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THE CONFORMATION OF DOPAMINE HYDROCHLORIDE

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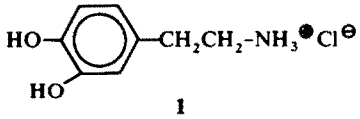
Abstract—A conformational analysis of dopamine hydrochloride has been performed by extended Hückel theory, potential energy functions and NMR spectroscopy. The results establish that dopamine hydrochloride has a *trans* population of 0·43 ± 0·05 over a 60° temperature range. Even though the *trans* conformer is the energetically preferred form, the experimental results show a small increase in the *trans* population with an increase in temperature, contrary to what would be expected from the Boltzmann law.

INTRODUCTION

CONSIDERABLE recent interest has centered on the role of conformation and biological activity which has stimulated the development of a number of semiempirical methods to determine preferred conformations. The conformation of dopamine hydrochloride (**1**), a hormone found in the central and sympathetic nervous systems of mammals,



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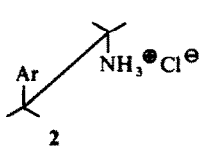
has been studied by both extended Hückel theory[1] (EHT) and X-ray analysis.[2] The solid state conformation has been shown to be exclusively *trans* (**2**) while the EHT study has been reported to reveal the complete absence of a *trans* conformation and predicts that only the *gauche* conformers (**3** and **4**) are significantly populated.



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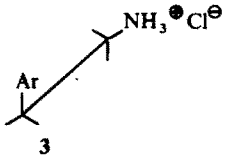

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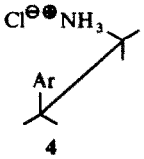

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Ar = 3.4-dihydroxyphenyl-

Since the latter result appeared to be rather unexpected, an investigation of the solution conformation of dopamine hydrochloride was undertaken both theoretically by EHT and other calculative techniques and experimentally by NMR spectroscopy.

EHT calculations

The EHT[3] calculations on dopamine hydrochloride were made substantially with an extended version of QCPE 64, a computer program available through the Quantum Chemistry Program Exchange. Matrix diagonalizations were performed using the Givens method (QCPE 62·3) rather than the Jacobi method used in the original

version of QCPE 64. Inclusion of a translation-rotation subroutine into QCPE 64 was made to permit a pre-determined set of conformations to be calculated solely from an initial data set. The only additional input required beyond a normal data set is the specification of twist axes, increments of twist angle per axis and a list of atoms to be rotated per axis. All bond angles and distances were taken from the tabulation of Pople and Gordon.[4] The atomic valence state parameters used in the calculations are listed in Table 1.

TABLE 1. EHT PARAMETERIZATION (α'S IN EV)

Atom	Slater exponent	α(2S)	α(2p)	α(1S)
C	1·625	−21·4	−11·4	
N	1·95	−26·0	−13·4	
O	2·275	−35·3	−17·8	
H	1·0			−13·6

The internal energy as a function of twist angle measured relative to the *trans* conformer is shown in Fig 1 which reveals that the *trans* conformer, represented by the deep minimum, is preferred on the basis of internal energy. Also shown are the two shallower minima which are representative of the less preferred *gauche* conformers. These latter extrema were the only ones reported in a prior study of dopamine hydrochloride by EHT.[1]

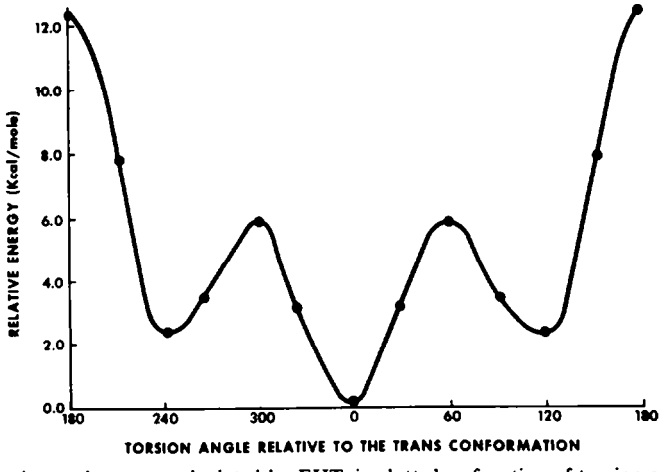
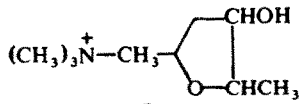


