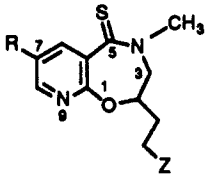
Optical Isomers of Rocastine and Close Analogues: Synthesis and H1 Antihistaminic Activity of Its Enantiomers and Their Structural Relationship to the Classical Antihistamines

Mark C. Sleevi,*,†,# Albert D. Cale, Jr.,† Thomas W. Gero,†,# Larry W. Jaques,† William J. Welstead,† Ashby F. Johnson,† Brian F. Kilpatrick,‡ Iulia Demian,§ Joseph C. Nolan,∥,# and Herndon Jenkins⊥

Research Laboratories, A. H. Robins Company, Inc., Richmond, Virginia 23261-6609. *Received January 24, 1990*

The enantiomers of 2-[2-(dimethylamino)ethyl]-3,4-dihydro-4-methylpyrido[3,2-*f*]-1,4-oxazapine-5(2*H*)-thione (rocastine) and two of its more potent analogues were prepared with an enantiomeric purity of >99.9%. The antihistaminic activity of these compounds was assessed by their ability to block histamine-induced lethality in guinea pigs and to inhibit [^{3}H]mepyramine binding to guinea pig cortex. In this series, compounds having the *R* configuration at the 2-position are at least 300 times more potent than the *S* isomers. Conformational analysis and molecular modeling suggest that rocastine can adopt a conformation in which the pyridine ring, ether oxygen, and protonated amine functions are positioned similarly to the corresponding elements of the probable binding conformers of some of the more classical antihistamines. This conformation, boatlike in the oxazepine ring with the side chain quasi-equatorial and folded back toward the ring, is the likely binding conformer at the histamine H1 receptor, and the available structure-activity relationship data is consistent with this interpretation.

Many histamine H1 antagonists contain chiral centers, and those that do often show marked differences in the antihistaminic potency of their optical isomers.[1] This paper describes the synthesis and pharmacological activity of the enantiomers of rocastine (1) and two of its more potent analogues (2 and 3). The racemic compounds are



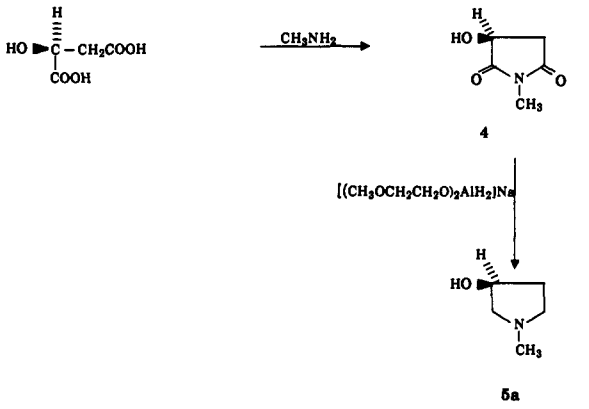
1: R = H, Z = $N(CH_3)_2$
2: R = Cl, Z = $N(CH_3)_2$
3: R = Cl, Z = N⬡

part of an unusual series of antihistamines prepared in these laboratories and recently reported[2] to include some of the most potent and selective H1 antagonists known. Conformational analysis, based on molecular mechanics calculations and NMR, and molecular modeling have indicated a probable binding conformation for 1 and likely points of common drug-receptor interaction for the active enantiomer of 1 with those of some of the more classical antihistamines. The results of these studies will be discussed along with structure-activity relationships (SAR) supporting the interpretations.

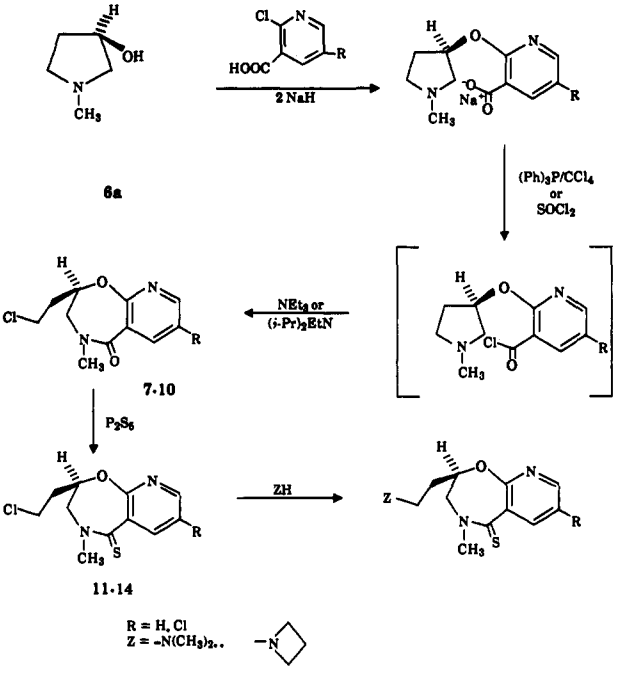
Chemistry

The general synthetic route described in the previous paper[2] is adaptable to the synthesis of the optical isomers of 1-3 since the starting material, 1-methyl-3-pyrrolidinol, is readily resolved via tartrate salts. The *S* isomer of 1-methyl-3-pyrrolidinol (**5a**) was also prepared (Scheme I) from commercially available (*S*)-malic acid by a modification of the method described by Bhat et al.[3] The remainder of the synthesis, shown for the *R* isomer in Scheme II, was accomplished without cleavage of any bond attached to the asymmetric carbon (Scheme II), thus establishing the absolute configuration of the target compounds. The intermediate 2-(2-chloroethyl)-3,4-dihydro-

Scheme I



Scheme II



4-methylpyrido[3,2-*f*]-1,4-oxazepin-5(2*H*)-ones and -thiones (7-14) are listed in Table I along with methods of prepa-

† Department of Chemistry.
‡ Department of Molecular Biology.
§ Department of Analytical Research.
∥ Department of Pharmacology.
⊥ Currently with Summit Industries, Altanta, GA.
Currently with Whitby Research, Inc., P.O. Box 27426, Richmond, VA 23261-7426.

(1) Casy, A. F. *Handbook of Stereoisomers: Therapeutic Drugs*; Smith, D. F., Ed.; CRC Press: Boca Raton, FL, 1989; pp 149-164.

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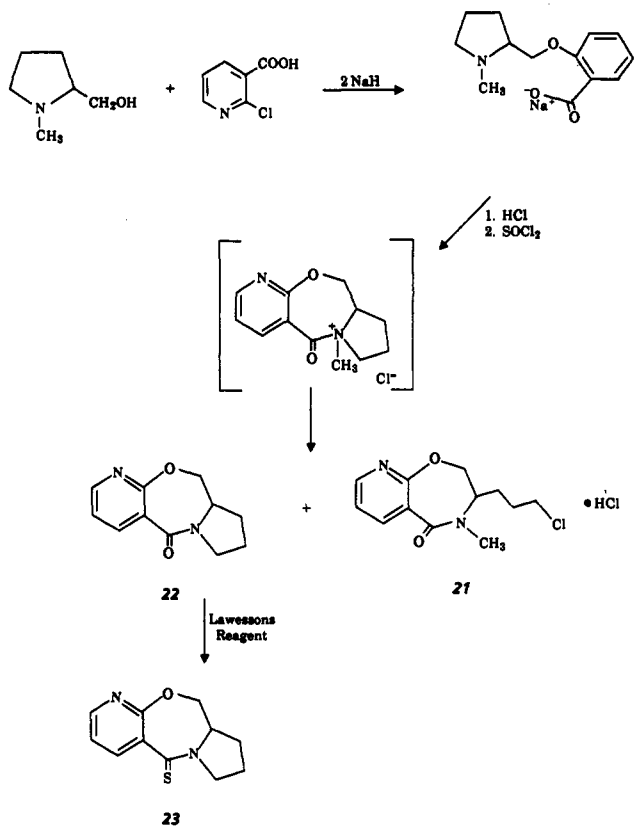
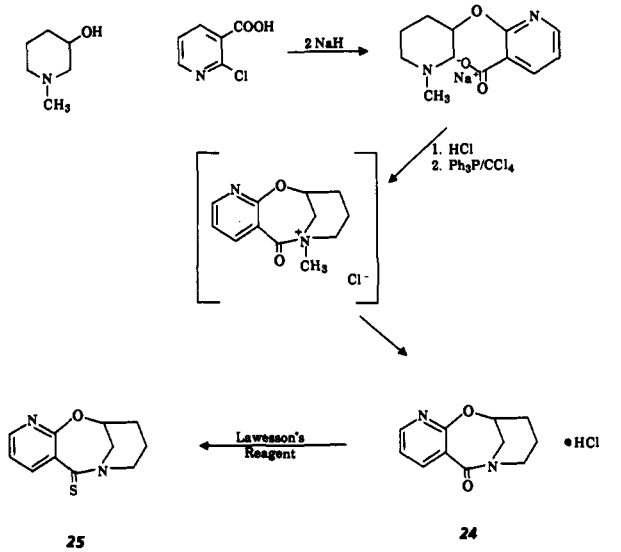
Scheme III



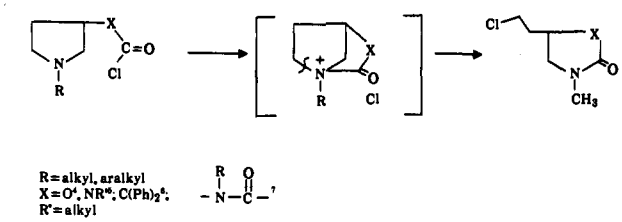
Scheme IV



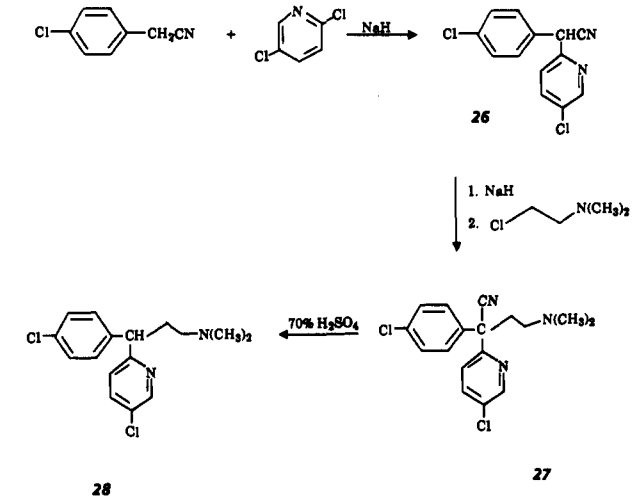
ration and physical data. Similar data for the target compounds (**15–20**), which were shown by HPLC to have enantiomeric purity of at least 99.9%, is presented in Table II.

Four tricyclic compounds (**22–25**), which were prepared in the course of the synthetic work on the rocastine series proved useful in the conformational analysis of **1**. Routes of these compounds, shown in Schemes III and IV, involve the intramolecular reaction of an acid chloride with a tertiary amine and can be viewed as proceeding through

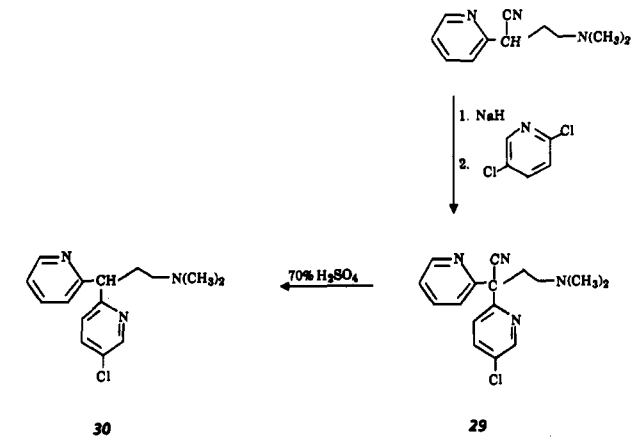
Scheme V



Scheme VI



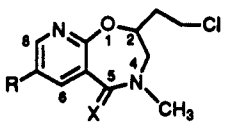
Scheme VII



an acylated quarternary ammonium intermediate. Nucleophilic attack by chloride ion on this intermediate cleaves one of three carbon–nitrogen bonds, with the composition of the product mixture reflecting the regioselectivity of the attack. Interestingly, in several previously reported[4–7] examples of this rearrangement (Scheme V) involving a pendant acid chloride at the 3-position of a pyrrolidine ring, the cleavage always occurs with ring opening of the pyrrolidine to produce a chloroethyl side chain. The same regioselectivity was observed in the preparation of chloroethyl intermediates (i.e., **7**) for the rocastine series (Scheme II). However, on replacement of the 1-methyl-3-pyrrolidinol in this sequence with 1-methyl-3-piperidinol (Scheme IV), the product results from N-demethylation rather than ring opening. None of the

(2) Cale, A. D., Jr.; Gero, T. W.; Walker, K. R.; Lo, Y. S.; Welstead, W. J., Jr.; Jaques, L. W.; Johnson, A. F.; Leonard, C. A.; Nolan, J. C.; Johnson, D. N. *J. Med. Chem.* **1989**, *32*, 2178.
(3) Bhat, K. L.; Flanagan, D. M.; Joullie, M. M. *Synth. Commun.* **1985**, *15* (7), 587.
(4) Fielden, M.; Welstead, W. J., Jr.; Dawson, N.; Chen, Y.; Mays, R.; DaVauzo, J.; Lunsford, C. *J. Med. Chem.* **1973**, *16*, 1124.
(5) Lunsford, C. D.; Cale, A. D., Jr. U.S. Patent 3,337,580, 1967.
(6) Lunsford, C. D.; Cale, A. D., Jr.; Ward, J. W.; Franko, B. W.; Jenkins, H. *J. Med. Chem.* **1964**, *7*, 302.
(7) Lunsford, C. D.; Cale, A. D., Jr. U.S. Patent 3,696,104, 1972.

