Conformational Populations for Antihistamines and Antihypertensives in Solution

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Abstract □ The conformations in aqueous solution were determined for the XCH_2CH_2N systems of some antihistamines (H_1-receptor antagonists) and some adrenergic neuron blocking agents, including the antihypertensive drugs guanethidine and guanoclor. The rotameric species thought to be the pharmacologically active one is often not the only rotamer present in solution.

Keyphrases ■ Antihistamines—containing the XCH_2CH_2N system, rotameric conformations in aqueous solution □ Antihypertensives—containing the XCH_2CH_2N system, rotameric conformations in aqueous solution □ Guanethidine—rotameric conformations in aqueous solution □ Guanoclor—rotameric conformations in aqueous solution

A considerable variety of pharmacological activity is observed among derivatives of 1,2-disubstituted ethanes, XCH_2CH_2Y, and this variety has led to many attempts to correlate the observed biological activity with the conformational preferences about the C—C single bond. In these compounds, three conformations are possible, depending on the relative dispositions of the substituents X and Y. In one, X and Y are in a *trans* relationship; and in two, X and Y are *gauche*. Generally, the energy differences between these rotamers are expected to be quite low (less than 42 kJ/mole), so all three rotamers may be present in solution at physiological pH.

Under these conditions, the proton magnetic resonance (PMR) spectrum is an average of the spectra of the individual rotamers (1). Although the spectral parameters can, in principle, provide rotameric populations, this is not always straightforward. The difficulties are partly experimental and numerical and partly interpretative (2). Recent work clarified the effects of the substituents X and Y on the parameters obtained from an analysis of the high-resolution PMR spectrum of the XCH_2CH_2Y system (3) (an *AA'BB'* spectrum), and quantitative rotameric populations can now be obtained.

Two clinically important examples of compounds containing the XCH_2CH_2N system are found among the antihistaminics and the antihypertensive agents. Conformational information for some representative molecules of these drug classes is presented here.

EXPERIMENTAL

The PMR spectra were recorded at 60, 90, and 100 MHz[1]. The spectral details for the antihistamines, aside from the chemical shift data, were reported earlier (4). The other compounds, except guanoclor, were run in the form of their salts as approximately 20% solutions in deuterium oxide. The solubility of guanoclor in deuterium oxide was only 0.25%.

The ethanic $-CH_2CH_2-$ portions of the PMR spectra were of the *AA'BB'* type. For many of the compounds, however, complete analysis of this part of the spectrum was made difficult by one or more complications due to overlapping absorptions, long-range couplings, unfavorable linewidths, or uncertainties in some parameters. However, the parameter N, the sum of the vicinal coupling constants J_{AB} and $J_{AB'}$, is estimated from the separation between the strongest pair of lines in one of the multiplets, either *A* or *B*, depending on which is clearer (2).

Pyrilamine Free Base—NMR ($CDCl_3$): δ 2.3 [s, 6H, $N(CH_3)_2$], 2.55 (m, 2H, CCH_2N, N = 14.6 Hz), 3.64 (m, 2H, NCH_2C, N = 14.6 Hz), 3.75 (s, 3H, OCH_3), 4.67 (s, 2H, benzyl CH_2), 7.0 (m, 7H, aromatic H), and 8.25 (m, 1H, pyridyl H_α).

Pyrilamine Maleate—NMR (D_2O): δ 3.06 [s, 6H, $^+N(CH_3)_2$], 3.8 (s, 3H, OCH_3), 3.48 (m, 2H, CCH_2N^+, N = 12.1 Hz), 4.02 (m, 2H, NCH_2C, N = 12.1 Hz), 4.67 (s, 2H, benzyl CH_2), 7.0 (m, 7H, aromatic H), and 8.25 (m, 1H, pyridyl H_α).

Tripelennamine Hydrochloride—NMR (D_2O): δ 3.17 [s, 6H, $^+N(CH_3)_2$], 3.53 (m, 2H, CCH_2N^+, N = 12.0 Hz), 4.08 (m, 2H, NCH_2C, N = 12.0 Hz), 4.83 (s, 2H, benzyl CH_2), 7.0 (m, 8H, aromatic H), and 8.3 (m, 1H, pyridyl H_α).

Methapyrilene Hydrochloride—NMR (D_2O): δ 3.07 [s, 6H, $^+N(CH_3)_2$], 3.45 (m, 2H, CCH_2N^+, N = 11.8 Hz), 3.98 (m, 2H, NCH_2C, N = 11.8 Hz), 7.1 (m, 6H, aromatic H and thienyl H), and 8.25 (m, 1H, pyridyl H_α).