FIG 1. The internal energy, calculated by EHT, is plotted as function of torsion angle. The difference in energy between *gauche* and *trans* is 2·3 Kcal/mole

Empirical potential energy calculations

In an attempt to circumvent the inherent inaccuracy of EHT energies, an empirical computational method was used which had been previously applied to conformational studies of macromolecules, and more recently to muscarine[5] (**5**) and acetylcholine[6] (**6**). As applied to these molecules, the method expresses the potential energy



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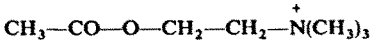

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as a function of the torsion angles about one or more bonds. There are two parts to the energy expression. the first of which is a sum of pairwise Van der Waals interactions. and the second. which is a sum of ethane-like torsional potentials over all rotation axes. In the case of dopamine hydrochloride. the expression for rotation about only the ethane fragment is

$$E(\vartheta) = \sum_i (a_i \exp(-b_i r_i) - c_i/r_i^6) + \frac{U_0}{2}(1 - \cos 3\vartheta) \qquad (1)$$

The summation is only over all pairs of non-bonded atoms whose interatomic distance. r_i. is dependent upon ϑ. measured from the *trans* conformation. The constant. U_0. was given a value of 2·7 Kcal/mole for the torsional term. with the constants a. b and c of the Buckingham potential being taken from Liquori *et al.*[5, 6]

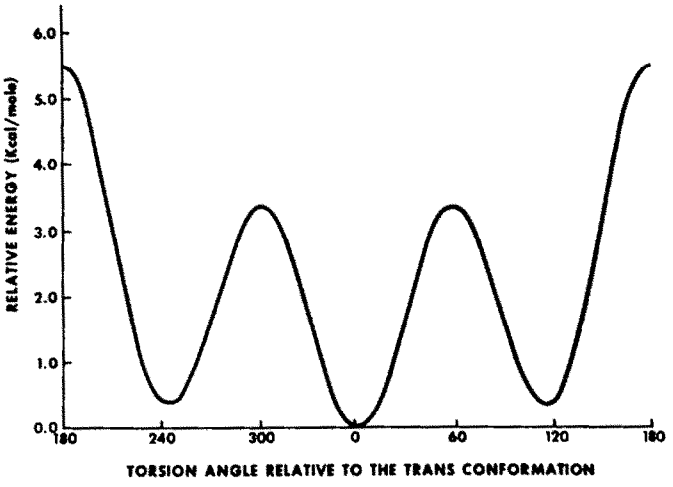


FIG 2. The internal energy. calculated from potential energy functions. is plotted as a function of torsion angle. The energy difference between *gauche* and *trans* is 0·39 Kcal/mole

The potential energy as a function of twist angle using equation (1) for dopamine hydrochloride is given in Fig 2. This diagram is qualitatively the same as that of Fig 1. but with much lower barrier heights and a smaller ground state energy difference between *gauche* and *trans* conformers. Since Eqn (1) contains empirical potentials. one can reasonably expect the resultant energy difference to approximate the true value. An estimate of the conformer populations can be made from these results

using the following expression for the free energy difference between *trans* and *gauche* conformers.[7]

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ = -\Delta E + RT \ln 2 \quad (2)$$

In this equation, the enthalpy difference, ΔH°, favoring the *trans* form, has been set equal to the negative of the potential energy difference between *gauche* and *trans*. The entropy term reflects only the double probability of finding a *gauche* form, which leads to its being favored with respect to entropy by $R \ln 2$. At 25°, ΔG° is 30 cal/mole, which implies a mole fraction of 0·49 for the *trans* conformer.

NMR spectra

In addition to aromatic resonances, the NMR spectrum of dopamine hydrochloride in D_2O consists of an AA'BB' multiplet centered at approximately 3·23 ppm (δ) at ambient probe temperature. The AA'BB' multiplet, arising from the two pairs of enantiotopic methylene protons of the ethane fragment, is not totally symmetrical as the transitions of the upfield half of the multiplet are more broadened than those of the downfield half (Fig 3).