Table I. 2-(2-Chloroethyl)-3,4-dihydro-4-methylpyrido[3,2-*f*]-1,4-oxazepin-5(2*H*)-ones and -thiones as Intermediates[a]



no.	config	R	X	method of prep	% yield[b]	mp,[c] °C	$[\alpha]_D$, deg (*c*) [solv][d]	recryst solvent	formula[e]
7	*S*	H	O	A	20	61–62	−22.95 (1.1) [MeOH]	$(i\text{-Pr})_2O$	$C_{11}H_{13}ClN_2O_2$
8	*R*	H	O	A	24	61–62	+22.4 (1.1) [MeOH]	$(i\text{-Pr})_2O$	$C_{11}H_{13}ClN_2O_2$
9	*S*	Cl	O	B	26	71–77	−26.6 (2.2) [MeOH]	$(i\text{-Pr})_2O$	$C_{11}H_{12}Cl_2N_2O_2$
10	*R*	Cl	O	B	36 (crude)	76–78	+26.9 (1.5) [MeOH]	$(i\text{-Pr})_2O$	$C_{11}H_{12}Cl_2N_2O_2$
11	*S*	H	S	C	73	168–170	+48.2 (1.0) [MeOH]	i-PrOH–$CHCl_3$	$C_{11}H_{13}ClN_2OS$
12	*R*	H	S	C	68	168–170	−47.4 (1.0) [MeOH]	i-PrOH–$CHCl_3$	$C_{11}H_{13}ClN_2OS$
13	*S*	Cl	S	C[f]	60	161–163	+139.7 (0.8) [MeOH]	$(i\text{-Pr})_2O$	$C_{11}H_{12}Cl_2N_2OS$
14	*R*	Cl	S	C[f]	75	160–162	−145.4 (0.5) [MeOH]	$(i\text{-Pr})_2O$	$C_{11}H_{12}Cl_2N_2OS$

[a] All compounds exhibited IR, ^1H NMR, and MS spectra consistent with assigned structures. [b] Isolated yields; no efforts were made to optimize yields. [c] Melting points were taken on a Thomas–Hoover apparatus in capillary tubes and are uncorrected. [d] Optical rotations were taken between 21 and 23 °C. [e] All compounds gave C, H, and N analysis within 0.4% of theoretical except where noted. [f] Reaction time was 8 h.

chloropropyl product analogous to **21** was isolated or detected in the reaction mixture by mass spectroscopy.

Two novel chlorpheniramine (**31**) analogues (**28** and **30**) were also prepared by the classical methods shown in Schemes VI and VII to extend the available SAR data for comparison with the rocastine series.

Biological Activity

The optical isomers (**15–20**) of rocastine and two of its more potent analogues were evaluated for antihistaminic activity. The biological data reported in Table II include oral ED_{50} values for protection against a lethal dose (twice the LD_{50}) of histamine in guinea pigs and IC_{50} values for the inhibition of [^{3}H]mepyramine binding to the H1 receptor in guinea pig cortex. In all cases, the *R* isomer is the more potent compound, with ED_{50} ratios (*S*/*R*) for **15**/**16**, **17**/**18**, and **19**/**20** of 328, 5138, and 1533, respectively. Although the values differ, the order of IC_{50} ratios remains the same for the in vitro binding (700, 6263, and 3269). For comparison, the active *S* isomer of chlorpheniramine (**31**) has been reported[8] to be about 100 times more potent than the *R* isomer, based on binding studies in tissue from a variety of mammalian brains. The ED_{50} values against histamine-induced lethality at 1, 6, and 24 h pretreatment times show that **20** has both a long duration of action and high potency.

Antihistaminic data for the two new analogues (**28** and **30**) of chlorpheniramine in Table III indicate that **30** is highly potent, while **28** has only weak activity.

Conformational Analysis and Relationship to Classical Antihistamines

Having both the previously reported SAR data[2] and knowledge of the absolute configuration required for antihistaminic activity in the rocastine (**1**) series, it is reasonable to question the relationship of these compounds with the more classical antihistamines. In the absence of detailed information on the actual structure of a particular receptor, a useful approach for comparison of different series of compounds that interact at that receptor involves the elucidation of a pharmacophoric model, or three-dimensional arrangement of chemical groups and features, which are collectively responsible for recognition and binding. The nature of the pharmacophoric groups included in the model is inferred from common elements of drugs interacting at the receptor. The spatial arrangement

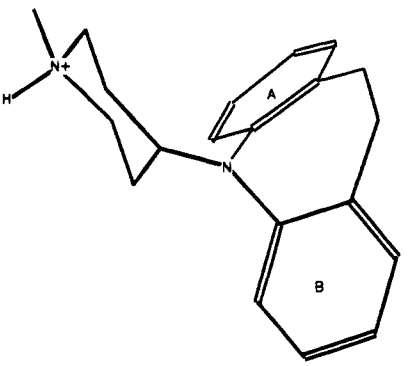


Figure 1. A simplified version of the histamine H1 pharmacophoric model proposed by Naturo et al.,[9] with sites for the aromatic rings (A and B) and potonated amine nitrogen (N^+) based on cyproheptadine (**2**) and including a site (N) for a hydrogen-bonding heteroatom.

of these groups can be deduced by examining low-energy conformations of different drugs to find those conformations in which the pharmacophoric groups are oriented in a manner achievable by all of the compounds. This process is greatly simplified if one or more of the drugs binding to the receptor is conformationally rigid, thus establishing the orientation required for binding.

Once a suitable pharmacophoric model is obtained, it serves as a template for topographical comparison of drugs in different series. Binding conformations for flexible drugs can be proposed if low-energy conformers are found in which the pharmacophoric groups are superimposable on those of the model. Pharmacophoric equivalence of chemical groups in different series of compounds can be inferred from overlays of their binding conformers. Since the equivalent groups are assumed to occupy the same site on the receptor, the validity of both the pharmacophoric model and the fit of various compounds to it can be verified in that identical chemical modification of equivalent groups in two series of compounds should produce roughly parallel changes in the biological activity.

Naruto et al.[9] proposed a four-element pharmacophore for the histamine H1 receptor, which consisted of two aromatic rings (A and B) and protonated amine nitrogen (N^+) positioned as in the rigid antihistamine cyproheptadine (**32**, Chart I) and a site for a heteroatom (N) capable of hydrogen bonding. Molecular mechanics and least-

(8) Chang, R. S. L.; Tran, V. T.; Snyder, S. H. *J. Neurochem.* **1979**, *32*, 1653.

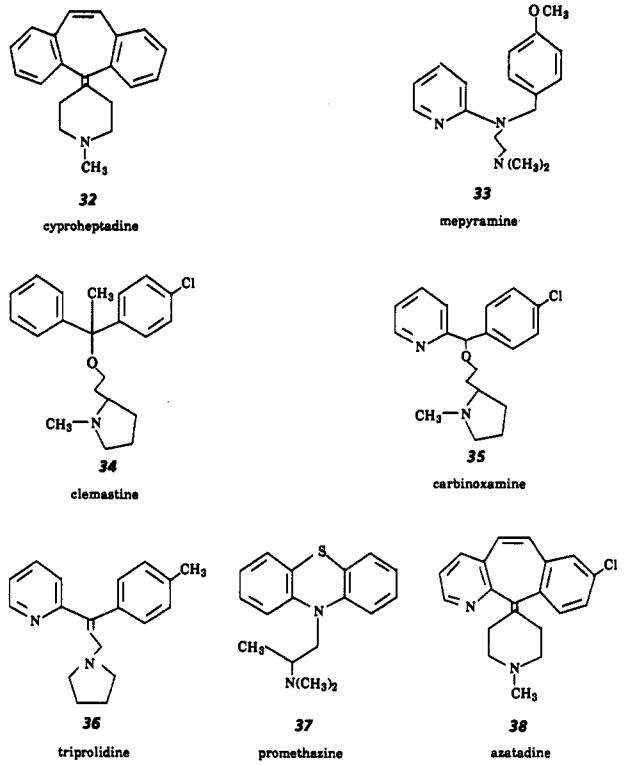
(9) Naturo, S.; Motoc, I.; Marshall, G. R. *Eur. J. Med. Chem.* **1985**, *20*, 529.

Table II. Physical and Antihistaminic Data on 2-[2-(Dimethylamino and azetidino)ethyl]-3,4-dihydro-4-methylpyrido[3,4-*f*]-1,4-oxazepine-5(2*H*)-thiones[a]

no.	config	R	Z	method of prep	reaction time, days	% yield[b]	mp,[c] °C	$[\alpha]_D$, deg (*c*) [solv][d]	recryst solvent	formula[e]	protection against histamine-induced lethality in the guinea pig: ED_{50}/mg/kg po (confidence limits); pretreatment time: 1 h	6 h	24 h	inhibition of [^{3}H]mepyramine binding to the histamine receptor in guinea pig cortex: IC_{50}, nM (av. of *n* experiment)
15	*S*	H	$N(CH_3)_2$	D	2	36	170–179	+20.8 (1.1) [H_2O]	(*i*-Pr)$_2$O	$C_{13}H_{19}CN_3OS$·2HCl	23 (17.7–29.9)	ND	ND	21000 (3)
16	*R*	H	$N(CH_3)_2$	D	2	50	170–176	−21.8 (1.1) [H_2O]	EtOH	$C_{13}H_{19}N_3OS$·2HCl	0.07 (0.05–0.09)	2.96 (1.45–6.58)	ND	30 (3)
17	*S*	Cl	$N(CH_3)_2$	D	6	76	181–182	+63.3 (3.0) [H_2O]	*i*-PrOH–(*i*-Pr)$_2$O	$C_{13}H_{18}ClN_3OS$·HCl·0.5H_2O	66.8 (37.8–100.1)	ND	ND	11900 (2)
18	*R*	Cl	$N(CH_3)_2$	D	7	68	181–182	−60.2 (2.3) [H_2O]	*i*-PrOH–(*i*-Pr)$_2$O	$C_{13}H_{18}ClN_3OS$·HCl·0.5H_2O	0.013 (0.0097–0.017)	0.15[f] (0.13–0.18)	58 (36.7–91.6)	1.9 (2)
19	*S*	Cl		E	3	53	109–110.5	+135 (0.5) [MeOH]	tol-(*i*-Pr)$_2$O	$C_{14}H_{16}ClN_3OS$[g]	8.28 (3.88–15.91)	ND	ND	870 (2)
20	*R*	Cl		E	3	57	109–111	−136.8 (1.0) [MeOH]	tol-(*i*-Pr)$_2$O	$C_{14}H_{16}ClN_2OS$	0.0054 (0.003–0.007)	0.0078 (0.0061–0.0107)	0.21 (0.05–3.91)	0.26 (2)

[a–g] See corresponding footnotes in Table I. [f] ED_{50} at 5 h pretreatment time. [g] C: calcd, 53.92; found, 53.47.

