The Hybridization State of Nitrogen as a Conformational Variable in Biologically Active Molecules

Peter R. Andrews,* Sharon L. A. Munro, Maruse Sadek, and Margaret G. Wong
School of Pharmaceutical Chemistry, Victorian College of Pharmacy Ltd., 381 Royal Parade, Parkville, Victoria, Australia 3052

A survey of the conformations found in the crystal structures of central nervous system-active drugs and related nitrogen-containing structures shows that there is a continuous distribution of conformations from that typical of the sp^3 (N-torsion 120°) to that typical of the sp^2 (N-torsion 180°) hybridization state. In general the presence of an adjacent carbonyl group favours a planar geometry, while the lack of any conjugating group favours the tetrahedral state, but nitrogens with aromatic substituents cover the entire range between the two states. Other than those cases where the nitrogen has aromatic substituents or is in a sterically constrained ring-system (*e.g.*, cage compounds), steric effects have relatively little influence on the conformation at nitrogen. In these cases calculations using various techniques suggest that simple non-bonded potential calculations may be the most practical approach to the evaluation of nitrogen geometry. Even in small molecules, however, calculated barriers to nitrogen inversion are generally low and poorly reproduced by either molecular orbital or classical calculations.

The conformational variables normally considered in conformation–activity studies are rotations around single bonds, frequently including changes in ring geometry (*e.g.*, chair–boat conversions).[1] In more detailed studies, bond lengths and angles are varied, but for conformational searches over many torsional variables in larger molecules, full geometry-optimization of this type rapidly becomes prohibitive.[1]

A factor which has not normally been regarded as a conformational variable is hybridization state, which is usually considered to be a static function of the valency of the central atom. While this may be true in the case of carbon, it does not appear to be justified for nitrogen atoms. Trivalent nitrogen atoms, for example, could be either sp^2- or sp^3-hybridized, or possibly in an intermediate hybridization state. The purpose of this paper is to highlight the importance of nitrogen hybridization as a conformational variable, and to formulate a rational approach to this problem. Although the term hybridization state is commonly used to interpret the geometry at the atom, strictly speaking it should be used only within the valence-bond model. For want of a better term, we shall use it here to describe the conformational relationship between the atoms attached to the nitrogen atom, regardless of whether the data discussed are derived from experiment, valence-bond considerations, or molecular orbital calculations.

Figure 1 illustrates how the choice of nitrogen hybridization state can bias modelling studies. It depicts the range of locations occupied by the terminal amine group of the antidepressant drug imipramine as the hybridization state of the nitrogen atom in the dibenzazepine nucleus changes from sp^3 through to sp^2 and back to sp^3. The distance between the terminal amine in the extreme positions is 9.40 Å, well beyond the tolerance usually considered in modelling studies. Since the terminal amine and the tricyclic nucleus are known to be the major structural requirements for antidepressant activity, this potential variation in their spatial relationship clearly has a substantial impact on conformation–activity studies.

Another example of how the nitrogen hybridization state can influence the outcome of a conformational study is provided by the rotational barriers around the nitrogen (N-5)–carbon (phenyl) bond in 2,4-diamino-6,6-dimethyl-5-(substituted phenyl)-5,6-dihydrotriazines. Thus, calculations in our laboratory[2] using classical potential-energy methods resulted in two energy wells (30, 150°), whereas those previously published by Hopfinger,[3] who used similar techniques, resulted in two double minimum-energy wells (30, 90°; −90, −150°). Rather than attribute this difference to the energy potentials and parameterizations used, we confirmed that it results from different initial geometries being used in each study. Whereas Hopfinger used structures with a tetrahedral 4-amino substituent, obtained from the optimization procedures of Allinger's MM1 program, our study used geometries based on crystal structures optimized by MINDO/3 which featured a planar 4-amino substituent on the dihydrotriazine ring. Careful consideration of nitrogen hybridization states is thus clearly necessary in the planning and interpretation of conformational studies.

In order to establish a rational basis for choosing the most appropriate nitrogen hybridization state, we have approached the question from three directions: (1) a survey of the hybridization state of nitrogen in the crystal structures of central nervous system (CNS)-active drugs and related

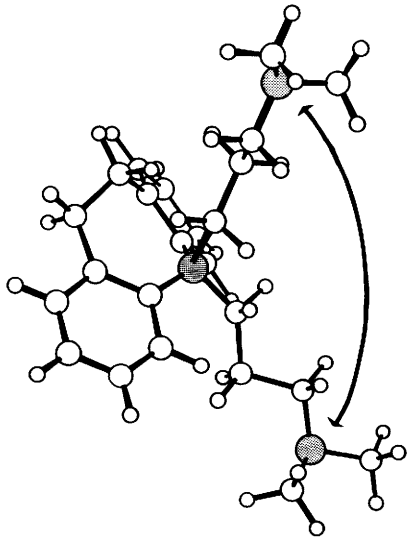
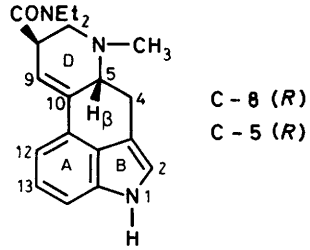
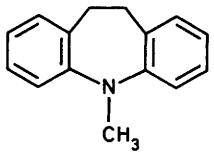


Figure 1. Graphic representation of range of locations covered by the terminal nitrogen in imipramine during inversion of the ring nitrogen