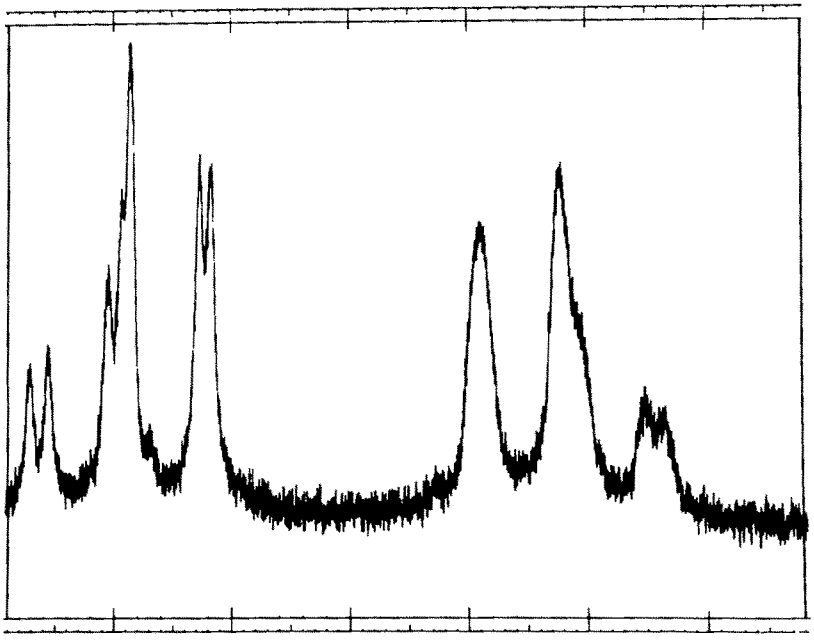


FIG 3. The AA'BB' spectrum of dopamine hydrochloride in D_2O at ambient probe temperature (31°)

The broadening was shown not to be caused by unresolved benzylic long-range coupling[8] when no significant improvement in line shape was observed during spin decoupling experiments in which the aromatic resonances were irradiated. The broadening was therefore ascribed to unresolved nitrogen quadrupole coupling. Since quadrupolar broadening in quaternary nitrogen compounds is largest when the N atom and protons are separated by three bonds,[9, 10] the downfield half of the

multiplet is reasonably assigned to the $^{\oplus}N—CH_2$ protons (H_A, $H_{A'}$) and the upfield half to the Ar—CH_2 protons (H_B, $H_{B'}$).

Analysis of the AA'BB' spectra

Two geminal coupling constants, J_A and J_B, two vicinal coupling constants, J and J', and the chemical shift difference between the non-equivalent nuclei, δ_ν, completely define an AA'BB' spin system.[11,12] In the most general case, the magnitudes of J_A and J_B are different and assumed to be negative while the magnitudes of J and J' are also different but positive. To assist in the analysis of such spin systems four additional dependent parameters have been defined—$N = J + J'$, $L = J - J'$, $K = J_A + J_B$, and $M = J_A - J_B$.

Twelve transitions are present in each half of an AA'BB spectrum in the most general case; however, when $|L| \ll |K|$, a condition generally present in the case of 1,2-disubstituted ethanes, two transitions are weak and not experimentally observable. Under such circumstances the spectrum cannot be readily analyzed to obtain K and therefore only the absolute value of the difference between the geminal couplings $|M|$ can be determined.[13] For emphasis, Table 2 lists calculated transition frequencies for such a system where N, L, and M remain constant while K is varied. It should be noted that only three transitions vary with a three-fold increase of K and the largest change is only 0·9 Hz.

TABLE 2. CALCULATED TRANSITION FREQUENCIES FOR AN AA'BB' SPIN SYSTEM WHEN N = 14·0, L = 2·0, $|M|$ = 0·9, δ_ν = 19·0, AND K IS VARIABLE

When $K = -24·3$ $J_A = -12·6$, $J_B = -11·7$	When $K = -44·3$ $J_A = -22·6$, $J_B = -21·7$	When $K = -64·3$ $J_A = -32·6$, $J_B = -31·7$
12·36	12·24	12·27
13·25	13·25	13·25
17·48	17·48	17·48
18·38	18·38	18·38
19·67	19·67	19·67
19·75	19·77	19·78
20·57	20·57	20·57
20·75	20·73	20·72
25·85	25·84	25·84
27·25	27·25	27·25

