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Molecular Orbital Studies on the Conformation of Hallucinogenic Indolealkylamines and Related Compounds. The Isolated Molecules and the Solvent Effect†

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Received October 4, 1973

The comparison of the available theoretical computations and X-ray crystal data for serotonin does not lead to an unambiguous proposal about the preferred conformation(s) of this type of compound. It is shown that more clear-cut conclusions can be reached by extending the computations (which are carried out by the molecular orbital PCILO method) to a larger number of indolealkylamines provided that these molecules are divided into subgroups corresponding to their possible occurrence in the neutral or cationic species with an amino or dimethylamino terminal grouping. Each subgroup has its conformational preferences with respect to the two principal torsion angles τ_1 and τ_2. The study accounts for the X-ray crystal conformation of the representatives of these differents groups. An exception is the planar extended structures observed (among other) for the two cationic species. The theoretical study is extended to the evaluation of the effect of water on the conformation of the cationic forms of indolealkylamines. For this sake the principal hydration sites of these molecules are determined and new conformational energy maps are constructed for the hydrated species using the "supermolecule" approach. The results indicate that the hydrated cations should not manifest any marked tendency for an exclusive conformation and should exist in solution as a nearly equivalent mixture of gauche and trans forms. This prediction is confirmed by recent nmr results on the conformation of serotonin in aqueous medium.

The structural properties of indolealkylamines have aroused recently a wide interest on behalf of quantum theoreticians, parallel to a very substantial development of X-ray crystal studies on these compounds. The best known in this series of molecules is serotonin and the published theoretical papers have centered essentially around the conformational properties of the cationic form of this compound. The computations involved both "empirical" (*i.e.*, using partitioned potential functions) and quantum mechanical methods. Within these restricted limits, the comparison of the theoretical results with the available experimental crystal data leads to uncertain conclusions. In the most recent appraisal of the situation Kang, Johnson, and Green[1] express the pessimistic view that "neither the experimental observation nor the theoretical calculations establish an unambiguous conformation of 5-HT" (5-hydroxytryptamine, serotonin). As the conformational properties of drugs are frequently considered essential for their activity, this is an unpleasant situation.

Before discussing it in more detail, we would like to restate the problem. It concerns essentially the conformation of the ethylamine side chain with respect to the indole ring. In principle three torsion angles have to be considered (Figure 1): τ_1 (C_2-C_3-C_{10}-C_{11}), τ_2 (C_3-C_{10}-C_{11}-$N^+{}_{12}$), and τ_3(C_{10}-C_{11}-$N^+{}_{12}$-H_{13}). [We recall that the

† This work was supported by the A.T.P. No. A 655-2303 of the C.N.R.S.

Table I. X-Ray Crystallographic Results on the Conformation of Indolealkylamines[a]

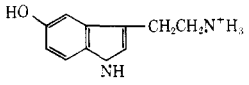
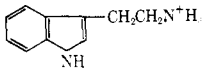
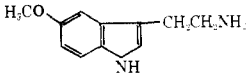
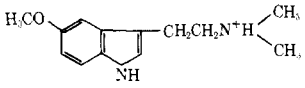
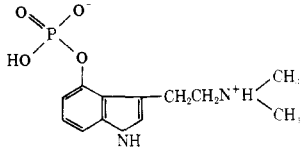
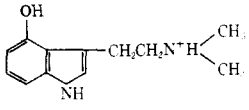
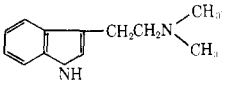
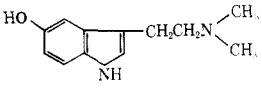
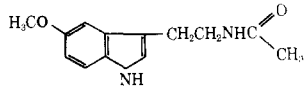
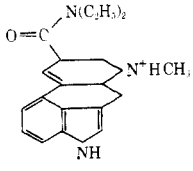
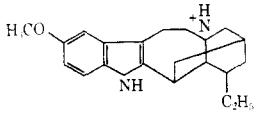
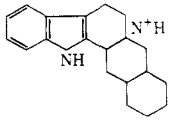
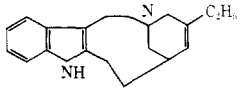
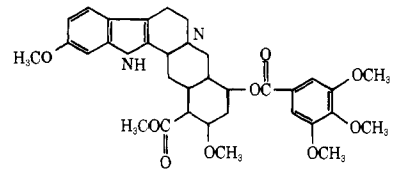
Compound	Formula		τ_1	τ_2	Symbol[b]
	Subgroup 1				
Serotonin (creatinine sulfate complex)			13.3	172.6	= t
Serotonin (picrate complex)			112.5	−66.6	⊥ g
Tryptamine hydrochloride			110.8	−60.5	⊥ g
	Subgroup 2				
5-Methoxytryptamine			116.3	−54.7	⊥ g
	Subgroup 3				
5-Methoxy-*N*,*N*-dimethyltryptamine			17.2	179.3	= t
Psilocybin		A	107	−174	⊥ t
		B	72	165	⊥ t
Psilocine					
	Subgroup 4				
N,*N*-Dimethyltryptamine		A	102.3	175.9	⊥ t
		B	89.9	−171.8	⊥ t
Bufotenine		A	86.5	175.3	⊥ t
		B	72.0	170.4	⊥ t
	Other				
Melatonin			5.2	−171.6	= t
LSD			150.3	174.2	= t
Ibogaine			36.2	−57.1	
Yohimban			8.9	−38.5	= g
Cleavamine			41.2	45.6	

Table I (*Continued*)

Compound	Formula	τ_1	τ_2	Symbol[b]
Reserpine		10.9	-40.1	= g

[a]For references, see text. [b] =, $\tau_1 \approx 0$ or 180° representing C_{10}–C_{11} approximately coplanar with the ring. ⊥, $\tau_1 \approx \pm 90°$ representing C_{10}–C_{11} approximately perpendicular to the ring. t, $\tau_2 \approx \pm 180°$ representing a trans, extended form. g, $\tau_2 \approx \pm 60°$ representing a gauche, folded form.

