LONG RANGE TRANSMISSION OF POLAR EFFECTS IN CHOLINERGIC 3-ARYLIDENEANABASEINES. CONFORMATIONS CALCULATED BY MOLECULAR MODELLING[a]

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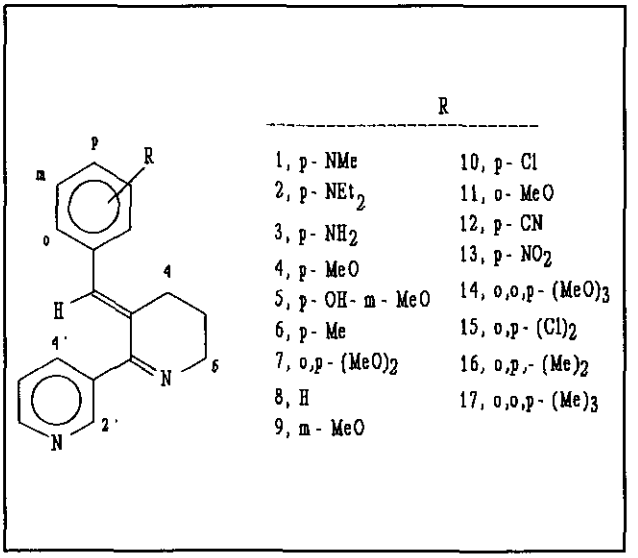
Abstract —— Benzylidene products resulting from acid-catalyzed aldol condensations of aryl aldehydes and anabaseine at position 3 of the tetrahydropyridine ring have E stereochemistry. In spite of a chemical shift scale ranging only over 0.11 ppm the methylene protons (H-6) adjacent to the imino group of the tetrahydropyridine ring show an excellent Hammett correlation using σ_M and σ_P values. Methoxy and chloro groups in an ortho position require a σ_I parameter. Substrates with one or two ortho methyl groups deviate. AM1 and MM2 calculations indicate that both the phenyl and pyridyl rings are twisted out of a common plane defined by the central ring.

[a]Dedicated to Professor Edward C. Taylor, Jr. on the occasion of his 70th birthday.

Anabaseine[1] (3,4,5,6-tetrahydro-2,3'-bipyridine) is a potent nicotinic receptor agonist in the central nervous system but its pharmacological properties have not yet been examined in any detail. Found naturally as a neurotoxin in nemertine sea worms[2] and in some species of ants,[3,4] it can be converted on reacting with an aromatic aldehyde into a valuable benzylidene derivative.[5] In view of their expected attractive pharmacological properties we have synthesized an extensive series of such benzylidene compounds **(1-17)** having one, two or three substitutents in the benzene ring. The resultant tricyclic systems demonstrate a linear free energy correlation of excellent quality between chemical shifts of the methylene protons (H-6) adjacent to the imino group and classical Hammett σ_M and σ_P as well as σ_F (field) or σ_I (inductive) substitutent constants for ortho groups including chloro and methoxy but not methyl. This correlation is likely to prove insightful when interpreting the results of structure-bioactivity relationships and in predicting the basicity of the imino nitrogen atom.

Synthesis: *p*-Dimethylaminobenzaldehyde and anabaseine undergo an acid-catalyzed mixed aldol-type of condensation at the acidic 3 position of anabaseine to give 3-([4-dimethylamino]benzylidene)anabaseine.[5] This approach proves to be satisfactory for other aryl aldehydes having electron-donating substitutents but new conditions are required when a strongly electron-withdrawing group such as nitro is present. A base such as acetate ion in aqueous acetic acid is necessary, presumably to aid dehydration of the carbinol intermediate.

Structure Determination: The ^1H chemical shifts of all the compounds present as the free base in $CDCl_3$ were measured at 300 MHz. They all exhibit a single structure; no evidence was found for a second ring-chain tautomeric component such as that observed for anabaseine itself in aqueous acid[6] or in mixed aqueous solvents.[7] Materials are easily characterized as the ring-closed imine by the absence of a carbonyl signal in



the ^{13}C spectrum at about 200 ppm and by the presence of the imino carbon signal at, for example, 167.30 (*p*-MeO) and 167.10 (*p*-Me) ppm, the most deshielded signals.

In addition to the obvious four signal pattern at low field for a 3-substituted pyridine there is a broad singlet for the olefinic proton near this region. This proton is weakly spin coupled to the H-4 methylene group but the coupling merely broadens the signal for the olefinic hydrogen and is unresolved. The signal due to H-4 does clearly show the four bond 2 Hz allylic coupling however and also the three bond 6.5 Hz coupling to H-5 to give a triplet of doublets. Also diagnostic are the triplet for H-6 and the apparent quintet due to H-5 at high field, the remaining methylene groups of the tetrahydro pyridine (1-piperideine) ring. The presence of three strongly coupled methylene groups demands that no isomerization in the position of either double bond took place; the molecule must have the extended conjugation as represented.