For this reason, when iterative computer techniques are used to analyze such a spectrum and the magnitudes of the geminal couplings are treated as variables, anomalous values of J_A and J_B with large probable errors may result, even though the calculation has minimized to a solution with a small RMS error. However, the absolute value of the differences between the calculated geminal couplings is an accurate measure of $|M|$. Alternately, the magnitude of $|M|$ can be determined directly from the spectrum and arbitrary values of J_A and J_B, having as their difference $|M|$, can be used as starting parameters but not treated as variables during the iterative calculation.

A deceptively simple 20 line AA'BB' spin system was present in the spectra of acetylcholine isologues and is responsible for the anomalously large geminal

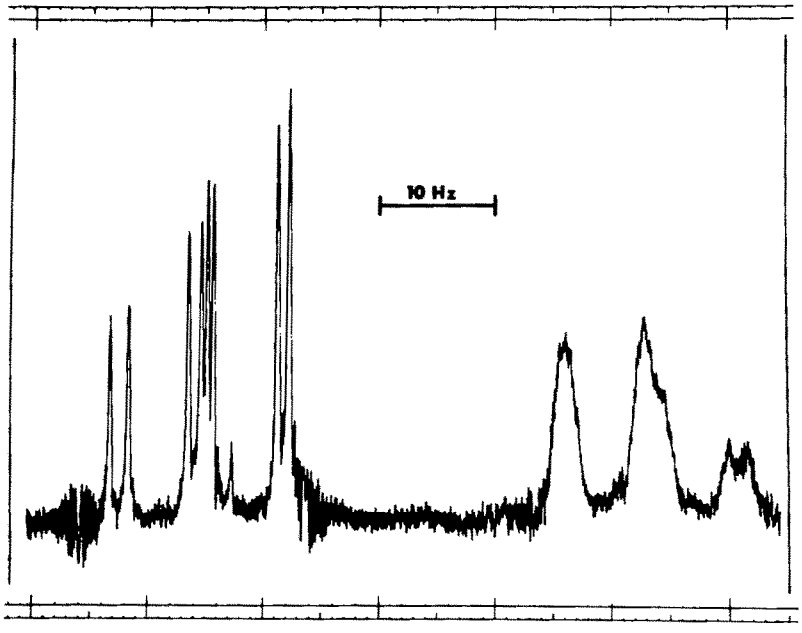


FIG 4. High temperature spectrum of dopamine hydrochloride in D_2O at 90°

couplings reported for these compounds.[14] The downfield half of the high temperature spectrum of dopamine hydrochloride (Fig 4) exhibits nine distinct transitions with a tenth obscured weak transition revealing that a deceptively simple spectrum is also observed for this compound.

The AA'BB' spectrum of dopamine hydrochloride in D_2O was analyzed at three different temperatures using the iterative computer program LAOCN3.[15] Calculations were made both in which the magnitudes of J_A and J_B were variables and when

TABLE 3. NMR PARAMETERS OF DOPAMINE HYDROCHLORIDE IN D_2O AT VARIOUS TEMPERATURES*

Temperature	31°	55°	90°
J	6·78	6·70	6·58
J'	7·60	7·80	7·99
N	14·38	14·50	14·57
$-L$	0·82	1·10	1·41
$\|M\|$	1·55	1·52	1·64
δr	18·04	18·03	18·69
$\|K\|$†	23·65	23·70	23·56
RMS error	0·017	0·022	0·032
$2J + J'$	21·16	21·20	21·15

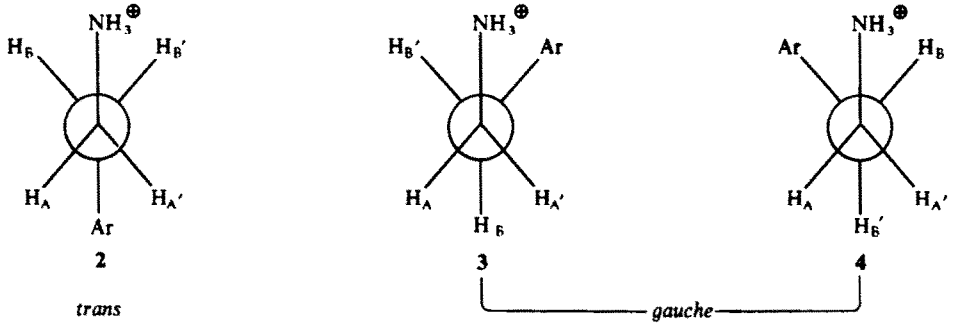
* All reported values are in Hz at 100 MHz. J and J' were not uniquely determined from analysis of the spectra nor was the sign of L—c.f. discussion.

