Structural Correlations of Choline Acetyltransferase Inhibitors: *trans*-*N*-(Carboxymethyl)-4-(β-1-naphthylvinyl)pyridinium Bromide and *cis*-*N*-(2-Aminoethyl)-4-(β-1-naphthylvinyl)-3-methylpyridinium Bromide Hydrobromide

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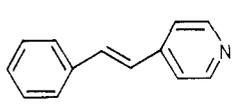
This paper correlates the X-ray structures of two 4-(β-1-napthylvinyl)pyridine analogues (one cis and one trans) with chemical and biological activity data for this class of cholineacetylase inhibitors. Our results suggest that one of the two proposed mechanisms for inhibition by this class of compounds better describes their efficacy. Previous arguments about coplanarity of the aromatic rings and nucleophilicty across the vinyl linkage need to be modified. Quantum calculations are also included and substantiate previous suggestions about the charge distribution across the vinyl linkages. An alternate new mechanism of inhibition is proposed to encompass the published data and more recent results discussed in this paper.

Acetylcholinesterase (EC 3.1.1.7, AChE) and choline acetyltransferase (EC 2.3.1.6, ChAT) are two major enzymes in nerve endings that are involved in the chemical transmission of cholinergic nerve impulses. AChE is responsible for hydrolysis of the neurotransmitter, acetylcholine, while ChAT mediates the synthesis of acetylcholine in nerve and other tissues. Whereas a great deal of information is available on AChE, much less has been reported on ChAT. Hundreds of inhibitors of ChAT have been synthesized in order to study some of the characteristics of this enzyme. Among the more potent inhibitors of ChAT are derivatives of 4-stilbazole (**1**)[1-3] and 4-(β-1-naphthylvinyl)pyridine (NVP, **2**).[4,5] Cavallito et al.[6] have reviewed structure–activity relationships (SAR) for these compounds. More recently, analogues of **1** and **2** were evaluated for inhibition and kinetic parameters on ChAT in various stages of purification.[7] Three of the four depicted regions of the NVP derivative **3** (i.e., regions a–c in **3**) have been subjected to rigorous investigation. Quaternization at the pyridine nitrogen to produce the cation NVP^+ (**3**) generally increased inhibitory action. We have also examined further details of the SAR of the side chain (region d in **3**).[8] Almost all compounds reported in the literature are purported to possess the trans geometry; the cis isomers are much less active and also difficult to obtain in a pure form. Unsaturation at region b in **3** is critical for inhibition. This report focuses primarily on the vinyl group, especially in terms of stereochemistry and possible participation in binding to ChAT.



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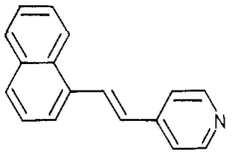

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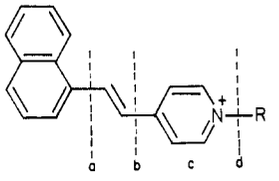

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Two major concepts concerning the activity of these inhibitors have arisen from previous SAR studies. One proposal[1,5] states that there is a requirement for coplanarity of the a–b–c system such that enzyme inhibition is favored by the presence of aromatic ring systems connected to a pyridine ring through the vinyl group. This hypothesis provides an overall coplanar molecule (with minimal three-dimensional structure), which includes a π-electron bridge between the two ring systems, suggesting the possibility of a charge-transfer complex with the enzyme. The second proposal by Baker and Gibson[3] focuses on the vinylic linkage b, since it might be involved directly in an interaction with the enzyme through a nucleophilic residue. This proposal envisions a mesomeric interaction between the carbocyclic nucleus and the pyridyl ring causing a partial positive charge to reside on the carbon adjacent to the benzene ring and a partial negative charge on the carbon next to the pyridine ring.

This paper deals with the investigation of these concepts using chemical, spectral, crystallographic, and quantum chemical techniques.[9] The first section deals with the crystal structures of both *cis*- and *trans*-NVP molecules and the subsequent two sections deal with the quantum chemical and chemical–spectral properties of these molecules. From these experiments, conclusions are drawn concerning the two major concepts of structure–activity relationships of this class of inhibitors.

Results

Crystal Structures. In related studies[8] we synthesized several NVP inhibitors. One of these derivatives, *trans*-

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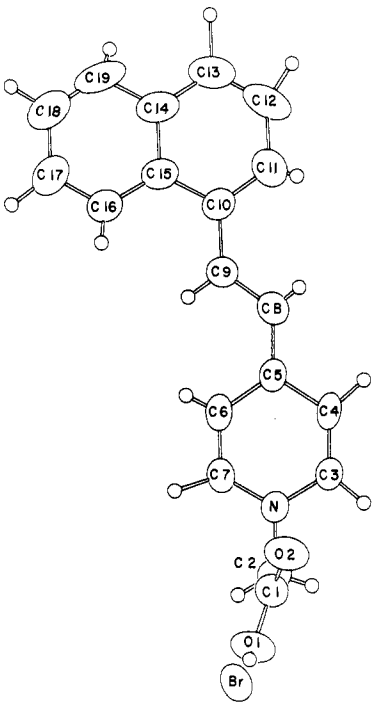
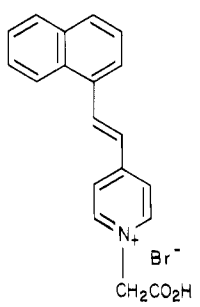


Figure 1. The atomic numbering system of 4 showing thermal vibration ellipsoids.