Chart I

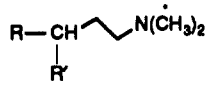


squares methods were used to develop a three-dimensional model of the pharmacophore. Each of the seven potent antihistamines included in the study could adopt a conformation fitting the model with only a modest energy cost, within the approximately 5 kcal/mol generally available from drug–receptor interactions. A somewhat simplified version of this model (Figure 1) used in the current work was constructed from the skeletal elements of cyproheptadine (**32**) minimized with molecular mechanics, to which has been added the heteroatom site. Molecular mechanics calculations were performed with the MMX force field,[10] which is an adaptation of Allinger's MM2 program[11] with the π routines from MMP1.[12] Overlays of antihistamines with the pharmacophroic model were created with PCMODEL.[10] Since the MMX force field lacked torsion parameters for bonds to a protonated amine, structures for protonated antihistamines for fitting purposes were generated within PCMODEL with the geometry of the corresponding free base minimized with MMX. The model of the histamine H1 pharmacophore in Figure 1 was readily generated with PCMODEL and MMX, and accommodates the binding conformers of the antihistamines suggested by Naturo et al.[9] An overlay of the active *S* isomer of chlorpheniramine (**31**), in the approximate binding conformation suggested by the previous authors, with the proposed pharmacophore is shown in Figure 2.

Conformational analysis of **1** and related compounds in the series was performed using a combination of molecular mechanics and ^{1}H NMR. Vicinal proton–proton coupling constants for conformations of interest were calculated for comparison with experimentally measured values using the generalization of the Karplus equation developed by Haasnoot et al.[13] with routines built into PCMODEL. Cou-

(10) Serena Software, Bloomington, IN.
(11) Allinger, N. L. *J. Am. Chem. Soc.* **1977**, *99*, 8127.
(12) Allinger, N. L.; Sprague, J. T. *J. Am. Chem. Soc.* **1973**, *95*, 3893.
(13) Haasnoot, C. A. G.; de Leeuw, F. A. A. M.; Altona, C. *Tetrahedron* **1981**, *36*, 2783.

Table III. Antihistamic Data for Chlorpheniramine Analogues



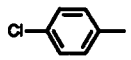
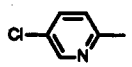
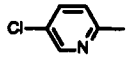
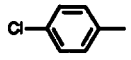
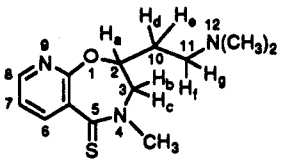
no.	R	R′	protection against histamine-induced lethality in the guinea pig: ED_{50}, mg/kg po at 1 h pretreatment (confidence limits)	inhibition of [^{3}H]mepyramine binding to the histamine receptor in guinea pig cortex: IC_{50}, nm (av of *n* experiments)
28			>10	430 (1)
30			0.009 (0.0055–0.0141)	24 (6)
31			0.18 (0.06–0.55)	8.8 (3)
	(chlorpheniramine)			

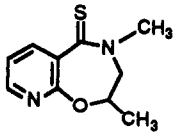
Table IV. Proton Chemical Shifts for 2-[2-(Dimethylamino)ethyl]-3,4-dihydro-4-methylpyrido[3,2-*f*]-1,4-oxazepine-5(2*H*)-thione Hydrochloride (Rocastine, 1)



H	chemical shift (ppm) in $CDCl_3$	chemical shift (ppm) in D_2O
a	4.954	5.038
b	3.664	3.855
c	3.824	3.945
d	2.405	2.063
e	2.091	2.150
f	3.560	3.436
g	3.447	3.641

pling constants and chemical shifts were determined from the 400-MHz spectra by computer simulation by using the standard software package on the FT-80A spectrometer. The empirical coupling constants have an accuracy of ±0.1 Hz. A summary of chemical shift data and proton–proton coupling constants for rocastine (1) is given in Tables IV and V.

To determine the conformational preferences of the heterocyclic ring system of rocastine (1), 3,4-dihydro-2,4-dimethylpyrido[3,2-*f*]-1,4-oxazepine-5(2*H*)-thione (II), which can be viewed as a fragment of 1 lacking most of the flexible side chain, was studied by use of molecular mechanics. The calculations indicated the existence of



II

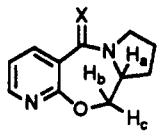
the two low-energy conformers shown in Figure 3 (III and IV), along with the calculated coupling constants and energy. The heterocyclic ring of each boatlike conformer bears a mirror image relationship with the other, the difference being in the orientation of the 2-methyl substituent. A similar boatlike conformation has been proposed on the basis of ^{1}H and ^{13}C NMR analysis by Duddeck and Levai[14] for some related 1,4-benzoxazepin-5-one and -thione derivatives. In addition, two model com-

(14) Duddeck, H.; Levai, A. *Arch. Pharm.* **1983**, *316*, 100.

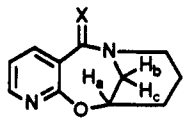
Table V. Proton–Proton Coupling Constants for 2-[2-(Dimethylamino)ethyl]-3,4-dihydro-4-methylpyrido[3,2-*f*]-1,4-oxazepine-5(2*H*)-thione Hydrochloride (Rocastine, 1)

J	coupling constants (Hz) in $CDCL_3$	coupling constants (Hz) in D_2O
ab	8.5	8.3
ac	3.9	3.9
bc	-15.3	-15.4
ad	2.5	2.4
ae	10.8	11.0
de	-13.2	-14.4
df	5.0	5.6
dg	11.0	9.9
ef	10.8	9.6
eq	4.8	5.8
fg	-12.8	-13.1

Table VI. Experimentally Determined Proton–Proton Coupling Constants for Protons on the Oxazepine Ring Portion of **22–25**, with Calculated Values for Their MMX Minimized Conformations in Figure 4



22 X = O
23 X = S



24 X = O
25 X = S

no.	conformer	J_{ab}(exp)	J_{ab}(calc)	J_{ac}(exp)	J_{ac}(calc)
22	XIX	8.3	8.7	1.3	0.8
23	XVII	11.6	11.1	4.3	6.6
24	XVIII	1.0	1.0	2.1	4.4
25	XVI	1.0	0.9	2.6	4.6

pounds (**23** and **25**) were prepared with saturated rings fused to the oxazepine-5(2*H*)-thione system. The added rings prevent the ring flipping which interchanges conformations such as III and IV, greatly simplifying the Karplus analysis of coupling constants. The experimental values for the coupling constants for both **23** and **25** are in reasonable agreement with those calculated (Table VI) for a boatlike conformation of the oxazepine-5(2*H*)-thione ring (Figure 4). The two conformers of 3,4-dihydro-2,4-dimethylpyrido[3,2-*f*]-1,4-oxazepine-5(2*H*)-thione (III and IV) are essentially identical energetically. The experimentally determined coupling constants (Table VI) for protons on the 2- and 3-positions of the oxazepine ring of 1 (J_{ab} and J_{ac}) are in agreement (4.2 and 8.6 calculated vs 3.9 and 8.5 experimental) with those calculated for a 3:1 mixture of the quasi-axial conformer IV with the quasi-equatorial conformer III. On addition of the full (di-

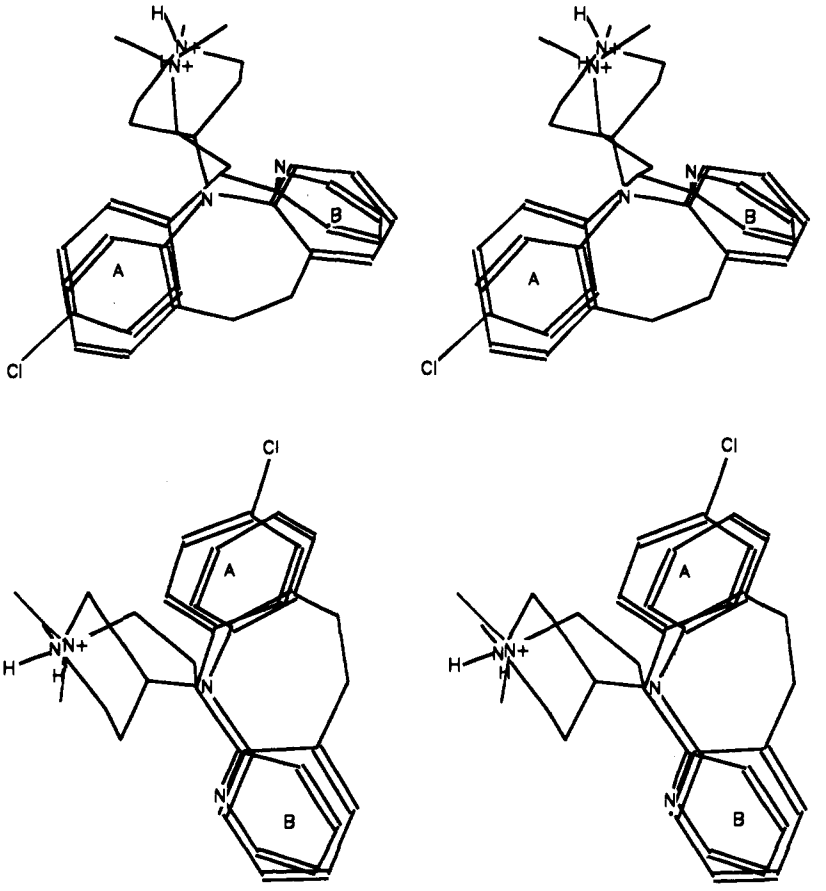
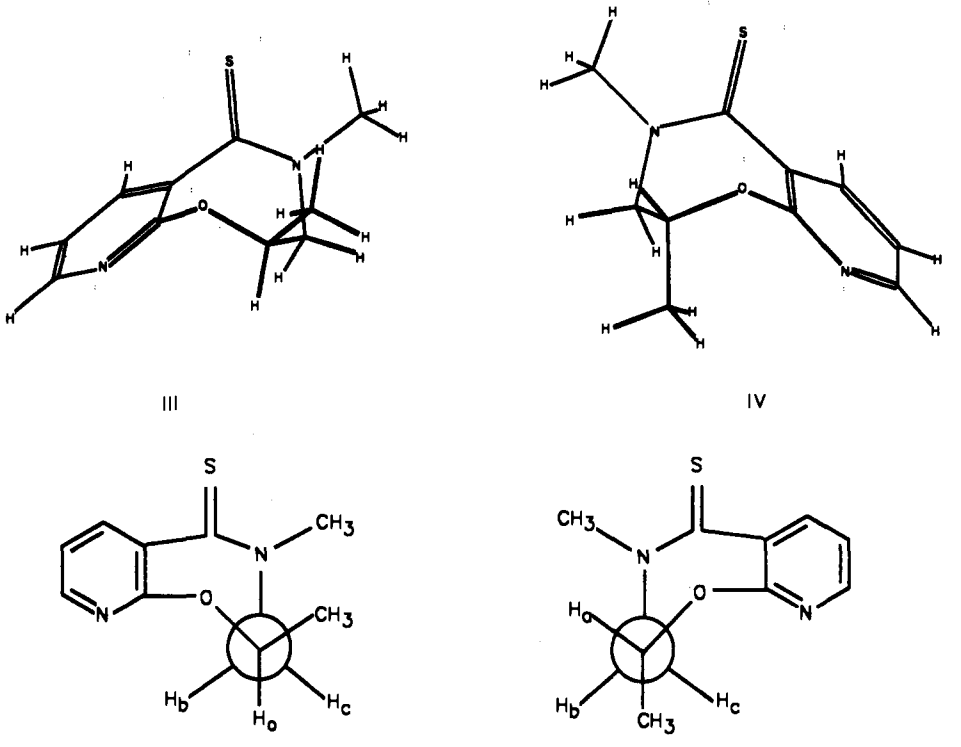


Figure 2. Two stereo views of the fit of chlorpheniramine (31) to the pharmacophoric model.



quasi-equatorial
MMX energy 25.71 kcal/mole
$J_{ab\ calc}$ 6.27 Hz (39 deg)
$J_{ac\ calc}$ 0.74 Hz (74 deg)

quasi-axial
MMX energy 25.85 kcal/mole
$J_{ab\ calc}$ 3.53 Hz (51 deg)
$J_{ac\ calc}$ 11.25 Hz (166 deg)

Figure 3. Low-energy conformers (III and IV) of 3,4-dihydro-2,4-dimethylpyrido[3,2-*f*]-1,4-oxazepine-5(2*H*)-thione, with Newman projections along the C2–C3 bond showing the approximate orientations of the ring protons in each.