† Arbitrarily assigned—c.f. discussion

assigned invariant arbitrary values differing by $|M|$. In either case the calculations smoothly minimized to solutions with RMS errors of 0·032 or less after 3–4 iterations. In every case in which J_A and J_B were variables, unreasonable values for the geminal couplings were obtained-both anomalously large or small as well as both positive and negative. However, the calculated value of $|M|$ was always in excellent agreement with the value measured directly from the spectra. Several different starting parameters were used for which the calculations minimized to identical solutions when care was taken to assign the proper identification number to the experimentally observed transitions. The final parameters obtained from these calculations are collected in Table 3 with their respective RMS errors. Computer plots of the final solutions were indistinguishable from the experimental spectra.

Conformational analysis

If the assumptions are made that a conformation is more stable when staggered rather than eclipsed and that the tetrahedral symmetry of carbon is maintained. then the three rotational conformers of dopamine hydrochloride are the *trans* rotamer **2** and the two enantiomeric *gauche* forms **3** and **4**.



Under conditions of rapid rotation, not necessarily implying equal populations. protons H_A and $H_{A'}$ as well as H_B and $H_{B'}$. are enantiotopic and only two vicinal coupling constants will be observed:[11,12]

$$J = J_{AB} = J_{A'B'} \tag{3}$$

$$J' = J_{A'B'} = J_{AB'} \tag{4}$$

The observed magnitudes of J and J' will be the weighted averages of the vicinal couplings between *gauche* protons (J_g) and *trans* protons (J_t) in the individual rotamers. Expressions for J and J' have been derived in terms of the populations of the *trans* and *gauche* rotamers expressed in mole fractions (n_t and n_g) with the assumption that J_g and J_t are constants and identical in all three rotamers:[13]

$$J = n_t J_g + \tfrac{1}{2} n_g (J_t + J_g) \tag{5}$$

$$J' = n_t J_t + n_g J_g \tag{6}$$

If the assumption is made that $J_t > J_g$, then equations (5) and (6) can be used to graphically reveal the relationship between J and J' with n_t as shown in Fig 5. The

population dependence of the parameters N and $|L|$ are also shown and reveal several conformationally useful correlations.

The magnitude of N linearly decreases simultaneously with n_t. Therefore the effect of perturbations on the rotamer populations arising from changes in temperature or solvent, etc. can be detected and the direction of the population redistribution directly determined. However, since J_g and J_t are not accurately known, only estimates for n_t can be obtained and the specific values of n_t remain undefined as does the identity of the preferred or predominant conformation.

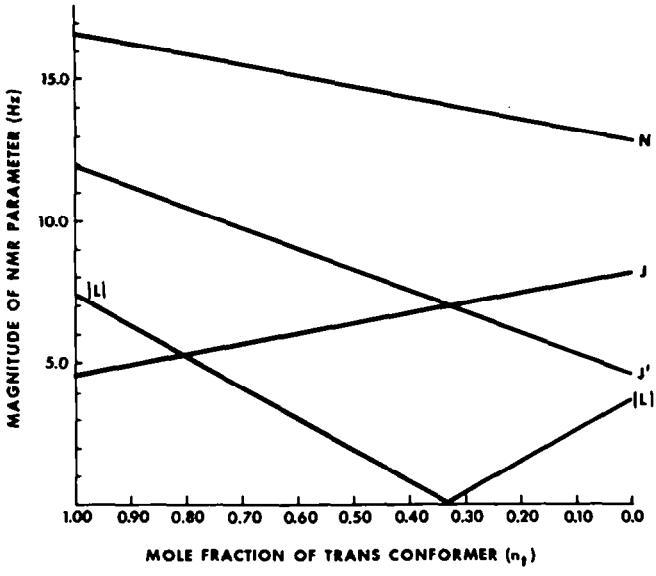


FIG 5. Variation of N,J,J' and $|L|$ with the mole fraction of the *trans* conformer, assuming $J_t = 12{\cdot}0$ and $J_g = 4{\cdot}6$