N-(carboxymethyl)-4-(β-1-naphthylvinyl)pyridinium bromide (4) was crystallized and subjected to a single



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crystal X-ray analysis. The molecular structure showing the thermal vibration ellipsoids for all atoms is shown in Figure 1, and bond distances and angles are listed in Table I. No significant differences were found between the dimensions of the naphthyl ring in this structure and others that have been reported.[10,11] The carboxyl group C(1)–O(1) and C(1)–O(2) bond distances [1.273 (9) and 1.207 (10) Å, respectively] on the carboxyl group compare favorably with other structures.[12,13] The bond lengths and angles of the pyridine ring likewise do not differ significantly from corresponding values in related structures.[14,15] In the area of the vinylic linkage, C(8)–C(9), which is of prime interest to this study, significant bond shortening does occur. The C(10)–C(9) bond distance of 1.442 (10) Å and the C(8)–C(5) bond distance of 1.427 (9) Å are shortened compared with a single bond between sp^2 carbons (1.488 Å).[16] Likewise, the C(8)–C(9) bond distance, 1.331 (11) Å, is comparatively short for a conjugated double

Table I. Bond Lengths and Angles in *trans*-*N*-(Carboxymethyl)-4-(β-1-naphthylvinyl)pyridinium Bromide

	bond length, Å		bond angle, deg
Br–O(1)	3.111 (6)	O(1)–C(1)–O(2)	127.3 (7)
O(1)–C(1)	1.273 (9)	C(2)–C(1)–O(1)	111.6 (6)
O(2)–C(1)	1.207 (10)	N–C(2)–C(1)	109.4 (6)
C(1)–C(2)	1.5222 (9)	C(3)–N–C(2)	120.9 (6)
C(2)–N	1.445 (9)	C(7)–N–C(2)	119.5 (6)
N–C(3)	1.364 (8)	C(7)–N–C(3)	119.2 (6)
N–C(7)	1.345 (8)	C(4)–C(3)–N	120.7 (6)
C(3)–C(4)	1.346 (10)	C(5)–C(4)–C(3)	122.0 (7)
C(4)–C(5)	1.417 (10)	C(6)–C(5)–C(4)	114.2 (6)
C(5)–C(6)	1.407 (9)	C(8)–C(5)–C(4)	122.1 (6)
C(5)–C(8)	1.427 (9)	C(8)–C(5)–C(6)	123.5 (6)
C(6)–C(7)	1.344 (9)	C(7)–C(6)–C(6)	122.1 (6)
C(8)–C(9)	1.331 (11)	N–C(7)–C(6)	121.4 (6)
C(9)–C(10)	1.442 (10)	C(9)–C(8)–C(5)	125.5 (6)
C(10)–C(11)	1.363 (12)	C(10)–C(9)–C(8)	128.5 (7)
C(10)–C(15)	1.437 (10)	C(11)–C(10)–C(9)	122.2 (7)
C(11)–C(12)	1.401 (14)	C(15)–C(10)–C(9)	120.2 (6)
C(12)–C(13)	1.351 (12)	C(15)–C(10)–C(11)	117.5 (7)
C(13)–C(14)	1.404 (12)	C(12)–C(11)–C(10)	123.1 (9)
C(14)–C(15)	1.423 (11)	C(13)–C(12)–C(11)	119.9 (8)
C(14)–C(19)	1.424 (12)	C(14)–C(13)–C(12)	120.3 (8)
C(15)–C(16)	1.397 (11)	C(15)–C(14)–C(13)	119.9 (7)
C(16)–C(17)	1.353 (11)	C(19)–C(14)–C(13)	121.6 (8)
C(17)–C(18)	1.393 (13)	C(15)–C(14)–C(19)	118.4 (8)
C(18)–C(19)	1.323 (13)	C(14)–C(15)–C(10)	119.0 (7)
		C(16)–C(15)–C(14)	117.0 (7)
		C(16)–C(15)–C(10)	123.9 (7)
		C(17)–C(16)–C(15)	122.3 (7)
		C(18)–C(17)–C(16)	120.2 (8)
		C(19)–C(18)–C(17)	120.0 (9)
		C(18)–C(19)–C(14)	121.8 (8)

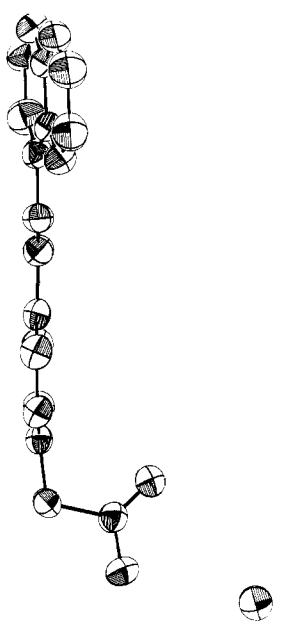

Figure 2. A view of 4 parallel to the plane of the pyridine ring, showing the coplanarity of the different segments of the molecule and the 12.6° deviation from planarity of the naphthyl ring.