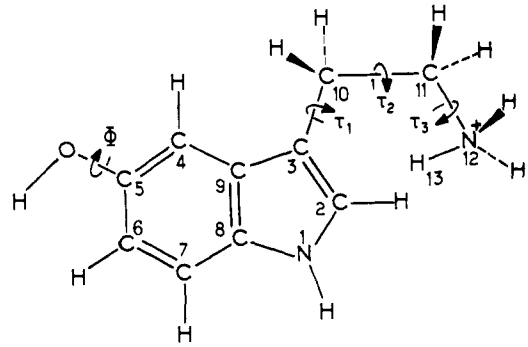


Figure 1. Torsion angles and atom numbering in indolealkylamines. The figure corresponds to $\tau_1 = \tau_2 = \tau_3 = 0, \Phi = 0$.

torsion angle τ (A–B–C–D) between the bonded atoms A–B–C–D represents the angle between the planes ABC and BCD. Viewed from the direction of A, τ is positive for clockwise and negative for counterclockwise rotations, the far end rotating with respect to the near end. The value τ = 0° corresponds to the cis-planar arrangement of the bonds AB and CD.] A large number of theoretical and experimental results on similar cases allow us to hold the N^+H_3 group in a staggered conformation with respect to the C_{10}–C_{11} axis (τ_3 = 60 or 180 or 300°) so that at least for serotonin the problem is reduced to one of two variables. A preliminary, separate study enables us to fix the preferred value of the torsion angle Φ (C_6–C_5–O–H) which appears to be 0°. The torsion angle τ_1 defines the overall arrangement of the side chain with respect to the plane of the indole ring while τ_2 defines the rotation of the cationic head about C_{10}–C_{11}.

The first quantum mechanical computation on serotonin carried out by Kier using the extended Hückel theory (EHT)[2] predicted only one stable conformation corresponding to τ_1 = 90°, τ_2 = 180°. A more precise EHT treatment[1] indicated, however, the existence of two local energy minima at τ_1 = 90°, τ_2 = ±60°, 1 kcal/mol above the global one. An "empirical" computation performed by the same authors[1,3] reversed the relative stabilities of these forms indicating the gauche forms to be 1–3 kcal/mol more stable than the trans one. Computations using more refined theoretical methods yielded quite different results. PCILO (Perturbative Configuration Interaction using Localized Orbitals) treatment[4] predicted as the most stable state a highly folded conformation with τ_1 = 140°, τ_2 = −20° (and τ_1 = −140°, τ_2 = 20° for the enantiomorph) with a secondary local energy minimum at τ_1 = 100°, τ_2 = 60° (τ_1 = −100°, τ_2 = −60°), 2 kcal/mol above the global one. INDO (Intermediate Neglect Differential Overlap) calculations[5] predicted a most stable form at τ_1 = 60°, τ_2 = 0° with the conformation at τ_1 = 90°, τ_2 = 180° as the least probable.

This confused theoretical situation may be compared

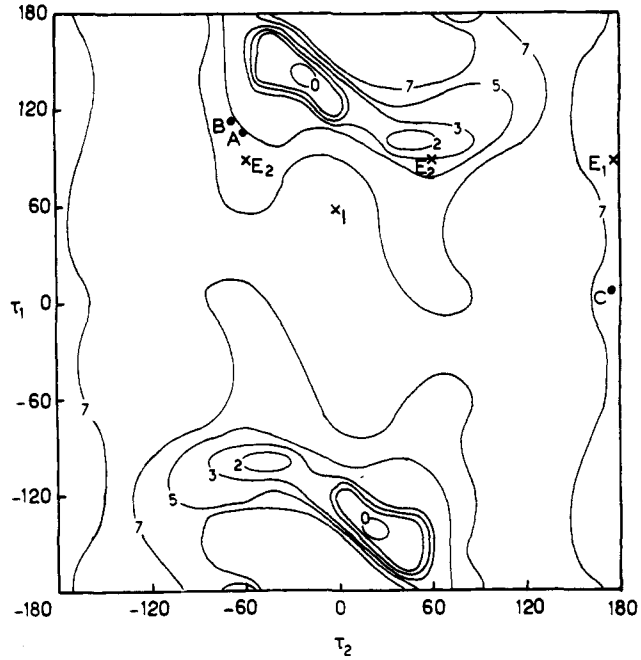


Figure 2. PCILO conformational energy map for cationic serotonin (representing compounds of subgroup 1). Isoenergy curves in kilocalories per mole with respect to the global energy minimum taken as energy zero. Also indicated: E_1, global minimum of EHT computations;[2] E_2, secondary minimum of EHT computations[1] (in empirical computations[1] these minima are reversed); *i*, global minimum of INDO computations.[5] X-Ray crystal conformation of A, tryptamine hydrochloride;[10] B, serotonin-picrate monohydrate;[7] C, serotonin-creatinine sulfate complex.[6]

with the available experimental data which come essentially from X-ray crystallographic studies. Two results are available for serotonin corresponding to different surroundings: one for serotonin–creatinine sulfate complex[6] with τ_1 = 13.3°, τ_2 = 172.6° and one for serotonin–picrate monohydrate[7,8] with τ_1 = 112.5°, τ_2 = −66.6°. For reasons which will become clear later in this paper we may consider here also the results on tryptamine hydrochloride[9,10] which indicate τ_1 = 110.8°, τ_2 = −60.5° (for the chemical formulas see Table I).

The theoretical and experimental data are plotted on Figure 2 from which it is obvious that the agreement between theory and experiment is a limited one. Although two of the three experimental conformations are close to the predicted PCILO global energy minimum, the remaining one (representing serotonin in the creatinine sulfate complex) does not even correspond to a local energy minimum and falls in a region of relatively high conformational energy, 7 kcal/mol above the global minimum. It represents an extended, trans form with the N^+ atom nearly coplanar with the ring.

