Table I. Energy Results for Cyclohexane

Conformation	Negative total energy, au[a]	Relative energies, kcal/mol FSGO	Hoyland[b]	Allinger et al.[c]	Exptl
Chair	198.040 024	0.00	0.00	0.00	0.00
Boat	198.016 753	14.61	7.19	6.59	4.8–5.9[d]
Half-chair (ideal)	198.014 148	16.24	11.22	11.04	10.8[e]
Planar	197.975 922	40.23			
Chair (flattened, 111.13°)	198.041 865	−1.15			
Half-chair (rocked)	198.014 521	16.01			

[a] 1 au = 627.503 kcal/mol. [b] Reference 3. [c] Reference 4. [d] References 5, 6, and 7. [e] Reference 8.

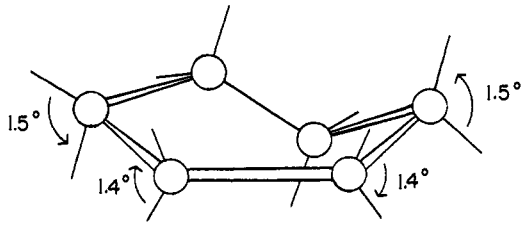

Figure 1. Half-chair conformation with minimum energy. Arrows indicate rock angles of methylene groups.

methylene groups relieves some of the eclipsing interactions. This is indicated in Figure 1. These results are in accord with those observed for cyclopentane[1] and cyclobutane.[11]

As can be seen from Table I, the relative energies of the various conformations give the correct ordering. However, the energy differences between the chair and the other conformations are larger than those obtained in previous calculations[12] and experimental studies. There is considerable eclipsing of methylene groups in all conformations other than the chair. The FSGO model is known to overestimate by approximately a factor of 2 such eclipsing interactions as in cyclopentane,[1] cyclobutane, and ethane.[11] Thus, the overestimation of the calculated energy differences between the various conformations and the chair conformation is not unexpected.

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Effect of Dielectric Constant on the Conformational Behavior of Tryptamine and Serotonin

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Abstract: Using an empirical method, the conformational behavior of tryptamine and serotonin has been investigated as a function of dielectric constant. The properties such as conformational energy, percent population, dipole moment, optical anisotropy, and depolarization ratio are considered. The shape of the contour maps changes considerably when the dielectric constant increases even though the position of the global minimum remains unaltered. The potential barrier between the trans and gauche forms also decreases. When the dielectric constant is low, the gauche forms dominate, and while it is high, the trans forms predominate due to conformational transition. The dipole moments slightly increase as the dielectric constant increases. The optical anisotropies and depolarization ratios increase sharply when the dielectric constant increases from 1.0 to 8.0, and then decrease steadily as the dielectric constant further increases. The range of 20 to 30 for the dielectric constant has been proposed for the conformational study of drug molecules. The difference between tryptamine and serotonin has been further explored in terms of intramolecular forces.

The methods that are available for the calculation of the conformational energies of any molecule can be classified into two categories: (i) the molecular orbital methods (MO), and (ii) the empirical method. The MO methods, such as EHT (Extended Hückel Theory), INDO (Intermediate Neglect of Differential Overlap), and PCILO (Pertur-

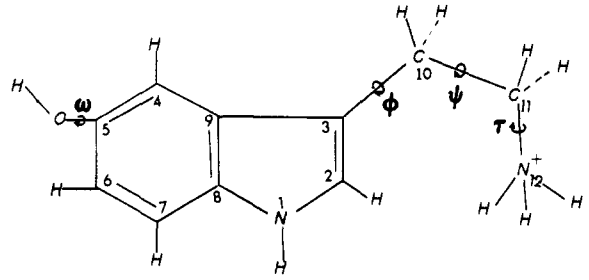

Figure 1. Definition of dihedral angles φ, ψ, τ, and ω. The angle $\varphi = 0$ when the C_{10}–C_{11} bond is in the planar cis position with the C_2–C_3 bond. The angle $\psi = 0$ when C_{11}–N_{12} is in the planar cis position with the C_3–C_{10} bond. $\tau = 0$ when N_{12}–H is in the planar cis position with the bond C_{10}–C_{11}. $\omega = 0$ when the O–H bond is in the same plane as the indole ring. In the present calculations, $\tau = \omega = 60°$ were set. The figure is drawn for $\varphi = \psi = \tau = 0$, and $\omega = 180°$.