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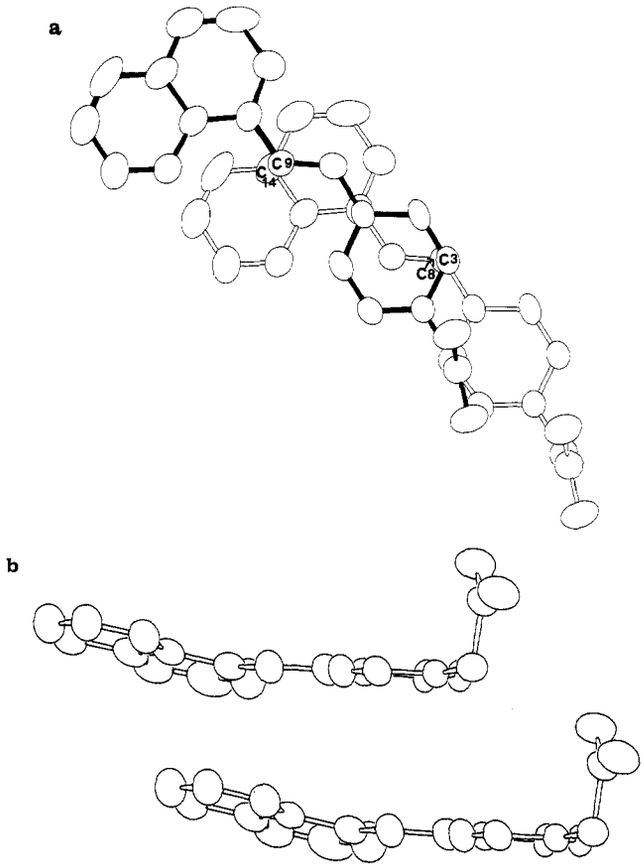


Figure 3. (a) Stacking in *trans-N*-(carboxymethyl)-4-(β-1-naphthylvinyl)pyridinium bromide. A view normal to the plane of the pyridine ring. Note the overlapping of the vinyl group and the naphthyl and pyridyl rings. Also note the close contact between C3 of one molecule with C8 of the next and the reciprocol close contact of the other vinyl group C(9) to C(14). (b) Stacking in *trans-N*-(carboxymethyl)-4-(β-1-naphthylvinyl)pyridinium bromide. A view parallel to the plane of the pyridine ring.

bond and lies close to that of an isolated double bond, 1.32 (3) Å.[17] A similar vinyl bond distance in *trans*-stilbene[18] was also found; however, the stilbene structure determination suffered from disorder, which could lead to the observed short bonds. There was, however, no apparent disorder in the present crystal structure. An interesting feature of this structure is the fact that the Br atom is associated with an oxygen rather than the quaternized N atom, indicating that the positive charge on N is distributed throughout the aromatic system [the Br···O(1) distance of 3.11 (6) Å is in agreement with other Br···O hydrogen-bonded systems]. This charge distribution was evident from the quantum calculations, as shown in the following section on quantum calculations.

Least-square planes calculated through the naphthyl and pyridine rings demonstrated that the pyridine ring and the vinyl group are completely coplanar and the naphthyl ring is out of the plane by 12.6° (cf. Figure 2). .

The stacking between the two molecules in the unit cell (Figure 3) shows that the vinyl group lies parallel to the pyridine ring of an adjacent molecule with a close intermolecular contact between C(3) and C(8) (3.55 Å), and the vinyl group of the other molecule reciprocates with a close naphthyl ring C(14) to C(9) double-bond interaction of 3.39 Å. Many aromatic hydrocarbons are observed to stack in

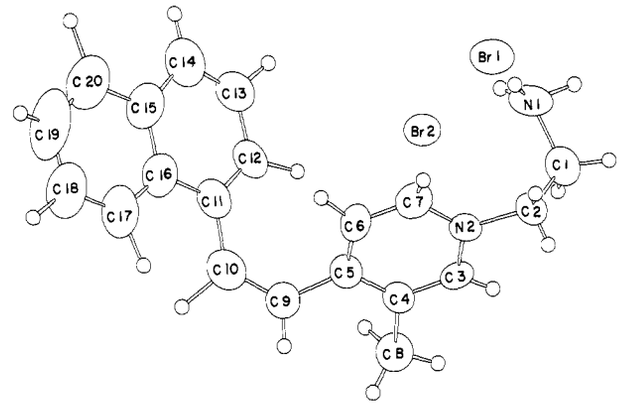


Figure 4. The atomic numbering system of **5** showing thermal vibration ellipsoids.