Truly, there is no stringent reason that computations

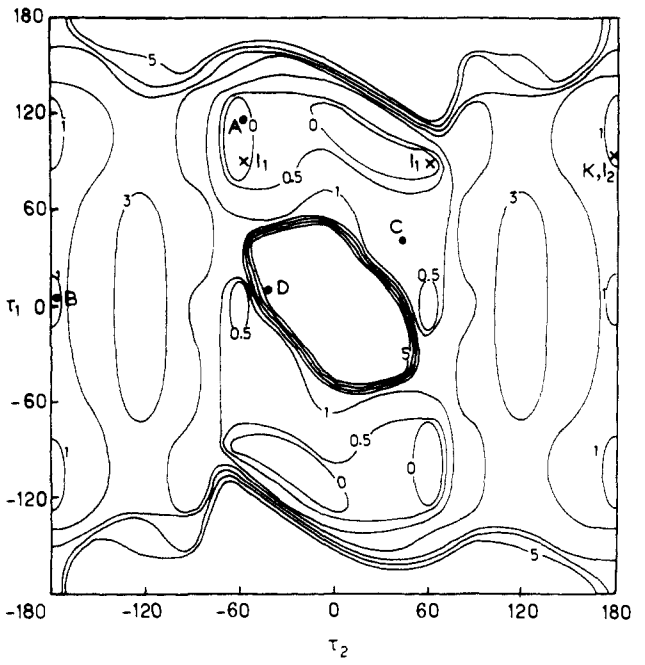


Figure 3. PCILO conformational energy map for neutral 5-methoxytryptamine and serotonin (representing compounds of subgroup 2). Isoenergy curves in kilocalories per mole with respect to the global energy minimum taken as energy zero: i_1, global minimum of INDO calculations; i_2, secondary minimum of INDO calculations.[5] X-Ray crystal conformations of A, 5-methoxytryptamine;[12] B, melatonin;[12] C, cleavamine;[21] D, reserpine.[22]

performed for isolated molecules should agree with conformations observed in crystals. An agreement is, however, frequently observed and a strong disagreement (in the sense of experimental conformations lying in theoretically high energy zones) is rare. (For a discussion of this point, see, *e.g.*, ref 11.) Before concluding that environmental factors are responsible for this situation, a deeper study is advisable.

It seems to us that such a study is possible by (1) extending the computations to a larger number of compounds of this series and (2) by subdividing the molecules studied into appropriate subgroups. Thus, a large number of indolealkylamines structurally related to serotonin have been investigated recently by X-ray crystallography (for a general review, see ref 10). The available results are summarized in Table I, largely inspired from ref 10. Provided that it is realized that these compounds may be subdivided into groups, each of which needs to be studied theoretically separately, definite progress seems to be possible in the understanding of the conformational properties of this general class of compounds and in determining the relationship between the theoretical computations and the crystallographic experimental findings.

The fundamental divisions into which it seems to us advantageous to separate the molecules consist of the neutral and cationic derivatives (in crystals) and derivatives with a serotonin type (amino) or a bufotenine-type (dimethylamino) terminal group. Four principal subgroups need thus to be considered.

(1) Ionic derivatives with N^+H_3 terminal group, represented by the serotonins and tryptamine discussed above.

(2) Neutral derivatives with an NH_2 terminal group. There is only one compound in this subgroup: 5-methoxytryptamine studied by Quarles.[12]

(3) Ionic derivatives with an $N^+H(CH_3)_2$ terminal, represented by 5-methoxy-*N,N*-dimethyltryptamine[13] and psilocybin.[14,15]

(4) Neutral derivatives with an $N(CH_3)_2$ terminal group, represented by *N,N*-dimethyltryptamine and bufotenine.[16,17]

Melatonin (studied also by Quarles, ref 12) represents a particular intermediate between the subgroups 2 and 4. To these may be added indoleamines with fused heterocyclic ring systems: LSD whose crystal structure has recently been determined,[15-18] ibogaine hydrobromide,[19] yohimban hydrobromide,[20] cleavamine methiodide,[21] and reserpine.[22] The first three of these compounds are ionic: the last two are neutral in the crystal. Although they do not classify strictly within our division and although obvious constraints are exerted on their "ethylamine" conformations, it seems nevertheless useful to include them in the overall discussion.

Method

The method utilized in the computation is the molecular orbital PCILO (Perturbative Configuration Interaction using Localized Orbitals) procedure.[23-25] The computational program may be obtained from Q.C.P.E. (Quantum Chemistry Program Exchange) at the Chemistry Department of Indiana University, Bloomington, Ind.

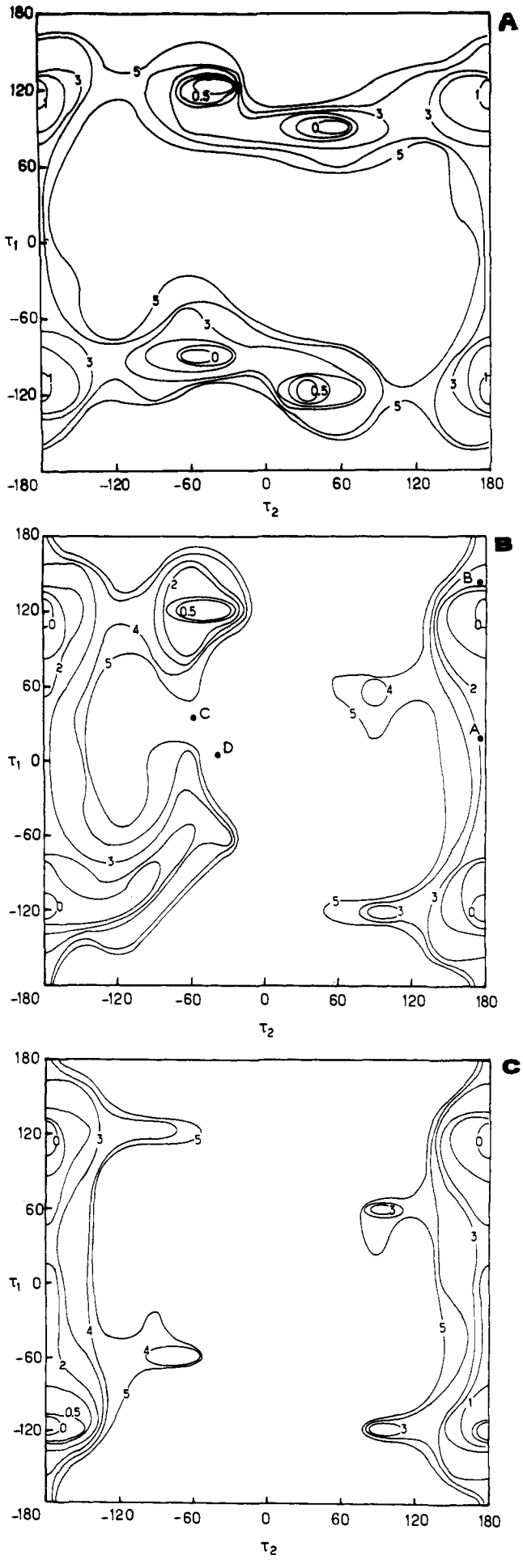
A conformational energy map has been constructed for each subgroup of indolealkylamines using geometrical input data of a representative compound from its subgroup. Thus, Figure 2 is based on the geometry of ref 7. Figure 3 on the geometry of 5-methoxytryptamine,[12] Figure 4 on the geometry of ref 13, and Figure 5 on the geometry of ref 17.