C - 8 (*R*)
C - 5 (*R*)

(1)



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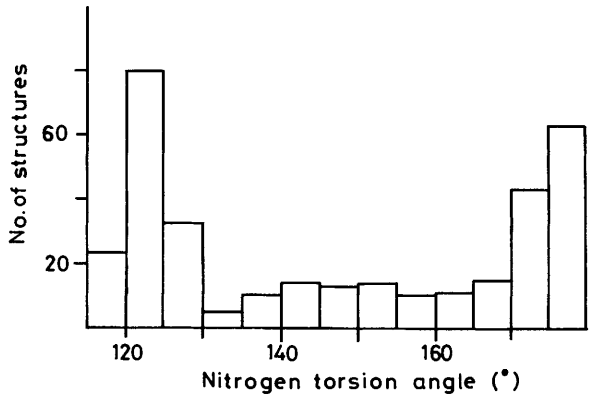

Figure 2. Histogram showing the distribution of nitrogen torsion angles found in the survey of crystal structures of CNS-active drugs and related structures

compounds listed in refs. 4 and 5; (2) a survey of the literature on theoretical and experimental barriers to nitrogen inversion in small molecules; (3) a series of classical molecular mechanics and semi-empirical molecular orbital calculations on two larger structures, lysergic acid diethylamide (LSD) (**1**) and *N*-methyl-10,11-dihydrodibenz[*b*,*f*]azepine (**2**).

Methods

In this work we define the nitrogen-torsion angle τ_N (R^1–N–R^3–R^2) as a conformational variable for the investigation of nitrogen hybridization. This serves as a more sensitive index of the hybridization state than the corresponding bond angles, since it extends from 120° for the sp^3 to 180° for the sp^2 state, whereas the bond angles (*e.g.*, R^1–N–R^2) only vary from 109 to 120°. Previous methods[6] used to define the nitrogen hybridization state have included those where the angles around the nitrogen have been summed; where the height of the nitrogen above the plane specified by the three attached atoms is given; and in theoretical calculations the percentage *s*-character has been calculated. All these methods give a much more limited and less obvious range of values to relate to the nitrogen hybridization state than the τ_N definition.

The derived formula [equation (1)[7]] relating the N-torsion angle, τ_N, to three bond angles around the nitrogen (α = R^3–N–R^2, β_1 = R^3–N–R^1, β_2 = R^2–N–R^1) caters for the special symmetrical case where $\beta_1 = \beta_2$ and α is kept constant. It thus approximates the common situation in which R^2 and R^3 form part of a ring system, as in imipramine above, while R^1 is a methyl group or longer alkyl chain. The main use of equation

$$-\cos\tau_N = (1 - 1/\cos\alpha)/(\tan\alpha\tan\beta) \qquad (1)$$

(1), however, is in building up the initial structures for full or partial (dihedral angles only) optimization. Without optimization the energies corresponding to each nitrogen-torsion value are prejudiced by the initial geometry of a structure. This bias may need to be overcome by subsequent geometry optimization, and is further discussed below.

The program COMOL,[8] run on a Cyber 835 computer at the Royal Melbourne Institute of Technology (RMIT), was used for the optimization of dihedral angles with fixed bond lengths and bond angles. For full optimization a semi-empirical molecular orbital calculation with MINDO/3 and/or MNDO parameterizations was used. The specific programs[9] were MINDO/3 on the Cyber 835 computer and the MOPAC package on a VAX 11/750 computer, also at RMIT. Calculations for each reaction pathway were carried out in a stepwise manner, with each calculation providing the starting geometry for the next point on the pathway. Although the stationary points were only characterized by first-derivative methods, this procedure effectively ensures that true minima, rather than transition states, are located. The crystallographic survey was done with the Cambridge Crystallographic Data Base (1983 release), implemented on a PDP 11/34 computer. The MORPHEUS package[10] on the PDP 11/34 was used for molecular modelling and comparison of molecular geometries.

Results and Discussion

It is generally thought that nitrogen hybridization can be affected by many factors, including electronic and steric effects. In the solid state there is also the possibility of effects from molecular packing forces. The initial survey of crystallographic data was therefore undertaken to establish if the experimental reality reflects these effects. Theoretical methods were then surveyed and compared with experimental data from crystallographic and other sources.

(1) *Crystal Structures.*—Initially the crystal structures of CNS-active drugs and closely related compounds listed in ref. 4 were examined. The co-ordinates of these compounds were extracted from ref. 5. This was supplemented by a keyword bibliographic search of ref. 5 for various CNS drug types (*e.g.*, anticonvulsant, antipsychotic, *etc.*) and by references from the more recent literature. The search yielded *ca.* 500 compounds, of which about 200 were not measured, as they contained only quaternary nitrogens. This left compounds containing either only tertiary nitrogens or both tertiary and quaternary nitrogens in the same molecule.

Closer consideration was given only to those compounds where the nitrogen was bonded to three non-hydrogen atoms (NR_3), where R was usually a carbon. This was done because the positions of nitrogen and carbon are much more precisely located in *X*-ray crystallographic studies than those of hydrogen.[11] For tertiary nitrogens with hydrogens as substituents, it was shown that the trends were similar, though the ranges were wider as would be expected for the lower positional precision for hydrogen atoms. There were insufficient neutron diffraction studies for more accurate comparisons between tertiary nitrogen of the type NR_3 and NR_xH_y.

