Crystal, Solution, and Molecular Modeling Structural Properties and Muscarinic Antagonist Activity of Azaprophen

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The structure of azaprophen, which was originally assigned by ^{1}H NMR analysis, was confirmed by X-ray crystallography. A comparison of ^{13}C NMR isotropic chemical shift data for azaprophen in the solid state and in $CDCl_3$ and DMSO-d_6 solution was used to correlate solution and solid-state conformation as determined by the X-ray data. The data suggested that the solid-state and solution conformation of azaprophen were similar. The observed solid-state structure was also compared to low-energy conformations identified by molecular-mechanics calculations. A comparison of azaprophen and atropine radioligand binding in guinea pig ileum, rat heart, rat brain, and in CHO cells expressing transfected m_1 and m_3 receptors was conducted. Azaprophen is more active than atropine in all preparations except the m_3 receptor expressed in CHO cells. However, like atropine, it does not provide major discrimination among the muscarinic receptor subtypes.

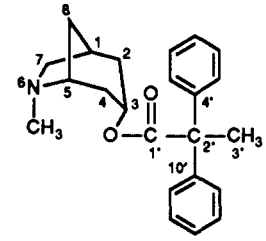
6-Methyl-6-azabicyclo[3.2.1]octan-3α-ol 2,2-diphenylpropionate (1, azaprophen) was found to be substantially more potent than atropine and other anticholinergics as an inhibitor of carbachol-induced α-amylase release and for inhibiting acetylcholine-induced contractions in the guinea pig ileum.[1] Since azaprophen was found to be about 10^6 times more potent than pirenzepine in blocking the contractile response in the guinea pig ileum and amylase secretion from pancreatic acini, we speculated that azaprophen was a strong anticholinergic at M_2 receptors. However, we pointed out that additional studies were required to establish the selectivity of azaprophen.[1] Interestingly, azaprophen has been shown to be less potent than atropine in behavioral studies using rats[2] and rhesus monkeys.[3] The structure and pharmacological profile of azaprophen suggested that it may interact with the muscarinic receptor in a unique manner.[1]

We have now obtained single-crystal X-ray data and solid- and solution-state ^{13}C NMR data which confirms the earlier structural assignment based on ^{1}H NMR analysis.[1] In addition, the conformational properties of azaprophen were studied by using solid-state and solution NMR techniques combined with molecular modeling methods. To address further the receptor selectivity of azaprophen, we have conducted radioligand binding in guinea pig ileum, brain, and heart, in rat brain, and in CHO cells bearing transfected m_1 and m_3 receptors.

Results

X-ray. The structure of azaprophen was solved by direct methods and difference Fourier techniques with SHELXTL[4] and refined by the blocked-cascade[5] least-squares refinement method using 1752 reflections with $I \geq 1.5\sigma(I)$ to $R = 0.060$ and $R_w = 0.075$. An ORTEP drawing of the solid-state conformation is shown in Figure 1. Bond length and angles are in accord with expected values.[6] The structure possesses the 3α orientation of the ester moiety as predicted by our earlier NMR studies.[1] More interesting is the endo orientation of the *N*-methyl group. The distance between the nitrogen and the C-3 oxygen is 3.26 Å. The dihedral angle between the two phenyl rings is 112.0°. One ring is folded beneath the azabicyclo ring with the distance of 4.48 Å from the nitrogen to the center of the aromatic ring.

Table I. Carbon-13 NMR Chemical Shifts for Azaprophen Hydrochloride as Solid and in $CDCl_3$ and DMSO-d_6



carbon no.	solid δ	solution	
		$CDCl_3$, δ	DMSO-d_6, δ
1	32.50	31.58	31.32
2	36.00	35.94	34.52
3	66.43	66.46	67.20
4	36.00	34.93	34.38
5	60.40	59.32	59.06
7	53.47	54.29	54.34
8	30.26	29.71	29.02
NCH_3	35.50	35.27	35.47
1′	174.80	173.33	173.27
2′	57.60	56.12	55.98
3′	27.40	27.29	26.79
4′,10′	141.99, 145.19	142.21, 143.30	143.83, 142.80