Guanethidine Sulfate—NMR (D_2O): δ 1.7 (m, 10H, CCH_2C), 3.35 (m, 6H, NCH_2), and 3.60 (m, 2H, CCH_2NH, N = 13.4 Hz).

N-(*2-Morpholinoethyl*)*guanidine Sulfate*—NMR (D_2O): δ 2.60 (m, 6H, NCH_2, N = 13.0 and 9.5 Hz), 3.35 (m, 2H, CCH_2NH, N = 13.0 Hz), and 3.74 (m, 4H, $-OCH_2$, N = 9.5 Hz).

Diphenhydramine Hydrochloride—NMR (D_2O): δ 2.9 [s, 6H, $^+N(CH_3)_2$], 3.36 (m, 2H, CCH_2N, N = 10.1 Hz), 3.76 (m, 2H, OCH_2C, N = 10.1 Hz), 5.55 (s, 1H, >CH), and 7.4 (m, 10H, aromatic H).

Guanoclor Sulfate—NMR (D_2O): δ 3.54 (m, 2H, CCH_2N, N = 10.0 Hz), 4.48 (m, 2H, OCH_2C, N = 10.0 Hz), and 7.5 (m, 3H, aromatic H).

Quantitative interpretation of N values is based on the correlation between the vicinal coupling constants in the individual rotamers and the electronegativities of substituents X and Y derived by Abraham and Gatti (3). Relations for the parameter N as a function of n_t, the proportion of *trans*-rotamer, can be derived for the OCH_2CH_2N and NCH_2CH_2N fragments and are given in Eqs. 1 and 2, respectively (2):

$$N = 9.63 + 8.17n_t \quad \text{(Eq. 1)}$$

$$N = 10.51 + 7.39n_t \quad \text{(Eq. 2)}$$

In the absence of a complete analysis, the N value may be overestimated and the errors inherent in the correlations are such that the n_t values listed in Table I may be in error by ±5%.

RESULTS AND DISCUSSION

Antihistamines—Until recently, the known antagonists of histamine acted only on H_1-receptors such as those found in the gut and bronchi (5). Tertiary amine derivatives, *e.g.*, pyrilamine and tripelennamine, are typical agents of this type. These antihistamines might now be called H_1-receptor antagonists, since the discovery of the gastric secretory inhibitors based on imidazole-substituted butylthioureas has provided the pharmacological classification of H_2-receptor antagonists (6). Rotameric populations for the H_1-receptor antagonists of the XCH_2CH_2N type, for which qualitative conclusions were presented earlier (4), are given in Table I.

For the antagonists of the ethylenediamine type, there is a fall in the percentage of *trans*-rotamer when the aliphatic side-chain nitrogen atom is protonated. At physiological pH, this side-chain nitrogen atom will be protonated; Table I shows that only 20% of the

[1] Varian A60, HA100, and Bruker WH90 NMR spectrometers.

Table I—Spectral Parameter N, Proportions (n_t) of *trans*-Rotamer, and Biological Activity Data for Antihistamines and Antihypertensives

Compound	N, Hz	n_t	H_1-Receptor Antagonist pA_2 Value (9)	Adrenergic Neuron Blocking Activity (13,14)
	NCH_2CH_2N Types			
Pyrilamine base (deuterochloroform)	14.6[a]	0.56	—	—
Pyrilamine maleate (deuterium oxide)	12.1[a]	0.22	9.36	—
Tripelennamine hydrochloride (deuterium oxide)	12.0[a]	0.21	9.00	—
Methapyrilene hydrochloride (deuterium oxide)	11.8[a]	0.18	8.63	—
Guanethidine sulfate (deuterium oxide)	13.4	0.4	—	Active
N-(2-Morpholinoethyl)guanidine sulfate (deuterium oxide)	13.0	0.34	—	No activity
	OCH_2CH_2N Types			
Diphenhydramine hydrochloride (deuterium oxide)	10.1[a]	0.06	8.14	—
Xylocholine bromide (deuterium oxide)	10.1[b]	0.06	—	Active
Guanoclor sulfate (deuterium oxide)[c]	10.0	0.04	—	High activity
N-(2-Morpholinoethyl)guanidine sulfate (deuterium oxide)	9.5	0	—	No activity

[a] From Ref. 4. [b] From Ref. 16. [c] Guanoclor is [[2-(2,6-dichlorophenoxy)ethyl]amino]guanidine.