In total, the structural geometries of 238 crystal structures

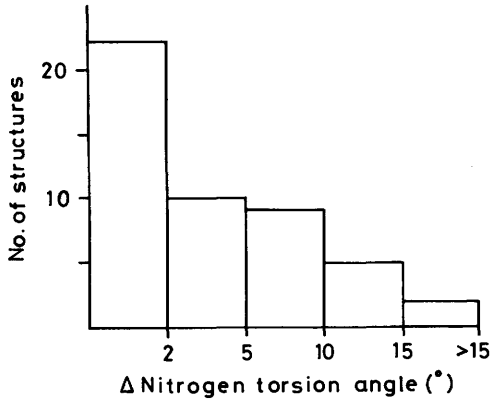

Figure 3. Variations in torsion angle observed in structures where the unit cell contained more than one molecule

containing 338 tertiary nitrogens were examined using the MORPHEUS package. The modulus of measured torsional angles around each nitrogen was averaged to give the torsion angle used for comparisons. A summary of the data (Figure 2) indicates that although most of the nitrogens have torsion angles of *ca.* 120° (*i.e.*, sp^3 hybridization) or 180° (*i.e.*, sp^2 hybridization), significant numbers are found with torsion angles throughout the range 115—180°. This continuous range of nitrogen torsion angles clearly indicates that intermediate hybridization states are energetically accessible in a significant number of molecules and, therefore, that the nitrogen hybridization state is an important conformational variable.

Molecular packing forces. Those crystal structures with more than one molecule in the unit cell were examined to see if crystal packing forces significantly alter the nitrogen-torsion angle. The variations in torsion angle between such molecules are summarized in Figure 3, and indicate that a difference of 10—15° between molecules in a unit cell is not uncommon. A difference of 40° was found in one crystal structure,[12] but was associated with some crystal-packing disorder. These data suggest that the energies generally associated with crystal packing forces (0—2 kcal mol^{-1} [13]) are insufficient to have a major effect on nitrogen hybridization. However, in the absence of accurate data for crystal packing forces in the individual molecules, this finding is not conclusive.

Conjugation. Since conjugation between nitrogen and other groups is clearly likely to affect nitrogen hybridization, the data were further classified according to the presence of an adjacent carbonyl group, the presence of an aromatic substituent, and the absence of any possibility of conjugation. These data are displayed in Figure 4.

The presence of an adjacent carbonyl group (Figure 4a) obviously has an over-riding influence on nitrogen hybridization (keeping it planar), presumably due to electron delocalization from the carbonyl group, as in the amide bonds of peptides. Two classes of exceptions to this rule were found, the first being the five-membered-ring phenylbutazone compounds (τ_N 138—147°), in which the relevant nitrogen is connected to a carbonyl group, an aromatic ring, and another nitrogen which also has a phenyl and a carbonyl group attached. Presumably, adverse steric interactions between the two adjacent phenyl rings are enough to overcome the effects of the adjacent carbonyl group. The second class of exceptions consists of strychnine-related compounds (τ_N 143—163°), where the effects of the cage system are dominant.

It might intuitively be expected that nitrogen with aromatic substituents would also tend towards sp^2 hybridization, due to the possibility of conjugation between the unshared electron-

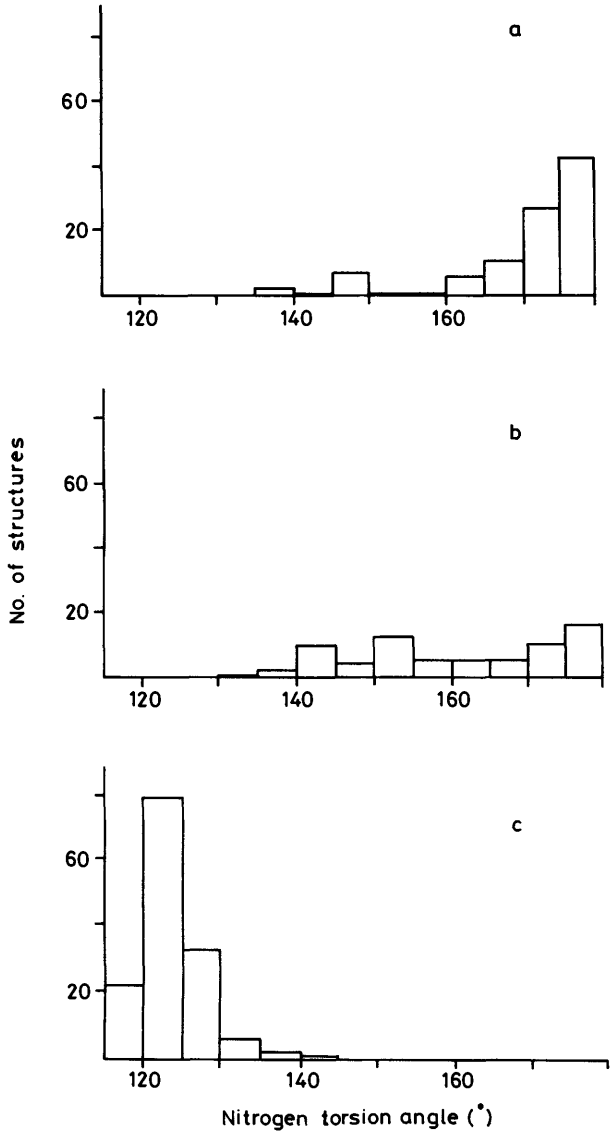

Figure 4. The effect of conjugation on nitrogen hybridization: (a) nitrogen with an adjacent carbonyl group; (b) nitrogen with one or more aromatic substituents but no other conjugating substituent; (c) nitrogen for which there is no possibility of conjugation