NMR. The ^{13}C chemical shift data for azaprophen in $CDCl_3$ and in the solid state are given in Table I. The solution assignments are based on chemical shifts comparisons and carbon multiplicities obtained from an attached proton test (APT) spectrum. All assignments are straight forward with the exception of carbons 2 and 4 which are not specifically assigned. The ^{13}C NMR solid-state chemical shift assignments are based on solution

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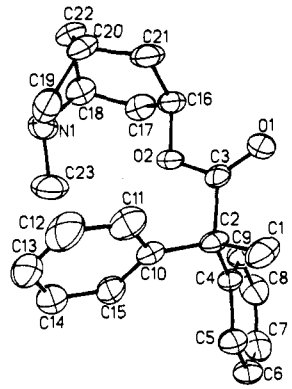
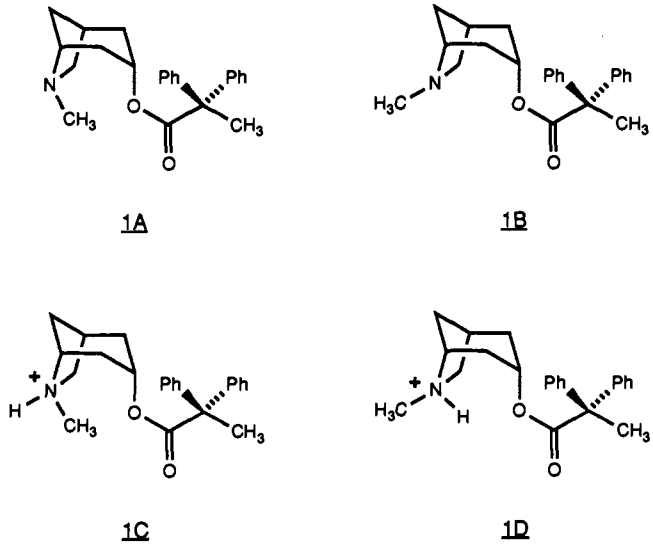


Figure 1. ORTEP view of azaprophen. The numbering of the atoms is arbitrary.

Chart I



assignments, integration of the solid-state spectrum, identification of nitrogen-bonded carbons and the use of the dipolar dephasing experiments which distinguished primary (methyl) and quaternary carbons. A comparison of the solution chemical shifts with the solid state chemical shifts shows these data to be almost identical for all carbons.

A 2-D NMR NOE (NOESY)[7] experiment was performed on **1** in $CDCl_3$. A cross peak between the N-CH_3 protons and the C-5 proton was observed.

Molecular Modeling. Four structural and conformational isomers of azaprophen, shown in Chart I, were studied by molecular mechanics methods: the *endo*- and *exo*-*N*-methyl conformers (**1A** and **1B**, respectively) of unprotonated azaprophen and the *endo*- and *exo*-*N*-methyl conformers (**1C** and **1D**, respectively) of protonated azaprophen. By using the program SYBYL,[8] the conformational global energy minima of the diphenylpropionic acid side chain was identified for each structure. the structures were then energy-minimized by using either the MAXIMIN2[8] or MM2(87)[9] force fields. The energies and geometries of these final structures were compared to the X-ray crystal structure of azaprophen. The results of these energy and structural studies are presented in Table II. Although the

Table II. Calculated Energies and Root-Mean-Square (RMS) Fits of Structures 1A–1D

	MM2(87)		MAXIMIN2	
structure	energy[a]	RMS fit[b]	energy[a]	RMS fit[a]
1A	24.813	0.2322	12.787	0.2854
1B	24.650		11.950	
1C	27.640	0.1988	17.756	0.2220
1D	21.725		14.383	

[a] Energy in kilocalories/mole. [b] RMS fit comparing all non-hydrogen atoms to the X-ray crystal structure.

conformation of the *N*-methyl group observed in the solid state is endo, the *exo*-*N*-methyl conformers (protonated or unprotonated) were calculated to have the lowest energy by either force field.

With the exception of *N*-methyl conformers, the calculation of the overall structure of azaprophen including the conformation of the ester side chain was quite successful. The torsional angles of the five rotatable bonds considered in the ester side chain were calculated to be within ±5° of the torsional angles observed in the X-ray structure. The overall accuracy of the conformational search/energy minimization procedure for predicting the structure of the endo isomers was determined by calculating the root-mean-square deviation of the atomic coordinates of the calculated structures compared to the X-ray crystal structure (Table II).