trans-rotamer is present in solution for the common antihistamine salts. In these compounds, which contain an α-pyridyl ring as well as a benzyl group, the *gauche*-form can be stabilized considerably by the hydrogen bond involving the heterocyclic nitrogen atom and the $^+NH(CH_3)_2$ group. This kind of intramolecular hydrogen bond, which was originally postulated to be a factor in determining the conformation of the histamine monocation (7), is more important with these antihistamines (80% *gauche*-rotamers) than with the corresponding methylated histamine derivative, 4(5)-(β-dimethylaminoethyl)imidazole (40% *gauche*-rotamers) (8).

Comparison of the ethylenediamine and ether type of antagonist shows that the order of decreasing pA_2 value (9) parallels the percentage of *trans*-rotamer. These observations lend support to the view that the *trans*-$XCCN^+$ conformation is the pharmacologically active one for H_1-receptor antagonism (10–12). However, the pharmacologically active species is then not the most abundant one in solution. This objection is not too serious because of the changes that may occur during drug–receptor binding and in view of the small energy differences between the rotamers (a population ratio of 4:1 implies an energy difference of about 4.2 kJ/mole).

Antihypertensives—Adrenergic neuronal blocking drugs form one group of clinically useful antihypertensive agents (13, 14). On nerve stimulation, these drugs act within the nerve terminal to prevent release of the adrenergic transmitter. Aside from xylocholine bromide (15, 16), the first compound with this action to be discovered, there is little information about either the solid-state or solution conformations of these compounds.

The common structural feature of these drugs seems to be a positively charged terminal group separated from a lipophilic ring structure (14); a number of these agents are 1,2-disubstituted ethanes. Table I summarizes the conformational populations for the ethanic chain of some molecules representative of these antihypertensives. The positively charged groups in these compounds are either trimethylammonium, the guanidinium ion, or the aminoguanidinium ion.

Augstein *et al.* (17), in discussing adrenergic neuron blocking activity of aryloxyethylguanidines and aryloxyethylaminoguanidines, suggested that activity results when the cationic head and the ring are separated by a distance similar to that found in sympathomimetic amines. Hydrogen bonds involving $O—HN^+$ were thought to be important in maintaining this distance, with a resulting O/N *gauche* disposition for the substituents of the ethanic side chain.

For compounds based on the OCCN system, Table I shows that the *gauche*-rotamers predominate either completely or very nearly so in aqueous solution. The observed *gauche* conformations of many choline derivatives have been ascribed to an electrostatic interaction between the oxygen and the cationic head and also to hydrogen bonds of the O—HC type (18). Although such a hydrogen bond has been inferred from the crystal structure of xylocholine (15), this type of hydrogen bond has been doubted (19). For other choline derivatives, recent opinion has favored an electrostatic interaction (2, 16, 20); but in the case of guanoclor, the NH group of the guanidinium head may provide extra stability through a six-membered ring involving the O—C—C—N—N—H atoms (17).

Comparison of the chemical shifts of phenocholine and xylocholine showed that for xylocholine in solution the aromatic ring was considerably restricted in rotation to a region orthogonal to the plane of the COC bond (2). The chemical shift of the OCH_2 protons for guanoclor indicates that the bulky *ortho*-dichloro substituents are also restricting rotation.

The NCCN system of guanethidine sulfate has about 40% of the *trans*-rotamer present in solution, whereas the morpholino compound has slightly less. These populations, when compared with those of the aryloxy compounds, can be understood in terms of weaker hydrogen bonds and/or weaker electrostatic effects, resulting from the replacement of an oxygen atom by a less electronegative nitrogen atom.

The percentages of *gauche*-rotamers deduced from the PMR spectra are in qualitative accord with the neuronal blocking activity; but the orientation and character of the ring structure are also important, as the inactivity of the morpholino compound shows. Whereas the lipophilic character of the morpholino ring is apparently unfavorable for adrenergic neuronal blocking activity, the ring inversions possible in the eight-membered ring in guanethidine can produce conformations similar to those found with the aryloxy compounds.

These results demonstrate the extent of the conformational flexibility of the antihistamines and antihypertensives in aqueous solution. Nevertheless, these results should not be applied directly to the *in vivo* situation of the drug–receptor complex, since there are probably a number of unknown conformational ramifications in the binding process.