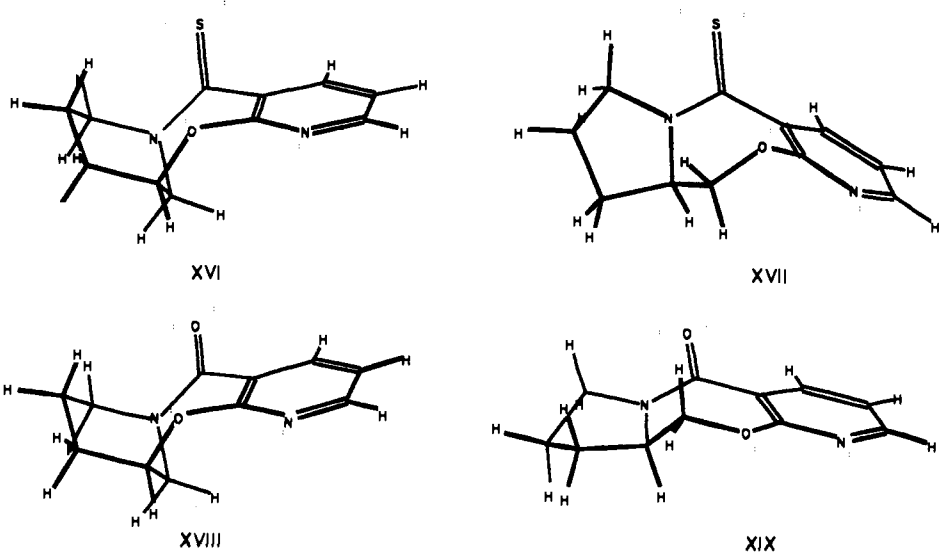


Figure 4. MMX minimized conformations of **25** (XVI), **23** (XVII), **24** (XVIII), and **22** (XIX). Calculated coupling constants are similar to those observed (Table VII).

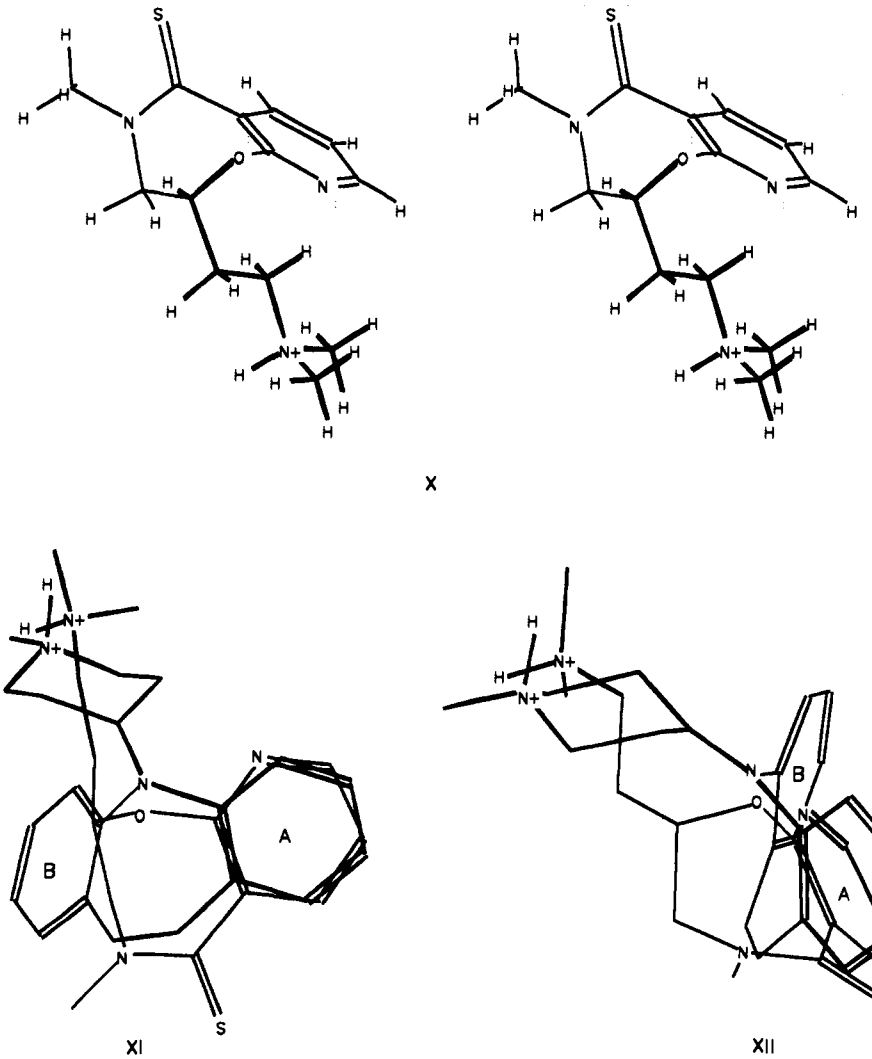
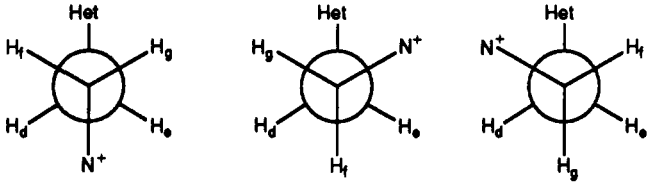


Figure 5. Stereo view of the major solution conformation (X) of 1 based on ^{1}H NMR and molecular mechanics calculations, with two views (XI and XII) of its fit to the H1 pharmacophoric model.

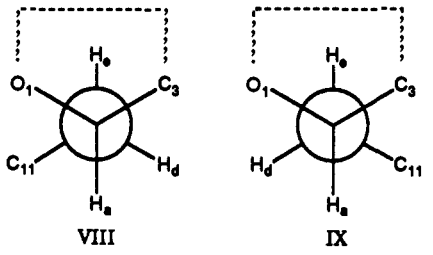
methylamino)ethyl side chain, the energy calculated for the quasi-axial conformer becomes slightly lower than that of the quasi-equatorial for similar side chain orientations, probably due to increased steric interaction between the larger substituent and the amide *N*-methyl group in the quasi-equatorial conformer.

The experimentally determined values for coupling between the protons of the dimethylene portion of the side chain (J_{dg}, J_{df}, J_{ef}, J_{eg}) of 1 indicate predominantly gauche relationship for H_d with H_f and for H_e with H_g, while an anti relationship is suggested for H_d with H_g and for H_e with H_f, consistent with the extended rotomer V being the

major species in either $CDCl_3$ or D_2O. With use of an approximation of 13 Hz for coupling of protons having an anti relationship and 3 Hz for gauche, the experimental values are in agreement with a 4:1:1 ratio of the extended rotomer V to the folded rotomers VI and VII.



The orientation of the side chain is further determined by the torsion angles of the substituents around the C2–C10 bond linking the heterocyclic ring with the side chain. The values of the coupling constants J_{ad} and J_{ae} suggest an anti relationship between H_a and one of the hydrogens on C10, and a gauche relationship with the other. MMX calculations on the two rotomers meeting these requirements for both the quasi-axial and quasi-equatorial conformations indicate that the orientation having C11 positioned anti to C3 in the ring (VIII) is favored by 1.5 to 2 kcal/mol over that having C11 positioned anti to ring oxygen (IX), probably due to steric interaction of the hydrogens on C11 with those on C3 of the ring in the latter.



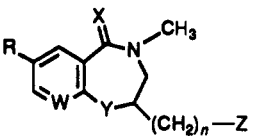
In Figure 5 is shown a stereo view (X) of the conformation of the active *R* isomer of rocastine (1) which represents the major species in solution on the basis of both on the NMR data and calculations described above. While the conformer binding to the histamine H1 receptor is not necessarily the major conformer in solution, it provides a useful reference point. As mentioned previously, the proton–proton coupling constants for 1 appear to require substantial proportions of minor conformers to account for their magnitude. These conformations contribute significantly to the time-weighted average value of the coupling constants and cannot be excluded energetically from consideration as potential binding conformers.

Figure 5 also shows two views (XI and XII) of the best fit found for the low-energy conformer with the model of the histamine H1 pharmacophore. In it, the pyridine ring of the *R* isomer of rocastine fits well over the aromatic ring (A) of the model, with the protonated amine nitrogen situated near the N^+ site and the ether oxygen filling the hydrogen bonding heteroatom (N) site. It is apparent from XII, however, that while the fit to the major elements of the pharmacophoric model is good, the overall spacial requirements of this conformation are quite different from those of the pharmacophore, with the bulk of the seven-membered heterocyclic ring and the side chain positioned well above the corresponding portions of the cyproheptadine derived model.

A much better fit (XIV and XV) was obtained for conformer XIII in Figure 6. As before, the pyridine ring of 1 overlays the aromatic ring (A) of the model, with the ether oxygen positioned at the hydrogen bonding (N) site and the protonated amine nitrogen located at the N^+ site. In addition, the planar arrangement of atoms formed by C13–N4–C3 about the thioamide nitrogen overlays and is approximately coplanar with the other aromatic ring (B). Atoms of the dimethylene portion of the side chain also fit well with elements of the piperidine ring of the pharmacophoric model. The conformation shown in XIII is a quasi-equatorial boat corresponding to III in Figure 3, with the side chain in a folded conformation. MMX calculations indicate that the energy of conformer XIII as the free base is only about 1.3 kcal/mol above the minimum represented by X, well within the range normally considered acceptable for a binding conformation. Protonation might even be expected to stabilize folded conformations as in XIII by charge–dipole interaction of the protonated amine group with the electronegative oxygen and nitrogen of the heterocyclic ring.

Discussion

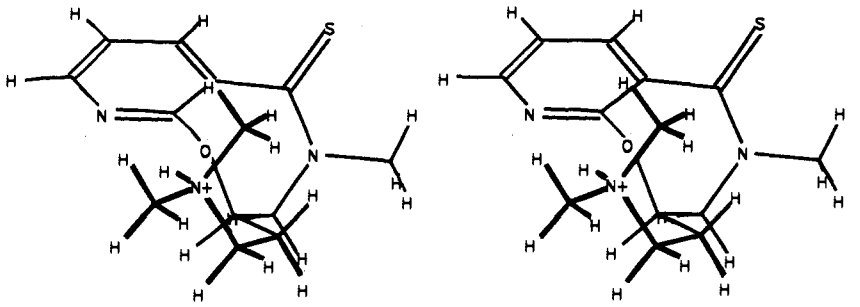
Conformer XIII in Figure 6 represents a low-energy conformation of 1 with a good fit to the pharmacophoric model. Assuming that the available pharmacological data (blockade of histamine-induced lethality in guinea pigs, po or ip) primarily reflect receptor affinity, the fit can be used to help rationalize much of the SAR associated with the rocastine series, and to provide a basis for comparison of this series with other antihistamines. Key elements of the previously reported[2] SAR data for the rocastine series are summarized with reference to the general structure XX as follows. In the seven-membered heterocyclic ring, the



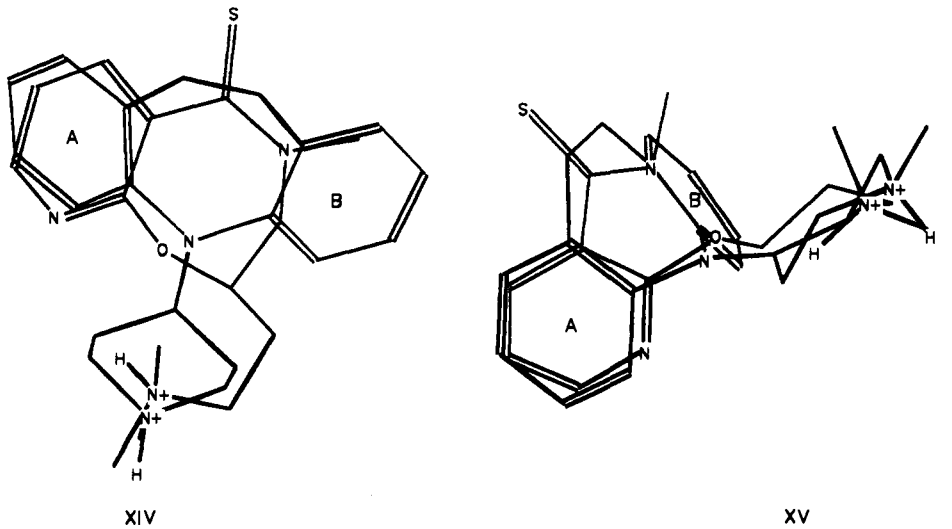
XX

ether oxygen (Y = O) is required for good activity. Compounds with other groups are much less potenti (Y = S or CH_2) or inactive (Y = NCH_3) compounds. Thioamides (X = S) are generally much more potent than the corresponding amides (X = O). The most potent compounds contained a small tertiary amine group (Z = $N(CH_3)_2$ or azetidinyl) on the side chain. Primary, secondary, or larger tertiary amine substituents were substantially less potent. The optimal length for the side chain was obtained with a dimethylene bridge (n = 2). Compounds with shorter (n = 1) side chains were inactive, while those with longer (n = 3) were less potent. For the aromatic portion of the system, pyridinyl compounds (W = N) are considerably more active than phenyl (W = CH) analogues, and a chlorine substituent at the 7-position (R = Cl) increased activity by about 4-fold over hydrogen (R = H).

