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CONFORMATION OF THE CYP2D6 MODEL SUBSTRATE SPARTEINE UNDER PHYSIOLOGICAL CONDITIONS

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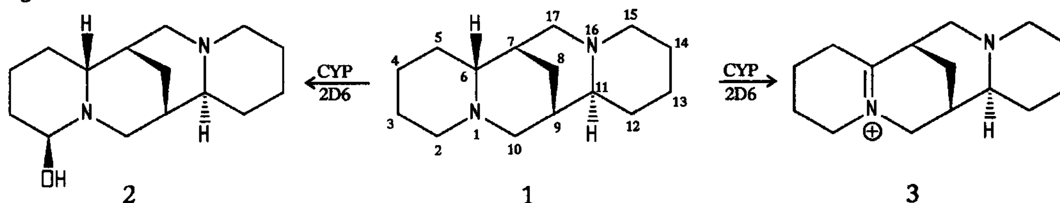
Abstract. The conformation of the CYP2D6 model substrate sparteine (1) is investigated by NMR techniques over a wide pH range. Regioselectively deuterated analogues are used to aid assignments. At pH > 6, *i.e.* under physiological conditions, 1 adopts an all-chair conformation (mono-protonated cage structure). Below pH 4, ring C is inverted to a boat conformation, allowing for double protonation at both nitrogens of 1.

The quinolizidine alkaloid sparteine (1), employed as an antiarrhythmic agent, is the first drug for which the extremely high variance, determined experimentally for human metabolism, could be related definitively to the genetically controlled polymorphism of the expression of a single hepatic cytochrome P-450 isozyme (CYP2D6).[1] Roughly 10% of the caucasian population is not, or only to a very limited degree, able to transform sparteine into the two principal renally excreted metabolites, (*2S*)-hydroxysparteine (2) and the 1,6-didehydrosparteinum ion (3) (Figure 1).[2]

Figure 1.

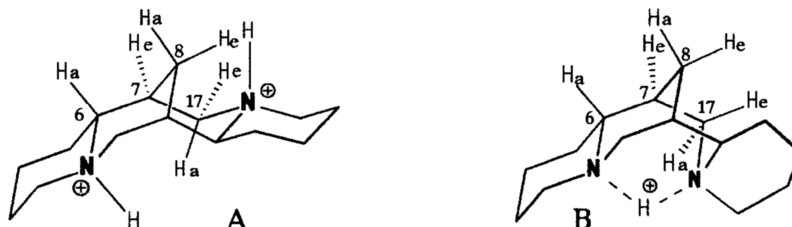


A large number of clinically relevant drugs has in the meantime been established as being metabolized *via* the same isozyme, CYP2D6. All recognized substrates of this enzyme possess a highly basic aliphatic nitrogen atom as a common structural feature. This basic center is requisite for substrate fixation; enzymatic oxidation then occurs at a position, 400-500 pm distant from this center.[3] Considerable insight into the geometry of the substrate binding site has been gained by molecular modelling. This approach so far was limited, however, to rather flexible substrates and CPY2D6 inhibitors, such as propanolol and quinidine. Because of its rigid structure, 1 should prove an especially suitable model substrate.[3,4a-d] We have therefore studied sparteine conformation under physiological conditions which alone are relevant for pharmacological activity.

NMR Results. NMR investigations (^1H,^{13}C) on sparteine so far were carried out almost exclusively in aprotic solvents (C_6D_6, $CDCl_3$). In these media, *i.e.* as the non-protonated free base, sparteine beyond any doubt adopts a chair-chair-boat-chair conformation (*cf.* A).[5a-f] We now report the results of a systematic ^1H and ^{13}C NMR study of (+)-sparteine in aqueous medium and under varying pH conditions.[6]

Sparteinium sulfate[7] was dissolved in D_2O/H_2O; the pH of such solutions is in the range of 3.5 - 4.5. To assure unified and reproducible pH conditions, the solution was adjusted to pH 1 with dilute sulfuric acid;[8] this results at the same time in appreciable line sharpening in the ^{13}C NMR. Individual assignments were cross-correlated by H,H and C,H COSY experiments, and also by a full analysis of the ^1H coupled ^{13}C NMR spectrum. A number of line assignments in fact requires selectively deuterated sparteines[7] for a comparison of the relative isotope shifts. From these painstaking experiments, sparteine in aqueous medium at pH 1 is assigned, unambiguously, the di-protonated form with conformation A (Figure 2).

Figure 2.



