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CONFORMATIONAL ANALYSIS OF AMPHETAMINE IN SOLUTION BASED ON UNAMBIGUOUS ASSIGNMENT OF THE DIASTEREOTOPIC BENZYLIC PROTONS IN THE ^{1}H NMR SPECTRA

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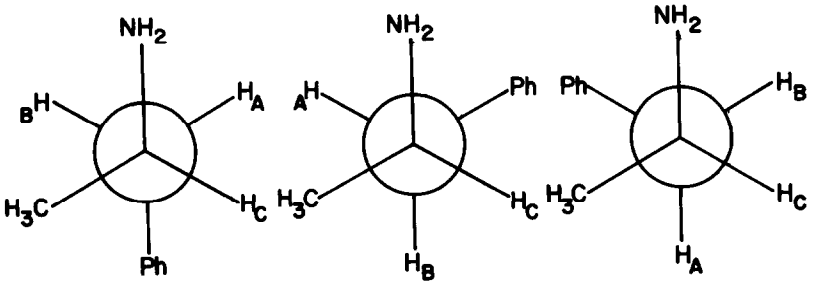
SUMMARY

The *erythro*- and *threo*-amphetamine-β-d diastereomers were synthesized and used for the unambiguous assignment of the diastereotopic benzylic protons and the measurement of vicinal ^{1}H-^{1}H coupling constants which were used to determine the distribution of rotamers around the central C_α-C_β bond in the side chain.

The conformation of a flexible drug in solution plays a role during its interaction with the receptor and may thus influence its pharmacological properties.[1,2] Amphetamine and some of its analogs are potentially flexible drug molecules endowed with interesting pharmacological properties and can exist as a number of conformers in equilibrium with one another. Of the conformers resulting from rotation around the central side-chain C_α-C_β bond, the most stable are the three perfectly staggered shown in Figure 1. The relative distribution of the above conformers in amphetamine[3] and some of its methoxy-substituted analogs[4,6] in solution has been studied using proton magnetic spectroscopy. These studies have relied on the measurement of vicinal proton-proton coupling constants and their correlation with the H-C-C-H dihedral angles.



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Measurement of vicinal coupling constants from the ^1H NMR spectra of amphetamine involves analysis of an ABX spin system including two diastereotopic benzylic protons (H_A,H_B) and one *alpha* proton (H_X). Within this AB spin system the spectral assignment of H_A and H_B (Fig. 1) is uncertain. Previous assignments in this[3] and similar spin systems[1] have depended on predicting trends in the relative distribution of conformers among a series of analogs based on stereochemical considerations. Assumptions were then made on the relative shielding and deshielding effects that *gauche* substituents have on H_A and H_B. However, for an unambiguous spectral assignment it is necessary to label H_A and H_B. This can be accomplished by substituting independently each of the two protons in question with its stable and magnetically different isotope deuterium.

We wish to report here the spectral assignment of H_A and H_B in the ^1H spectra of amphetamine and its salt in D_2O and $CDCl_3$ solutions (Table 1), using stereoselectively synthesized *erythro*- and *threo*-amphetamine-β-d. The individual synthetic sequences[7] are outlined below:

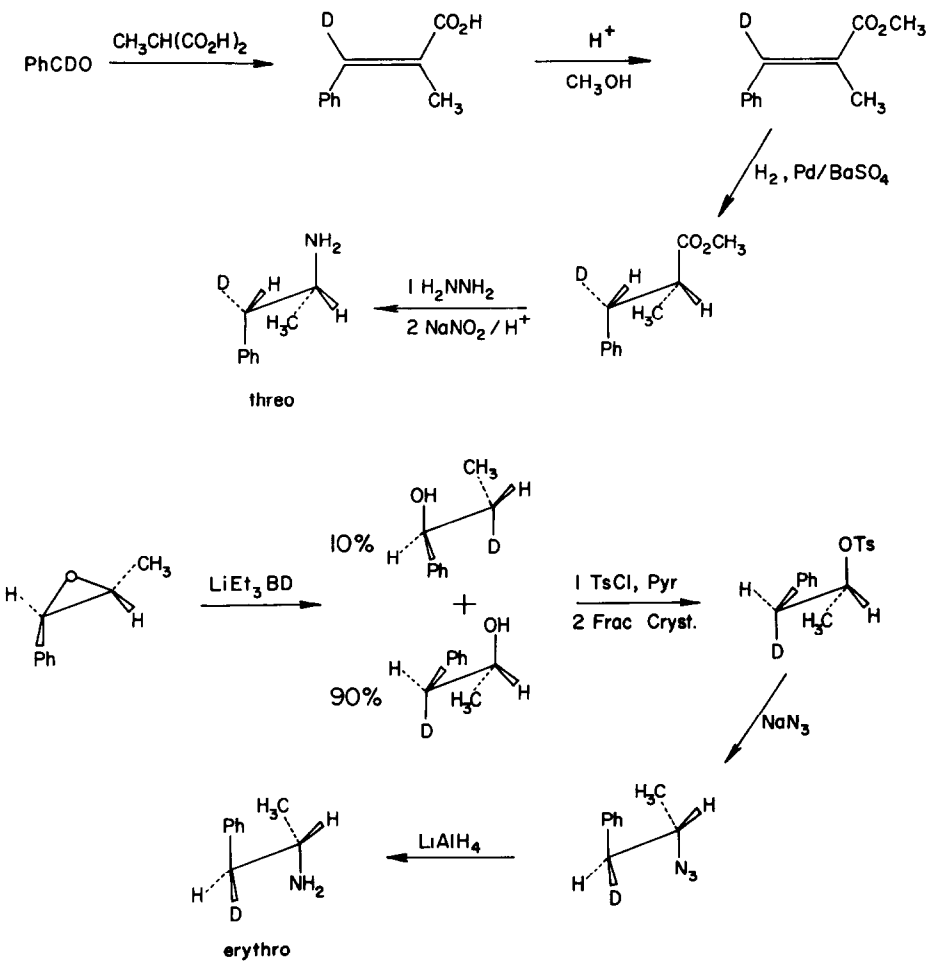

Table 1

Spectral Assignments[a] from Amphetamine-β-d Analogs

Compound	Solvent	Chemical Shift[b,c,d] (ppm)		Vicinal Coupling Constants[b] (Hz)		Conformational Distribution[e]		
		H_A *(threo)*	H_B *(erythro)*	J_{AX}	J_{BX}	pI	pII	pIII
Free base	$CDCl_3$	2.50	2.69	8.06	5.50	.27	.61	.12
	D_2O	2.55	2.70	7.70	6.23	.36	.56	.08
Hydrochloride	$CDCl_3$	3.22	2.84	5.13	9.16	.76	.21	.03
	D_2O	2.92	2.94	7.5	6.9	.45	.53	.02

a) ^1H spectra were obtained at 270 MHz using .05M solutions; T = 298°; b) Measured from the ^2H-decoupled ^1H spectrum assuming AX or BX spin systems; c) Relative to TMS ($CDCl_3$) or DSS (D_2O); d) The reported chemical shifts are 0.015 ppm upfield from the observed corresponding chemical shifts in undeuterated amphetamine due to a geminal deuterium isotope effect; e) Calculated assuming J_t = 11.0 Hz and J_g = 3.5 Hz, obtained from a model compound. Values for conformer distribution should be considered as approximate with a possible error of about 10%. The uncertainties involved in these calculations are described in ref. 6.

The H_A and H_B assignments allowed us to measure unambiguously the J_{AX} and J_{BX} vicinal coupling constants (Table 1) from which the relative distribution of amphetamine conformers in solution was calculated (Table 1). The benzylic protons in *threo-* and *erythro*-amphetamine-β-d are designated as H_A, H_B respectively. We find that under this designation H_A is the upfield proton while H_B is downfield in the spectra of amphetamine free base in D_2O and $CDCl_3$. However, in the spectrum of amphetamine hydrochloride in $CDCl_3$, the relative positions of the benzylic protons change and H_A becomes the downfield proton. The spectrum of the same salt in D_2O at 25° has the two benzylic protons with almost identical chemical shifts, H_A being only slightly upfield. This corresponds well with the deceptively simple benzylic proton doublet observed in the spectrum of the fully protonated amphetamine hydrochloride in D_2O at 25°.

The unambiguous assignments of the benzylic amphetamine proton resonances allowed us to correct previously made assignments. We, thus, found that the reported H_A, H_B assignments in the spectrum of amphetamine free base in D_2O[3] are incorrect. In a different report[5] dealing with ^1H spectra of several ring substituted amphetamines, we found that the benzylic proton assignments for the hydrochlorides in $CDCl_3$ were consistent with those of amphetamine hydrochloride. However, the assigned benzylic resonances in the ^1H spectra of the free bases of all the compounds in $CDCl_3$ did not correspond with those we observed in the two diastereomeric amphetamine-d free bases in the same solvent. It is doubtful that this is a consistent reversal in the relative position of H_A, H_B due to ring substitution but rather a chemical shift misassignment. We are currently studying the effects of ring substitution on the ^1H NMR spectra of amphetamine.

Following a different approach, Wright[8] used a lanthanide shift reagent to assign the H_A, H_B protons in the spectrum of amphetamine free base in $CDCl_3$. He observed that, in the presence of $Eu(fod)_3$, one of the benzylic protons exhibited a larger downfield change in its chemical shift than the other. Assuming that the $Eu(fod)_3$ complexes with the free amino group of amphetamine, he concluded that the proton with the largest chemical shift change was the one which resided for the longer time nearest to the amino group. This was the H_A proton which was gauche to the amino group in the two major amphetamine rotameters I and II (Fig. 1). The proton with

the smaller chemical shift change was the H_B proton which is gauche in only one of the two major amphetamine rotamers. To test this hypothesis we repeated the experiment with the amphetamine-β-d diastereomers and were able to confirm Wright's H_A, H_B assignments. However, we found the reported conformer distribution to be incorrect because of a misassignment in J_{AX} and J_{BX}.

The present study leads to the following conclusions:

a. Amphetamine free base in D_2O and in $CDCl_3$ exists predominantly as the conformer in which the phenyl group is gauche to the amino group and trans to the methyl (II). However, when amphetamine hydrochloride is dissolved in $CDCl_3$ rotamer I predominates. This presumably happens because in hydrophobic media the amine salt exists as a bulky ion pair which is preferentially forced away from the aromatic ring (trans I). In aqueous media[9] the fully ionized salt exists as an approximately equal mixture of the two major conformers (I and II).

b. The chemical shift assignments indicate that free amino groups have a shielding effect on gauche vicinal protons while ammonium salts in $CDCl_3$ have a deshielding effect. Ammonium salts in aqueous media have a smaller shielding effect on vicinal gauche protons.

We are currently extending the above methods for assigning H_A, H_B geminal protons in other ABX systems.

ACKNOWLEDGEMENTS

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7. The diastereomers were obtained in ≥ 95% isotopic purity. *Erythro*-amphetamine-β-d is a racemic mixture of RR and SS optical isomers while the *threo* analog is a racemic mixture of the RS and SR isomers. All the products gave satisfactory spectroscopic (^1H and ^2H, nmr, ir, ms) and physical (mp, bp) data.
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9. In aqueous media the amphetamine rotamer distribution as well as the H_A, H_B chemical shifts are temperature dependent. Such studies will be reported elsewhere.

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