The fit shown in Figure 6 locates the ether oxygen of 1 at the N site of the model, where hydrogen bonding to the receptor presumably can stabilize drug–receptor binding. The presence of a similarly positioned hydrogen-bonding heteroatom can be inferred from the structures of many common antihistamines including mepyramine (**33**), clemastine (**34**), and carbinoxamine (**35**), although it is not an absolute requirement (i.e., **31** and **32**). A heteroatom at this site may be required to compensate for greater side chain flexibility, since potent antihistamines lacking this feature generally have either fewer atoms separating the aromatic portion of the molecule from the protonated amine (i.e. **31**) or have a rigid carbon–carbon double bond incorporated in the side chain (i.e., **32** and **36**). The SAR data suggest that this interaction is critical in the rocastine series in that replacement of the ether oxygen with a methylene (non-hydrogen

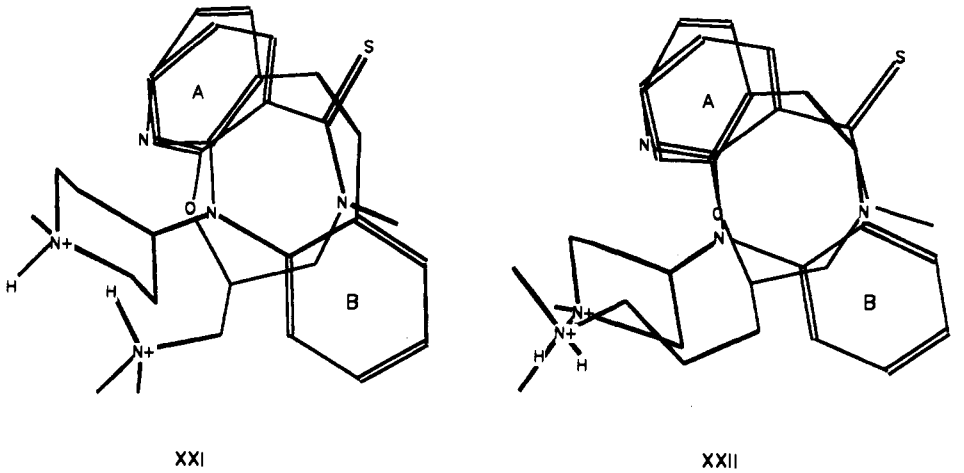


XIII



XIV XV

Figure 6. Stereo view of the proposed binding conformation (XIII) of 1, with two views (XIV and XV) of its fit to the H1 pharmacophoric model.



XXI XXII

Figure 7. Effect of changing the length of the side chain of 1 on the fit to the H1 pharmacophoric model.

bonding) or sulfur (weakly hydrogen bonding) afforded compounds with greatly reduced activity. This effect may be magnified in the current series due to the absence of a second aromatic ring.

The folded binding conformation (XIII) is supported by SAR data, in that substitution of a methylamino group for the ether oxygen (O1) led to a complete loss of antihistaminic activity. Steric interaction between the methyl group of the methylamino substituent and the side chain prevents adoption of the folded conformation proposed for binding. In the proposed binding conformation (XIII) of 1, the hydrogen of the protonated amine is located approximately 2.1 Å from the ether oxygen (O1). The folded conformation of the side chain might be stabilized by a hydrogen bond between these two atoms. The importance of this feature is, however, questionable in light of the predominance of the extended orientation indicated by the NMR.

The superimposition of the proposed binding conformers of 1 with the pharmacophoric model (Figure 6) suggests that the tertiary amine group of 1 occupies the same region of the histamine H1 receptor as similar groups found in the side chains of the more classical antihistamines. This is supported by the similar requirement of small tertiary amine substituents[2,15] for optimal activity in both.

(15) Witiak, D. T. *Medicinal Chemistry*, 3rd ed., Part II; Burger, A., Ed.; Wiley-Interscience: New York, 1970; pp 1645–1668.

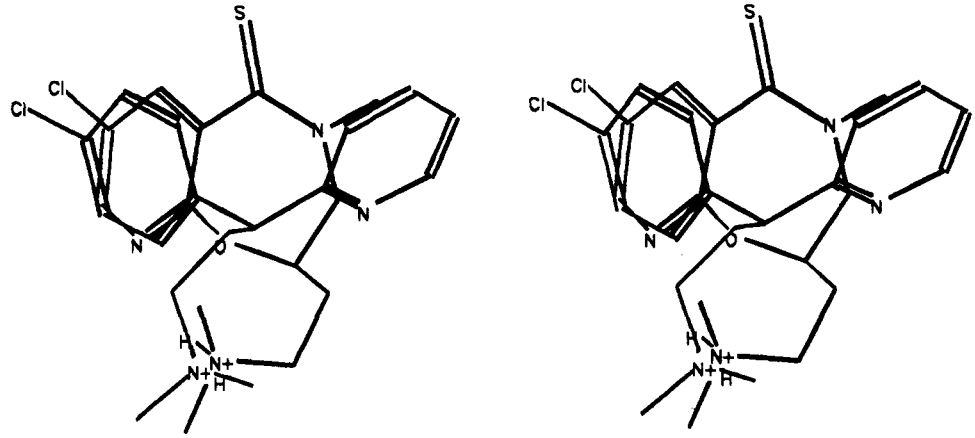


Figure 8. Superimposition of the proposed binding conformers of the active enantiomers of chlorpheniramine (31) and the chlorinated analogue 2 of rocastine (1), showing the similar positioning of the chloro groups.

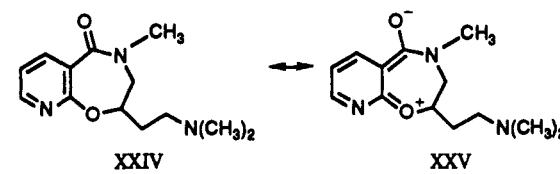
The effect of the fit to the model pharmacophore of changing the length of the side chain in the rocastine series is shown in Figure 7. As is apparent from XXI, when the side chain has only one methylene unit, it is impossible to position the protonated amine nitrogen at the N^+ site while keeping the heterocyclic ring positioned as in XIV, accounting for the lack of activity. With three methylene units in the side chain, a good fit (XXII) can be obtained. The conformation in XXII was calculated to be approximately 1.3 kcal/mol higher than the quasi-axial extended conformer of the same compound and thus not energetically unlikely. Either the slightly greater distance between the aromatic ring and the protonated amine in XXII relative to XIV, or the increased flexibility of the longer side chain may explain the lower potency of the former. Greater conformational flexibility may reduce the free energy of binding to the histamine H1 receptor via an unfavorable entropy effect.[16]

The cyproheptadine-based pharmacophoric model is achiral. In antihistamines such as chlorpheniramine (31), however, a chiral carbon bridges the two aromatic rings, and the histamine H1 receptor can clearly distinguish between the two enantiomers. For chlorpheniramine, the *S* isomer is approximately 100-fold more potent than its enantiomer.[8] Many related antihistamines share this enantiomeric preference.[1] In addition, the nature of substitution on the aromatic rings strongly influences the properties of drugs in this series and other similar antihistamines. Replacement of the 4-chloro substituent with hydrogen, for instance, leads to a 16–19-fold reduction in potency,[17] and substitution of phenyl for the 2-pyridyl group affords compounds which are slightly less potent, less selective, and perhaps more likely to cause sedation.[15]

In comparing possible binding modes of chlorpheniramine (31) and rocastine (1), it is reasonable to suggest that the pyridyl group of 1 might occupy the same receptor site as one of the aromatic rings in 31, although it is not obvious from the SAR data which rings to superimpose. In the rocastine series, the greater potency of the pyridyl compounds relative to their phenyl analogues suggests that the pyridyl compounds relative to their phenyl analogues suggests that the pyridyl ring of 1 may fit the same receptor site as the pyridine ring in 31, while the increase in potency on introduction of a chloro substituent at the 7-position of 1 (analogue 2) parallels the SAR associated with the chlorphenyl group of 31, suggesting a similar receptor fit for the pyridine ring of 1 and the chlorophenyl group of 31. Superimposition of the binding conformation of the active *R* isomer of the chlorinated analogue (2) of rocastine over the *S* isomer of chlorpheniramine (31) in Figure 8 supports the latter interpretation and shows that the chloro substituent of each is, in fact, simlarly positioned.

To further validate this interpretation, two novel analogues of chlorpheniramine were prepared and tested for antihistaminic activity. If the pyridine ring of 31 occupies the same receptor site as the pyridine ring of 1, then its dichloro analogue 28 should be highly potent in parallel with the SAR observed for 1. In fact, 28 has only weak activity. On the other hand, replacement of the chlorophenyl group of 31 with a chloropyridyl substituent afforded the highly potent 30, paralleling the SAR for 1. Thus, both the comparison of SAR data and the superimposition of the active enantiomers indicate that pyridine ring of 1 binds at the same site on the histamine H1 receptor as the chlorophenyl group of 31.

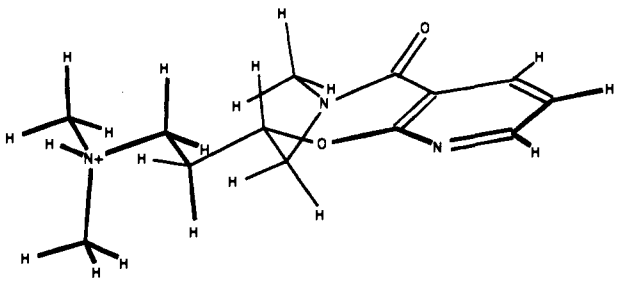
A rationale for the diminished activity of the pyrido[3,2-*f*]-1,4-oxazepin-5(2*H*)-one analogues of 1, which generally have one-tenth or less the potency of the corresponding thione, is less apparent. It may be due to a decrease in the ability of the ether oxygen to participate in hydrogen bonding. The substitution of oxygen for the sulfur of the thioamide is likely to reduce the availability of the lone pairs on the ether oxygen for hydrogen bonding via increased donation of electron density into the carbonyl.



XXIV XXV

The lower potency of the amides relative to the thioamides in this series does not appear to be due to inability to adopt the binding conformation. Molecular mechanics calculations indicate that the lowest energy conformation of the free base of 2-[2-(dimethylamino)ethyl]-3,4-dihydro-4-methylpyrido[3,2-*f*]-1,4-oxazepin-5(2*H*)-one (39) is similar to X, boatlike with a quasi-axial extended side chain, with the conformer corresponding to XIII being about 1.5 kcal/mol higher in energy. There is an alternate conformation of the seven-membered heterocyclic ring of 39 close in energy to the boatlike minimum, in which the O1–C2 bond is nearly coplanar with the pyridine ring. This conformer (XXVI in Figure 9) is about 1.5 kcal/mol above

(16) Andrews, P. R.; Craik, D. J.; Martin, J. L. *J. Med. Chem.* **1984**, *27*, 1648.
(17) Labelle, A.; Tislow, R. *J. Pharmacol. Exp. Ther.* **1955**, *113*, 72.