Biological. We employ the nomenclature for muscarinic receptor subtypes recommended by The Fourth International Symposium on Subtypes of Muscarinic Receptors.[10] This nomenclature uses M_1, M_2, and M_3 defined on the basis of antagonist action and m_1 to m_5 defined according to sequence. There is a likely correspondence.

The data are summarized in Tables III and IV. Table III characterizes the binding of [^{3}H]QNB to the five preparations studied and of [^{3}H]pirenzipine to rat brain. Our binding constant of 4.8×10^{-11} M for [^{3}H]QNB to the m_1 receptor is in good agreement with that recently reported by Fraser et al.,[11] and a similarly good agreement exists for the K_I value for atropine (Table IV). Binding of [^{3}H]QNB is of approximately 3-fold lower affinity to m_3 receptors than to m_1 receptors or to the receptors of ileum, heart, and brain.

Discussion

Comparison of the binding constants of azaprophen and atropine (Table IV) against [^{3}H]QNB binding in the five preparations studied reveals that, as generally recognized, atropine is a nonselective ligand. Azaprophen is approximately 2–3 times more active than atropine in all preparations except the m_3 receptor expressed in CHO cells (Table IV). Additionally, azaprophen is some 7 times more potent than atropine against [^{3}H]pirenzepine binding in brain. The K_I values that we report for azaprophen inhibition of [^{3}H]QNB binding are in good accord with that first reported, 2.7×10^{-10} M, for the inhibition of [^{3}H]*N*-methylscopolamine binding to neuroblastoma cells.[1] These values also accord with those reported earlier of 3.9×10^{-11} [pA_2 10.4] and 6.8×10^{-11}M [pK_I 9.6] for inhibition of guinea pig ileal contractions and α-amylase release, respectively, in physiologic media at 37 °C.[1]

Azaprophen shows no dramatic selectivity between the various muscarinic receptor subtypes studied. The K_I

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Table III. Characterization of Radioligand Binding at Muscarinic Receptors

tissues	[^{3}H]QNB			[^{3}H]Pirenzepine		
	$K_D \times 10^{-11}$ M	B_{max}, pmol/mg	n	$K_D \times 10^{-9}$ M	B_{max}, pmol/mg	n
guinea pig ileum	5.40 ± 0.51	4.17 ± 0.63	6			
rat heart	5.33 ± 0.53	0.42 ± 0.03	6			
rat brain	5.32 ± 0.42	1.52 ± 0.06	6	4.27 ± 0.33	0.64 ± 0.08	3
CHO m_1	4.81 ± 0.29	8.63 ± 0.33	5			
CHO m_3	12.1 ± 1.1	4.44 ± 0.70	5			

Table IV. Inhibition of [^{3}H]QNB and [^{3}H]Pirenzepine Binding by Azaprophen and Atropine

tissues	azaprophen			atropine		
	$K_I \times 10^{-10}$ M	n_H	n	$K_I \times 10^{-10}$ M	n_H	n
[^{3}H]QNB						
guinea pig ileum	2.79 ± 0.69	0.81 ± 0.05	8	6.83 ± 1.45	1.08 ± 0.09	4
rat heart	1.96 ± 0.56	0.80 ± 0.03	6	5.45 ± 0.59	1.03 ± 0.08	4
rat brain	1.30 ± 0.18	0.86 ± 0.04	6	3.71 ± 0.35	0.94 ± 0.07	3
CHO m_1	0.88 ± 2.11	0.84 ± 0.04	9	3.29 ± 0.21	1.06 ± 0.08	5
CHO m_3	4.72 ± 0.35	1.02 ± 0.04	6	3.44 ± 0.46	1.03 ± 0.04	4
[^{3}H]pirenzepine						
rat brain	1.18 ± 0.18	1.25 ± 0.14	4	8.34 ± 0.05	0.81 ± 0.02	3

values for M_1 and M_2 receptor subtypes in ileum, heart, and brain and the m_1 subtype expressed in CHO cells are not significantly different. However, azaprophen, like [^{3}H]QNB, is slightly less active against the m_3 receptors. These results do not support the thesis that azaprophen is a receptor-selective ligand.