bative Configuration Interaction using Localized Orbitals), have been extensively employed in conformational analysis of pharmacologically important molecules in understanding the drug-receptor interactions.[1,2] On the other hand, the empirical method has scarcely been applied[3] in the conformational study of small molecules, but extensively used in the study of proteins and polypeptides.[4-6]

Even though the empirical method is based on empirical potential functions, it has a great advantage over the MO theories: (a) the MO theories are not adequate to compare with the crystal conformation which occurs as a result of Coulombic interactions with gegenions, since these theories do not deal with these forces, (b) MO theories have no way of accounting for the pH or the temperature or the solvent effect, which are of course important in biological systems. The empirical method, on the other hand, has a way of treating these effects.

In the framework of an empirical method, the conformational energies are determined primarily as the sum of the contributions from the nonbonded, torsional, electrostatic interactions. The potential functions for the nonbonded and torsional interactions have been established fairly well.[4-6] The potential function for the electrostatic interactions has been described by Coulomb's law, which unfortunately and critically depends on an ill-defined dielectric constant. Since this dielectric constant is an effective dielectric constant rather than the bulk dielectric constant, it is not understood properly.[7] Due to this reason, there is a considerable doubt as to the value of this constant which must be used. The range of 1 to 5 has been suggested for the conformational study of proteins and polypeptides.[4-7] Since the macromolecular systems are quite different than the small molecular systems, one cannot consider that this range is also applicable to small molecules. Therefore, it is necessary to establish a range for the effective dielectric constant for the study of small molecules. The conformational behavior of a molecule depends on the nature of the solvent medium, which might be very critical in biological systems. Thus, it is essential to understand the conformational behavior of drug molecules as a function of solvent (dielectric constant).

Method of Calculation

The conformational energies are computed using the following relation:

$$E(\varphi,\psi) = E_{\text{nonbonded}} + E_{\text{torsional}} + E_{\text{electrostatic}} \quad (1)$$

where φ and ψ are the dihedral angles (Figure 1). The nonbonded energy between the pair of interacting atoms is calculated by using the Lennard-Jones 6-12 potential function, which is

$$E_{\text{nonbonded}} = \sum_{i,j} (d_{ij} r_{ij}^{-12}) - (e_{ij} r_{ij}^{-6}) \quad (2)$$

where r_{ij} is the distance between the ith and the jth interacting atoms. The e_{ij} and d_{ij} are the coefficients, whose values for various atoms have been tabulated elsewhere.[3]

The torsional potential energy is calculated making use of the following equation.[4-6]

$$E_{\text{torsional}} = (E_\varphi^0/2)(1 + \cos 3\varphi) + (E_\psi^0/2)(1 + \cos 3\psi) + (E_\tau^0/2)(1 + \cos 3\tau) \quad (3)$$

$E_\psi^0 = 2.8$ kcal mol^{-1} (ref 6) and $E_\varphi^0 = 0.5$ kcal mole^{-1} (ref 3 and 8) are used. Since the τ was fixed at 60° in a staggered position with the preceding group, the last term in eq 3 vanishes.

The electrostatic energy for the nonbonded pairs has been evaluated using the formula

$$E_{\text{electrostatic}} = 332 e_i e_j / \epsilon r_{ij} \quad (4)$$

where ϵ is an effective dielectric constant, and e_i is the charge on the ith atom, which is determined as the sum of π and σ charges. The π charges are computed by the Hückel method[9] and the σ charges by the method suggested by Del Re.[10] The φ and ψ are rotated in 10° intervals. The necessary geometric input is taken from Falkenberg.[11]

Let n_t, n_{g1}, and n_{g2} represent the mole fractions (or the percent population) of three stable rotamers one trans and two gauches, respectively. Then,

$$\begin{aligned} n_t &= 1/(1 + f_1 + f_2) \\ n_{g1} &= f_1/(1 + f_1 + f_2) \\ n_{g2} &= f_2/(1 + f_1 + f_2) \end{aligned} \quad (5)$$

where $f_i = 1.0/\exp(-\Delta G_i/RT)$. In this expression, the ΔG_i is the free-energy difference between the trans and the ith gauche rotamer, which is,

$$\Delta G_i = \Delta H_i - T\Delta S_i = \Delta E_i - RT \ln (\Omega_t/\Omega_{gi}) \quad (6)$$