Further, Fig 5 reveals that when $|L| = 0$ the three conformers are of equal energy and $n_t = 0{\cdot}33$.[13] As the populations (more precisely, the relative energies) of the conformers change in either direction, the magnitude of $|L|$ increases. However, the sign of L would be required to know which specific conformer was of lower energy when $|L| > 0$. Since analysis of an AA'BB' spectrum gives values for two vicinal couplings but does not uniquely define J and J', the sign of L cannot be directly obtained.

However, the identity of the lowest energy conformation can be determined when the magnitudes of both N and $|L|$ are known under two or more conditions for which n_t is different. For example, if the conformationally mobile system is perturbed and the magnitudes of both N and $|L|$ change in the same direction (increase or decrease) then $1{\cdot}0 > n_t > 0{\cdot}33$ (Fig 5). Therefore since n_t is greater than the population of either of the two individual *gauche* conformers, the *trans* conformer must be of lowest energy. If however the magnitudes of N and $|L|$ change in opposite directions (one increases while the second decreases) then similar reasoning reveals that the *gauche* conformers are of lowest energy. This analysis requires only that the perturba-

tions be suitably minor so that changes in the magnitude of $|L|$ are sufficiently small to prevent unrecognized crossing of the $|L| = 0$ condition.

It should be emphasized that this analysis defines only the conformer(s) of lowest energy and does not necessarily determine the most populated (predominant or favored) conformation. Since there are two enantiomeric *gauche* conformers, a favorable entropy factor permits the total population in this conformation to predominate even though the *trans* conformer is of lower energy (i.e. as n_t varies between 0·33 and 1·0 the *trans* conformer is of lower energy, but when $0{\cdot}50 > n_t > 0{\cdot}33$ the *gauche* conformation predominates). In cases where the *gauche* conformers are of lower energy, n_t varies between 0·33 and 0·0 and therefore the *gauche* conformers predominate.

The values for N and $|L|$ determined for dopamine hydrochloride in D_2O at three different temperatures are collected in Table 3. Since both N and $|L|$ increase with temperature the *trans* conformer **1** is shown to be of lowest energy. This in turn requires that, since n_t must be between 0·33 and 1·0, the larger of the two experimentally determined vicinal couplings must be J' while the smaller must be J and L must therefore be negative (Fig 5). Consistent with this analysis is the observation that J' increases and J decreases with increased temperature. The increase of J', N, and $|L|$ clearly reveals that the population of the *trans* conformer increases with temperature even though this conformer is of lowest energy.

It has been further shown that a population independent relationship can be derived from Eqns (5) and (6) which relates J and J' to J_g and J_t:[13]

$$2J + J' = 2J_g + J_t \tag{7}$$

Similarly by definition a relationship exists between J and J' with the dependent parameters N and L:[13]

$$2J + J' = \tfrac{3}{2}N + \tfrac{1}{2}L \tag{8}$$

Therefore Eqns (7) and (8) define a quantity that is not only population but also temperature independent (assuming J_t and J_g are invariant with temperature) and therefore is a physical constant of the compound being examined. The values determined for dopamine hydrochloride in D_2O at three different temperatures (Table 3) are indeed constant within experimental error (21·17 ± 0·03 Hz), confirming that J,J', and the sign of L have been correctly assigned and the three sets of data are self-consistent and therefore suitable for comparison.

In addition, Eqns (5)–(7) can be used to determine reasonable ranges for n_t and n_g. For example, if any value of J_g is substituted into Eq (7) with the observed values for J and J', a correspondingly related value for J_t is determined. This value can be substituted into Eqn (5) and (6) to obtain the corresponding values for n_t and n_g. Although the exact values of J_g and J_t remain unknown, reasonable extreme values can be assigned from those reported for 1,2-disubstituted ethanes[16] or from the Karplus relationships,[17] thereby giving a range of possible values for n_t and n_g. When the extreme values $J_g \leqslant 5{\cdot}0$ Hz and $J_t \leqslant 13{\cdot}0$ Hz are assumed, the permitted values of n_t and the corresponding ranges of J_g and J_t which are actually possible for dopamine hydrochloride are collected in Table 4. Specific values for n_t are also shown for the case where J_t and J_g were arbitrarily assigned to be 12·0 and 4·6 Hz, respectively.