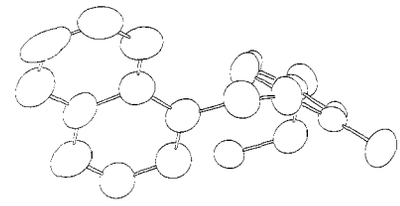
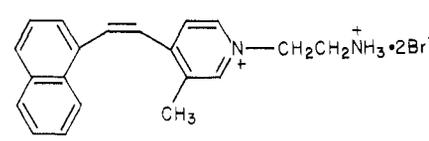


Figure 5. View of **5** parallel to the plane containing the vinyl bond and its adjacent bonds, showing the nonplanarity of the molecule as a whole.

a similar fashion with similar interplanar spacing distances, for example, 3.46,[19] 3.53,[20] 3.33,[21] and 3.55 Å.[22] It has been reported[23] that carbon–carbon double bonds which are polarized by their environment can also interact with aromatic rings in adjacent molecules. This result would then be in agreement with Baker and Gibson's views about polarization across the C(8)–C(9) bond.

Another crystal structure determination performed on *cis-N*-(2-aminoethyl)-4-(β-1-naphthylvinyl)-3-methylpyridinium bromide hydrobromide (**5**) showed some in-



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teresting results in comparison with 4. A computer plot of the molecule is shown in Figure 4, and the intramolecular bond distances and angles are given in Table II. Since the molecule has the cis configuration, the steric interactions between aromatic groups causes significant differences in planarity, as seen in Figure 5. Calculations of least-squares planes show that the pyridine ring is rotated by 42.4° out of the plane of the double bond C(9)–C(10), and the naphthyl ring is rotated out of the plane of the double bond by 36.8°. The naphthyl and pyridinium rings are situated at an angle of 62.5° from each other. The C(5) and C(11) atoms that are normally coplanar with the double bond in nonsterically hindered cis olefins are even forced apart by 12°. In addition to the strain evidenced in the C(12)–C(11)–C(10)–C(9), C(11)–C(10)–C(9)–C(5),

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Table II. Bond Lengths and Angles in *cis*-*N*-(2-Aminoethyl)-4-(β-1-naphthylvinyl)-3-methylpyridinium Bromide Hydrobromide

	bond length, Å		bond angle, deg
Br(1)–N(1)	3.275 (9)	N(1)–C(1)–C(2)	110.4 (7)
Br(2)–N(1)	3.288 (9)	N(2)–C(2)–C(1)	113.5 (7)
N(1)–C(1)	1.516 (12)	C(2)–N(2)–C(3)	121.2 (6)
C(1)–C(2)	1.511 (12)	C(2)–N(2)–C(7)	120.1 (6)
C(2)–N(2)	1.482 (10)	C(3)–N(2)–C(7)	118.5 (6)
N(2)–C(3)	1.326 (9)	C(4)–C(3)–N(2)	123.3 (7)
N(2)–C(7)	1.360 (9)	C(5)–C(4)–C(3)	118.4 (7)
C(3)–C(4)	1.353 (10)	C(3)–C(4)–C(8)	117.7 (6)
C(4)–C(5)	1.384 (10)	C(5)–C(4)–C(8)	123.8 (6)
C(4)–C(8)	1.518 (10)	C(6)–C(5)–C(4)	118.4 (7)
C(5)–C(6)	1.382 (11)	C(9)–C(5)–C(4)	118.9 (7)
C(5)–C(9)	1.475 (11)	C(9)–C(5)–C(6)	122.4 (7)
C(6)–C(7)	1.365 (11)	C(7)–C(6)–C(5)	121.4 (7)
C(9)–C(10)	1.339 (12)	N(2)–C(7)–C(6)	119.7 (7)
C(10)–C(11)	1.493 (12)	C(10)–C(9)–C(5)	127.8 (8)
C(11)–C(12)	1.386 (11)	C(11)–C(10)–C(9)	127.9 (8)
C(12)–C(13)	1.439 (12)	C(12)–C(11)–C(10)	118.7 (7)
C(13)–C(14)	1.360 (13)	C(16)–C(11)–C(10)	120.2 (7)
C(14)–C(15)	1.444 (13)	C(16)–C(11)–C(12)	120.6 (8)
C(15)–C(16)	1.446 (13)	C(13)–C(12)–C(11)	119.4 (8)
C(15)–C(20)	1.422 (15)	C(14)–C(13)–C(12)	123.2 (8)
C(16)–C(11)	1.415 (12)	C(15)–C(14)–C(12)	116.9 (9)
C(16)–C(17)	1.394 (13)	C(16)–C(15)–C(14)	121.4 (8)
C(17)–C(18)	1.311 (15)	C(20)–C(15)–C(16)	118.1 (9)
C(18)–C(19)	1.427 (17)	C(20)–C(15)–C(14)	120.4 (9)
C(19)–C(20)	1.379 (17)	C(15)–C(16)–C(11)	118.2 (8)
		C(17)–C(16)–C(11)	124.0 (8)
		C(17)–C(16)–C(15)	117.7 (8)
		C(18)–C(17)–C(16)	124.5 (9)
		C(19)–C(18)–C(17)	118.5 (10)
		C(20)–C(19)–C(18)	120.0 (11)
		C(19)–C(20)–C(15)	119.6 (11)

