Bioorganic & Medicinal Chemistry Letters, Vol 2, No.12, pp. 1681-1684, 1992
Printed in Great Britain

0960-894X/92 $5.00 + .00

USE OF TRANSFERRED NUCLEAR OVERHAUSER EFFECTS TO DETERMINE THE CONFORMATION OF 1-(3,4-DICHLOROPHENYL)-2-AMINOPROPANE WHEN BOUND TO THE ACTIVE SITE OF PHENYLETHANOLAMINE *N*-METHYLTRANSFERASE (PNMT)

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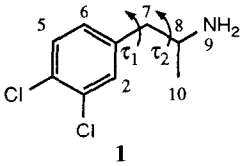
(*Received* 20 *July* 1992)

Abstract: Transferred two dimensional nuclear Overhauser effect spectroscopy (transferred NOESY) was used to show that the side chain of 1-(3,4-dichlorophenyl)-2-aminopropane (**1**) exists when bound at the active site of PNMT in an extended conformation (τ_2 = 167 - 180°) with the aromatic ring rotated out of the plane of the ethylamine side chain (τ_1 = 29 - 45°).

The role played by epinephrine (Epi) in the central nervous system remains unclear. Epi has been shown to play a role in the regulation of α_2-adrenergic receptors,[2] the release and inhibition of release of pituitary hormones,[3] and the regulation of both blood pressure[4] and body temperature.[5] Epi is synthesized by PNMT (EC 2.1.1.28) from norepinephrine (NE).[6] In order to design a potent and selective inhibitor for PNMT, it would be useful to know the binding requirements for both substrates and inhibitors. We have previously assessed the conformational requirements of ligands at the active site of PNMT by using conformationally-restricted and conformationally-defined analogues.[7] These studies have led to the conclusion that 2-phenylethylamines prefer an extended conformation about the side chain C-C bond (τ_2 = 180°) with the aromatic ring somewhat out of the plane of the side chain (τ_1 = 10-35°). However, in the conformationally-defined analogues in which the values of τ_1 and τ_2 were fixed, there was additional steric bulk present which did not allow optimal binding at the active site of PNMT. The reduced affinity of these compounds for the enzyme thus made the conformation-activity relationship somewhat ambiguous. We describe here our use of transferred NOESY for a determination of the conformation of 1-(3,4-dichlorophenyl)-2-aminopropane (**1**), a good inhibitor[8] of PNMT (K_i = 6.7 ± 0.3 μM), when bound at the active site of the enzyme.

The bovine adrenal PNMT used for these studies was isolated[9] and purified[10] as previously described. Inhibitor **1** was synthesized from 3,4-dichlorobenzaldehyde.[8] Because PNMT shows a low enantioselectivity toward 1-phenyl-2-aminopropanes,[11] these nmr studies were done using racemic **1**. The transferred nuclear Overhauser effect (TRNOE) technique[12,13] has been previously described and used to study the conformation of small molecules bound to macromolecules. The TRNOE technique has been performed in both one[13] and two[14] dimensions.



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All NMR experiments were carried out on a Bruker AM-500 operating at 500.139 MHz for protons. The spectra were recorded nonspinning at 297 °K using a proton-only probe optimized for water suppression. In a typical experiment, in 500 μl of degassed 0.1 M (pH 7.25) phosphate buffer in D_2O were dissolved 2.5 mg of protein (≈0.15 μM) and 0.56 mg of **1** (≈5μM). A phase sensitive (TPPI) NOESY spectrum was recorded using a 2 second delay during which the residual HDO peak was suppressed by presaturation, 4 dummy scans and