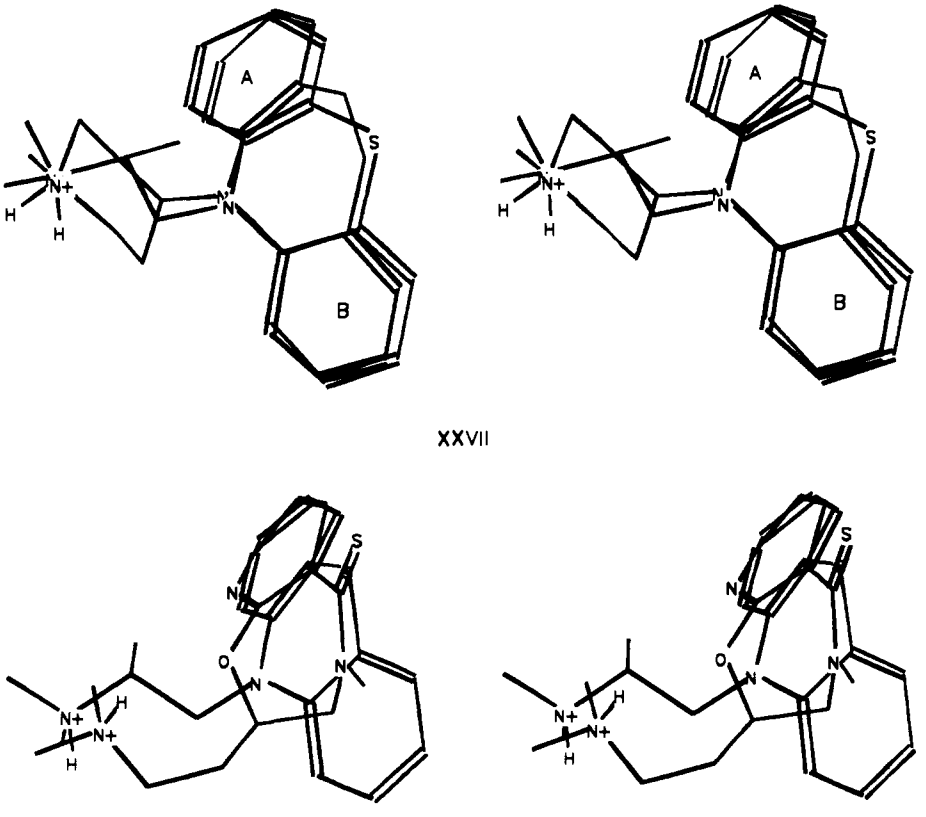
XXVI

Figure 9. Alternate low-energy ring conformation of the lactam analogue of rocastine (1).

the minimum. The coupling constants between protons on the 2- and the 3-position of the oxazepine ring of **39** (3.23 and 7.80 Hz) are smaller than those for 1 (3.9 and 8.5 Hz, Table VI). This may reflect the contribution of XXVI (calculated values are 1.73 and 7.24 Hz) to the time-weighted average. Greater conformational flexibility of **39** relative to 1 may explain in part the reduced potency of **39**. Further evidence for an alternate low-energy conformation in the 3,4-dihydropyrido[3,2-*f*]-1,4-oxazepin-5-(2*H*)-one ring system can be seen in the comparison of experimental and calculated proton–proton coupling constants (Table VI) for compounds **22** and **24**, which are oxo analogues of **23** and **25**. In the case of the thiolactams, the coupling constants for both **23** and **25** are consistent with a boatlike conformation, as are those for lactam **24** (Figure 4). In the case of **22**, however, a ring conformation similar to that in XXVI is indicated. This preference suggests that the more planar ring conformation as in XIX or XXVI is stabilized in the amides relative to the thioamides. Such stabilization may be rationalized in that the more planar conformation allows a greater contribution from resonance forms like XXV, which should be more important for the amides than for the thioamides. A comparison of the ^{13}C NMR spectra of the more planar **22** with the boatlike **24** supports the increased contribution of this resonance form in **22**. The spectrum of **24** shows an amide carbonyl resonance at 170.8 ppm, while the spectrum of **22** shows an unusually high field amide carbonyl at 162.3 ppm, indicating higher electron density in the amide group of **22** relative to **24**.

The reduced potency of the amides may also reflect a specific interaction of the thioamide with the receptor. Figure 10 shows an overlay of the binding conformers of promethazine (**37**) and 1. The thiolactam sulfur of 1 should occupy roughly the same region of the receptor as the thioether of **37**. While there are numerous examples of potent tricyclic antihistamines having soft polarizable goups in this region, those having hard polar oxygen functions are noticeably lacking.

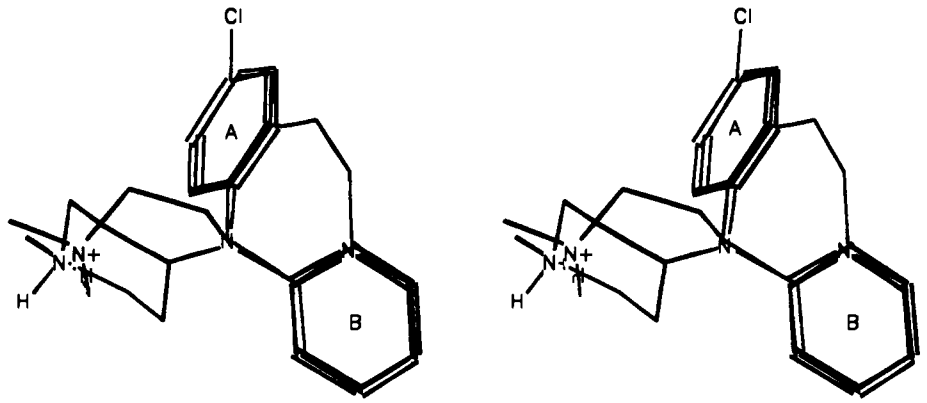
Interestingly, a modification of the binding conformation of chlorpheniramine (**31**) leads to an additional point of overlap with 1. An approximately 180 deg rotation of the 2-pyridyl ring of **31** allows for the overlap of the thiolactam nitrogen of 1 with the pyridine nitrogen of **31** (Figure 11). The calculated difference in energy of the two conformers of **31** as the free base is 1.00 kcal/mol with the conformation in Figure 11 being the lower in energy at 1.99 kcal/mol above the global minimum. There is support for a binding conformation of **31** as in Figure 2 in rigid tricyclics such as azatadine (**38**), but no direct comparison with a tricyclic having the pyridine nitrogen in the alternate orientation is possible, since this would require a quarternized pyridine at the ring junction.



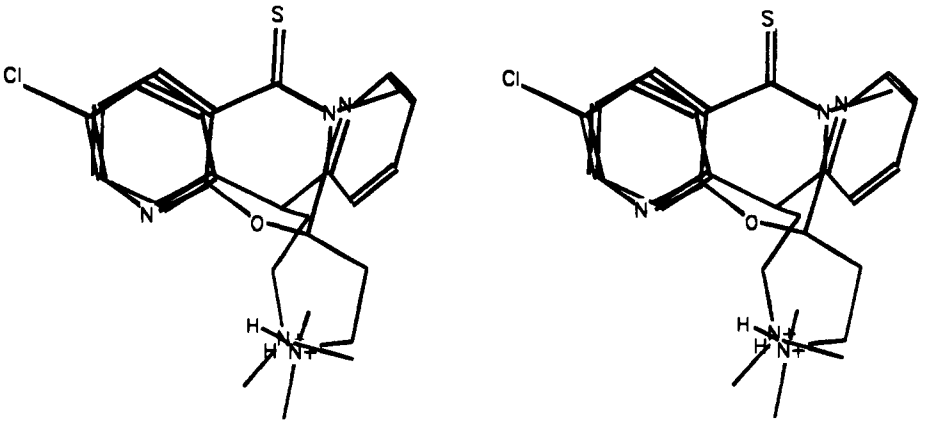
XXVII

XXVIII

Figure 10. Stereo views of the fit of promethazine (**37**) in the approximate binding conformation suggested by Naturo et al.[9] to the H1 pharmacophoric model (XXVII) and superimposition of the binding conformers of 1 and **37** (XXVIII).



XXIX



XXX

Figure 11. Stereo views of an alternative binding conformer of chlorpheniramine (31) to that in Figure 2 superimposed on the pharmacophoric model (XXIX) and on the proposed binding conformer (XXX) of rocastine (1) showing the similar positioning of the pyridine nitrogen of 31 and the thiolactam nitrogen of 1.

Conclusions

From the preceding discussion it is clear that essentially all of the antihistaminic activity associated with rocastine (1) and its analogues resides in isomers having the *R* configuration at the 2-position of the heterocyclic ring. Fruthermore, in a low-energy conformation available to 1, boatlike in the heterocyclic ring with side chain quasi-equatorial and folded back toward the ring, the relative positions of the pyridine ring, ether oxygen, and protonated amine functions of 1 are similar to those for like elements in the proposed binding conformers of the more classical antihistamines. This is a likely binding conformer for 1, and the known SAR data is consistent with this interpretation.

Experimental Section

Melting points were determined in open capillary tubes with a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses (C, H, and N) were performed in house and were within 0.4% of the theoretical values except where noted. IR spectra were obtained on a Perkin-Elmer Model 297 spectrophotometer. ^{1}H NMR were recorded either on a Varian EM-360, Varian FT-80A, or an XL-400 spectrometer. For salts, the spectra were recorded in D_2O, which served as field/frequency lock for the 80-MHz and 400-MHz spectrometer. TSP served as the internal standard at all three field strengths and the field/frequency lock for the 60-MHz spectrometer. For free bases, the spectra were recorded in $CDCl_3$, which served as field/frequency lock for the 80-MHz and 400-MHz spectrometer. TMS served as the internal standard at all three field strengths and the field/frequency lock for the 60-MHz spectrometer. ^{13}C NMR were recorded on a Varian FT-80A instrument using tetramethylsilane as the internal standard. Electron-impact (EI) mass spectra were obtained at 70 eV on a Hitachi RMU-6 spectrometer. Chemical-ionization spectra were obtained on a Varian MAT-44 using isobutane as the reagent gas. Analytical TLC were carried out on Analtech Uniplate silica gel GF. Preparative HPLC separations were performed on a Waters Model 500 system using a 5.5 × 30 cm silica gel column. In multistep sequences the purity and structure of intermediates were verified by ^{1}H and ^{13}C NMR. Optical rotations were measured with a Perkin-Elmer Model 241 MC polarimeter set at 589.6 nm (Na D line).

Enantiomeric purities were determined by HPLC on a Varian 5500 LC equipped with a variable-UV detector and by using an Daicel Chiralcel OG column (25 cm × 4.6 mm) and eluting with the required solvent blend (Table VII) at 1 mL/min. (The column packing is a derivatized form of cellulose coated on Lichrospheric Si-100 silica.) A Spectra Physics SP4270 Integrator was used for data assimilation.

Racemic mixtures of the compounds were used to establish k' values and separation factors (Table VII). In prepared mixtures 0.1% of the minor enantiomer was easily detected. When the individual enantiomers were subjected to the above conditions, none of the undesired enantiomer was detected.

Molecular Modeling. Molecular modeling with PCMODEL[10] and molecular mechanics calculations using the MXX[10] force field were performed on a Tandy 4000 microcomputer using the standard parameters supplied with the software. Overlays of the various antihistamines and the pharmacophoric model were generated within PCMODEL by choosing several pairs of atoms, one atom from each structure, to be superimposed. An overlay was then created in which the distances between the atoms in the

Table VII. HPLC Separation Parameters for the Enantiomers of Rocastine (1) and Close Analogues

compound (0.1–0.5 mg/mL in the mobile phase)	mobile phase (flow rate 1 mL/min) (%)	k′ of enantiomeric forms		separation factor, α = k′(S)/k′(R)
		R	S	
15/16	10 i-PrOH 10 EtOH 80 hexane–0.1% diethylamine	6.82	9.70	1.42
17/18	20 i-PrOH 80 hexane	6.16	7.07	1.14
19/20	20 i-PrOH 80 hexane	7.02	7.99	1.13

selected pairs were minimized by a least-squares procedure.

Preparation of Chiral 1-Methyl-3-pyrrolidinols. (*S*)-3-Hydroxy-1-methyl-2,5-pyrrolidinedione (4). To a stirred solution of 134 g (1 mol) of (*S*)-malic acid in 700 mL of toluene was added 97 mL of 40% aqueous CH_3NH_2. After 0.5 h the mixture was heated to reflux in flask equipped with a Dean–Stark trap and 93 mL of H_2O was collected over a 48-h period. EtOH (300 mL) was added, the mixture was concentrated, and the residue was distilled to give 101 g (78%) of 4: bp 140 °C (2 mm)–145 °C (0.5 mm).

A sample of the above compound was crystallized from EtOAc–(*i*-Pr)$_2$O: mp 87–89 °C; $[\alpha]^{22}_D$ −79.2° (methanol, c 1.7). Anal. ($C_9H_{17}NO_7$) C, H, N.