pair on nitrogen and the delocalized π-orbitals of an aromatic ring. However, examination of the data (Figure 4b) shows a fairly even number of nitrogens with torsion angles in every category from 135—140° up to 175—180°. In fact, almost all the 'intermediate' hybridization values found in this study arise from such compounds, as can be seen by comparison of Figures 2 and 4b. It is noteworthy that this finding is consistent with experimental studies on anilines[14] where nitrogen-torsion values of 140—160° are common. In triphenylamine,[15] on the other hand, the nitrogen is more planar, with nitrogen-torsion angles varying between *ca.* 160 and 180° for the four molecules in the unit cell. A similar case is that of diphenylaminotriphenylmethane,[16] in which the nitrogen-torsion angle is 179.6°, indicating sp^2 hybridization. The reverse situation is found in *NN*-dimethyl-8-nitro-1-naphthylamine, where seven different crystal modifications have been observed by *X*-ray diffraction.[17] Here there appears to be very little conjugation with the naphthyl ring, as the nitrogen torsion angles vary from

118.6 to 138.9°; *i.e.*, the nitrogen hybridization of the amine group is tending towards sp^3.

Nitrogens with no possibility of conjugation show a remarkably limited range of torsion angles (Figure 4c). Indeed, this range is far smaller than might be expected in light of the common belief that sterically hindered nitrogens tend away from sp^3 hybridization and towards planarity.[18] Inter- and intramolecular hydrogen bonding may partially account for the small range observed, but cannot be the only factor.

Influence of ring size. Sub-division of nitrogens into five-, six-, and seven-membered rings and aliphatic acyclic compounds (Figure 5) shows that the range of nitrogen hybridization in the total sample is closely matched only by nitrogens located in six-membered rings, probably due both to this being by far the largest sub-category and to the inherent flexibility of the six-membered ring. Nevertheless, the smaller sub-sets of data for the five- and seven-membered rings and aliphatic acyclic compounds follow the same general trends as those observed for the complete set. Thus, for molecules where there is no possibility of conjugation, only hybridization states close to sp^3 need be considered; nitrogens with an adjacent carbonyl group are almost invariably sp^2, but for nitrogens with a phenyl substituent a very much wider range of nitrogen torsion angles must be considered.

Steric effects. In order further to investigate the effects of steric hindrance on the nitrogen hybridization state, an additional search of ref. 5 was conducted for nitrogens lacking any possibility of conjugation but which might be regarded as sterically hindered. Two sets of such compounds were identified. The first set has nitrogen attached to three carbons, each of which is in turn connected to two other carbons. The second set has nitrogen attached to three carbons, only two of which are attached to a further two carbons. The results given in Figure 6 show that in the first, more sterically hindered, set the average