Stereochemistry About the Double Bond: Both E and Z configurations are possible according to molecular models, not just the intuitively expected E form where the small olefinic hydrogen is adjacent to the bulky pyridine ring.

The compound having a *p*-Cl group was selected for NOE studies to establish the stereochemistry because the signal for the olefinic site is clearly separated from other signals. The results summarized in the Table show that the olefinic hydrogen must be syn to the freely rotating pyridine ring. The identity of the carbocyclic aromatic multiplets is established on irradiation of H-4. Those protons closest to H-4 show a positive NOE while those further away demonstrate a negative NOE ("three-spin" effect[8,9]). The stereochemistry therefore is E as drawn.

Additional studies based on NOE experiments seem to be unnecessary for the other derivatives in light of the linear free energy correlation found for proton chemical shifts and Hammett substitutent constants described below. Since all the compounds in the correlation respond to substituent effects in the same way, all probably have the same stereochemistry.

Table. Results of NOE experiments on **10**.

Irradiated Proton	Observed Proton	% NOE
H-2'	olefinic	6
H-4'	olefinic	6
H-4'	H-5'	21
H-4	o-phenylene	6.5
H-4	m-phenylene	- 1.5
H-4	H-5	2

Linear Free Energy Correlation: Chemical shifts for the protons of the methylene group (H-6)adjacent to the imino nitrogen atom only vary about 0.11 ppm when substituents on the benzene ring are changed. Nevertheless these shifts show an excellent correlation with Hammett substitutent constants[10] σ_M and σ_P and with σ_F or σ_I. The numbered points and associated substituents are identified in the columns near the general structural formula. This extensive and comprehensive series of 17 compounds consists mostly of meta and para monosubstituted substrates, including those ranging from the highly electron-donating *p*-Me_2N (**1**), *p*-Et_2N (**2**) and *p*-H_2N (**3**) to the strongly electron-withdrawing *p*-CN (**12**) and *p*-NO_2 (**13**) groups as well as the parent unsubstituted compound (**8**) itself and even a meta (**9**, *m*-MeO) and a few disubstituted (**5**, *p*-HO-*m*-MeO, **7**, *o*,*p*-$(MeO)_2$, and **15**, *o*,*p*-Cl_2) and one trisubstituted (**14**, *o*,*o*,*p*-$(MeO)_3$) substrates. Remarkably, ortho substituted compounds (**7**, *o*,*p*-$(MeO)_2$; **11**, *o*-MeO; **14**, *o*,*o*,*p*-$(MeO)_3$) and **15**, *o*,*p*-$(Cl)_2$) give chemical shift values that fall on the correlation line, provided their electronic effect at the ortho position is expressed by an inductive substitutent constant σ_I[11] or the equivalent field constant σ_F.[10,12] Also noteworthy is the additivity of the electronic effects of the di- and trisubstituted compounds having both ortho and para groups. The normal Hammett para value and the value for the inductive effect of an ortho group were added together algebraically to gauge the combined electronic effects of the two groups. This additivity suggests that the ortho group does not induce a serious change in conformation about the single bond to the aromatic ring compared with substrates lacking such a group.

Curiously, however, compounds with *o*-methyl groups that are similar in size to the methoxy and chloro substituents do deviate from the line by 0.02 to 0.03 ppm, being at too low a field and are not included.

The correlation of the first 15 points is expressed by Eq. 1 with an excellent correlation coefficient, r, of 0.991. The calculated intercept value is essentially that observed (3.892) for the unsubstituted compound.

$$\delta = 0.0679\ \Sigma(\sigma) + 3.884 \quad (1)$$

Similar correlations involving other positions are of poorer quality and therefore are not reported.

Structure in Aqueous Acid: In view of the ability of aqueous acid to hydrolyze the imine bond of anabaseine,[6,7] the structure of the benzylidene derivatives under similar conditions is of some interest. Selected compounds were examined in both dilute and concentrate aqueous acids. The chemical shift of the methylene protons adjacent to the nitrogen atom serves as a convenient indicator of the two possible ring-chain tautomers. The more electronegative sp^2 hybridized nitrogen atom of the cyclic protonated imine is considerably more deshielding than the sp^3 nitrogen of the acyclic protonated primary amine, 3.90 vs 3.07 ppm, respectively, for anabaseine.[6] Protonation of the imine also causes the olefinic hydrogen to become deshielded.