In eq 6, we have set $\Delta H_i = \Delta E_i = E_t - E_{gi}$ and $T\Delta S_i = RT \ln (\Omega_t/\Omega_{gi})$. The Ω_t and Ω_{gi} are the degeneracy factors or the number of trans and gauche rotamers having the same energy E_t and E_{gi}, respectively.

The dipole moment of an ith rotamer is predicted using the relation $\mu_i = \Sigma e_i r_i$. Then the average dipole moment of a mixture is given as $\langle\mu\rangle = \Sigma n_i \mu_i$, where n_i is the mole fraction of the ith rotamer. The optical anisotropy and the depolarization ratios are evaluated according to the valence optical scheme.[6,12] The average anistropy and the depolarization ratio for a mixture is given below.

$$\langle\gamma^2\rangle = \Sigma n_i \gamma_i^2 \quad (7)$$

$$\langle\rho\rangle = 6\Sigma n_i \gamma_i^2/(45\bar{\alpha}^2 + 7\Sigma n_i \gamma_i^2) \quad (8)$$

where γ_i^2 is the anisotropy of an ith rotamer, and $\bar{\alpha}$ is the average molecular polarizability.

Results

The conformational energies are computed using eq 1 as a function of dielectric constant ϵ for tryptamine and serotonin. The energy maps (contour diagrams) are reproduced in Figures 2 to 4. The contours are drawn in 1 kcal mol^{-1} interval from the most stable state (global minimum). This minimum lies at $\varphi = 90°$ and $\psi = 60°$ when $\epsilon = 1.0$, corresponding to the gauche 1 (g1) conformation. However, this minimum has shifted to $\varphi = 80°$ and $\psi = 70°$ when $\epsilon = 4.0$. Then the position of the minimum remains unaltered as $\epsilon \rightarrow \infty$. In addition to the global minimum, there are two secon-