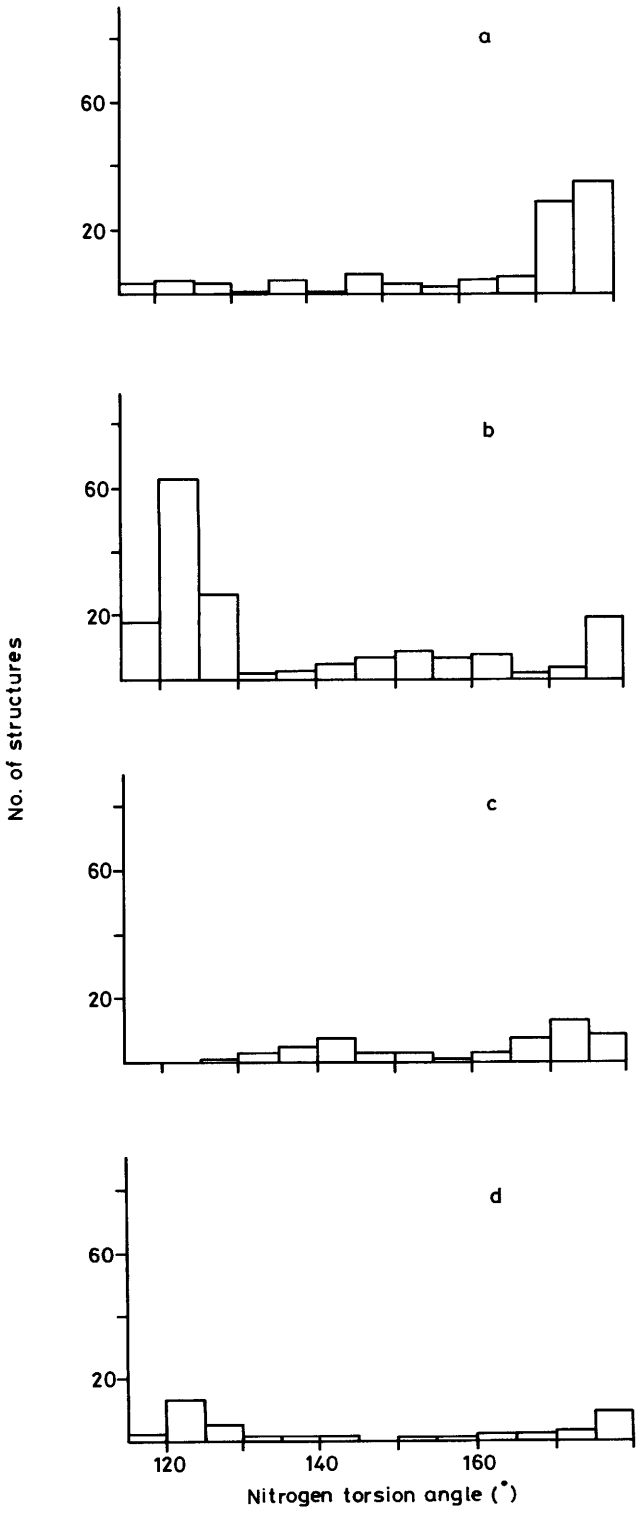

Figure 5. The effect of ring size on nitrogen hybridization: (a) five-membered rings, (b) six-membered rings, (c) seven-membered rings, (d) aliphatic compounds

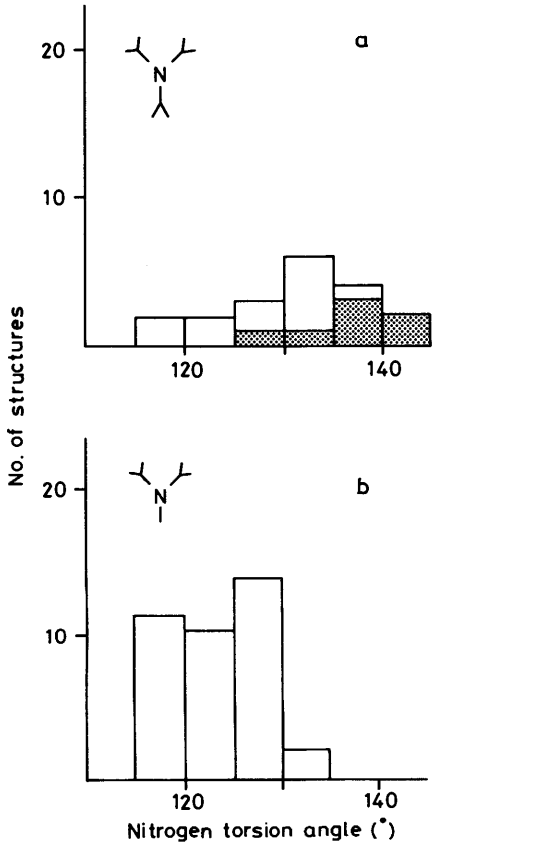

Figure 6. The effect of steric hindrance on nitrogen hybridization. (a) Compounds with nitrogen surrounded by three ternary carbons. The hatched region in the more sterically hindered set refers to nitrogens in cage structures. (b) Compounds with nitrogen surrounded by two ternary carbons

Table 1. Summary of the experimental and calculated values for the nitrogen inversion barriers (kcal mol^{-1}) in ammonia and methylamines

	Exp.	MINDO[a,i]	MINDO[c,j]	MNDO[c,j]	E_{mb}[a,k]	PRDDO[b,l]	(DZP)[b,l] *ab initio*
NH_3	5.8[d]	3.7	6.1	11.5	5.8	10.0	4.8[m]
$MeNH_2$	4.8[e]	5.8	2.1	7.6	2.4	6.4	5.1
Me_2NH	4.4[f]	6.7	0.0	4.1	2.1	4.5	5.4
Me_3N	8.2[g] / 6.7[h]	6.5	0.0	1.7	1.3	5.4	9.6

[a] Standard geometries; tetrahedral and planar bond angles are used. [b] Experimental geometries for the ground state and optimized for the planar state are used. [c] Fully optimized geometries for both ground and transition states are used. [d] Ref. 18. [e] Ref. 24. [f] Ref. 25. [g] Ref. 26. [h] Ref. 27 (the value is for dibenzylmethylamine). [i] Ref. 21. [j] Ref. 19. [k] Ref. 28. [l] Ref. 22. [m] Ref. 20. The inversion barriers calculated with STO-3G, 5-31G, and DZP basis sets, and near the Hartree–Fock limit, are 11.1, 0.4, 5.0, and 5.2 kcal mol^{-1}, respectively.

nitrogen torsion angle is increased by *ca.* 15° relative to the total set of unconjugated nitrogens (compare Figure 4c). Excluding cage compounds, which are shown cross-hatched in Figure 6a, this difference is only 10°, while for the second, less sterically hindered set of structures (Figure 6b), there is no significant movement towards sp^2 hybridization. It thus appears that in the absence of any possibility of conjugation, steric effects have a minimal influence on nitrogen hybridization.

(2) *Calculated Barriers to Nitrogen Inversion.*—Various methods are available for the experimental determination of atomic inversion. Those most often used for small molecules are based on the study of vibrational spectra from the i.r. to microwave region, depending on the compound.[18] Dynamic n.m.r. methods are usually applied to medium-sized molecules with inversion barriers of 5—25 kcal mol^{-1},[19] and n.m.r. or *X*-ray analyses are also suitable for conformational studies of stable isomers. However, because of difficulties in differentiation between atomic inversion and other dynamic processes by some experimental methods, theoretical calculations are often employed to estimate inversion barriers. A summary of these methods as applied to nitrogen inversion is presented below.

