The Stereochemistry of *N-tert*-Butyl-4-(4-hydroxyphenyl)-4-phenyl-2-butylamines

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The pairs of enantiomers 2*R*,4*R*/2*S*,4*S* and 2*R*,4*S*/2*S*,4*R* of *N-tert*-butyl-4-(4-hydroxyphenyl)-4-phenyl-2-butylamine, which are the main metabolites formed from terodiline, *N-tert*-butyl-4,4-diphenyl-2-butylamine, have been separated and isolated by reversed phase HPLC. The structure of the diastereomers was determined by ^{1}H NMR spectroscopy using nuclear Overhauser enhancement (NOE) measurements. The NOE interactions were compared with distances between hydrogens in molecular models generated by modification of the crystal structure of terodiline.

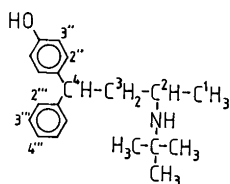
Studies of the biotransformation of terodiline, (*R*,*S*)-*N-tert*-butyl-4,4-diphenyl-2-butylamine (**I**), showed that the dominant metabolic pathway in rats,[1,2] dogs[3,4] and man[4] is hydroxylation and subsequent glucuronidation of one of the prochiral aromatic rings. Both *in vitro* studies with dog and human liver microsomes and *in vivo* studies in rats, dogs and humans showed that *N-tert*-butyl-4-(4-hydroxyphenyl)-4-phenyl-2-butylamine (**II**) was the major primary metabolite. In urine, **II** was mainly found as a conjugate with β-D-glucuronic acid. After removal of the β-D-glucuronyl group, two products, **II:1** and **II:2**, possessing identical mass spectra were obtained. Their *O*-trifluoroacetates could be separated by GC. Terodiline has an asymmetric center at C-2, and these products are consequently the diastereomers (e.g. 2*S*,4*S* and 2*S*,4*R*) formed by hydroxylation of one of the aromatic rings.

In vitro studies in rats[2] and in man[4] showed that (*R*)-terodiline is oxidized to give preferentially one diastereomer (corresponding, according to GC, to the *O*-trifluoroacetate of **II:2**), whereas no selectivity is observed for the metabolism of (*S*)-terodiline. In order to determine the products formed by the stereoselective metabolism, the stereochemistry of **II:1** and **II:2** has been established.

Experimental

Separation of the enantiomeric pairs by HPLC. A mixture of *N-tert*-butyl-4-(4-hydroxyphenyl)-4-phenyl-2-butylamine hydrochloride isomers was synthesized as earlier described.[3] The enantiomeric pairs of amines, **II:1** and **II:2**, were separated on a reversed phase C_{18} column (Nucleosil, 7 μm, 250×10 mm i.d.) eluted isocratically with 40 % methanol in a 0.1 M sodium phosphate buffer (pH 2.5) using a flow rate of 4 ml min^{-1}. The injection volume was 200 μl, and the injected amount of **II** was about 1 mg. The compounds **II:1** and **II:2**, which were collected from several runs, were eluted after 20 and 26 min, corresponding to capacity factors of 5.3 and 7.2, respectively. Separation was monitored by UV-detection at 260 nm.

The substances were checked for identity and purity by GC/MS before being converted into hydrochlorides and subjected to NMR analysis.



II

Table 1. ^{1}H NMR data for **II:1** and **II:2** as hydrochlorides. Assignments, chemical shifts (ppm) and coupling constants (Hz).

Assignment	**II:1**, HCl δ, *J*	**II:2**, HCl δ, *J*
H-1	1.42, $J_{1,2}$ 6.6	1.42, $J_{1,2}$ 6.6
H-2	3.08, $J_{2,3}$ 10.6, $J_{2,3'}$ 3.3	3.05, $J_{2,3}$ 10.5, $J_{2,3'}$ 3.2
H-3	2.21, $J_{3,3'}$ −13.2, $J_{3,4}$ 4.6	2.24, $J_{3,3'}$ −13.4, $J_{3,4}$ 5.0
H-3′	2.53, $J_{3',4}$ 11.2	2.55, $J_{3',4}$ 11.0
H-4	3.98	4.00
H-2″/H-6″	7.15, $J_{2'',3''}$ 8.5	7.13, $J_{2'',3''}$ 8.5
H-3″/H-5″	6.77	6.73
H-2‴/H-3‴/H-5‴/H-6‴	7.28	7.32
H-4‴	7.18	7.22
t-Bu	1.25	1.25