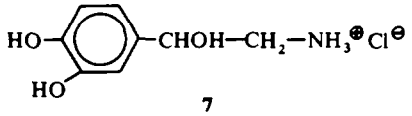
TABLE 4. CALCULATED RANGES OF n_t FOR DOPAMINE HYDROCHLORIDE AT VARIOUS TEMPERATURES WHEN $J_g \leqslant 5{\cdot}0$ HZ AND $J_t \leqslant 13{\cdot}0$ HZ

Temperature	31°	55°	90°
n_t	0·39–0·42	0·42–0·45	0·44–0·48
permitted range J_g		4·10–5·00	
permitted range J_t		13·00–11·20	
n_t when $J_g = 4{\cdot}6$ and $J_t = 12{\cdot}0$ Hz	0·41	0·43	0·46

DISCUSSION

The preceeding theoretical and experimental analyses, in contrast to a previous report,[1] have conclusively shown that the *trans* conformer is of lowest energy in aqueous solution. Further, our results indicate that in solution appreciable amounts of the *gauche* conformers are also present, $n_t = 0{\cdot}43 \pm 0{\cdot}05$, the result of a favorable statistical term in ΔG° and the possible intervention of other factors (*vide infra*).

The most readily apparent discrepancy in the previously reported calculations[1] for dopamine hydrochloride is the presence of only a two-fold rotational barrier with respect to twist around the ethane-like single bond. Indeed, previously reported EHT calculations[18] on the structurally similar compound norepinephrine hydrochloride (**7**) does show the expected three-fold rotational barrier, with the *trans* conformer as the most stable.



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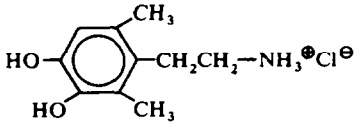
We have performed additional EHT calculations on the 2,6-dimethyl substituted analogue of dopamine hydrochloride (**8**) in an attempt to remove the three-fold character of the energy function with respect to internal rotation. The resultant energy minima for the *gauche* forms are much elevated above that of the *trans* conformer as expected, but more importantly, the three-fold nature of the energy function has been maintained. We can only conclude that the original work of Kier and Truitt is in error.



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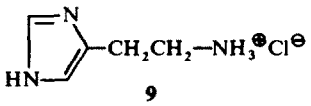
Our calculated results are in general agreement with X-ray results which showed that dopamine hydrochloride exists preferentially in the *trans* form in the solid state.[2] Unfortunately, while EHT can dependably predict the most stable conformer, accurate barrier heights or ground state energies are generally not obtained. Usually these quantities are overestimated resulting in unreliable calculated conformer

populations. For example, our calculated conformational energy difference of 2·3 Kcal/mole (Fig 1) obtained for dopamine hydrochloride by EHT when used in Eq. (2), predicts that the mole fraction of the *trans* conformer will be greater than 0·95. In contrast, calculations involving empirical potential energy functions (Fig 2) give values for n_t which agree remarkably well with the experimentally observed populations determined from the NMR results. Further, the estimate of the *trans* conformer population is rather insensitive to error in ΔE and temperature, provided that the *only* temperature dependence that $\Delta G°$ exhibits is the explicit one in Eq. (2). For example, at 25° n_t is 0·49, while at 60°, the mole fraction decreases to 0·47. An overestimation of ΔE by 50% results in a mole fraction of 0·43, thus the calculated value of n_t is in reasonable agreement with the NMR results. Through use of Eq. (2) and the equilibrium constant relationships, $n_t/(1 - n_t) = K_{eq.} = \exp(-\Delta G/RT)$, ΔE can be calculated from the experimental value of n_t assuming that the only entropic term is $RT \ln 2$. The calculated value of ΔE from the experimental n_t is 0·20 Kcal/mole compared to 0·39 Kcal/mole obtained from potential energy functions. The disparity between the two calculations may be attributed to solvent stabilization of the dipole moment of the *gauche* form,[19] thereby reducing the value of ΔE calculated by potential energy functions, since solvent effects were not included in these calculations.