The computations of the torsion angles τ_1 and τ_2 have been carried out in increments of 30° As already said above, τ_3 for the $-N^+H_3$ group is held in a staggered conformation. τ_3 for the $-N^+H(CH_3)_2$ group presents a problem and different positions have been investigated, in particular, those corresponding to τ_3 = 0 (eclipsed), 180 (staggered), and 60° (gauche).

Results and Discussion

(A) Conformational Energy Maps and Crystal Structures. As already indicated in the introduction, Figure 2 constructed for cationic serotonin represents the conformational possibilities and preferences of the compounds of subgroup 1. PCILO, in contrast to EHT, predicts a strong preference for a gauche form with the plane of the side chain inclined with respect to the ring. The comparison with the X-ray results is at this stage inconclusive although two of the three experimental conformations are close to the PCILO global energy minimum and substantiate its significance. The "abnormally" behaving third example, which represents serotonin in the creatinine sulfate complex, does not correspond to any energy minimum in any of the available computations. It represents an extended form in which, moreover, the side chain is coplanar with the ring. It is therefore quite different from the most probable form predicted by the EHT procedure which, although extended, corresponds to the side chain perpendicular to the ring. The global energy minimum predicted by the INDO method does not seem to be significant.

Figure 3 presents the conformational energy map for neutral 5-methoxytryptamine (which represents also neutral serotonin), the only compound of subgroup 2 whose crystal structure is known. The map is substantially different from that of Figure 2, not so much in the position of the global energy minimum (which is degenerate and corresponds to τ_1= 90–130°, τ_2 = −60° and 0–60°) than in the occurrence of low-lying, local energy minima (1 kcal/mol above the global ones) corresponding to extended forms (τ_2 = 180°) both for $\tau_1 \approx 100°$ and τ_1 = 0°. INDO



computations are also available in that case[5] and they give results identical with PCILO.

The experimental conformation of neutral 5-methoxytryptamine, which corresponds to a folded structure, falls within one of the global energy minima. On the other hand, melatonin, which may be considered as somewhat related to this subgroup, has an extended planar conformation corresponding to one of the local energy minima. We have also plotted on Figure 3 the experimental conformations of reserpine and cleavamine. They are situated in close vicinity to local energy minima, although in the case of reserpine the conformation appears quite strained.

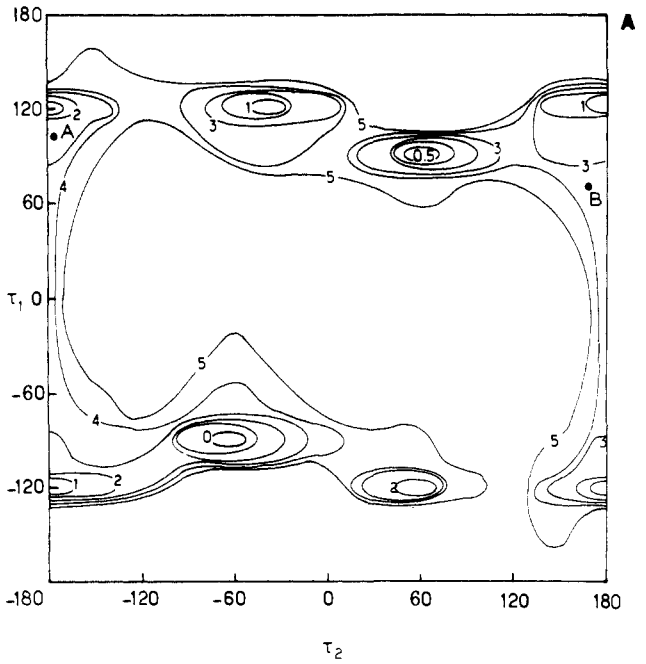
Compounds of subgroup 3 which have an $-N^+H(CH_3)_2$ terminal group present a complication because of the possible effect upon the conformational energy map of the orientation of the cationic head. In order to explore this influence three conformational energy maps have been constructed for cationic bufotenine (based on the geometry of 5-methoxy-*N*,*N*-dimethyltryptamine[13]) corresponding to τ_3 (C_{10}-C_{11}-N^+_{12}-H_{13}) = 0, 60, and 180°. These maps are presented in Figure 4. The most stable global energy minimum is associated with τ_3 = 60° but the global energy minima of the two remaining maps are only within a few tenths of a kilocalorie per mole above it. It is seen that the maps corresponding to τ_3 = 60 or 180° predict both as the most stable conformation an extended one with the side chain appreciably inclined with respect to the ring (τ_1 = ±120°, τ_2 = 180°). On Figure 4B (τ_3 = 60°) there is, however, also a local energy minimum for a gauche form (τ_1 = 120°, τ_2 = −60°) only 1 kcal/mol above the global one. On the other hand, the most stable conformation associated with τ_3 = 0° should be a folded one (τ_1 = −90°, τ_2 = −60° or τ_1 = 90°, τ_2 = 60°) with, however, a local energy minimum at τ_1 = ±120°, τ_2 = 180° for an extended form only 1 kcal/mol above.