Our findings differ both quantitatively and qualitatively from those reported recently by Gordon et al.[12] which claim azaprophen to be a selective antagonist for the M_3 muscarinic receptor subtype in rat pancreas. Their data reveal, however, major quantitative discrepancies between the previously reported affinities[1] for azaprophen and those reported in this paper. The pA_2 value, 8.21, reported by Gordon et al.[12] is much lower than that originally reported[1] in which the same guinea pig ileum preparation was used although the pA_2 value for atropine is the same in both reports. The basis for these differences is not clear. Furthermore, their data show that azaprophen reveals identical activities against [^{3}H]*N*-methylscopolamine binding in neuroblastoma cells, inhibition of ileal smooth muscle contraction and inhibition of α-amylase release [pK_I 8.32, pA_2 8.21, and pIC_{50} 8.31, respectively]. Their claim of selectivity for azaprophen derives rather from a comparison against atropine activities which reveal an anomalously low potency against α-amylase release.

In our earlier study,[1] the structure of azaprophen was deduced by an analysis of the chemical shift and observed half-height width ($W_{1/2}$) of the C-3 proton.[1] To make the assignment unambiguous and to provide conformational information, an X-ray crystal structure was obtained that confirmed the structure assignment. The single-crystal X-ray analysis also showed that a conformer possessing an endo methyl conformation was a low-energy conformer in the solid state (see Figure 1). In addition, the close similarities of the ^{13}C NMR chemical shifts (<1 ppm) in the solid state and the solution, especially for carbons 4, 5, and 7 and the *N*-methyl carbon, suggest that the solution structure of 1 is dominated by the *endo*-methyl conformation.[13] If the *exo*-methyl conformation were contributing to the solution structure of 1, one would expect a measurable ^{13}C chemical shift difference for these carbons between the solid state and solution because of likely differences in steric interactions between the *N*-methyl group and carbons 4, 5, and 7. The observed NOE between the N-CH_3 protons and the C-5 proton provides additional evidence that the N-CH_3 group of 1 is in the endo conformation in solution.

A stepwise systematic conformational search of the diphenylpropionate side chain located a global energy minimum that was very close to the observed conformation. With the exception of *N*-methyl conformer prediction, molecular mechanics calculations on azaprophen have been found to produce highly accurate models. The difference in the *N*-methyl conformation is not surprising since the force-field calculations model the behavior of the isolated molecule in vacuo while the X-ray structure is that of the ammonium compound associated with an anionic counterion in the solid state. Furthermore, the determination of the relative energies of *N*-methyl conformations of cyclic systems is a well-known problem.[14]

With the validation provided by this comparison of observed and modeled structures, application of this computational model to comparisons of the steric and electrostatic fields around azaprophen and other muscarinic agents is now possible. In particular, a computer-aided molecular-modeling examination of the isomeric azabicyclic head groups of azaprophen and atropine is expected to provide insight into the exact structural requirements for binding to muscarinic receptors.

Conclusion

Azaprophen is a potent muscarinic antagonist. However, in contrast to an earlier report, it shows little selectivity between the various muscarinic receptor subtypes studied.[12] Single-crystal X-ray analysis confirmed the structure of azaprophen as 6-methyl-6-azabicyclo[3.2.1]octan-3α-ol 2,2-diphenylpropionate. A correlation of the solid state and solution ^{13}C NMR data to the X-ray data showed that the solid and solution conformational properties of azaprophen were similar. The computational and experimentally observed structures of azaprophen reveal close agreement in overall shape and conformation. Of the two sites of conformational flexibility in azaprophen, a combined conformational search/force-field calculation was

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quite successful in mimicing the observed conformation of the highly flexible ester side chain. Molecular mechanics prediction of the endo/exo preference of the *N*-methyl group in this ring system has proven to be a more subtle problem and will be the subject of further investigation.

Experimental Section

Single-Crystal X-ray Analysis of Azaprophen. Crystal Data. Single-crystal X-ray data for azaprophen hydrochloride $C_{23}H_{28}NO_2Cl$, fw = 385.94, were obtained from a colorless crystal (recrystallized from methanol and ether) fragment 0.53 × 0.40 × 0.32 mm^3. The crystal belongs to the monoclinic space group $P2_1/c$ with a = 8.468 (8), b = 14.300 (10), c = 16,802 (10) Å, β = 93.85 (4)°, V = 2030 (3) Å^3, Z = 4, D_{calc} = 1.26 g cm^{-3}.