Ammonia. From the summary in Table 1 it is apparent that the experimental nitrogen inversion barrier[20] for NH_3 is not well reproduced by either semi-empirical molecular orbital (MO) calculations with full geometry-optimization[21] (bond lengths, bond angles, and dihedral angles varied until minimum energy is obtained), or by *ab initio* calculations using small basis sets.[22] The failure of these methods seems to be due to incorrectly optimized geometries, as they give reasonable agreement with experimental values if fixed geometries[23,24] (either standard or experimental) are used. Boggs and Niu[25] have shown in their recent work that very large basis sets are required to obtain correct geometries for ammonia and compounds containing amine nitrogen, and they recommend care when calculated structures for biologically important nitrogen-containing compounds are used.

Methylamines. The experimental results for methylamines show[26–29] that methyl substitution decreases the nitrogen inversion barrier slightly, but in trimethylamine the barrier is again increased, apparently due to methyl stabilization of the pyramidal ground state. This trend of nitrogen inversion barriers in methylamines is again reasonably reproduced by MO calculations if standard or experimental geometries are used.[23,24] The barriers calculated from non-bonded potential-energy calculations[30] reflect the steric effects only, and fail to account for the increase of the barrier in NMe_3.

The summary in Table 1 shows that for calculations with geometry optimization the MINDO method fails[21] primarily because of its tendency to favour the planar nitrogen configuration. The MNDO method gives somewhat better

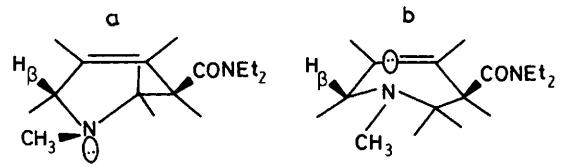

Figure 7. Possible conformations of the D-ring in LSD: (a) flap-up β-*N*-methyl (equatorial), (b) flap-down α-*N*-methyl (equatorial)

results,[21] although the discrepancy between the calculated and experimental values is still large. Similar results are obtained by the PRDDO-SCF-MO method.[24]

Anilines. The nitrogen torsion found by microwave spectroscopy is *ca.* 143° for aniline (inversion barrier[14a] 1.5 kcal mol^{-1}), and *ca.* 160° for *N*-methylaniline (inversion barrier[14b] 0.5 kcal mol^{-1}). The flattening of the nitrogen configuration and the decrease of the nitrogen inversion barrier with increasing substitution at the nitrogen is reproduced only qualitatively by MO calculations. On the evidence of increasing π-charge transfer in *N*-methylanilines,[31] and the rise of the N-torsion barrier in *p*-methyl-*N*-methylaniline,[30] this flattening is attributed to electronic (hyperconjugation) rather than steric effects. Both calculations with the STO-3G basis set[31–33] and geometry optimization, and those with higher polarization function[34] sets (4-21N*, 4-21**), grossly exaggerate non-planarity and nitrogen torsion barriers at the nitrogen, whereas calculations with a 4-21G set[35] converge on completely planar aniline.

The failure of the MO methods to calculate accurate nitrogen inversion barriers with simultaneous geometry optimization is of special importance when considering molecular modelling, since the geometrical parameters for large molecules are often unavailable and the use of *ab initio* methods for full geometry-optimization is prohibitively expensive. It was thus of interest to compare the results of semi-empirical MO calculations, which should cover both steric and electronic effects, with those of much more rapid classical potential-energy calculations, which usually consider steric effects alone. Either method can be used with or without full geometry optimization.

(3) *Calculations on Large Molecules.—LSD* (**1**). The hallucinogen lysergic acid diethylamide (**1**) is a relatively large structure (49 atoms) with a tertiary nitrogen in the conformationally restricted D-ring. The conformation with the D-ring flap-up and the diethylamido group equatorial (Figure 7a) is thought to be implicated in biological activity.[36]

No crystal structure of the LSD free base has been reported so far, although the crystal structure of LSD *o*-iodobenzoate monohydrate has been determined.[37,38] The molecule of LSD used as a starting point for the calculations was built up using

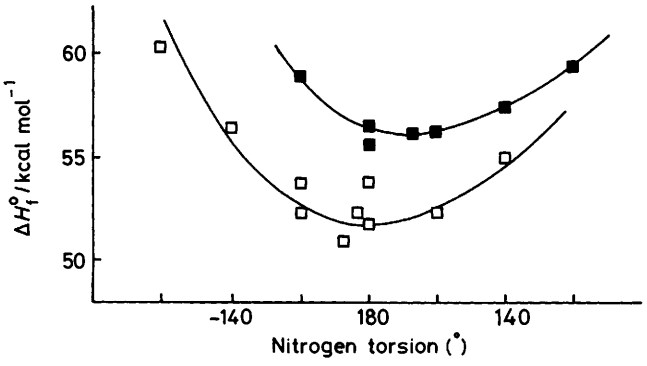

Figure 8. MINDO/3-calculated changes in heats of formation, ΔH^0_f, along the nitrogen torsion reaction co-ordinate for D-LSD (**1**). □ Is the flap-up and ■ the flap-down conformation of the D-ring