When ^1H and ^{13}C spectra are recorded at increasing pH (ΔpH ~ 1), NMR signals begin to broaden extremely. At pH 5.5, the proton spectrum displays a more or less unfeatured hump, and in the ^{13}C NMR spectrum there appear no lines any more. This behaviour is typical for a coalescence phenomenon, *i.e.* for the substrate molecule undergoing *e.g.* conformational changes at a rate roughly equivalent to the NMR relaxation time.

At pH 6, a new set of resonances appears which become increasingly better defined if the pH is raised to 10 (above pH 10.3, sparteine is no longer soluble in aqueous medium). Table 1 gives the ^{13}C and ^1H NMR shifts for this form (pH~10). Both line positions and apparent multiplicity in the ^1H NMR spectrum (Figure 3) do not change between pH 6.5 and 10; thus, the structural arguments from the pH 10 spectrum likewise hold under physiological conditions.

Again, H,H and C,H correlation experiments, including the selectively deuterated analogues, are required for an unambiguous assignment. All NMR evidence indicates that, above pH 6, sparteine adopts an all-chair conformation. The inversion of the conformation of ring C (structural formula in Figure 3) is demonstrated most explicitely by the changes in H,H coupling constants for the methylene protons 17-H_e and 17-H_a.

In the di-protonated form A, with ring C in boat conformation, the dihedral angle 17-H_e/7-$H_{(e)}$ is 0°. The respective large $^3J_{cis}$ coupling (11.4 Hz) and the geminal coupling to 17-H_a result in a well-defined doublet of doublets for the 17-H_e signal; no long-range splitting is visible (for full data set and spectrum, see ref. 6). For the all-chair conformation B, the dihedral angle between the CH_2 protons at C-17 and 7-H is ~ 60°, with a concomitant sharp reduction in vicinal coupling (2.4 Hz and 3.0 Hz). Ring C inversion also reduces the $^2J(17\text{-}H_{a,e})$ value: 14.4 Hz (A) ⟶ 12.9 Hz (B). The all-chair form likewise provides for planar W-pathways, long-range coupling therefore being observed for $^4J(17e,8a) = 2.4$ Hz, $^4J(17a,6) = 1.2$ Hz.

Figure 3. ^{1}H NMR Spectrum (250MHz) of (+)-sparteinium hydrogensulfate in D_2O / pH 10 (a-axial, e-equatorial position at the respective carbon atom)

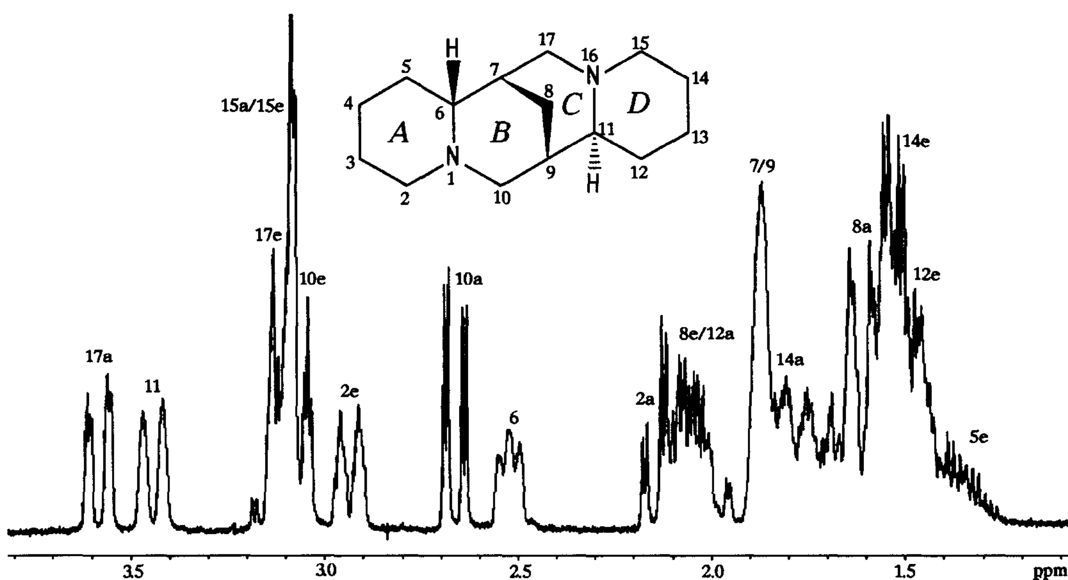


Table 1. ^{1}H and ^{13}C NMR data of (+)-sparteinium hydrogensulfate in D_2O / pH 10

