscribe Houston recorder for fraction collecting. The chiral column (250×4.0 mm) used was a Chiralpak AD (amylose tris-3,5-dimethylphenyl carbamate) coated on 10 μm silica gel, from Daicel (Tokyo). Column void time (t_0) was measured by injection of tri-*tert*-butylbenzene as a nonretained sample.[19] HPLC chromatographic parameters are given as usual.[20] Experiments were performed at 19°C. Electronic absorption spectra were measured in various solvents using a Beckman DU-60 spectrophotometer. ^{1}H NMR spectra were obtained in $CDCl_3$, C_6D_6 and D_2O solutions at 250 MHz using a Bruker AC-250 spectrometer with TMS as internal standard.

^{1}H NMR ($CDCl_3$) δ 7.04 (narrow m, 4H), 5.79 (s, 1H, exchangeable with D_2O), 3.80 (t, 1H, J=6.6 Hz), 3.47 (m, 4H), 2.72 (m, 2H), 1.88 (m, 2H), 1.93–1.68 (m, 2H); (C_6D_6) δ 7.16 (m, 1H), 6.95 (m, 2H), 6.89 (m, 1H), 3.86 (t, 1H, J=6.6 Hz), 3.62 (broad s, 1H, exchangeable with D_2O), 3.17 (m, 4H), 2.47 (m, 2H), 1.86 (m, 2H), 1.69–1.38 (m, 2H); (C_6D_6 and NaOD) δ 7.18 (m, 1H), 6.97 (m, 2H), 6.90 (m, 1H), 5.80 (broad s), 3.86 (t, J=6.6 Hz), 3.65 (m, 2H), 2.66 (m, 2H), 2.48 (m, 2H), 1.86 (m, 2H), 1.70–1.38 (m, 2H).

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