In the crystal of 5-methoxy-*N*,*N*-dimethyltryptamine, τ_3 = 52°. The map most representative of this compound is thus that of Figure 4B. The experimental conformation represents an extended nearly planar structure which, although included within the 4 kcal/mol isoenergy limit, does not correspond to any energy minimum. This case is thus similar to that of cationic serotonin in its creatinine sulfate complex.

We have also plotted on Figure 4B the crystal conformation of LSD, ibogaine, and yohimban. While the conformation of LSD is close to the local energy minimum for the extended form, those of ibogaine and yohimban lie in high energy zones of this conformational energy map. Because of their particular structure this is in no way astonishing.

In Table I a second compound, psilocybin, belongs to subgroup 3. This molecule carries, however, a ring substituent at C_4 instead of C_5 as in serotonin, bufotenine, or 5-methoxy-*N*,*N*-dimethyltryptamine and it is possible that this different positioning of the ring substituent may have an influence on the conformational energy map. For this reason we have investigated the conformational energy map for psilocine, the analog of bufotenine with the OH substituent at position 4 instead of 5 and which in fact is considered to be the likely active metabolite of psilocybin derived from it by enzymic hydrolysis. Two maps have

Figure 4. (A) Conformational energy map of cationic bufotenine (representing compounds of subgroup 3). Isoenergy curves in kilocalories per mole with respect to the global energy minimum taken as energy zero. τ_3 = 0°. (B) Same as Figure 4A with τ_3 = 60°. X-Ray crystal conformations of A, 5-methoxy-*N*,*N*-dimethyltryptamine;[13] B, LSD;[18] C, ibogaine;[19] D, yohimban.[20] (C) Same as Figure 4A with τ_3 = 180°.



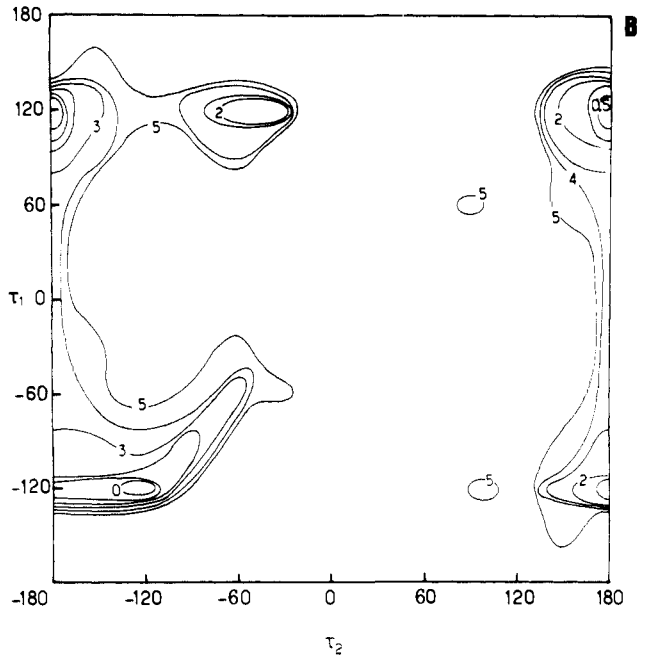


Figure 5. (A) Conformational energy map of cationic psilocine with τ_3 = 20°. Isoenergy curves in kilocalories per mole with respect to the global energy minimum taken as energy zero. X-Ray conformations of A, psilocybin A; B, psilocybin B. (B) Same as Figure 5A with τ_3 = 60°.

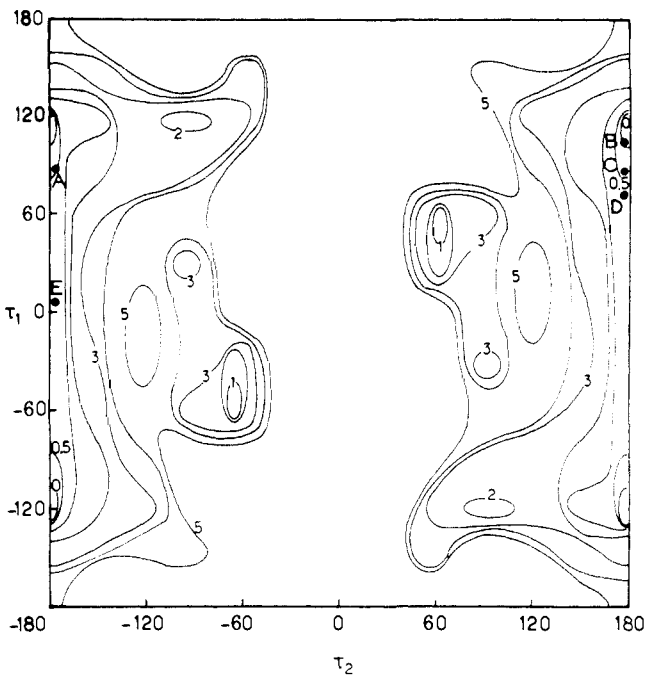


Figure 6. Conformational energy map of neutral bufotenine (representing compounds of subgroup 4). Isoenergy curves in kilocalories per mole with respect to the global energy minimum taken as energy zero. X-Ray crystal conformations of A, *N,N*-dimethyltryptamine molecule A; B, *N,N*-dimethyltryptamine molecule B;[16] C, bufotenine molecule A; D, bufotenine molecule B;[17] E, melatonin.[12]

been built, one with τ_3 = 20° (Figure 5A) which is approximately the value of this torsion angle in the crystal of psilocybin[18] and one with τ_3 = 60° (Figure 5B), in order to see, by comparison with Figure 4B, the effect of the displacement of the OH group from C_5 to C_4 for a similar value of τ_3. It is seen that the global energy minimum corresponds to a folded form on Figure 5A (τ_1 = −90°, τ_2 = −60°) and to a relatively but not completely extended form on Figure 5B (τ_1 = −120°, τ_2 = −120°). The comparison of Figures 4B and 5B shows that at least for τ_3 = 60° the variation of the position of the ring OH has a certain influence on the conformational energy map. On the other hand, Figure 5A contains also the experimental conformations of the two psilocybins, A and B, found in the asymmetric unit of the crystal. They represent both extended structures and are close to the local energy minimum at τ_1 = 120°, τ_2 = 180° which is only 1 kcal/mol above the global energy minimum. Although it must be remembered that the map refers to psilocine, while the experimental results concern psilocybin, it is also possible that the crystal packing forces are responsible for the occurrence of the compound in the extended form.