^{13}C NMR	δ [ppm]	δ [ppm] $^{1}H_{ax}$	$^{1}H_{eq}$	^{1}H NMR $^{2}J_{gem}$	J [Hz] $^{3}J_{vic}$	^{4}J
C-2	58.45	2.10	2.92	11.9	(2a,3a) 12.0, (2a,3e) 3.0	
C-3	27.50	1.50 [a]	1.62 [a]		(3a,2a) 12.0, (3e,2a) 3.0	
C-4	26.00	1.40 [a]	1.77 [a]			
C-5	31.88	1.55 [a]	1.38 [a]			
C-6	68.70	2.55			(6,17a) 1.2	
C-7	35.35		1.87 [a]		(7,17a) 3.0, (7,17e) 2.4	
C-8	28.90	1.60 [a]	2.07 [a]			(8a,17e) 2.4, (8e,10e) 2.4
C-9	35.51		1.89		(9,10a) 2.8, (9,10e) 2.4	
C-10	63.88	2.64	3.04	11.9	(10a,9) 2.8, (10e,9) 2.4	(10e,8e) 2.4
C-11	65.85		3.44		(11,12a) 12.5	
C-12	25.46	2.03 [a]	1.48 [a]		(12a,11) 12.5	
C-13	25.75	1.52 [a,b]	1.87 [a,b]			
C-14	20.69	1.77 [a]	1.52 [a]			
C-15	55.49	3.1 [a]	3.1 [a]			
C-17	49.87	3.58	3.11	12.9	(17a,7) 3.0, (17e,7) 2.4	(17a,6) 1.2, (17e,8a) 2.4

[a] Chemical shifts determined from $^{1}H,^{1}H$ correlation spectra (COSY).
[b] Axial and equatorial assignment may have to be exchanged.

Discussion. Sparteine is an extremely strong base (pK_{a1} 11.4),[9] compared to other tertiary aliphatic amines. This is readily understood in terms of the adamantoid cage structure **B** (Figure 2), with a proton-sponge type bifurcate N···H···N bridge. The same structure has been ascertained for a number of metal complexes where **1** serves as a bidentate ligand.[10] Di-protonation of course is not feasible in this conformation.

Both substrate and product stereoselectivity of an enzyme may serve as a stringent criterion for its characterization. It is remarkable, therefore, that **1** is transformed *in vivo* by human CYP2D6 into two metabolites (Figure 1).[11] It was demonstrated with isotopically (^2H) labelled substrate **1** that H abstraction by CYP2D6 is exclusively from the axial position at C-2 (*2S*).[12] *In vivo*-metabolism of the (+)-enantiomer of **1**, pachycarpin, in the rat affords stereospecifically (*4S*)-hydroxypachycarpin.[13] All these regio, substrate, and product stereoselectivities clearly prove the mono-oxygenase character of CYP2D6. Even though a conformational re-orientation upon fixation at the active site cannot be ruled out absolutely, the structural information, derived from the NMR spectra of sparteine as a respresentative substrate, now can serve as a basis for modelling the active site of the enzyme.

Acknowledgement. This work was supported by the Robert Bosch Foundation, Stuttgart, FRG

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7. Materials: Sparteine hydrogen sulfate pentahydrate is commercially available (Fluka, Neu-Ulm, FRG). The following deuterated analogues are used to aid assignments: 2,2-$^2H_2$-1; 3,3-$^2H_2$-1; 5,5-$^2H_2$-1; 5e,6-$^2H_2$-1; 15,15-$^2H_2$-1; 17,17-$^2H_2$-1; 5,5,6-$^2H_3$-1; 5,5,7-$^2H_3$-1 (sulfates or perchlorates). Their synthesis is discribed elsewhere: Meese, C. O.; Ebner, T. *J. Lab. Comp. Radiopharm.* **1987**, *25*, 329 - 334; Ebner, T.; Ph.D. Thesis, Universität Tübingen, FRG, 1989.
8. Methods: The pH of the solutions was monitored by micro-pH-electrode (Ingold, Steinbach, FRG). All NMR spectra were recorded with a Bruker AC-250 spectrometer (Bruker, Karlsruhe, FRG) (298K; ^1H spectra: 250 MHz, 0.05 molar; ^{13}C spectra: 63 MHz, 0.2 molar; for details see Ref.[6]).
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11. For an extensive metabolizer (EM), the 2:3 ratio is roughly 5:1; rats excrete only 2.
12. Comparable selectivity was observed for benzylic hydroxylation of debrisoquine by CYP2D6. The major metabolite, (*4S*)-hydroxydebrisoquine, is formed with >96% e.e. by EM individuals: Meese, C. O.; Thalheimer, P.; Eichelbaum, M. *J. Chromatogr.* **1987**, *423*, 344 - 350.
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