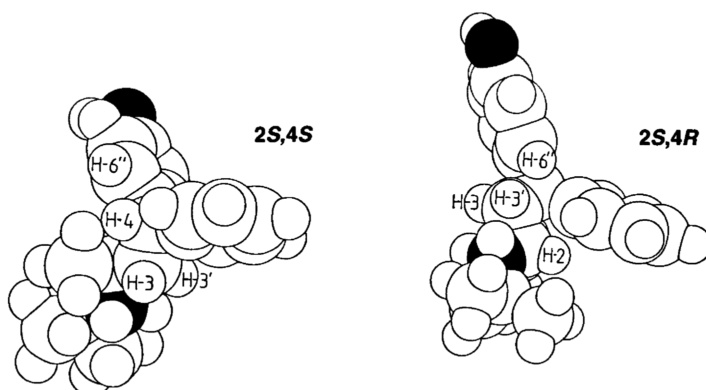


Fig. 1. Computer generated molecular models of (2*S*,4*S*)- and (2*S*,4*R*)-*N*-*tert*-butyl-4-(4-hydroxyphenyl)-4-phenyl-2-butylamine obtained by modification of crystal data[7] for terodiline. The atoms in the models are shown with 3/5 of the real van der Waals radii.

NMR spectroscopy. ^{1}H NMR spectra were recorded at 400 MHz on a JEOL GX-400 spectrometer. Spectra were obtained for solutions in CD_3OD at 30 °C using tetramethylsilane (TMS) as internal reference. NOE-difference experiments[5] were performed using 0.5, 1.0 and 2.0 s pre-irradiation of H-2″/H-6″ of the 4-hydroxyphenyl group for **II:1** and **II:2**. A total of 800 accumulations were used in each experiment, using cycles of 8 accumulations for on and off resonance, respectively, with a 10 s delay for relaxation between the pulses. The enhancements were measured relative to the saturated signal of the pre-irradiated protons in the difference spectrum.

Table 2. Interatomic distance (Å) between H-2″/H-6″ and the chain hydrogens H-2 to H-4 in the molecular models of (2*S*,4*S*)- and (2*S*,4*R*)-*N*-*tert*-butyl-4-(4-hydroxyphenyl)-4-phenyl-2-butylamine.

Hydrogens	(2*S*,4*S*)	(2*S*,4*R*)
H-2″/H-6″−H-2	2.3/2.7	3.2/4.6
H-2″/H-6″−H-3	3.9/3.6	2.8/2.8
H-2″/H-6″−H-3′	2.5/3.6	1.6/3.6
H-2″/H-6″−H-4	3.7/1.9	3.1/2.1

Molecular modelling using Chem-X.[6] The coordinates for **I** were obtained from X-ray data for (*S*)-terodiline.[7] The calculation of hydrogen coordinates was based on C−H bond distances of 1.08 Å and tetrahedral geometry. The C−CH_3 group was rotated to avoid hydrogen-hydrogen contact with the *tert*-butyl group. The coordinates for the