The conformation of psilocine has also been studied by the classical potential function calculations.[3] This study indicates a triply degenerate global energy minimum at (τ_1, τ_2) = (−120°, 60°), (60°, 60°), (120°, 180°) and a triply degenerate secondary energy minimum, 2 kcal/mol above the global one at (τ_1, τ_2) = (−120°, 180°), (−60°, −90°), (120°, −90°). These results bear an overall resemblance to the PCILO ones.

Finally Figure 6 presents the conformational energy map for neutral bufotenine, representing compounds of subgroup 4. This map is significantly different from that of neutral serotonin (Figure 3) or from cationic bufotenine (Figure 4B). It presents a clear-cut global energy minimum for an extended form with the ethylamine side chain nearly perpendicular to the indole plane (τ_1 = 100–120°, τ_2 = 180°). According to crystallography data the four molecules of this subgroup (two bufotenines and two *N,N*-dimethyltryptamines) whose crystal structures are known have a conformation of that type. We have also plotted in Figure 6 the experimental conformation of melatonin, which falls on this map in the low-energy zone, within 1 kcal/mol above the global minimum.

We may now summarize the general situation and try to evaluate the significance of the results obtained. Manifestly, the subdivision of the indolealkylamines into subgroups and the construction of separate conformational energy maps for each subgroup clarify to some extent at least the understanding of the conformational properties of these molecules as observed in crystals. Both ionization and dimethylation of the cationic head have an influence

on the conformational possibilities and preferences of the compounds studied.

The following conclusions seem to be substantiated by a comparison between theory and experiment.

(1) Neutral indolealkylamines with an amino terminal seem to prefer an approximately perpendicular and gauche conformation of the side chain ($\tau_1 \approx 100°$, $\tau_2 = \pm 60°$), although a number of extended, both perpendicular and coplanar conformations are within 1 kcal/mol above the most stable one (one of these secondary conformations is occupied by melatonin). At present experimental data are, however, available for only one compound of this subgroup.

(2) Neutral indolealkylamines with a dimethylamino terminal group show a strong preference for a perpendicular and fully extended conformation ($\tau_1 \approx 100°$, $\tau_2 \approx 180°$). The substitution of two methyl groups on the amino end favors thus the stretching out of the molecule.

(3) Ionic indolealkylamines with an N^+H_3 terminal show a preference for a perpendicular and gauche conformation. The computed ($\tau_1 = 140°$, $\tau_2 = -20°$) and observed ($\tau_1 \approx 100°$, $\tau_2 \approx -60°$) such conformations are somewhat different.

(4) Ionic indolealkylamines with an $N^+H(CH_3)_2$ terminal are predicted to prefer a perpendicular or at least strongly inclined arrangement of the side chain with respect to the ring ($\tau_1 \approx$ 90–120°). The gauche or trans orientation of the cationic head with respect to the ring depends somewhat on the value of τ_3: small values of τ_3 ($\approx$0–20°) favor the gauche arrangement while larger values (60–180°) favor the trans arrangement. Thus, dimethylation of the amino group seems to favor the stretching out of the molecule as is the case in the neutral indolealkylamines with a diamino terminal group. Among the two known compounds of this class one, psilocybin, seems to adopt a conformation corresponding to a local energy minimum, 1 kcal/mol above the global one.

(5) While theory predicts for all these molecules a preference for an inclined arrangement of the side chain with respect to the ring ($\tau_1 \approx$ 90–120°), experimentally cationic indolealkylamines of both types seem to adopt in the crystal also a planar and extended conformation. These last conformations do not correspond to an energy minimum on the corresponding conformational energy maps but lie in relatively high energy zones, 4–7 kcal/mol above the global minima. At present it seems plausible to admit that this type of conformation does not correspond to an intrinsic preference of the compounds but owes its stability in the crystals to environmental packing forces, possibly stacking interactions between the planar conjugated rings.

(B) Influence of the Solvent. In the preceding pages the theoretical computations concerned the isolated molecules and the comparison with the experimental data was carried out with respect to the X-ray crystal results, quite abundant in these series and until recently the only available ones.

For the pharmacological activity of drugs, it is, however, of importance to have information about the conformation of drugs in solution. We have therefore extended our study to the evaluation of the influence of water on the conformation of the two pharmacologically important cationic species previously discussed and upon which the influence of water may be expected to be particularly significant. For this sake we have adopted the "microscopic super-molecular" approach which consists of fixing water molecules in the most favorable hydration sites of the cation and calculating the conformational map of the new "super-molecule." The most favorable hydration sites are determined by *ab initio* studies on model compounds, following the procedure indicated in ref 28–30. The construction of the conformational map of the new "super-molecule" representing the hydrated indolealkylamine was carried out by the PCILO method since the compounds are then too large for an *ab initio* computation.

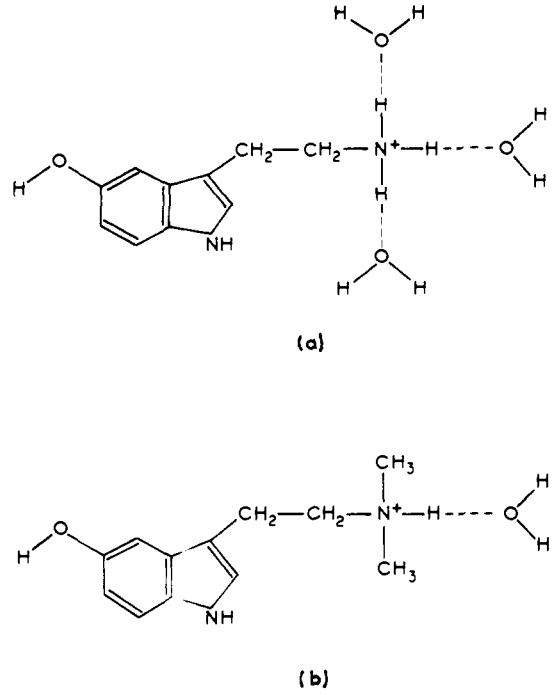


Figure 7. The principal hydration sites in (a) serotonin and (b) bufotenine.