and C(10)–C(9)–C(5)–C(6) linkages (torsion angles of 41.3, 12.6, and 36.1°), the deviations from planarity of C(17)–C(16)–C(11)–C(10) (6.9°) and C(8)–C(4)–C(5)–C(9) (7.1°) are also noteworthy. Such deviations from planarity show the strain of the bulky cis structure, which should not be attributed to crystal packing forces, since the trans isomer does not show such nonplanarity. It is also interesting that the two charged nitrogens are in a gauche conformation [N(2)–C(3)–C(1)–N(1) = 67°] rather than the extended conformation as might have been expected.

Quantum Calculations. The possibilities that the partial positive charge on the vinyl bridge may have a strong interaction with a nucleophilic residue on the enzyme[3] and that the bond interaction between the phenyl moiety and the enzyme might be hydrophobic rather than a charge-transfer complex, or a combination of both, and the observation of close intermolecular contacts observed in the crystal structure of **4** all provide good reasons to inspect the results of quantum chemical calculations for these molecules. The net atomic charges were calculated by MNDO (modified neglect of diatomic overlap, an approximate SCF-MO method)[9] with the atomic coordinates from the crystal structures, except for the atoms C(16)–C(19) and carboxyl group in **4** and the atoms C(1), C(2), C(17)–C(20), and N(1) in compound **5** because of computer program input limitations. The results for **4** (Figure 6) clearly show the double bond to be polarized: C(9), +0.16; C(8), −0.18. The cis structure, compound **5**, also indicated a similar charge separation between the vinyl atoms: C(10), +0.11; C(9), −0.16 (see Figure 7). It is also worth noting that in the trans structure, the reciprocating vinyl-group interactions of the two stacked molecules discussed earlier, i.e. atoms C(9) and C(14) as well as C(3) and C(8), are attracted by opposite charges (see Figures 3 and 6).

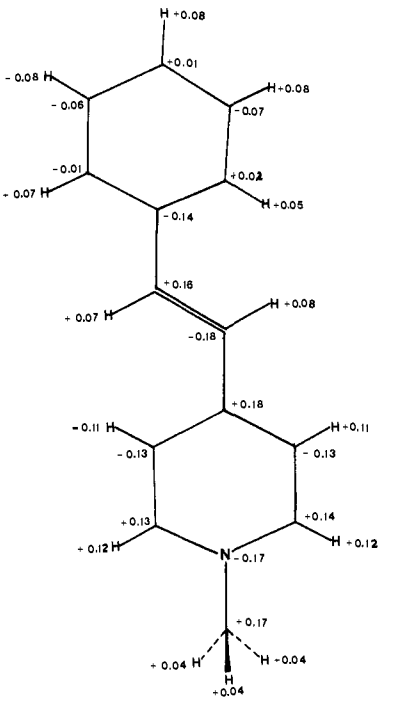

Figure 6. Electron charge distribution of **4** predicted by the MNDO molecular orbital calculations. The naphthyl ring was replaced by a phenyl group, and the carboxymethyl side chain was replaced by methyl.

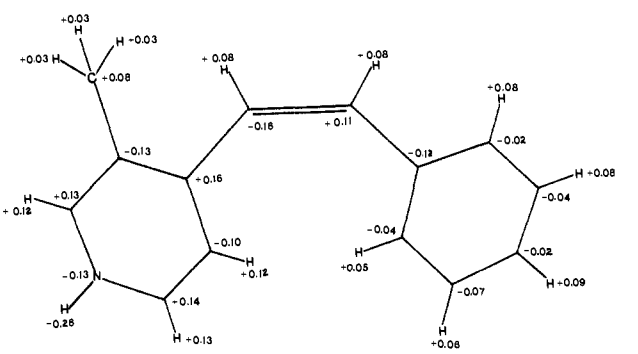

Figure 7. Electron charge distribution of **5** predicted by the MNDO molecular orbital calculations. The naphthyl ring was replaced by a phenyl group, and the 2-aminoethyl side chain was replaced by hydrogen.