The results of the variable temperature NMR experiments reveal a small but significant increase in the mole fraction of the *trans* conformer with temperature (Table 4) even though the *trans* conformer is of lowest energy. This observation is unexpected since the Boltzmann law predicts increased population of the higher energy conformer with increased temperature, provided that the energy levels and molecular interactions (intermolecular and molecule-solvent) remain constant over a temperature range. Although further investigation would be required to completely understand this phenomenon, several explanations are possible.

The *gauche* conformers could be stabilized through an intra- or intermolecular interaction of the ammonium group with the hydroxyl substituents on the phenyl ring, presumably involving H-bonding. The effective H-bonding distance has been estimated to be ~2·8 Å,[20] which implies that the proposed intramolecular interaction is solvent mediated, since the closest approach of interacting groups is 4·8 Å in the *gauche* conformer. Increased temperature would then be expected to break the weak bonding involved and result in destabilization of the *gauche* form. If the stabilization is intermolecular, the proposed interaction could be through dimerization between *gauche* conformers. Increased temperature would then result in dissociation of the dimer and destabilization of the *gauche* conformers with a concomitant increase in the population of the *trans* conformer. The dramatic decrease of the transition line widths with temperature observed in the NMR spectra (compare Figs 3 and 4) is compatible with such a decrease of intermolecular association.

A second possible explanation which cannot be rigorously eliminated is inhibition of the rotational freedom in the *trans* conformer by solvation, thus introducing a small, temperature dependent, negative entropy term into the free energy expression.



9

The solution conformation of histamine hydrochloride (9) has recently been determined by NMR[21] and in view of its structural similarity to dopamine hydrochloride similar conformer populations can reasonably be expected. Indeed, the preliminary results indicated nearly equal populations of *gauche* and *trans* conformers, in agreement with recent EHT calculations[22] and our results with dopamine hydrochloride.

There has been a resurgence of attempts to assign some *a priori* significance to calculated preferred conformations of potentially mobile systems.[23] In light of our results the general significance of these attempts is questionable for two reasons. In the case of dopamine hydrochloride, ascribing biological activity to *only* the lowest energy conformer is not justified since experimental evidence has shown that significant populations of both the *gauche* and *trans* conformations are present. However, in the case of acetylcholine (6)[24] and its isologues[14] (10 and 11), experimental evidence has been obtained which reveals that only one conformation of these compounds

$$CH_3CO—X—CH_2CH_2—N(CH_3)_3{}^{\oplus}Cl^{\ominus}$$

6 X = O
10 X = S
11 X = Se

is significantly populated in solution. Therefore, unless some experimental evidence has been obtained to corroborate that the calculated preferred conformation is *largely* predominant in solution, no special significance can really be attached to it in itself.

Moreover, measured conformer populations in themselves may not be sufficient to draw valid conclusions with regard to the role played between the preferred conformation and biological activity in potentially mobile systems. We face a dilemma which arises from a lack of information about the transition state formed between a drug and its site of action. If the equilibrium between the various drug conformers and the receptor (active site) is the slow step in the reaction sequence necessary to elicit a response, then the rate constant is directly dependant upon the conformer populations. Alternatively, biological activity cannot be ascribed to a particular conformer which is involved in a rapid step prior to equilibrium. Furthermore, if there is a fast equilibrium between conformers, the assignment of biological activity on the basis of populations is without foundation.

Therefore, since we have restricted ourselves to the physical and chemical aspects of the conformation of dopamine hydrochloride, we feel that it is inappropriate to attempt to attribute biological significance to our study.

EXPERIMENTAL

The dopamine hydrochloride used in the NMR study was purchased from Aldrich Chemical Co., Inc. Milwaukee, Wis. and used without further purification. Nuclear magnetic resonance spectra were obtained on a Varian Associates HA-100 spectrometer operating in the frequency sweep mode. Temps were determined from ethylene glycol calibration curves and are accurate to $\pm 5°$. Duplicate spectra were obtained at each temperature and averaged experimental line transitions were used in the computer calculations.

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