The ^1H nmr spectra of the *p*-MeO and the *p*-Me_2N benzylidene compounds in aqueous (D_2O) phosphate buffers at pD 7.35 and 6.53, respectively, appeared to be those of a single substance. The observed signal at 3.88 ppm (*p*-MeO, **4**), for example, is similar to that for the iminium ion of anabaseine[6] in water. The olefinic signal now has moved down to 7.21 ppm. Therefore, in phosphate solution the conjugate acids of the two benzylidene compounds remain cyclic as in chloroform.

The *p*-Cl compound was added to a a mixture of CD_3OD and DCl/D_2O. Signals were deshielded with respect to those for samples in aqueous phosphate, indicating that both nitrogen atoms were converted to their conjugates acids. Thus, for example, the signals for H-6' and H-2' of the pyridinium ring moved down to 9.26 and 9.14 ppm, respectively. The resultant dication was quite stable, no change took place even after heating at 60 °C for 3.5 hours.

In contrast with anabaseine, the benzylidene compounds remain in their cyclic imine form in aqueous solution even when they are converted to their dicationic states.

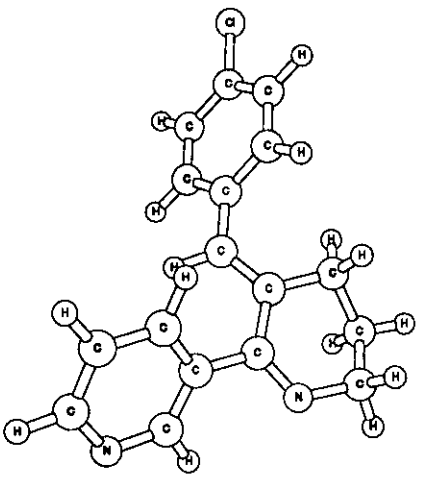
pKa Values: The pKa values for the imino site of the *p*-CN (6.55) and unsubstituted benzylidene substrates (7.32) were determined spectrophotometrically in H_2O at room temperature. The latter value is not unlike that for its anabaseine precursor lacking the benzylidene group.[6] These values and that already available for the *p*-Me_2N substrate (8.35[5]) provide a preliminary correlation of pKa *vs* Hammett sigma values. The Me_2N and CN substituents represent extremes in terms of their electronic effects and these two along with the parent compound, an approximate midpoint, provide a meaningful series to gauge the response to the effects of structure on basicity. The preliminary three point correlation is described by the same Hammett substituent constants used for the proton shifts and is of high quality (r = 0.999), having an intercept of 7.34 and a rho value of -1.21, some 17 times larger than that for the proton shifts.

The two linear relationships may be combined to give a useful correlation between chemical shifts for H-6 and pKa, Equation 2, and provides an easy way to estimate the pKa of the imino group.

$$\delta(\text{H-6}) = -0.055\ \text{pKa} + 4.29 \quad (2)$$

Conformations: Considerable insight is found in the results of force field (MM2) and semi-empirical (AM1) calculations that provide information about preferred conformations. The central ring is partially puckered, C-5 is out of the approximate plane established by the other five ring atoms and both aromatic rings are twisted out of this central plane (MM2). While the angle of twist varies with the nature of the computation and the ortho substituent, all the results indicate substantial twisting about the single bond for both aromatic rings. The phenyl ring makes a dihedral angle defined by its two carbon atoms and the vinyl group on the order of 40° to 50°. With two ortho chlorine atoms the preferred angle is some 80° but there is a broad range where the angle of twist has little influence on the heat of formation (AM1). Such a large angle of twist seriously reduces the degree of conjugation between the phenyl ring and the imine double bond and would be reflected in a small Hammett rho value. The pyridyl ring makes an angle of about 50° with the central ring (MM2). But it is likely that the calculations overestimate the angle of twist for both rings. The two nitrogen atoms are separated by about 4.2 Å as illustrated below in the conformation generated by an MM2 calculation. In the Z isomer the two aromatic rings are stacked more

or less face to face. The Z isomer of the unsubstituted molecule is higher in energy by about 2 kcal/mol (AM1).



EXPERIMENTAL SECTION

Compounds: The literature method employing anabaseine and an aryl aldehyde in a mixture of ethanol and hydrochloric acid[5] is described below in detail for the preparation of the unsubstituted compound. The physical properties of other benzylidene compounds will be reported elsewhere. Those aldehydes having electron-withdrawing groups, including the *p*-chloro substrate, require the acetic acid-acetate method illustrated for the preparation of the *p*-nitro derivative.