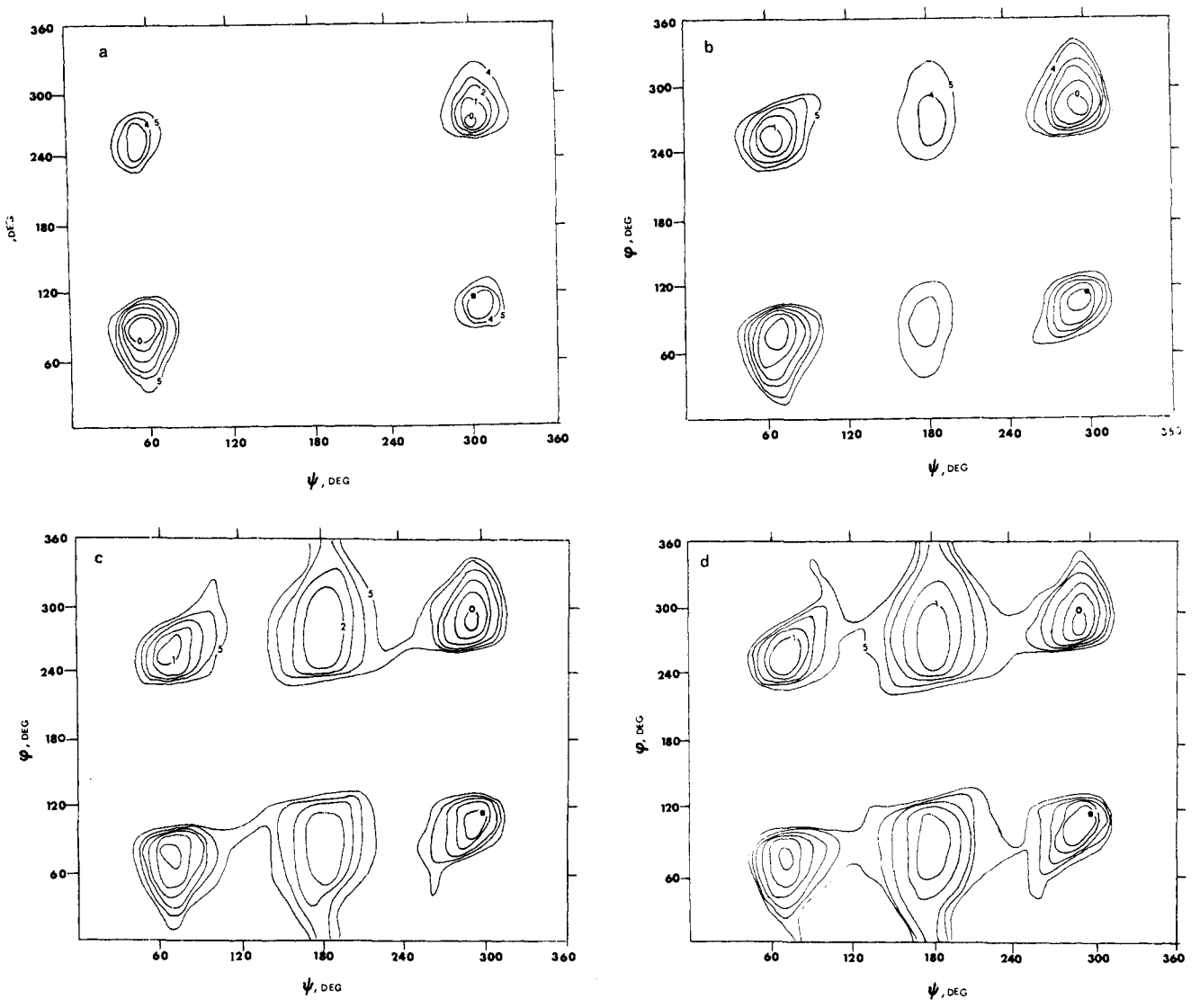

Figure 2. Contour diagrams for tryptamines (a) $\epsilon = 1.0$, (b) $\epsilon = 4.0$, (c) $\epsilon = 10.0$, and (d) $\epsilon = 20.0$. (■) X-ray conformation of tryptamine HCl.

dary minima corresponding to one trans (t) and one gauche (g2) forms. The trans form exists at $\varphi = 100°$ and $\psi = 180°$ for $\epsilon = 1.0$, and has shifted to $\varphi = 90°$ and $\psi = 180°$ for the range $4 \leq \epsilon \leq \infty$. Similarly, the g2 form which has the value of $\varphi = 110°$ and $\psi = 290°$ when $\epsilon = 1.0$ has changed to $\varphi = 110°$ and $\psi = 310°$ for the range $4 \leq \epsilon \leq \infty$. The energy difference between the global minimum and two secondary minima depends on the dielectric constant (Figures 5a and 5b). Since there is one trans and two gauche forms, Figure 5a is conveniently represented in the form of the difference between the trans and two gauche forms rather than the g1 − t and g1 − g2 difference. It does not make any difference as far as the qualitative behavior is concerned. As the dielectric constant increases, the energy difference between the global minimum (g1) and the two secondary minima (t and g2) narrows for both molecules.

The predicted dipole moments for tryptamine and serotonin are placed in Table I. Dipole moments of serotonin are slightly higher than those of the tryptamine indicating an increased ionic character. The anisotropies and depolarization ratios are determined using the bond polarizabilities given by Stuart.[13] These properties have also been included in Table I.

Table I. Dipole Moments, Anisotropy, and Depolarization Ratio of Rotamers Calculated from Equations 7 and 8

Torsional angles		Tryptamine			Serotonin		
φ, deg	ψ, deg	μ, D	$\gamma^2 \times 100^{48}$, cm^6	$\rho \times 100$	μ, D	$\gamma^2 \times 100^{48}$, cm^6	$\rho \times 100$
80	70 (gauche1)	9.32	51.11	1.57	9.89	56.15	1.64
80	180 (trans)	10.73	50.42	1.54			
90	180 (trans)				11.75	54.52	1.59
100	290 (gauche2)	10.27	53.89	1.65	10.47	59.25	1.73