Procedure A. (*S*)-1-Methyl-3-pyrrolidinol (2*S*,3*S*)-2,3-Dihydroxybutanedioate (5). A solution of 25.3 g (0.2 mol) of 4 in 150 mL of THF was added dropwise (maintain reflux) to a stirred solution of 226 g (0.78 mol) of Red-Al (Aldrich) [3.4 M solution of sodium bis(2-methoxyethoxy)aluminum hydride in toluene] in 500 mL of THF. Refluxing was continued 1 h after the addition was complete. The solution was cooled with an ice bath and 30 mL of H_2O was added dropwise, followed by 30 mL of 15% NaOH and then 90 mL of H_2O. The resulting mixture was heated at reflux for 0.5 h and cooled to 30 °C, and the layers were separated. The aqueous layer was extracted with $CHCl_3$ (5 × 100 mL). The organic extracts were combined, dried (Na_2SO_4), and concentrated. The residue was distilled to give 13 g (64%) of 5a: bp 95–100 °C (90 mm); $[\alpha]^{22}_D$ +0.74° (neat).

A 1-g sample of this material was treated with 1.5 g of (2*S*,3*S*)-tartaric acid in MeOH to afford 2 g of 5: mp 129–131 °C; $[\alpha]^{22}_D$ −11.5° (H_2O, c 1.0).

Procedure B. To a solution of 420 g (4.16 mol) of racemic 1-methyl-3-pyrrolidinol in 1 L of dry MeOH cooled in an ice bath was added a solution of 500 g (3.33 mol) of (2*S*,3*S*)-tartaric acid in 1 L of MeOH. At 50 °C the solution was seeded and cooled. The precipitate which formed was collected and recrystallized from MeOH (3×) to give 235 g (45%) of 5: mp 125–129 °C; $[\alpha]^{22}_D$ −11.6° (H_2O, c 9.8).

The free base of 5 was prepared as follows: A 235-g sample of 5 was treated with 135 g of KOH in 200 mL of H_2O, and the solution was continuously extracted with $CHCl_3$ for 24 h. The organic solution was dried (Na_2SO_4), concentrated, and distilled to give 80 g of 5a: bp 103–106 °C (37–40 mm); $[\alpha]^{22}$ +0.817° (neat). Anal. ($C_5H_{11}NO$·0.025$CHCl_3$) C, H, N. (Presence of $CHCl_3$ was confirmed by ^1H NMR.)

(*R*)-1-Methyl-3-pyrrolidinol (2*R*,3*R*)-2,3-dihydroxybutanedioate (6) was prepared from (*R*)-3-hydroxy-1-methyl-2,5-pyrrolidinedione as in procedure A: mp 124–127 °C; $[\alpha]^{22}$ + 11.1 (H_2O, c 9.57).

The free base of 6 was prepared in the same manner as its enantiomer 5a to give 6a: bp 103–106 (35 mm); $[\alpha]^{22}_D$ −0.852° (neat). Anal. ($C_5H_{11}NO$·0.025$CHCl_3$) C, H, N. (Presence of $CHCl_3$ was confirmed by ^1H NMR.)

Method A. (*R*)-2-(2-Chloroethyl)-3,4-dihydro-4-methylpyrido[3,2-*f*]-1,4-oxazepin-5(2*H*)-one (8). A solution of 47.4 g (0.3 mol) of 2-chloronicotinic acid and 30 g (0.3 mol) of 6a in 400 mL of THF was added over a period of 1 h to a stirred suspension of 26.4 g (0.66 mol) of 60% NaH/mineral oil in 500 mL of THF at 55–60 °C. The mixture was heated at reflux for 2.5 h and then allowed to cool to 25 °C. About 400 mL of CH_2Cl_2 was added to the slurry, followed by a dropwise addition of 34.5 g (0.36 mol) of CH_3SO_3H in 100 mL of CH_2Cl_2. The mixture was stirred for 10 min and 157 g (0.6 mol) of triphenylphosphine was added along with 200 mL of CCl_4. The mixture was heated to reflux for 4 h and then cooled (25 °C), and 100 mL of NEt_3 was added. The solution was concentrated and the residue was partitioned between CH_2Cl_2 (300 mL) and dilute HCl (300 mL). The layers were separated, and the CH_2Cl_2 layer was extracted with dilute HCl (6 × 100 mL). The acid extracts were combined, made basic with NaOH, and extracted with $CHCl_3$. The organic solution was dried (Na_2SO_4) and concentrated. The residue was dissolved in *i*-PrOH and treated with a solution of HCl in *i*-PrOH to give 25 g (30%) of the hydrochloride. A 1-g portion was recrystallized from *i*-PrOH: mp 152–154 °C; $[\alpha]^{22}_D$ + 36.2° (H_2O, c 0.3).

The above salt was partitioned between $CHCl_3$ and dilute NaOH. The organic phase was dried (Na_2SO_4) and concentrated. The residue crystallized from (*i*-Pr)$_2$O to afford 19 g (24%) of 8: mp 61–62 °C.

Method B. (*R*)-7-Chloro-2-(2-chloroethyl)-3,4-dihydro-4-methylpyrido[3,2-*f*]-1,4-oxazepin-5(2*H*)-one (10). To a suspension of 43.6 g (1.09 mol) of 60% NaH/mineral oil in 400 mL of THF heated at reflux under N_2 was added, dropwise, a solution of 95 g (0.495 mol) of 2,5-dichloro-3-pyridinecarboxylic acid and 50 g (0.495 mol) of 3a in 400 mL of THF dropwise at a rate to maintain reflux (~1 h). Heating at reflux with mechanical stirring was continued for 2 h until the reaction mixture became thick. After the mixture cooled to room temperature, 3 mL of H_2O was added, which resulted in solidification of the mixture. THF (300 mL) was added to break up the cake and the suspended solid was collected by filtration. After drying overnight at room temperature, 173 g of the crude sodium salt was obtained.

To 500 g of $SOCl_2$ was added 173 g of the above sodium salt. After stirring at room temperature for 10 min, the $SOCl_2$ was removed under reduced pressure. The residue was treated with 500 mL of toluene and reconcentrated and dissolved in 600 mL of CH_2Cl_2, and the solution was made basic by careful dropwise addition of diisopropylethylamine. The mixture was washed 2 × 100 mL with 1 N HCl and 2 × 100 mL with 2 N NaOH, dried (Na_2SO_4), filtered, and concentrated. Repeated triturations of the dark residue with (*i*-Pr)$_2$O gave 50 g (36%) of 10, which was recrystallized from (*i*-Pr)$_2$O: mp 76–78 °C.

Method C. (*R*)-2-(2-Chloroethyl)-3,4-dihydro-4-methylpyrido[3,2-*f*]-1,4-oxazepine-5(2*H*)-thione (12). To a suspension of 9 g (0.02 mol) of P_2S_5 in 75 mL of CH_3CN was added 18 g (0.075 mol) of 8, and the mixture was heated at reflux for 2.5 h, cooled, and diluted with 60 mL of toluene. The suspension was filtered, and the solid was washed twice with 30 mL of CH_3CN–toluene (1:3). The volume was made to about 400 mL with toluene–CH_3CN (1:1). The solution was washed with saturated $NaHCO_3$, treated with $MgSO_4$ and charcoal, and filtered. The filtrate was concentrated, and the residue was recrystallized from *i*-PrOH–$CHCl_3$ to give 13 g (68%) of 12: mp 168–170 °C.

Method D. (*R*)-2-[2-(Dimethylamino)ethyl]-3,4-dihydro-4-methylpyrido[3,2-*f*]-1,4-oxazepine-5(2*H*)-thione Dihydrochloride (16). A 10 g (0.042 mol) sample of 12 was treated with 50 mL of Me_2NH and sealed in a flask. The resulting solution was stirred at 25 °C for 48 h. The excess amine was allowed to evaporate and the residue was partitioned between $CHCl_3$ and dilute NaOH. The $CHCl_3$ solution was dried (Na_2SO_4) and concentrated. The residue was dissolved in CH_3CN and treated with a solution of HCl in CH_3CN. The resulting crystals were collected and recrystallized from EtOH to give 7 g (50%) of 16: mp 170–176 °C.

Method E. (*R*)-2-[2-(1-Azetidinyl)ethyl]-7-chloro-3,4-dihydro-4-methylpyrido[3,2-*f*]-1,4-oxazepine-5(2*H*)-thione (20). To 3.5 g (0.012 mol) of 14 in 20 mL of DMSO was added 2.0 g (0.035 mol) of azetidine and 6 g of K_2CO_3. The reaction mixture was stirred at room temperature for 3 days and then poured into 500 mL of H_2O. The suspension was extracted 2 × 200 mL with toluene. The combined organic extracts were washed 2 × 100 mL with H_2O, dried (Na_2SO_4), and concentrated. The residue crystallized from toluene–(*i*-Pr)$_2$O to yield 2.1 g (57%) of 20: mp 109–111 °C.

Miscellaneous Procedures. 3-(3-Chloropropyl)-3,4-dihydro-4-methylpyrido[3,2-*f*]-1,4-oxazepin-5(2*H*)-one Hydrochloride (21) and 6a,7,8,9-Tetrahydro-6*H*,11*H*-pyrido-

[3,2-*f*]pyrrolo[2,1-*c*][1,4]oxazepin-11-one (22). A solution of 50 g (0.43 mol) of 2-(hydroxymethyl)-1-methylpyrrolidine and 67.8 g (0.43 mol) of 2-chloronicotinic acid in 250 mL of dry DMF was added over a period of 45 min to a stirred suspension of 36.1 g (0.9 mol) of 60% NaH-mineral oil in 300 mL of dry DMF at 55 °C. Mild cooling was required to maintain a reaction temperature of 50–60 °C. After the addition, the mixture was heated to 60 °C for 1.5 h, cooled in the freezer for 2 h, and filtered. The filter cake was washed with EtOAc and dried to give 100 g of a solid. A 20-g sample of this material was suspended in 400 mL of $CHCl_3$ and made weakly acidic (pH meter reading 5.3) with anhydrous HCl. To this was added 21 g of $SOCl_2$, the mixture was heated at reflux for 2 h and cooled in an ice bath, and 100 mL of NEt_3 was added dropwise. The suspension was stirred overnight and then washed with dilute NaOH and concentrated. The residue was partitioned between $CHCl_3$ (1 L) and dilute HCl (1 L), and the layers were separated. The organic layer was extracted with additional dilute HCl (3 × 250 mL). The acid layers were combined, made basic with NaOH, and extracted with $CHCl_3$ (2 × 500 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated. Preparative HPLC of the residue on silica eluting with EtOAc afforded a material of molecular weight 240 (MS), which crystallized as the hydrochloride from HCl–*i*-PrOH to give 1.1 g (4.5%) of **21**: mp 162–164 °C. Anal. ($C_{12}H_{16}Cl_2N_2O_2$) C, H, N; C: calcd 49.50, found 50.15.

In addition, the chromatographic separation afforded a material of molecular weight 204 (MS), which crystallized from a solution of (i-Pr)$_2$O–EtOAc (4:1) to give 1.2 g (7%) of **22**: mp 113–116 °C. Anal. ($C_{11}H_{12}N_2O$) C, H, N. The *R* isomer of **22** was also prepared according to the method of Schultz et al.[18] Both **22** and its *R* isomer prepared by the alternate procedure had identical NMR spectra.

6a,7,8,9-Tetrahydro-6*H*,11*H*-pyrido[3,2-*f*]pyrrolo[2,1-*c*][1,4]oxazepine-11-thione (23). To a 3.5 g (0.017 mol) of **21** in 50 mL of toluene was added 5.7 g (0.014 mol) of 2,4-bis(4-methoxyphenyl)-2,4-dithioxo-1,2,3,4-dithiodiphosphetane (Lawesson's reagent), and the mixture was heated at reflux for 17 h. The toluene was decanted and washed once with diluted NaOH and twice with H_2O. The organic layer was dried (Na_2SO_4), filtered, and concentrated. Preparative HPLC of the residue on silica eluting with EtOAc gave an oil, which subsequently crystallized from EtOAc–(i-Pr)$_2$O to afford 1.5 g (49%) of **23**: mp 102–105 °C. Anal. ($C_{11}H_{12}N_2OS$) C, H, N; C: calcd 59.98, found 59.37.

2,3,4,5-Tetrahydro-7*H*-2,6-methanopyrido[2,3-*b*][1,5]oxazonin-7-one Hydrochloride (24). A solution of 100 g (0.87 mol) of 1-methyl-3-piperidinol and 137 g (0.87 mol) of 2-chloronicotinic acid in 300 mL of dry DMSO was added dropwise over 2.5 h to a suspension of 77 g (1.9 mol) of 60% NaH-mineral oil in a stirred mixture of 1 L of DMSO and 80 mL of isooctane at 50 °C. The addition rate was controlled to maintain a temperature of 50–58 °C with intermittent cooling. After addition was complete, the temperature was maintained at 55 °C for 1.5 h. About 1 L of EtOAc was added, and the mixture was cooled and filtered. The filter cake was thoroughly washed with EtOAc and dried to give 271 g of the crude sodium salt of 2-[(1-methyl-3-piperidinyl)-oxy]-3-pyridinecarboxylic acid.