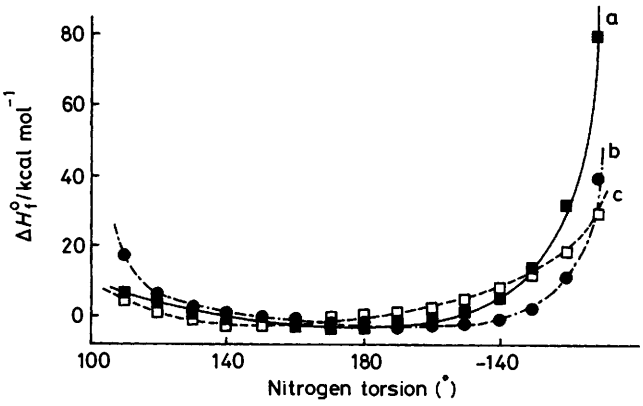

Figure 9. Changes in non-bonded energies, E_{nb}, along the nitrogen torsion reaction co-ordinate for D-LSD (**1**), MNDO-optimized conformations: (a) flap-down β-*N*-methyl, (b) flap-down α-*N*-methyl, (c) flap-up β-*N*-methyl

the available co-ordinates from the crystal structure of bromocriptine methanesulphonate[39] and geometrical parameters given for LSD *o*-iodobenzoate.[38]

This initial structure was fully optimized by the MINDO/3 method and converged on a structure with τ_N 167°, which corresponds to β-*N*-methyl (*i.e.*, with the α-face exposed). From this structure τ_N was changed in both directions in 20° steps, fixed at each step, and the rest of the structure minimized. However, more than one optimized structure was found for τ_N 180—200°. Also, when starting the full minimization from the structure optimized for τ_N 220°, a new global minimum with τ_N 188° was located at *ca.* 4 kcal mol^{-1} below the above-mentioned first minimum.

Close inspection of all optimized structures using the MORPHEUS package revealed that between τ_N 180—200° the D-ring also undergoes a conformational change. Thus the results in Figure 8 fall approximately on two curves, the higher-energy one corresponding to the flap-up D-ring conformation, and one of *ca.* 4 kcal mol^{-1} lower energy corresponding to the flap-down D-ring conformation (Figure 7b). There is no barrier to nitrogen inversion in either of these curves, with conformations of τ_N 215—145° and 205—125° falling within 3 kcal mol^{-1} of the minima. However, it is apparent (and the change of D-ring conformation during optimization also indicates) that the *N*-methyl prefers the pseudoequatorial position in both D-ring conformations, and that the flap-down conformation of the D-ring is the more stable according to MINDO/3 calculations.

This result is contrary to all findings and expectations from crystal structures[36] and n.m.r. studies[40] of similar compounds. The free-base bromocriptine is reported[41] to adopt the α-*N*-methyl D-ring flap-down conformation (τ_N 124°), presumably stabilized by internal hydrogen bonding between the lone pair on the nitrogen of the D-ring and NH of the *N*-alkylamido substituent. Such interactions are not possible in LSD, which is a diethylamide. *X*-Ray analysis of D-LSD *o*-iodobenzoate[38] shows that protonated LSD adopts the β-*N*-methyl D-ring flap-up conformation, the same conformation as bromocriptine salt. Another closely related alkaloid, ergotamine[42] is shown by *X*-ray analysis to be in the flap-up conformation, with β-*N*-methyl and CONHR equatorial, both as a salt and a free base. In summary, two discrepancies with the experimental data are apparent from the MINDO/3 calculations: first, the nitrogen lone-pair in the LSD D-ring cannot be conjugated, yet the N-torsion obtained is very close to sp^2 hybridization; secondly, the flap-down conformation of the D-ring is found to be *ca.* 4 kcal mol^{-1} more stable, yet all the crystal structures except bromocriptine free base adopt the flap-up conformation.

Since MNDO parameterization is now generally preferred to MINDO/3, we optimized the structures corresponding to the minima on the curves in Figure 8 by this method. β-*N*-Methyl was found in both the D-ring flap-up and flap-down conformations, with nitrogen-torsion angles of 138 and 148°, respectively. The latter conformation was shown still to be 0.6 kcal mol^{-1} more stable (with the *N*-methyl in the pseudoaxial position), but the energy difference in comparison with MINDO/3 calculations was significantly reduced. The values for N-torsion by the MNDO method are certainly more acceptable in view of the above survey of crystal structures; however, it should be remembered that in most of these crystal structures the N-lone-pair is engaged in either intra- or intermolecular hydrogen bonding, which tends to reinforce sp^3 hybridization.

The steric interactions in all MINDO- and MNDO-optimized structures of the N-torsion reaction co-ordinate seem to be well minimized, all having non-bonded steric energies[8], E_{nb}, within 1 kcal mol^{-1}. In the absence of suitable crystal structures, we used several of these optimized geometries for LSD (**1**) to evaluate the application of simplified molecular mechanics to the study of N-inversion.

In each of these structures nitrogen-torsion dihedral angles were systematically varied and the rotational position of *N*-methyl and diethylamide groups optimized at each step. As shown in Figure 9, the energy minimum is very broad, covering conformations with τ_N from 120 to 270°. However, the position of the global minimum and the shape of the well is influenced by the initial optimized structure used. Thus it seems that E_{nb} without full geometry optimization gives approximately the same information as lengthy MO calculations, *i.e.*, it indicates that a large range of conformations round nitrogen is accessible.