Chemical Investigations. Various chemical reagents were interacted with *trans*-NVP derivatives in order to test the reactivity of the bridging double bond. Treatment of the nucleophiles, sodium azide and sodium ethyl mercaptide under various conditions produced no reaction. Addition of cold Br_2 to NVP was also negative, indicating a sluggishness with electrophilic reagents toward this double bond. However, treatment of $NVP^+CH_3I^-$ (**3**, R = CH_3) with ethyl mercaptan and elemental sulfur, similar to other literature conditions,[24] produced the sulfide product **6** in 67% yield. The ethyl mercaptan addition occurred on the carbon atom α to the naphthyl group. The structure of **6** is consistent with the NMR, high-resolution mass spectrum (Table III), quantum mechanical calculations, and a reasonable mechanistic pathway.

Treatment of $NVP^+CH_3I^-$ with elemental sulfur and cysteine under the same conditions which afforded **6** was

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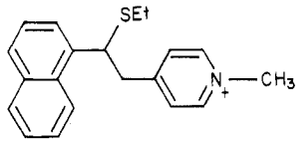

6

negative. Attempts to form sulfide addition products with NVP^+CH_3 using dithiothreitol and methyl disulfide were also negative. Treatment of NVP^+CH_3 with cold permaganate, however, showed an instant decolorization. It was further discovered by UV and chemical studies that the double bond is reactive toward water and light as previously indicated,[1] and we have found it to be extremely reactive in the presence of light and 50% acetic acid. The latter conditions produced a marked trans to cis isomerization within $^1/_2$ h.

Discussion

The aformentioned studies bring to light the fact that some of the proposed SAR correlations by previous workers may need to be revised and or incorporated under one umbrella encompassing the results of published data.

Planarity. The need for a *completely* coplanar system might be argued against or modified to say nearly coplanar. The crystal structure of the trans derivative showed that the entire aromatic conjugated system is not entirely planar (12°) in the crystalline state. Electronic spectra of NVP compounds in solution also have demonstrated that the naphthyl group is not coplanar with the vinylpyridine moiety.[25,26] The cis compounds are reported to be active as inhibitors,[27] and these most certainly cannot tolerate, to any great extent, a completely planar conjugated system between both aromatic moieties. Again, solution spectra bear this out. This raises the question of how nonplanar cis compounds can function as inhibitors. Aquilonius[27] and co-workers reported an I_{50} of 5×10^{-5} M for *cis*-NVP (isolated and purified) and 5×10^{-6} M for *trans*-NVP. Cavallito et al.[6] reported an I_{23} of 4×10^{-4} M for *cis*-NVP and an I_{50} of 2.5×10^{-5} M for *trans*-NVP·HCl, with the following statement: "The essentially inactive nature of the cis isomer...". An alternate explanation for the activity of the cis form is that it isomerizes to the trans form during the assay, but this has not been proven unequivocally in the literature. The distance from the center of the naphthyl ring to the center of the pyridine ring in the trans compound is 6.91 Å, whereas in the cis compound it is 5.80 Å. The relative positions of the rings and intramolecular distances between the rings are so different that it is difficult to imagine how both cis and trans molecules could satisfy the same binding site(s) if that site is stringent in its steric requirement and specificity. The X-ray results of the sterically strained cis form indicates that it should easily isomerize to the more sterically compatable trans form, which can be further stabilized by charge delocalization over the entire molecule.

Nature of the Vinyl Group. Baker and Gibson[2] suggest that polarization across the vinyl bond makes it susceptible to nucleophilic addition. Our laboratory chemical studies using nucleophilic reagents under various conditions do not support this conclusion. The quantum chemical calculations do show a charge distribution across these atoms as suggested by Baker and Gibson[3] and there seems to be an attraction of this double bond to the naphthyl ring of the next molecule (cf. Figure 3a); however, similar charge differences between these vinyl atoms [*trans*-**4**, C(9)–C(8), 0.34; *cis*-**5**, C(9)–C(10), 0.27] are found when looking at other adjacent atoms in the two molecules [for example, the charge difference between the carbon atoms C(5)–C(4) in *trans*-4 is 0.31, and between the carbon atoms C(6)–C(7) in *cis*-**5** is 0.26]. Considering the facts that (1) these cis–trans compounds interconvert easily in the presence of light, (2) react with mercaptan and sulfur to give an addition product, and (3) react with cold permanganate suggests a radical or radical anion attack and not a purely nucelophilic type.