Discussion

From the contour diagrams of tryptamine presented in Figures 2 and 4, it is obvious that the shape of the contours truely depends on the value of the dielectric constant. When $\epsilon = 1.0$ (Figure 2a), only four fragments appear corresponding to two gauche conformations ($\varphi = 90°$, $\psi = 60°$, and $\varphi = 110°$, $\psi = 310°$). Both these gauche forms differ in energy by about 3 kcal mol^{-1}. The region corresponding to trans conformation does not appear indicating that the trans forms, for this particular ϵ value, have energy greater than 5 kcal mol^{-1}. The preponderance of the gauche forms for $\epsilon = 1.0$ can be explained in terms of the high electrostatic interactions between the side chain and the indole ring, especially the protonated nitrogen and the ring portion. However, from the calculated percent population (Figure 5c), it seems that only the gauche 1 is dominant (99%) over the gauche 2 form (1%). When the dielectric constant is increased to 4.0 (Figure 2b), six fragments appear in contour

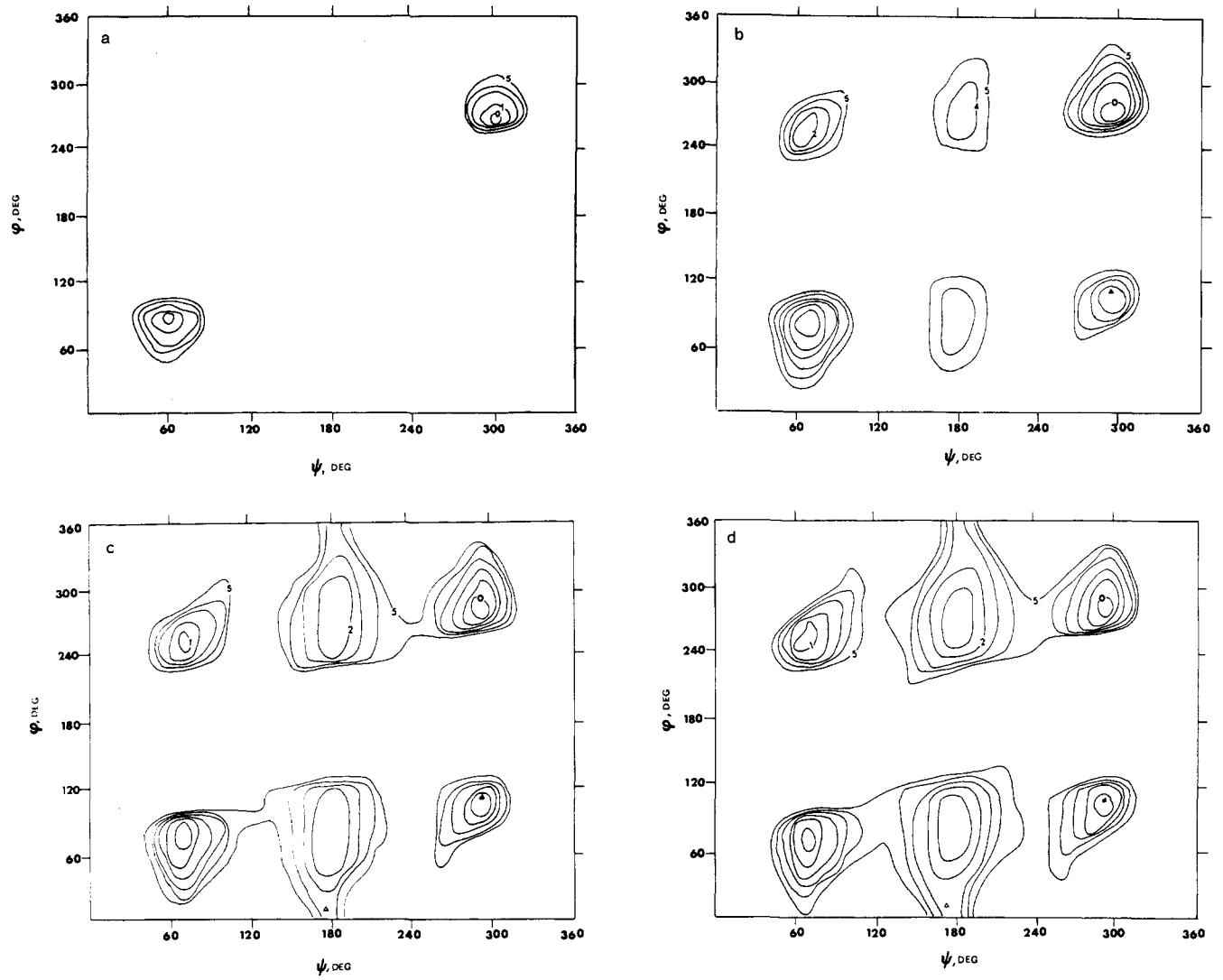

Figure 3. Contour diagrams for serotonin (a) $\epsilon = 1.0$, (b) $\epsilon = 4.0$, (c) $\epsilon = 10.0$, and (d) $\epsilon = 20.0$. (▲) X-ray conformation of serotonin picrate H_2O. (△) X-ray conformation of serotonin creatinine $H_2SO_4 \cdot 2H_2O$.

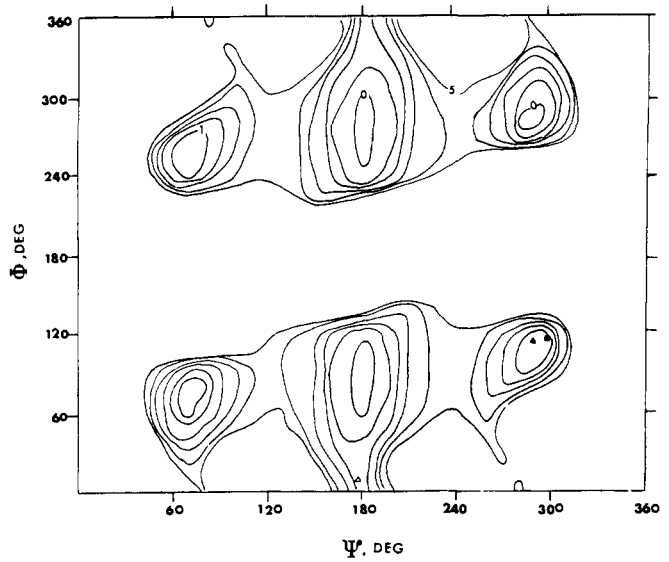

Figure 4. Contour diagram for tryptamine and serotonin when $\epsilon \geq 30.0$. See legends of Figures 2 and 3 for the x-ray conformations.