Preparation of (3-Benzylidene)anabaseine Dihydrochloride (8). A mixture of 0.30 g (3.9 mmol) of anabaseine dihydrochloride,[1] 1 ml (9 mmol) of benzaldehyde, 5 drops of conc. HCl and 7 ml of absolute ethanol was heated at 60 °C for 3 days. A white precipitate was obtained on adding 40 ml of ethyl acetate. Recrystallization from methanol-ether gave the analytical sample, 0.33 g (87%), mp 196-200 °C.(decomp.) Anal. Calcd for $C_{17}H_{18}N_2Cl_2.H_2O$: C,60.18; H, 5.94; N, 8.25. Found: C, 60.08, H, 5.78; N, 8.05. The free base was extracted into $CDCl_3$ from an alkaline solution when the nmr

spectrum was recorded.

Preparation of 3-(4-Nitrobenzylidene)anabaseine (13). Anabaseine dihydrochloride[1] was dissolved in methanolic mixture of 0.6 M acetic acid/0.2 M sodium acetate along with two equivalents of *p*-nitrobenzaldehyde. The volume was such that the concentration of acetic acid was 2.5 times that of the anabaseine. After heating for about 24 h at approximately 60 °C the cooled mixture was extracted with ethyl acetate to remove aldehyde. Solid Na_2CO_3 was added and the alkaline solution was extracted again with ethyl acetate. Drying (Na_2SO_4) and evaporating the extract gave an oil (70%) that was made to solidify by scratching with some cyclohexane. Recrystallization from methanol gave a pale yellow product, mp 125-128 °C. Anal. Calcd for $C_{17}H_{15}N_3O_2$: C, 69.61; H, 5.15; N, 14.33. Found: C, 69.18; H, 5.10; N, 14.12.

pKa Determinations. The spectrophotometric method of Albert and Serjeant[13] used buffers at a total ionic strength of 0.12 M and include formate, acetate, carbonate and those of Good *et al.*[14] (MES, HEPES and bicine). The analytical waves lengths were 328 for the unsubstituted compound and 330 nm for the *p*-cyano substrate.

NMR Measurements. ^1H and ^{13}C chemical shifts were recorded using Varian VXR 300 or G. E. QE 300 instruments operating at ambient temperature, usually 22 ± 2 °C with TMS (tetramethylsilane) as a reference in $CDCl_3$ along with TSP (sodium trimethylsilylpropionate) in D_2O. Spectra were recorded using a 37° pulse with no delay between transients.

Average ^1H chemical shifts ($CDCl_3$/TMS) in ppm for the 17 compounds are as follows (standard deviation). Pyridine protons: H-2', 8.74 (0.03); H-4', 7.83 (0.01); H-5', 7.32 (0.02); H-6', 8.63 (0.02). Tetrahydro ring: H-4, 2.84 (0.02) not including *o*-MeO 2.74, *o,p*-$(MeO)_2$ 2.75, and *o,o,p*-$(MeO)_3$ 2.37, *o,p*-$(Me)_2$ 2.62, *o,o,p*-$(Me)_3$ 2.22; H-5, 1.84 (0.01). Olefinic hydrogen: 6.61 (0.06) not including *o*-MeO 6.83, *o,p*-$(MeO)_2$ 6.78, *o,o,p*-$(MeO)_3$ 6.47, and *p*-NO_2 6.70.

The chemical shifts for H-6 are (δ) **1**, 3.831; **2**, 3.828; **3**, 3.839; **4**, 3.862; **5**, 3.864; **6**, 3.872; **7**, 3.882; **8**, 3.892; **9**, 3.887; **10**, 3.893; **11**, 3.906; **12**, 3.929; **13**, 3.945; **14**, 3.902, **15**, 3.925, **16**, 3.894, and **17**, 3.891.

For NOE difference measurements on the VXR using the p-Cl compound the sample first was subjected to six freeze-pump-thaw cycles, the thaw portion being carried out under argon. The probe temperature was set at 25 °C and the sample kept at this temperature for 20 min prior to making measurements. A preliminary determination of T_1 values using a composite 180° pulse indicated that the longest value of 4.4 sec is associated with the protons ortho to the chlorine atom. The decoupler was shifted off resonance when not saturating a signal during NOE measurements. The difference in signal intensity found on alternating between FID's for saturation and non-saturation was recorded. Intensities for the methylene group were normalized for two protons.

Computations: Either a fortran compiled version of AM1 (MOPAC ver 5.0) for a 486 microcomputer or a Silicon Graphics instrument running MACROMODEL (ver 3.5) or a Tektronix CAChe system (MOPAC ver 6.1 or AMPAC ver 2.1) was used (PRECISE for AMPAC or MOPAC). Heats of formation often were calculated for every 10° angle of twist.

ACKNOWLEDGMENT

A grant from Taiho Pharmaceutical Company, Ltd., Tokyo, Japan supported this work. Marshall Cory provided considerable assistance with the CAChe computer.

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Received, 7th September, 1992