Table III. High-Resolution Mass Spectral Data for 6

fragment	ion	elem comp	M_r found	M_r calcd
a	Np	$C_{10}H_7$	127.05480	127.05477
b	NpCHSEt	$C_{13}H_{13}S$	201.07397	201.07380
c	H_2C-Py	C_6H_6N	92.05080	92.05002
d	MeI	CH_3I	141.92864	141.92795

In conclusion, any final understanding of SAR studies with NVP analogues must probably await a more detailed knowledge of ChAT, which includes the need for further purification of the enzyme and possible crystallization for X-ray studies. In contrast to what has been previously published, we find that nucleophilic additions across the vinyl linkage are not likely. Also, past mechanisms requiring planarity throughout the inhibitor may be modified to say "nearly" coplanar. If the cis molecules are isomerized to the trans forms prior to binding to the enzyme, our results support the Cavallito model over the Baker–Gibson model. If the cis compounds do not isomerize before binding to the enzyme, the following mechanism for binding which encompasses the known facts can be envisioned. The first step would involve the initial binding of one of the two terminal aromatic groups of the cis inhibitor to an aromatic pocket, followed by the approach of a protein residue (such as OH or SH) or H_2O to the electropositive carbon of the double bond. A solvent molecule or protein residue may then interact with the double bond of the inhibitor (by radical or radical ion) at the electropositive vinyl carbon in a manner capable of producing a free rotation about the vinyl group. This attack need not necessarily lead to a covalent linkage but rather provide sufficient energy to open the vinyl bond and permit the second aromatic moiety (cis) to rotate to the trans-like conformation for more complete binding. Precedent for opening the vinyl linkage exists. Photohydration of *trans*-1,2-di-4-pyridinioethylenes has been observed;[28] however, such addition products were not detected after the brief exposures to the enzyme assay solutions. Such a mechanism would explain the weaker activity of the cis inhibitors due to a lesser affinity for the initial binding of only one aromatic group, whereas the trans inhibitors are favored due to simultaneous binding of both aromatic groups. Besides, if the slow step is the attack of a radical (R–S) on the double bond by a protein sulfur residue, for example, the irreversibility of these agents upon longer incubation might be expected, as is the case. The inhibitory action of the acetylenic compounds[5] can also be explained in a manner consistent with the discussion above. The acetylenes have a considerably free

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Table IV. Crystal Data for Compounds 4 and 5

	4	5
space group	$P2_1$	$P2_1/c$
a, Å	7.961	17.732
b, Å	5.067	7.607
c, Å	20.452	15.694
β, deg	95.0	113.6
ρ_{obsd}, g cm^{-3}	1.458	
ρ_{calcd}, g cm^{-3}	1.480	
Z (no. of molecules in unit cell)	2	4
no. of reflections collected	2010	3989
no of observed reflections	1885	2855
final R value	0.038	0.097

steric nature, with the aromatic groups almost superimposable (by model building) with those of the trans derivatives. This should permit simultaneous binding of both aromatic groups, which should make them similar in potency to the trans compounds, which is also found. In summary, if cis compounds are active, one can propose binding of one of the aromatic groups first, followed by opening of the double bond, rotation, and binding of the second aromatic group. The planar trans and acetylene inhibitors, on the other hand, can simply bind to the aromatic pockets directly. The binding of the skewed aromatic ring systems and opening of the double bond by an enzyme group are both suggestions from the previous SAR observations. The above hypothesis attempts to provide a unifying mechanism of action for this class of inhibitors, which would include an explanation for the activity of cis compounds.

Experimental Section

Melting points were determined with a Thomas-Hoover Uni-Melt apparatus and are uncorrected. IR data were obtained with a Perkin-Elmer Model 267 utilizing KBr pellets. NMR spectra were recorded on a Varian A-60 instrument, with spectra in organic solvents obtained with Me_4Si as an internal standard, whereas D_2O-soluble samples were run with DDS as an internal standard. UV and visible spectra were recorded on a Perkin-Elmer Instrument, Model 202. Graphite monochromated Cu $K\alpha$ X-ray intensities were collected on a Nonius-Cad-4 diffractometer using a θ–2θ scan. Analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN.

***trans*-*N*-(Carboxymethyl)-4-(β-1-naphthylvinyl)-pyridinium Bromide (4).** A solution of α-bromoethyl acetate (2.29 g, 0.013 mol) in 10 mL of dioxane was added to a solution of NVP (1.5 g, 0.0065 mol) in 15 mL of dioxane, and the resulting solution was refluxed. After 15 min, precipitation occurred, and the solution was cooled and then filtered. Recrystallization of the yellow solid from absolute ethanol–ether afforded 1.1 g (43% yield) of the analytically pure ester: mp 195–196; IR 3020, 2930, 1744, 1640, 1605, 970, 798, 770 cm^{-1}; NMR (Me_2SO-d_6) δ 1.3 (t, 3 H, CH_3), 4.3 (q, 2 H, CH_2), 5.8 (s, 2 H, CH_2), 7.4–9.2 (12 H, Ar H, vinyl); UV max (EtOH) 285, 402 nm. Anal. ($C_{21}H_{20}BrNO_2$) C, H, N. The I_{50} with ChAT was found to be 4.6×10^{-5} (see ref 8).