Crystallographic Measurements. A total of 2368 data were collected between $5 \leq 2\theta \leq 42°$ using monochromatized Mo K_α radiation (λ = 0.71069 Å) by the $\theta/2\theta$ scan method on a Siemens/Nicolet R3m/μ diffractometer. Data were corrected for the Lorentz and polarization effects but not for absorption (μ = 2.0 cm^{-1}). The structure was solved by direct methods and difference Fourier techniques with SHELXTL[4] and refined by the blocked-cascade[5] least-squares refinement method using 1752 reflections with intensities, $I \geq 1.5\sigma(I)$ to R = 0.060 and R_w = 0.075. $\sigma(I)$ values were derived from counting statistics, and weights were assigned according to $w = 1/[\sigma^2(F) + 0.003F^2]$. Non-hydrogen atoms were refined with anisotropic thermal vibration parameters. Hydrogen atoms were located from a difference Fourier map and were refined with isotropic thermal parameters. The maximum and minimum difference electron density residuals at the end of refinement were +0.37 and −0.41 e Å^{-3}, respectively. An ORTEP drawing of azaprophen is shown in Figure 1. Tables of structure amplitudes, fractional coordinates, anisotropic thermal parameters, and bond lengths and bond angles are deposited as supplementary material.

The dihedral angle between the two benzene rings is 112.0°. The chloride ion is hydrogen-bonded to the protonated nitrogen with N–H = 1.17 (5), H—Cl = 1.80 (5) N—Cl = 2.961 (3) Å, and the angle N–H—Cl = 175 (4)°.

NMR Experimental Methods. The ^{13}C cross polarization, magic angle spinning (CP/MAS) NMR spectra were obtained on about 100 mg of solid compound using a Chemagnetics CMC-200S (^{13}C at 50 MHz) and IBM NR100AF (^{13}C at 25 MHz) NMR spectrometers. The samples were spun about an axis oriented at 54° 44′ to the static field with rates from 2.5 to 6.5 KHz. All CP/MAS spectra were acquired with a CP time of 2000 μs and 3-s relaxation delay. At 200 MHz a 3.5-μs proton pulse (H_1 = 17 gauss, 72 KHz) was used, and two 1 K data blocks zero filled to 4 K were employed. At 100 MHz the proton pulse was 6.0 μs (H_1 = 10 gauss, 40 KHz) and two 2 K data blocks zero filled to 4 K were used. During the dipolar dephasing (DD) experiments at 200 MHz, a 68-μs delay in the proton pulse was inserted immediately following the carbon pulse (CP). At 100 MHz a 60-μs delay with a 12-μs 180° pulse to refocus chemical shifts was used. All signals were referenced to the most upfield resonance (31 ppm) observed in the spectrum of 1,4-di-*tert*-butylbenzene.

The solution ^{13}C NMR data were acquired by using a Bruker WM250 spectrometer. The NOESY experiment was run on a General Electric GN500 NMR spectrometer with use of a 2D NOE sequence in magnitude mode.

Molecular Modeling. Molecular modeling was performed by using SYBYL software package version 5.32 (Tripos Associated, St. Louis) running on Digital Equipment Corporation microVAX II's and VAXstation 3110 workstations. An Evans & Sutherland PS330 graphics workstation and a Macintosh IIcx interfaced to the VAX cluster were used for display and real-time manipulation of three-dimensional molecular models.

For comparison purposes, the coordinates of the X-ray crystal structure of azaprophen were input using the CRYSIN command. The computational models of **1A-D** were constructed from molecular fragments contained in the SYBYL package. Systematic conformational searches of the five rotatable bonds of the diphenylpropionate side chain (rotation of the terminal methyl group was not considered) were performed by first using 30° increments over 360° ranges, except for the phenyl groups which were allowed to rotate over a 180° range. The lowest energy conformations of the 30° search were then submitted to a search using 1° increments over ranges of ±15° around the starting angle for each rotatable bond. The global energy minimum conformations identified by this procedure were then submitted to further energy minimization under the MAXIMIN2 or MM2(87) force fields.

The final structures were compared to the X-ray crystal structure by using the FIT command of SYBYL. The relative position of all the corresponding non-hydrogen atoms of the calculated structure and the X-ray crystal structure were compared, and the extent of the superimposition of all such pairs of atoms is expressed as a root-mean-square deviation.