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Determination of Hyoscyamine in BPC Mixtures

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Abstract □ The hyoscyamine contents of four BPC mixtures (containing either belladonna or hyoscyamus tincture) were determined using the acid-dye technique. A sample size of 10 ml was required. The mean percentage recovery of hyoscyamine ranged from 99.73 to 101.03 from three mixtures; from the magnesium trisilicate and belladonna mixture, it was 94.8. The effects of pH and adsorption on the extraction of the alkaloid–dye complex from the mixtures examined are discussed.

Keyphrases □ Hyoscyamine—colorimetric determination using acid-dye technique, mixtures containing belladonna or hyoscyamus tincture □ Colorimetry—determination, hyoscyamine in mixtures, acid-dye technique

The current edition of the British Pharmaceutical Codex (BPC) lacks a quantitative method for the estimation of hyoscyamine content in mixtures containing either belladonna or hyoscyamus tincture. Instead, a TLC method is specified for testing the presence of tropane alkaloids in these mixtures (1). Recently, a colorimetric method based on the acid-dye technique was suggested for the microdetermination of hyoscyamine and scopolamine in synthetic mixtures (2).

The present work reports the suitability of the suggested method for the determination of hyoscyamine content in some BPC mixtures. The following mixtures were examined: aluminum hydroxide and belladonna; belladonna mixture, pediatric; magnesium trisilicate and belladonna; and potassium citrate and hyoscyamus.

EXPERIMENTAL

Materials—The materials used in preparation of the BPC mixtures were of either BP or BPC grade[1]. Belladonna and hyoscyamus tinctures BP[2], bromcresol purple[1], and reagent grade chloroform[1] were used.

Procedures—Hyoscyamine determination was carried out on the freshly prepared mixtures as follows:

Aluminum Hydroxide and Belladonna Mixture—Measure 10 ml of the mixture, add 10 ml of water (used to rinse the pipet), and centrifuge the diluted mixture for 3 min at 4000 rpm. To a 5-ml aliquot of the clear supernate, add 10 ml of McIlvaine buffer solution (pH 6.60 ± 0.05) and 10 ml of chloroform solution of bromcresol purple (4×10^{-4} *M*). Shake the mixture for 1 min and allow the layers to separate for 10 min.

Separate the chloroform layer containing the hyoscyamine–dye complex and reextract the aqueous layer with 3 × 10 ml of chloroform. To the combined chloroform extracts, add 15 ml of 0.1 *N* NaOH and shake to liberate the combined dye. Dilute the aqueous phase to 25 ml with 0.1 *N* NaOH and measure the absorbance at 580 nm[3].

Carry out a standard run under the same conditions using 0.25 ml of the standard hyoscyamine sulfate solution in 70% alcohol (containing the equivalent of 30 mg % of hyoscyamine base). Compare using a blank similarly prepared.

Belladonna Mixture, Pediatric—Measure 10 ml of the mixture and add about 0.5 ml of 0.1 *N* NaOH to adjust the pH to 6.6. Follow the procedure for the aluminum hydroxide and belladonna mixture, starting from "... add 10 ml of McIlvaine buffer solution...." Use 0.3 ml of the standard hyoscyamine sulfate solution containing the equivalent of 30 mg % of hyoscyamine base.

Potassium Citrate and Hyoscyamus Mixture—Measure 10 ml of the mixture, add 10 ml of water and 5 ml of ammonia T.S., and extract with 4 × 10 ml of chloroform. Evaporate the combined chloroform extracts and dissolve the residue in the 10 ml of McIlvaine buffer solution used in the assay. Follow the procedure for the aluminum hydroxide and belladonna mixture; use the 3 × 10-ml chloroform aliquots to rinse the container previously washed with the buffer.

For the standard run, use 2 ml of the standard hyoscyamine sulfate solution in 70% alcohol (containing the equivalent of 5 mg % of hyoscyamine base).

Magnesium Trisilicate and Belladonna Mixture—Measure 10 ml of the mixture, add 40 ml of 0.5 *N* HCl, and heat at 50° for 30 min with shaking. Cool and then centrifuge for 3 min at 4000 rpm. To a 10-ml aliquot of the supernate, add a few drops of 0.1 *N* NaOH to adjust the pH to 6.6. Follow the procedure for the aluminum hydroxide and belladonna mixture, starting from "... add 10 ml of McIlvaine buffer solution ...." Use 0.1 ml of the standard

[1] British Drug Houses Ltd., Poole, England.
[2] William Ransom and Son Ltd., Hitchin, Hertfordshire, England.
[3] Unicam SP 500 Series 2.