The corresponding carboxylic acid was prepared by hydrolysis of the ester in boiling HBr. After the solution was boiled for 20 min, needle crystals began to separate from the solution. Heating was discontinued, and the flask was allowed to stand at room temperature overnight. Upon filtration, long yellow needle crystals of analytically pure 4 were obtained in 60% yield: mp 233–235 °C; IR 3030, 1750, 1640, 1611, 975, 960, 795, 770 cm^{-1}; NMR (Me_2SO-d_6) δ 5.7 (s, 2 H, CH_2), 7.3–9.2 (13 H, Ar H, vinyl); UV max (EtOH) 285, 392 nm. Anal. ($C_{19}H_{16}BrNO_2$) C, H, N.

***cis*-*N*-(2-Aminoethyl)-4-(β-1-naphthylvinyl)-3-methylpyridinium Bromide Hydrobromide (5).** The trans form of compound 5 was prepared as follows: 4-(1-napthylvinyl)-3-methylpyridine (3 Me-NVP) was dissolved in dioxane and quaternized with a half-molar excess of 2-bromo-1-aminoethane hydrobromide. The product was recrystallized from an ethanol–methanol mixture.

Very thin, flat single crystals were grown in 95% ethanol solution with ethyl acetate at room temperature and found to be unsuitable for X-ray analysis. These crystals were redissolved in the solution by heating and three drops of 2-propanol, dioxane, and water were added. Total volume of the solution was about 2 mL. One needle-shaped crystal was obtained from this, which was shown by X-ray analysis to be the cis compound 5. Since only one crystal of cis-5 was isolated, no inhibitory properties were measured for it and no attempt to analyze the amount of *cis*-5 in solution were made.

Crystallization and X-ray Analyses. Crystals of 4 were grown in a methanol solution by vapor diffusion of methyl bromide at room temperature. A single crystal of *cis*-5 was obtained after 10 days by recrystallization of *trans*-5 from an aqueous solution containing 2-propanol and dioxane as described above. The unit cell parameters for both compounds, 4 and 5, were measured on a diffractometer. The crystal data for the respective compounds are given in Table IV.

The heavy atom positions were determined from the respective Patterson syntheses. The positions of the remaining atoms in the compounds, 4 and 5, were determined by Fourier methods. All hydrogen atoms for compound 4 were determined from a difference Fourier map, while 16 of the 22 hydrogen atoms for compound 5 were directly determined from a difference Fourier map. The remaining six hydrogen atoms in 5 were generated based on the assumed hybridization of the heavy atom to which they were attached.

The positional and thermal parameters were refined by full-matrix least-squares methods. In the case of 4, the y coordinate of the bromine atom was held fixed throughout the refinement, fixing the origin along the twofold screw axis. In both refinements, a weighing scheme based on counting statistics was employed.

The scattering factors used for the heavy atoms were those from the International Tables for X-ray Crystallography (1968) and the scattering factors for the hydrogen atoms were those of Stewart, Davidson, and Simpson.[29]

For the quantum calculations all aromatic and vinyl hydrogens atoms were given a standard C–H distance of 1.08 Å. All other hydrogens (e.g, CH_3) have a C–H distance of 1.09 Å. Bond angles and distances of all non-hydrogen atoms are the same as derived from the X-ray sructures.

***N*-Methyl-4-(1-naphthylvinyl)pyridinium Ethyl Sulfide Addition Product (6).** Compound 3 (R = Me) was prepared according to a previously reported procedure.[1] To an ethanolic solution of the pyridinium compound 3 (R = Me) (1 g, 0.0027 mol) was added 12 mg of elemental sulfur and ethyl mercaptan (1.66 g, 0.027 mol). The solution was refluxed for 5 h and then filtered, and the filtrate was evaporated in vacuo. The resulting solid was recrystallized from absolute ethanol–ether to afford pale yellow needles in 67% yield: mp 159–160 °C; IR 3020, 2960, 2920, 2860, 1640, 780 cm^{-1}; NMR (CD_3OD) δ 1.15 (t, 3 H, CH_3), 2.52 (q, 2 H, CH_2), 3.70 (d, 2 H, CH_2), 4.27 (s, 3 H, CH_3), 5.27 (t, 1 H, CH), 7.29–8.77 (11 H, Ar H). Anal. ($C_{20}H_{22}NIS$). For High-resolution mass spectrum results, see Table III.

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Registry No. *cis*-2, 20111-31-3; *trans*-2, 16375-78-3; 3 (R = Me), 20144-05-2; 4, 89711-10-4; 4 (α-bromoethyl ester), 89711-15-9; *cis*-5, 89711-12-6; *trans*-5, 89711-17-1; 6, 89711-13-7; *trans*-3-Me-NVP, 89711-14-8; ChAT, 9012-78-6; α-bromoethyl acetate, 40258-78-4; 2-bromo-1-aminoethane hydrobromide, 2576-47-8.

Supplementary Material Available: Atomic parameters (Tables 2 and 3) and observed and calculated structure factors (Appendixes I and II) for compounds 4 and 5 (45 pages). Ordering information is given on any current masthead page.

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