Biological. Cell Cultures. Chinese hamster ovary (CHO) cell lines specifically expressing transfected m_1 or m_3 muscarinic receptor subtypes were employed. These lines were supplied by Dr. C. Fraser, Section on Receptor Biochemistry, NINCDS, NIH.[11] Cells were cultured in monolayer in 100-mm plastic culture dishes in medium containing nutrient mixture F-12 (Ham's) 90%, fetal bovine serum 10%, L-glutamine 2 mM, penicillin 50 units/mL, and streptomycin 50 μg/mL in a humidified atmosphere of 95% O_2/5% CO_2. Geneticin 50 μg/mL was added to the medium to prevent the loss of cell lines expressing the cloned receptor genes. Cells were subcultured at a density of (2–5) × 10^4 cells per mL of medium, and the cultures became confluent by day 3 with a density of (2–5) × 10^6 cells per dish containing 10 mL of medium.

Preparation of Cells and Tissues for Radioligand Binding. Microsomal fractions of guinea pig ileum, rat heart, and rat brain were prepared as described previously in our laboratory.[15,16] In brief, male albino guinea pigs and male Sprague-Dawley rats were killed by decapitation and guinea pig ileal longitudinal smooth muscle of the intestine was stripped away. The smooth muscle and brain were minced with scissors in 15 vol/g wet weight in ice-cold 50 mM Tris buffer (pH 7.2, 15 °C) with 10 passes of a motor-driven (TRI-R-Stirrer) glass–Teflon homogenizer at setting 7. Rat heart homogenate was prepared in similar fashion save that it was first homogenized in a Brinkman Polytron at setting 7 for 5 s. The m_1 or m_3 expressing cells were suspended in 50 mM ice-cold Tris buffer and homogenized with a motor-driven glass–Teflon homogenizer at setting 5 with 10 passes.

The homogenates were centrifuged at 1100g for 20 min, and the supernatants were recentrifuged at 45000g for 45 min at 4 °C. The resultant pellet was resuspended in ice-cold 50 mM Tris buffer at a concentration of 10–20 μg, 20–30 μg, 120–150 μg, 5–8 μg, 10–15 μg per 5 mL binding assay volume for ileum, brain, heart, m_1, and m_3 cells, respecitively, for the [^{3}H]QNB binding assay. For the [^{3}H]pirenzepine binding assay, 50–80 μg of brain protein per 1 mL assay volume was used. Protein concentrations were measured by the method of Bradford[17] with bovine serum albumin as a standard.

[^{3}H]QNB and [^{3}H]Pirenzipine Radioligand Binding. The membrane fractions were incubated with various concentrations of [^{3}H]QNB or [^{3}H]pirenzipine to define saturation curves for these ligands. The incubation times at 25 °C were 60 min for the tissue preparations, 90 min for the m_1 cells, and 120 min for the m_3 cells. For competition experiments 4.56 × 10^{-11} M [^{3}H]QNB and 2.44 × 10^{-9} M [^{3}H]pirenzepine were employed with various concentrations of azaprophen or atropine. Nonspecific binding was defined by 10^{-6} M atropine. After incubation, samples were filtered over Whatman GF/B filters and washed twice with equal volumes of ice-cold Tris buffer by using a cell harvester (model M-24R, Brandel Instruments, Gaithersberg, MD). *Materials.* [^{3}H]QNB [L-[*benzilic*-4,4-^{3}H]quinuclidinyl benzilate), specific activity 43.9 Ci/mmol and [^{3}H]pirenzipine ([*N-methyl*-^{3}H]pirenzepine), specific activity 87.1 Ci/mmol were purchased from DuPont-New England Nuclear (Boston, MA). Tissue culture media and supplements were obtained from GIBCO Laboratories, Grand Island, NY. *Data Analysis.* Radioligand binding data were analyzed by using a nonlinear curve fitting program (BDATA,

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CDATA, EMF Software, Knoxville, TN) implemented on an IBM personal computer. K_I values were calculated by the method of Cheng and Prusoff.[18]

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Registry No. 1, 107010-27-5.

Supplementary Material Available: Tables of structure amplitudes, fractional coordinates, anisotropic thermal parameters, and bond lengths and bond angles (6 pages). Ordering information is given on any current masthead page.