diagrams, four for gauche and two for trans forms suggesting that the potential barrier between the gauche and the trans forms has lowered considerably (less than 5 kcal mol^{-1}), and the trans forms begin to appear (Figure 5c, 1%). There is a sudden decrease in g1 population and a sharp increase in g2 population. This indicates that the conformational transition from g1 to g2 and t commences around $\epsilon = 4.0$, which can be seen more clearly in Figure 5c. A further increase in ϵ decreases the g1 population monotonically and increases that of the trans. Contrary to this, g2 population reaches its maximum at about $\epsilon = 10$, then decreases slowly as $\epsilon \rightarrow \infty$. Thus the conformational transition becomes a function of the dielectric constant. An increase in the t population as ϵ increases can be interpreted as due to an increased interaction between the side chain and the solvent medium. The potential barrier between the gauche and the trans forms also decreases to a greater extent (Figure 5a). From Figure 5c, few interesting observations can be noted: (a) the t population becomes greater than g2 and g1 respectively when $\epsilon \geq 15$, and $\epsilon \geq 26$; (b) if we consider the entire gauche population ($n_{g1} + n_{g2}$), then the t population becomes greater than the gauche population when $\epsilon \geq 43$. Therefore, these values $\epsilon = 15$, 26, and 43 may be considered as the transition points.