N-*Methyl* 10,11-*dihydrobenzazepine* (**2**). This analogue of the antidepressant drug imipramine was selected for study because the amount of computer time required to consider the entire imipramine molecule during nitrogen inversion with MINDO/3 would have been both prohibitive and impractical. In particular, an *N*-methyl group was used as the alkyl substituent instead of the propylamino side-chain of imipramine on the premise that the inherent flexibility of the side-chain would, in any event, allow the minimization of any steric interactions otherwise encountered. At an intuitive level, a low barrier to nitrogen inversion would be expected for this system as a result of anticipated steric and conjugation effects. These were evaluated by both molecular orbital and classical potential energy calculations.

In the molecular orbital study, full geometry-optimization of the tricyclic nucleus was allowed during a series of MINDO/3

Table 2. Results of MINDO/3 calculations for nitrogen inversion in *N*-methyldibenzazepine derivative (**2**)

τ_N (°)	$\Delta H_f^{0\,a}$	$E^b_{electronic}$	$E^b_{repulsion}$	ω^c (°)
240 (sp^3)	91.4	−14 923.8	12 610.6	59.8
210	79.4	−14 875.3	12 561.7	53.1
180 (sp^2)	78.1	−14 877.6	12 563.9	53.1
150	78.9	−14 874.3	12 560.6	50.2
120 (sp^3)	90.4	−14 916.1	12 602.9	49.8

[a] Units are kcal mol^{-1}. [b] Units are eV molecule^{-1}. [c] Torsional angle across the dimethylene bridge in (**2**).

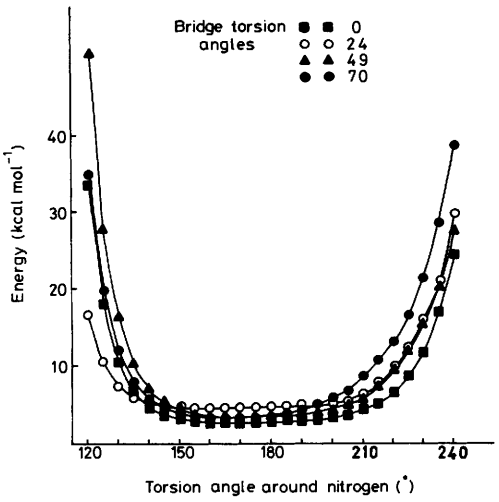

Figure 10. Graphical representation of the relative energies, E_{nb}, of different conformers during a simulated nitrogen inversion for four fragments of (**2**), each with a different degree of skew across the dimethylene bridge

calculations in which the torsion angle around the nitrogen was incremented between 120 and 240° to simulate a nitrogen inversion (see Table 2). This series of calculations indicated the absence of a barrier to nitrogen inversion within this system. Instead there was a broad area of low-energy conformations when the hybridization of the nitrogen was between 150 and 210°.

Owing to the interdependence of the torsion angle across the dimethylene bridge upon the hybridization of the nitrogen,[7] a series of four different benzazepine fragments was included in the simplified molecular mechanics study, each with a different torsion angle across the bridge. Then, in a similar fashion to the MINDO/3 calculation (except that no geometry-optimization was undertaken), the torsion angle around the nitrogen was stepped from 120 to 240°, simulating the nitrogen inversion (see Figure 10). Once again, no barrier to nitrogen inversion was calculated and a broad region of low-energy conformers was indicated. The relatively high calculated energies of the sp^3 hybridization states result from steric interactions with either the dimethylene bridge protons (in the case of 240°) or the aromatic electrons under the plane of the tricyclic nucleus (when the torsion angle is 120°). As in the case of LSD, the results are similar to those seen with MINDO/3 calculations.

Conclusions.—Nitrogen inversion is clearly a conformational variable, with many compounds being capable of adopting sp^2, sp^3 or intermediate hybridization states. Taking 5—6 kcal mol^{-1}, the energy barrier to nitrogen inversion in ammonia, as an estimate of the purely electronic component in this process, the preceding data indicate that this barrier is reduced in many N-substituted compounds by delocalization of the lone pair, and thus stabilization of the planar transition state. Bulky substituents also decrease the nitrogen inversion barrier by destabilization of the pyramidal ground state (by opening the bond angles), but steric effects alone have relatively little influence on hybridization state, except in those cases where the inversion barrier is already reduced by conjugation, particularly to aromatic rings. The latter finding is particularly significant in studies of biologically active compounds, where an attempt should be made to take all alternative low-energy conformations into account in determining the biologically active form.

Experimental methods such as *X*-ray crystallography or n.m.r. spectroscopy offer relatively little help in this regard, since only a single low-energy conformation is generally revealed. Molecular orbital calculations, on the other hand, supply energies for all conformations but generally give a poor picture of the barriers to nitrogen inversion. Large basis set *ab initio* calculations are possible exceptions to this rule, but the time required for these calculations is prohibitive for biologically active molecules of any reasonable size.

The comparisons between the results of molecular orbital and classical calculations on larger molecules presented here suggest that a simple molecular mechanics calculation is probably as good a method as any for estimating the barrier, although this too is somewhat dependent on the initial geometry selected around the nitrogen atom.

As a rule of thumb, it appears that non-conjugated acyclic nitrogen atoms are sp^3 and amide nitrogens sp^2, with a range of 15° either side of the strict hybridization state being allowable. Those with phenyl substituents may, however, adopt the full range of hybridization states from sp^2 to sp^3. The latter class thus requires special consideration in studies of conformation–activity relationships.

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