Thienotriazolodiazepines as Platelet-Activating Factor Antagonists. Steric Limitations for the Substituent in Position 2

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The preparations of thienotriazolodiazepines bearing a substituted ethynyl group at the 2-position, and the corresponding *cis*-olefins and fully saturated analogues are described. The compounds were evaluated as potential antagonists of platelet-activating factor (PAF) in in vitro and in vitro test models. The new thienotriazolodiazepines are compared with known related compounds such as WEB 2086 (compound **6**) and the phenylethyl derivatives **27** and **28**.

In a previous publication[1] we reported the preparation of a series of triazolobenzodiazepines and thienotriazolodiazepines carrying a functionalized propynyl group at the 8- and 2-position, respectively. Some of these compounds were among the most potent, orally active platelet-activating factor (PAF) antagonists yet described. When the site of attachment of the propargylic side chain was shifted from the 8-position to the 9-position of the triazolobenzodiazepine system, the activity was essentially lost. We ascribed this result to lack of binding to the PAF receptor brought about by the bulky substituent protruding linearly from the 9-position. We now wish to report on the observation of similar steric limitations for the 2-position substituent of some 2-ethynyl-substituted thienotriazolodiazepines.

Chemistry

The preparation of the unsaturated analogues of WEB 2086[2] (compound **6**) is illustrated in Scheme I. Brotizolam, (**1a**)[3] was coupled with propargyl alcohol by palladium catalysis to give **2**. Oxidation of this propargylic alcohol with activated manganese dioxide in the presence of morpholine led directly in moderate yield to the amide **3**. Hydrogenation of the triple bond to the *cis*-olefin **4** was achieved by using 5% palladium on carbon as catalyst and thiophene as a poison. The known saturated compound **6** (WEB 2086) was obtained by standard hydrogenation of **3** over palladium on carbon. The *trans*-olefin **5** resulted from the reduction of **3** with sodium borohydride in methanol. As suggested by this hydride reduction, the triple bond in compound **3** is quite reactive toward nucleophiles and reacted smoothly with hydrazine at room temperature to form the hydrazone **7**, which was thermally converted to the pyrazolone **9**. Addition of sodium azide to the triple bond led to the vicinal triazole **8** (Scheme II).

The ethynyl derivatives **10–21** were accessible by the coupling of the iodo compound **1b**[1,4] with various acetylenes as described in our previous paper[1] (Method A). The monosubstituted acetylene **10** was prepared by coupling of **1b** with (trimethylsilyl)acetylene to give **11** and subsequent hydrolysis of the trimethylsilyl group. Compound **10** could then be functionalized by coupling with aryl iodides as shown by the reaction of **10** with 1-iodonaphthalene to yield **13**, and by coupling with 1-(4-iodophenyl)imidazole (**45a**) to yield the acetylenic precursor of **30** (Method A'). It was generally preferable to prepare the substituted acetylenes prior to the coupling with the triazolothienodiazepine **1b**. The required acetylenes **41–43**, **47b–d**, and **50** were formed by reaction of the appropriate aryl halide with (trimethylsilyl)acetylene and subsequent hydrolysis (Method E). The aryl iodides **36** and **45a**, as well as the known compounds **45b**[5] and **45c**[6] were prepared by Sandmeyer reaction of the corresponding anilines (Method D). 1-(4-Aminophenyl)imidazole (**44a**) was accessible by reduction of the nitro compound as described in the literature.[7] The iodides **37** and **38** were obtained by iodination (Method F) of the dihydrobenzofurans **39** and **40**,[8] respectively (Scheme III). The known 4-iodoisobutylbenzene[9] (**45d**) was accessible by direct iodination of commercially available **46** with iodine monochloride.

While the hydrogenation of the triple bond to the single bond (Method C) presented no problems, the partial hydrogenation (Method B) to the *cis*-olefins was more difficult and compounds **22–26** generally had to be isolated chromatographically from the mixture containing some starting material and some over-hydrogenated saturated product. The saturated analogue **6**, **27**, and **28** had been previously prepared by different syntheses as disclosed in

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(3) Weber, K.-H.; Bauer, A.; Danneberg, P.; Kuhn, J. US patent 4,094,984; June, 1978.
(4) This compound was first prepared by M. Gerecke at Hoffmann-La Roche & Co, AG., Basle, Switzerland.
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