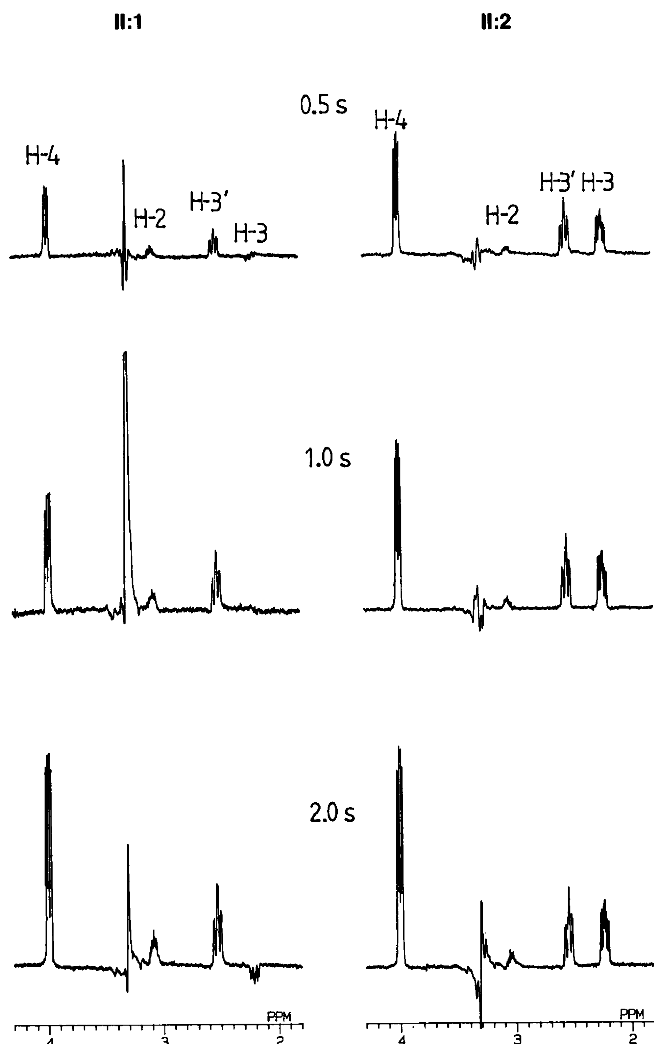


Fig. 2. NOE-difference spectra showing the signals for H-1 to H-4 of **II:1** and **II:2**, as hydrochlorides, at pre-irradiation times 0.5, 1.0 and 2.0 s.

diastereomers were then constructed by addition of a hydroxy group in one of the aromatic rings. Interatomic distances between H-2''/H-6'' and the chain hydrogens H-2, H-3, H-3' and H-4 were then calculated. Calculations of energy levels for different conformations when rotating the C-2–C-3 and C-3–C-4 bonds were performed.

Results and discussion

The isomeric mixture of *N-tert*-butyl-4-(4-hydroxyphenyl)-4-phenyl-2-butylamine (**II**) was separated by reversed-phase chromatography into the enantiomeric pairs **II:1** and **II:2**, which were converted to their hydrochlorides. ^{1}H NMR spectra of **II:1** and **II:2** showed only minor differences (Table 1). The $^{3}J_{2,3}$, $^{3}J_{2,3'}$, $^{3}J_{3,4'}$ and $^{3}J_{3',4}$ values indicate that the dominant conformation of both **II:1** and **II:2** in solution is similar to that in the reported crystal structure of (*S*)-terodiline[7] with respect to the C-2–C-3 and C-3–C-4 bonds. Calculation of the energies for the conformations generated by rotation of these bonds also gives the lowest energy level for this conformation. The values further support the postulate that this conformation predominates in solution. Mole-

Table 3. Enhancements observed for H-2–H-4 in **II:1** and **II:2** after pre-irradiation of H-2''/H-6'' for different times (s). Values (%) are given relative to the irradiated protons using NOE-difference spectroscopy.

Hydrogen	**II:1**			**II:2**		
	0.5	1.0	2.0	0.5	1.0	2.0
H-2	2	2	3	0	1	1
H-3	0	0	−1	2	2	2
H-3'	3	4	5	2	2	2
H-4	4	7	9	3	4	5

cular models of the diastereomers (Fig. 1) show different atomic distances between H-2''/H-6'' of the 4-hydroxyphenyl group and the chain hydrogens H-2, H-3, H-3' and H-4, respectively (Table 2). As the nuclear Overhauser enhancement is proportional to the dipole-dipole relaxation between the protons in questions and to r^{-6} it should be obtained only for signals from protons close to the irradiated protons. Because of the dynamics of the molecule the calculated interatomic distances are only approximate, but as the conformation existing in the crystal should be highly populated due to its low energy, no major differences are expected. Thus, enhancements are expected for H-2, H-3' and H-4 of the 2*R*,4*R*/2*S*,4*S* enantiomers and for H-3, H-3' and H-4 of the 2*R*,4*S*/2*S*,4*R* enantiomers due to short interatomic distances (Table 2). In order to measure the rather small enhancements, NOE-difference spectroscopy[7,8] was employed, using different pre-irradiation times (Fig. 2).

NOE-difference spectra for **II:1** and **II:2** showed, in addition to a strong enhancement of the signal from the *ortho* protons H-3''/H-5'', the expected enhancements for signals from H-3' and H-4 (Table 3). For **II:1**, enhancement was also observed for the signal from H-2, whereas H-3 showed a negative signal at prolonged irradiation, indicating spin-diffusion from H-3'. This result is in accord with the enhancements predicted for the 2*R*,4*R*/2*S*,4*S* enantiomers. For **II:2**, the signal from H-3 was enhanced, whereas only minor enhancement for the signal from H-2 at longer irradiation times was observed. These effects are expected for the 2*R*,4*S*/2*S*,4*R* enantiomers.

The results obtained show that formation of (2*R*,4*S*)-*N*-*tert*-butyl-4-(4-hydroxyphenyl)-4-phenyl-2-butylamine dominates in the metabolism of (*R*)-terodiline.

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