It cannot be expected that the entire solution behavior of indolealkylamines will be explained by such a reduced treatment. Our aim is to obtain a reasonable indication of the direction and magnitude of changes in conformational preferences of the isolated molecule when it enters aqueous solution and from this point of view the inclusion of the essential water molecules of the first hydration shell should be particularly significant. The procedure has been applied recently with a striking success to the study of the conformational properties of histamine cations in solution.[29]

The *ab initio* calculations on model compounds of the ethylammonium series, carried out in a STO 3G basis[30] using the program Gaussian 70,[31] indicate that the principal hydration sites are located along the N^+–H bonds of the cationic head. The two cationic species under investigation in this paper can therefore form energetically very favorable hydrogen bonds to water following the scheme indicated in Figure 7, with three water molecules fixed at the cationic head of the N^+H_3 terminal of tryptamine and one molecule fixed at the cationic head of the $N^+H(CH_3)_2$ terminal of bufotenine. The energies computed for these interactions are of the order of 20 kcal/mol per hydrogen bond. The hydrated N^+H_3 group is considered to be staggered with respect to the C_{11} methylene group. For the hydrated $N^+H(CH_3)_2$ group explicit computations indicate that the value of $\tau_3 \approx 60°$ remains the preferred one and the results presented correspond therefore to this value.

With the water fixation scheme of Figure 7, the PCILO conformational energy maps for the hydrated cations of serotonin and bufotenine are given in Figures 8 and 9, respectively.

They show different effects with respect to the corresponding nonhydrated molecules. The map for hydrated bufotenine is nearly identical with that for the nonhydrat-

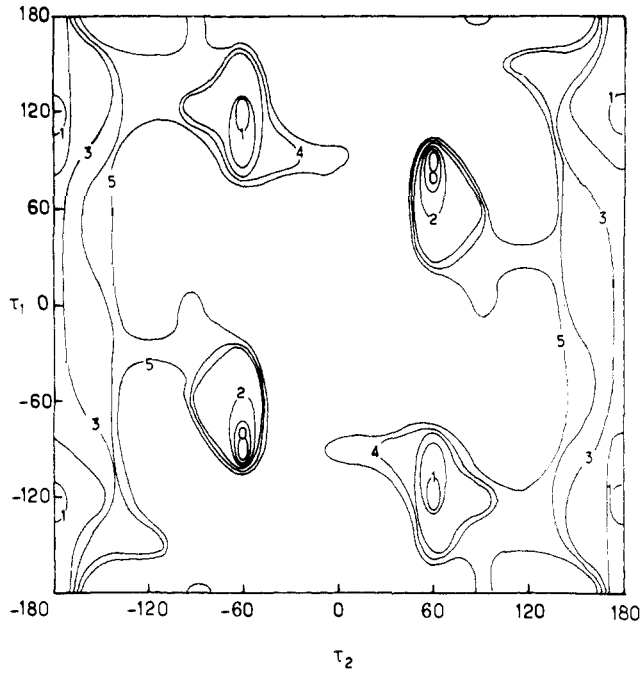


Figure 8. The conformational energy map of hydrated cationic serotonin. Isoenergy curves in kilocalories per mole with respect to the global energy minimum taken as energy zero.

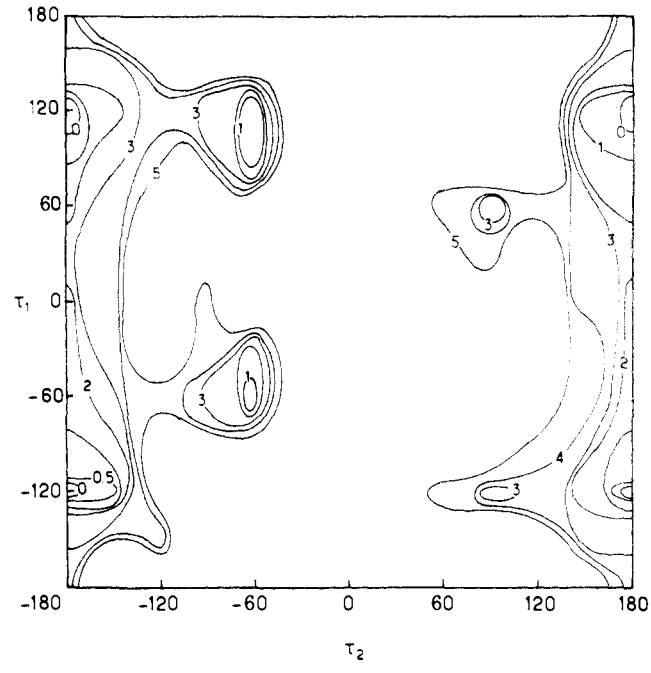


Figure 9. The conformational energy map of hydrated cationic bufotenine. Isoenergy curves in kilocalories per mole with respect to the global energy minimum taken as energy zero.

ed species (Figure 4b) while the map for hydrated serotonin is profoundly different from that of the nonhydrated species (Figure 2). The high selectivity for a gauche form, which was one of the outstanding features of the conformational energy map for isolated serotonin, disappears for the hydrated form. The essential result on both maps for the hydrated species is the near equivalence of the energy minima related to the gauche and trans forms which may therefore be expected to coexist in solution in comparable amounts.