Tryptamine and serotonin differ only in the substitution of the OH group at the 5 position (Figure 1). Since the activities are associated with the conformational behavior,[14,15] it is interesting to compare the conformational behavior of these two molecules. The contour diagrams of serotonin differ from those of tryptamine only for $\epsilon \leq 30$. Therefore, for

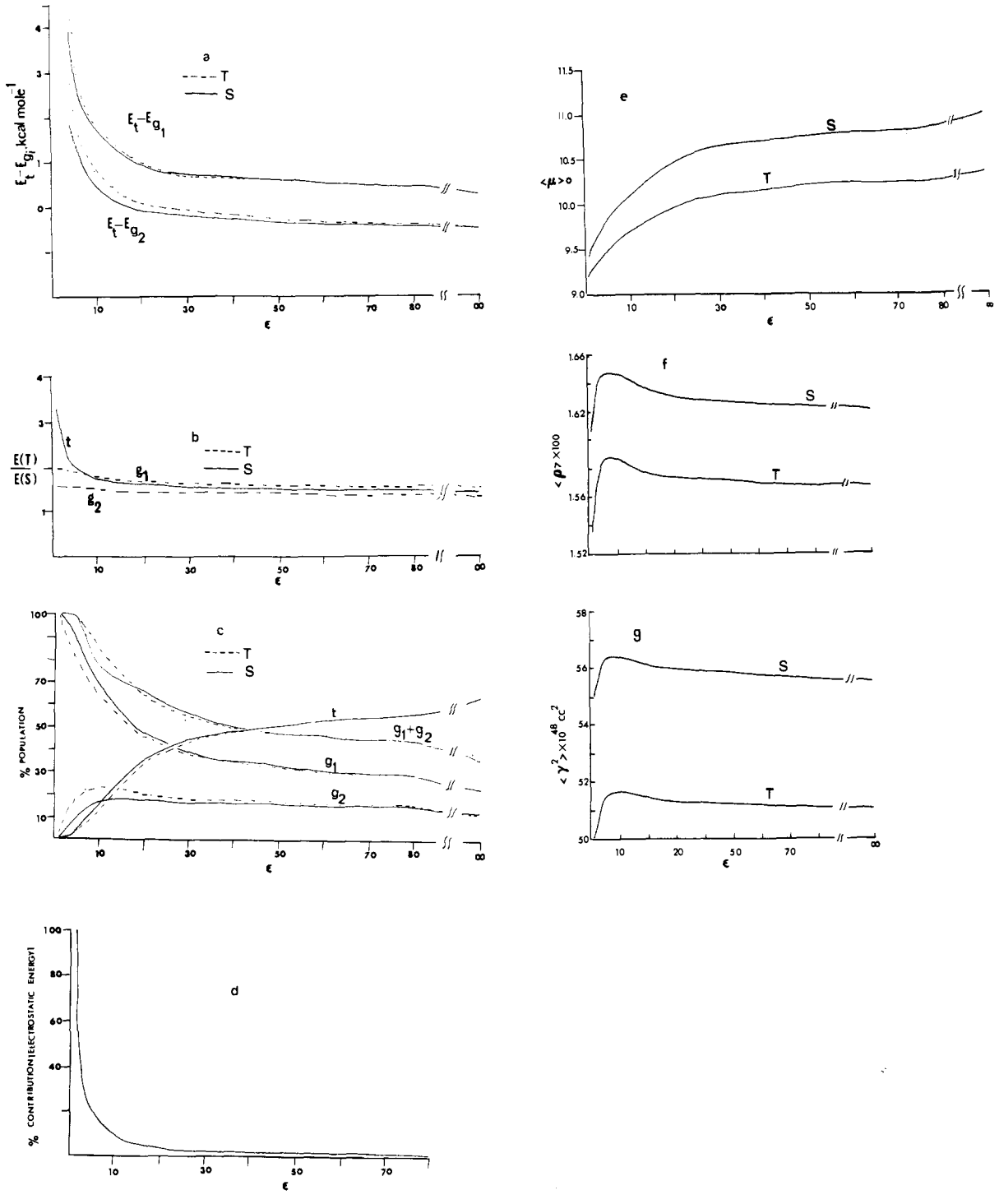

Figure 5. Effect of variation of dielectric constant on various properties: (a) the potential barrier between the trans and gauche forms, (b) the ratio of energies of rotamers of tryptamine (T) and serotonin (S) (in general tryptamine rotamers have higher energy than serotonin), (c) percent population, (d) percent decrease in contribution of electrostatic interaction assuming 100% contribution when $\epsilon = 1.0$ (e) dipole moment, (f) optical anisotropy, and (g) depolarization ratio.