HCl was bubbled into a suspension of 231 g (~0.9 mol) of this material in 1500 mL of $CHCl_3$ until the mixture was weakly acidic (pH meter reading 6.0). To the resulting suspension was added 524 g (2 mol) of triphenylphosphine and 524 g of CCl_4. The solution was heated at reflux for 1.5 h. To the hot suspension was added 100 mL of EtOH (caution: exothermic), and the solution was cooled to room temperature. About 1 L of isooctane was added, and the resulting solution was extracted with 6 N HCl (1 × 1 L, 3 × 250 mL). The pH of the acid solution was adjusted to 0.5 with NaOH and the resulting mixture extracted with EtOAc. The aqueous layer was made basic with NaOH and the mixture extracted with $CHCl_3$ (3 × 500 mL), which was dried (Na_2SO_4) and concentrated. The residue was dissolved in 1 L of i-PrOH and the solution treated with anhydrous HCl until strongly acidic. About 500 mL of (i-Pr)$_2$O was added and the precipitate which formed was collected and recrystallized once from a solution of EtOH–MeOH (3:1) and then from EtOH to give 44 g (21%) of **24**: mp 148–149 °C. Anal. ($C_{11}H_{13}ClN_2O_2$) C, H, N.

2,3,4,5-Tetrahydro-7*H*-2,6-methanopyrido[2,3-*b*][1,5]oxazonine-7-thione (25). A solution of 14.4 g (0.06 mol) of the free base of **24** in 200 mL of dry toluene was treated with 16 g (0.04 mol) of Lawesson's reagent. The mixture was heated at reflux for 5 h, cooled, and washed with 100 mL of dilute K_2CO_3. The aqueous layer was extracted with $CHCl_3$ (2 × 100 mL), and all of the organic layers were combined, dried (Na_2SO_4), and concentrated. The residue was dissolved in EtOAc (200 mL) and extracted with dilute HCl (3 × 100 mL). The combined acid extracts were made alkaline with NaOH and extracted with $CHCl_3$ (200 mL), which was dried (Na_2SO_4) and concentrated. The residue crystallized from i-PrOH to afford 6.5 g (49%) of **25**: mp 141–143 °C. Anal. ($C_{11}H_{12}N_2O$) C, H, N.

5-Chloro-α-(4-chlorophenyl)-2-pyridineacetonitrile (26). To a suspension of 8.8 g of 60% NaH–mineral oil (0.22 mol) in 400 mL of DMSO was added a solution of 30 g (0.2 mol) of 4-chlorobenzonitrile in 50 mL of DMSO. The reaction mixture was stirred at room temperature until H_2 evolution ceased. A solution of 37 g (0.25 mol) of 2,5-dichloropyridine in 50 mL of DMSO was added in a fine stream. The suspension was stirred at room temperature for 24 h and then at 70 °C for 24 h. The mixture was cooled, an additional 8.8 g of 60% NaH was added, and heating was continued at 60 °C for 3 h. After cooling, the mixture was poured into 1.5 L of H_2O and extracted with 2 × 600 mL with EtOAc. The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated. The resulting semicrystalline residue was purified by preparative HPLC on silica eluting with 15% EtOAc in *n*-hexane. Trituration in hexane gave 28 g of **26**: mp 99–101 °C. Anal. ($C_{13}H_8Cl_2N_2$) C, H, N.

5-Chloro-α-(4-chlorophenyl)-α-[2-(dimethylamino)-ethyl]-2-pyridineacetonitrile (*Z*)-2-Butenedioate (1:1) (27). To a suspension of 5.0 g of 60% NaH–mineral oil (0.125 mol) in 500 mL of THF and under N_2 was added 18 g (0.125 mol) of (dimethylamino)ethyl chloride–HCl. The mixture was stirred vigorously for 2 h.

Meanwhile, in another reaction vessel, to a suspension of 5 g of 60% NaH–mineral oil (0.125 mol) in 1 L of toluene was added 26.3 g (0.10 mol) of **26** portionwise over 5 min. The mixture was stirred vigorously under N_2 for 30 min. The suspension containing (dimethylamino)methyl chloride prepared above was added slowly to this over 2 min. The entire reaction mixture was heated at reflux for 12 h and then cooled and quenched carefully with 250 mL of H_2O under N_2. The contents were transferred to a separatory funnel and the layers were separated. The organic phase was washed with 500 mL of H_2O and extracted 2 × 400 mL with 1 N HCl. The combined aqueous acidic extracts were washed with 300 mL of toluene, made basic with concentrated NH_4OH, and extracted 2 × 400 mL with CH_2CL_2. The combined organic extracts were dried (Na_2SO_4) and concentrated. The residue was passed through a short bed of silica gel eluting with EtOAc to give 14 g (34%) of a colorless oil. A 3.5-g sample of this material was treated with maleic acid in i-PrOH to give the salt (**27**): mp 152–154 °C. Anal. ($C_{17}H_{17}Cl_2N_3 \cdot C_4H_4O_4$) C, H, N.

5-Chloro-γ-(4-chlorophenyl)-*N*,*N*-dimethyl-2-pyridinepropanamine Ethanedioate (1:1) (28). To a cooled solution of 65 g of 75% H_2SO_4 was added 10.5 g (0.031 mol) of **27**. The resulting two-layer system was stirred in an oil bath at 120 °C for 2 h and then at 135 °C for 8 h. The cooled reaction solution was then carefully poured over ice and made basic with concentrated NH_4OH while the temperature was moderated with ice. The emulsion was extracted with 2 × 100 mL EtOAc, dried (Na_2SO_4), and concentrated to give 8 g (84%) of a mobile oil. The oil was treated with oxalic acid in i-PrOH to give **28**: mp 154–155 °C.

5-Chloro-α-[2-(dimethylamino)ethyl]-α-(2-pyridinyl)-2-pyridineacetonitrile Ethanedioate (1:1) (29). To a suspension of 0.62 g of 60% NaH–mineral oil (0.016 mol) in 20 mL of DMSO was added 2.5 g (0.013 mol) of a α-[2-(dimethylamino)ethyl]-2-pyridineacetonitrile[19] in three portions with stirring over 5 min. After H_2 evolution ceased, 1.9 g (0.013 mol) of 2,5-dichloropyridine

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was added all at once, and the reaction mixture was heated to 60 °C in an oil bath for 18 h. The reaction mixture was cooled and poured into 300 mL of H_2O, which was extracted with 2 × 200 mL with EtOAc. The combined organic extracts were dried (Na_2SO_4) and concentrated. The residue was treated with oxalic acid in *i*-PrOH to give 3.5 g (69%) of **29**: mp 176–178 °C. Anal. ($C_{16}H_{17}ClN_4 \cdot C_2H_2O_4$) C, H, N.

5-Chloro-*N*,*N*-dimethyl-γ-(2-pyridinyl)-2-pyridinepropanamine (30). A mixture of 18 g (0.06 mol) of the free base of **29** and 85 g of 75% H_2SO_4 was heated to 135 °C in an oil bath for 8 h. After cooling, the reaction mixture was poured over ice. The solution was neutralized carefully with concentrated NH_4OH while the temperature was moderated with ice. The resulting emulsion was extracted 2 × 200 mL with EtOAc. The combined organic extracts were dried (Na_2SO_4) and concentrated to give 8 g (48%) of an oil. A portion of this oil was treated with 2 equiv of maleic acid in *i*-PrOH to yield the salt: mp 108–109 °C.

Pharmacology. Histamine-Induced Lethality in Guinea Pigs. Female, English short-hair guinea pigs (250–500 g) were obtained from Hazelton Research Products, Denver, VA. The animals were fasted overnight prior to testing. Drugs were administered orally by gavage to the animals at various times prior to the histamine challenge. Histamine was injected intravenously (1.2 mg of histamine/kg). Survival of the animals (at 24 h postchallenge) was indicative of antihistaminic activity. To determine ED_{50} values, groups of 5–10 guinea pigs per treatment were used, and ED_{50}s were calculated by the method of Litchfield and Wilcoxon[20] or by a computer probit analyses method.

In Vitro Binding. Membrane Preparation. Female Dunkin–Hartley guinea pigs weighing 200–500 g were used in vitro [^{3}H]mepyramine binding to the H1 histamine receptor in cerebral cortex according to a modification of the method described by Wallace and Young.[21] The cerebral cortex was homogenized in 30 volumes of cold buffer (50 mM sodium potassium phosphate, pH 7.5) with a polytron homogenizer (10 s, setting 6). The homogenate was centrifuged at 48000*g* for 10 min at 4 °C and the supernatant discarded. The resultant pellet was suspended in 30 volumes of fresh buffer and centrifuged at 48000*g* for 10 min at 4 °C. The resultant pellet was washed once more by resuspension and centrifugation. The final pellet was suspended in 4.0 mL of buffer (50 mM sodium potassium phosphate, pH 7.5)/g of tissue. The membrane preparations were prepared from fresh tissue and used on the day of preparation.

[^{3}H]Mepyramine Binding. [^{3}H]Mepyramine binding to the H1 histamine receptor in guinea pig cerebral cortex was investigated according to the method described by Chang et al.[22] The assay consisted of 100 μL of 15 nM [^{3}H]mepyramine (NEN, 23 Ci/mmol), 100 μL of the indicated compound concentration, and 700 μL of buffer (50 mM sodium potassium phosphate, pH 7.5) in a total volume of 1 mL. After incubation at 25 °C for 20 min, bound radioactivity was separated from free by rapid filtration onto GF/b glass-fiber filters with a Brandell M-48R harvester. Radioactivity trapped on the filters was determined by liquid scintillation counting with a Packard 2200Ca liquid scintillation analyzer (65% counting efficiency).

Data Analysis. For IC_{50} value determinations, six concentrations of the indicated compounds were assayed in triplicate. Competition data was analyzed by means of the Lundon 2 computer program that consisted of linear and nonlinear curve-fitting procedures (Lundeen and Gordon[23]).

Registry No. **1**, 104609-87-2; **4**, 104612-35-3; **5**, 104712-66-5; **5a**, 104641-59-0; **6**, 104712-67-6; **6a**, 104641-60-3; **7**, 104641-62-5; **8**, 104713-31-7; **8**·HCl, 104641-63-6; **9**, 131321-33-0; **10**, 131346-29-7; **11**, 104641-64-7; **12**, 104641-65-8; **13**, 131321-34-1; **14**, 131321-35-2; **15**, 104641-56-7; **16**, 104641-57-8; **17**, 131321-36-3; **18**, 131321-37-4; **19**, 131321-38-5; **20**, 131321-39-6; **21**, 131250-08-3; **22**; **23**, 131250-09-4; **24**, 131237-71-3; **25**, 131237-72-4; **26**, 131237-73-5; **27**, 131237-75-7; **28**, 131237-77-9; **29**, 131237-79-1; **30**, 131237-80-4; (*S*)-malic acid, 97-67-6; (±)-1-methyl-3-pyrrolidinol, 99445-21-3; (*R*)-malic acid, 636-61-3; (*R*)-3-hydroxy-1-methyl-2,5-pyrrolidinedione, 131237-81-5; 2-chloronicotinic acid, 2942-59-8; 2,5-dichloro-3-pyridinecarboxylic acid, 59782-85-3; azetidine, 503-29-7; 2-(hydroxymethyl)-1-methylpyrrolidine, 30727-24-3; 1-methyl-3-piperidinol, 3554-74-3; 2-[(1-methyl-3-piperidinyl)-oxy]-3-pyridinecarboxylic acid, 131250-10-7; 4-chlorobenzonitrile, 623-03-0; 2,5-dichloropyridine, 16110-09-1; (dimethylamino)ethyl chloride hydrochloride, 4584-46-7; 5-chloro-α-(4-chlorophenyl)-α-[2-(dimethylamino)ethyl]-2-pyridineacetonitrile, 131237-74-6; α-[2-(dimethylamino)ethyl]-2-pyridineacetonitrile, 131237-82-6; 3,4-dihydro-2,4-dimethylpyrido[3,2-*f*]-1,4-oxazepine-5(2*H*)-thione, 131250-11-8.

Supplementary Material Available: Cartesian coordinates for the histamine H1 pharmacophoric model and Cartesian coordinates for the proposed binding conformer of rocastine (**1**) (3 pages). Ordering information is given on any current masthead page.

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