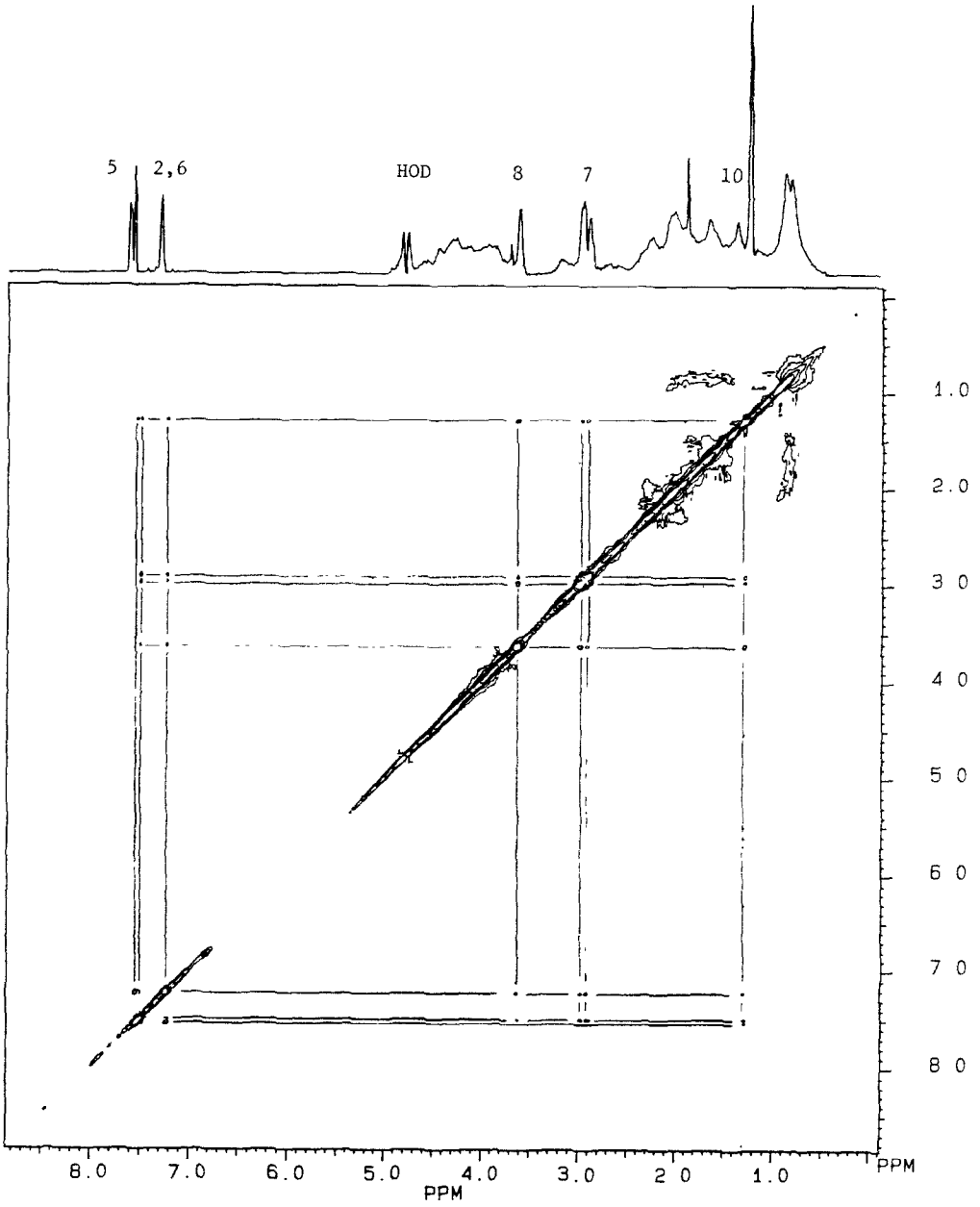

Figure 1. NOESY spectrum of **1** and PNMT. Note the NOEs between the CH_3 of **1** and each of the three aromatic protons. There are also NOEs between the methylene and methine protons with the ortho aromatic protons (H2 and H6) These characteristic NOEs are absent in the NOESY spectrum of **1** with inactive PNMT (data not shown).

48 accumulations per block and 512 t_1 increments. This was repeated for mixing times of 50, 150, 300, 500, 700 and 1000 ms. The data sets were processed on a Bruker Aspect 3000 or 1000 computer and phase corrected in both dimensions. The NOESY spectrum of **1** and PNMT recorded at 300 ms mixing time is shown in Figure 1. For the various mixing times, diagonal and cross peak intensities were extracted from integrals of the appropriate matrix rows and columns.[14] The distances between H5 and H6, H6 and H2, H8 and H10 (methyl) were considered as fixed and used as reference distances. The distances between aliphatic and aromatic protons were estimated based on the two-spin approximation[12] and classified as strong (≤ 3 Å), medium (3 - 4 Å) or weak (4 - 5 Å) depending on the size of the NOE observed. Once the range of distances was defined, they were used as distance constraints in the SYBYL conformational search program.[15]

Figure 2 shows the range of bound conformations of **1** that satisfy the distance constraints noted above. Torsion angle τ_1 ranges from 29 - 45° and τ_2 from 167 - 180°. Thus, the TRNOE method used here defines, within a narrow range, the conformation for the side chain of **1** at the active site of PNMT. The bound conformation is clearly a fully extended one (τ_2 near 180°) with the aromatic ring slightly twisted out of the plane of the ethylamine side chain. That this is consistent with our previous results obtained with conformationally-defined analogues of 1-phenyl-2-aminopropane (amphetamine) supports the validity of the published conclusions made from those results.[7]

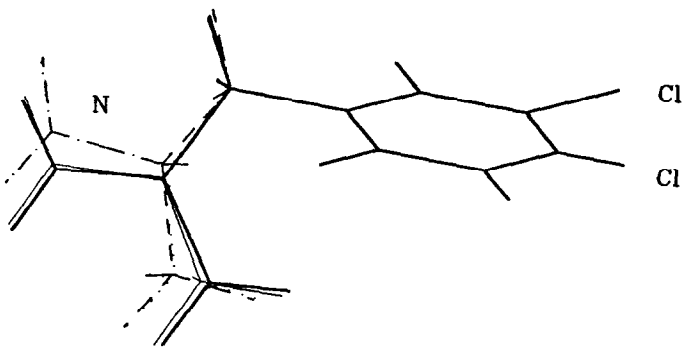

Figure 2. The range of conformations of the propylamine side chain in **1** that are consistent with the NOESY data.

Acknowledgements. We gratefully acknowledge the support of this work by the National Institutes of Health (HL 34193). We thank Dr. Charles Decedue, Mr. Richard Freeman and Ms. Karen Trubitt of the Biochemical Research Services Laboratory for the enzyme purification, Mr. Kevin Criscione for the enzymatic assays, Mr. Christopher Gunn of the Molecular Graphics and Modeling Laboratory for his assistance with the use of SYBYL, Dr. David Craik of the Victorian College of Pharmacy (Parkville, Australia) for helpful discussions, and Dr. Donna Wong of Stanford University (Palo Alto, CA) for advice on the affinity chromatographic purification of PNMT.

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