the sake of comparison, the contour maps for serotonin are produced in Figures 3 and 4. Since the contour diagrams for $\epsilon > 30$ are almost identical for both molecules due to a small amount of contribution from the electrostatic forces (Figure 5d), Figure 4 applies to both molecules. From Figure 5c, it is clear that the conformational behavior of serotonin becomes identical to that of tryptamine only when $\epsilon > 30$. In the region $\epsilon \leq 30$, the trans and g1 populations of serotonin are slightly higher than those of tryptamine. However, g2 population is less than that of the tryptamine. The percent population of g2 of serotonin does not decrease as fast as that of tryptamine. Most importantly, the rotamers of both molecules are energetically quite different for the entire range of the dielectric constant chosen here (Figure 5b), even though the difference is more noticeable for the range $\epsilon \leq 30$. The NMR study of serotonin in D_2O solvent[16] has indicated about 44% for the trans population. This value, in our case, corresponds to $\epsilon \approx 30$. Hence from the present study, it is revealed that the rotamer population definitely depends on the dielectric constant, that is, on the nature of the solvent. The crystal conformations are best generated when ϵ is high (Figure 2 to 4). In Table II, we

Table II. Summary of the Preferred Conformations of Serotonin According to Various Methods

Author	Method	Stability	
		Serotonin	Tryptamine
	Theoretical		
Kier[a]	EHT	Extended	
Kang et al.[b]	EHT	Extended, folded	
Courriere et al.[c]	PCILO	Folded	
Kang and Cho[d]	INDO	Extended, folded	
Port and Pullman[e]	SCF	Folded	
Pullman et al.[f]	PCILO with solvent effect	Extended, folded	
Present Study	Empirical	Folded when ϵ is low Folded, extended when ϵ is high	Folded, when ϵ is low Extended, folded when ϵ is high
	Experimental		
Karle et al.[g]	X ray	Extended	
Bugg and Thewalt[h]	X ray	Folded	
Wakahara et al.[i]	X ray		Folded
Ison et al.[j]	NMR	Extended, folded	

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have compared our results with results of various other techniques.

The rotamers of any molecule can be characterized by the values of torsional angle ψ or the dipole moment or the optical anisotropy or the depolarization ratio. The effect of dielectric constant on isomerism has been examined in Figures 5e, 5f, and 5g. The $\langle\mu\rangle$ slightly increases as ϵ increases owing to an increase in t population. Unlike $\langle\mu\rangle$, the $\langle\gamma^2\rangle$ and $\langle\rho\rangle$ first increase and then steadily decrease as ϵ increases. This behavior might be attributed to the dominance of the g2 form when $\epsilon \leq 10$, and to the t form when $\epsilon > 10$. Even though the $\langle\mu\rangle$, $\langle\gamma^2\rangle$, and $\langle\rho\rangle$ of both molecules follow the same trend, their values at any particular ϵ differ exhibiting the intrinsic difference between them; serotonin is more ionic and more easily polarizable than the tryptamine.

After having gathered all this information, it might be possible to prescribe the value or the range of ϵ for the study of drug molecules under physiological conditions. Since the biological activities are the statistical values, the rotamer population might play an important role in drug-receptor interaction. It is difficult to obtain rotamer population pertaining to the physiological conditions. Nevertheless, the vivo conformers are approximated by the solution conformers in vitro. Then referring to the percent trans NMR results,[16] the range of 20 to 30 appears to be suitable for the study of biological molecules.

Thus from the present study, the following conclusions can be drawn. (a) The potential barrier between the trans and gauche conformations truely depends on the magnitude of ϵ (the nature of the solvent medium). (b) In a low dielectric medium, only the folded forms are stable, while, in high dielectric medium, both folded and extended forms exist in equilibrium mixture due to the low potential barrier between them. This observation is of course very important in drug-receptor interaction. (c) The magnitude of the effective dielectric constant is immaterial as far as the stable conformation is concern, but it is critical in predicting the percent population. (d) There exists intrinsic difference between tryptamine and serotonin, the latter being more ionic and better polarizable than the former. (e) The value of the effective dielectric constant of about 20 to 30 appears to be suitable for the study of biological active molecules. (f) It is demonstrated that the empirical method has the ability of generating the x-ray as well as solution results.

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