It is particularly satisfying to quote a very recent experimental result[32] based on nmr spectroscopy which confirms this theoretical evaluation. The measurement of vicinal coupling constants for the protons of the $C_\alpha H_2$-$C_\beta H_2$ bond of cationic serotonin leads to two possible proportions of the gauche and trans forms, one corresponding to a slight preponderance of the trans rotamer (n_t = 0.45, n_g = 0.55) and the other to a slight preponderance of the gauche rotamers (n_t = 0.28, n_g = 0.72) (when the three rotamers are of equal energy: n_t = 0.33, n_g = 0.67). The experiment does not permit to decide between these two possibilities but in any case it indicates that the energy difference between the trans and gauche rotamers in solution is very small (for n_t = 0.45, n_g = 0.55, $E_g - E_t$ = 0.3 kcal/mol) which is in agreement with the results of our computations. Thus, the effect of the solvent is, as it is also in the case of the histamine cations,[29] to diminish the energy gap between the possible rotamers making thus their coexistence in solution possible.

Conclusion

The principal conclusions which can be drawn from this study appear to be the following.

(1) The conformational energy maps constructed by the PCILO method for the isolated indolealkylamines seem to account satisfactorily for the preferred conformations of these compounds in crystals provided that a distinction is being made between the neutral and ionic species and inside them between those which have an amino or a dimethylamino terminal. As in many other cases[11] the conformational properties of these drugs as observed in the solid state may be considered to be due predominantly to intramolecular interaction. There is, however, an exception in this case: the cationic forms [whether involving N^+H_3 or $N^+H(CH_3)_2$] exist in some of their crystals in extended planar forms which do not correspond to an energy minimum on the conformational maps but fall in a relatively high energy region (5–7 kcal/mol above the global minimum).‡ These conformations must be considered presently as due to the action of environmental forces.

(2) The effect of the solvent water, as studied by the "microscopic supermolecular" approach, brings about a smoothing out of the energy differences between the preferred conformers of the pharmacologically important cationic forms, especially those with a N^+H_3 cationic head. The trans (τ_2 = 180°) and gauche (τ_2 = ±60°) conformers are now of nearly equal energy and should coexist in solution. This theoretical result is confirmed by the nmr study of serotonin in water.

Acknowledgment. The authors thank Professor D. Carlström for communicating to them, prior to publication, the manuscript of a review paper (in collaboration with Dr. Bergin and Falkenberg) to appear in *Quarterly Review of Biophysics* [Added in proof: 6, 257 (1973)].

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‡ This result was recently confirmed by *ab initio* computations.[33]

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Inhibitors of Polyamine Biosynthesis. 1. α-Methyl-(±)-ornithine, an Inhibitor of Ornithine Decarboxylase

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α-Methyl-(±)-ornithine (**5**) was obtained by two independent syntheses. In the first synthesis 1-phthalimidopentan-4-one was subjected to the Bucherer–Lieb reaction to provide 5-(3-phthalimidopropyl)-5-methylhydantoin. The latter was hydrolyzed to produce **5**. The second synthesis involved the reaction of 3-imino(4-nitrobenzyl)piperidin-2-one with phenyllithium to form a resonance-stabilized anion which on treatment with methyl iodide and acid hydrolysis provided **5** in good yields. α-Methyl-(±)-ornithine monohydrochloride was found *in vitro* to be a potent, reversible, competitive inhibitor of ornithine decarboxylase obtained from the prostate glands of rats. This inhibition was not abolished at high concentrations of pyridoxal phosphate.

The diamine putrescine and the polyamines spermidine and spermine are present in all animal and plant tissue tested and at least one of these is present in all microorganisms.[1] The exact physiological role of the polyamines is not known at present. Recent studies, however, afford evidence that these amines may control cell division and growth and may participate in many steps in the biosynthesis of protein and RNA.[2] Studies of both normal and neoplastic rapid-growth systems indicate that the synthesis and accumulation of polyamines are elevated shortly after a stimulus inducing proliferation. Furthermore, tissues which actively synthesize protein as prostate, bone marrow, and pancreas contain higher concentrations of polyamines than most other mammalian tissues.[3]

The biosynthesis of polyamines in mammalian tissue, plant tissue, and bacteria has been extensively studied. In mammalian tissue the decarboxylation of L-ornithine to produce putrescine is catalyzed by the enzyme ornithine decarboxylase (ORD). This enzyme requires pyridoxal phosphate (PLP) and has no activity toward L-lysine, L-arginine, or D-ornithine.[4] Spermidine is formed from putrescine by an enzyme system which catalyzes the decarboxylation of *S*-adenosylmethionine and the transfer of the propylamine moiety to putrescine. It appears that the same enzyme system catalyzes the formation of spermine from spermidine.[5]

In spite of numerous studies on the biosynthesis and accumulation of polyamines in proliferating tissue, it is not certain if the increase in polyamine levels in these tissues mediates the elevated rate of protein synthesis or if the elevated rate of protein synthesis produces the increase in polyamine levels. One way of elucidating the role of polyamines in proliferating tissue would be to block their biosynthesis and to determine if this causes inhibition of cellular proliferation. A likely candidate for this blockade is the enzyme ornithine decarboxylase since the decarboxylation of L-ornithine appears to be the rate-limiting step in polyamine synthesis,[6] and the activity of ORD is sharply increased in rapidly growing tissue.

There are very few reports in the literature of inhibitors of the enzyme ornithine decarboxylase. L-Canaline was found to inhibit pyridoxal-dependent enzymes including ornithine decarboxylase but this inhibition was reversed by excess pyridoxal phosphate.[7] A number of ornithine analogs were prepared in an attempt to obtain specific inhibition of ornithine decarboxylase. None of these compounds were found to be effective inhibitors.[8] Recently, α-hydrazino-L-ornithine was reported to be a potent inhibitor of ORD but a much less effective inhibitor of other pyridoxal-dependent enzymes. The inhibition of ORD by α-hydrazino-L-ornithine is completely abolished at high concentrations of PLP.[9] Also, inhibition by α-methylornithine of ORD obtained from regenerating rat liver was briefly reported in a recent symposium.[5]

The present communication describes the synthesis of α-methylornithine and the evaluation of its inhibitory effect on ornithine decarboxylase obtained from rat prostate gland.

Results and Discussion

The target compound, α-methylornithine (**5**), was obtained *via* two independent syntheses. In the first synthe-