ated with two ions out of 6 $Rb^+$ ions at Rb(2) because of repulsive interactions with $Rb^+$ ion at Rb(4): two adjacent 6-ring sites of a $Rb^+$ ion at Rb(4) should be vacant. Therefore, in this case, a cluster of $(Rb_3)^{4+}$ may also be formed with much lower symmetry.

In this result, the reaction went to completion with 0.1 Torr of Rb(*g*) at 250℃ for 2 hrs and 24 hrs, respectively, and all $Ca^{2+}$ ions in dehydrated $Ca_6$-A were reduced and replaced by $Rb^+$ ions. The Ca metal was not found within the zeolite but was seen coating the external surface of the zeolite crystals.

**Acknowledgement.** The present studies were supported in part by the Basic Research Institute Programs, Ministry of Education, 1992, project No. BSRI-92-306.

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# Conformation of Antifungal Agent Fluconazole

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Conformational free energy calculations using an empirical potential function and a hydration shell model (program CONBIO) were carried out on antifungal agent fluconazole in the unhydrated and hydrated states. The initial geometry of fluconazole was obtained from two minimized fragments of it using a molecular mechanics MMPMI and followed by minimizing with a semiempirical AM1 method. In both states, the feasible conformations were obtained from the calculations of conformational energy, conformational entropy, and hydration free energy by varying all the torsion angles of the molecule. The intramolecular hydrogen bonds of isopropyl hydroxyl hydrogen and triazole nitrogens and the structural flexibility are of significant importance in stabilizaing the conformations of fluconazole in both states. Hydration is proved to be one of the essential factors in stabilizing the overall conformation in aqueous solution. Two F atoms of phenyl ring are not identified as an essential key in determining the stable conformations and may be responsible for the interaction with the receptor of fluconazole.

## Introduction

Fluconazole (UK-49,858, 2-(2,4-difluorophenyl)-1,3-bis(1*H*-1,2,4-triazol-1-yl)propan-2-ol) is a novel and water-soluble triazole antifungal agent, and has shown activity in several animal models of infection,[1] which is readily absorbed orally and has a plasma half-life of 25 h, high blood levels, low levels of protein binding, and high urinary recovery.[2] In spite of the progress in the clinical evaluation against a range of fungal infections, any structural information of fluconazole is not available yet.

In the present study, the conformations of fluconazole in free space and aqueous solution were studied to determine its detailed structure and the hydration effect as a first step

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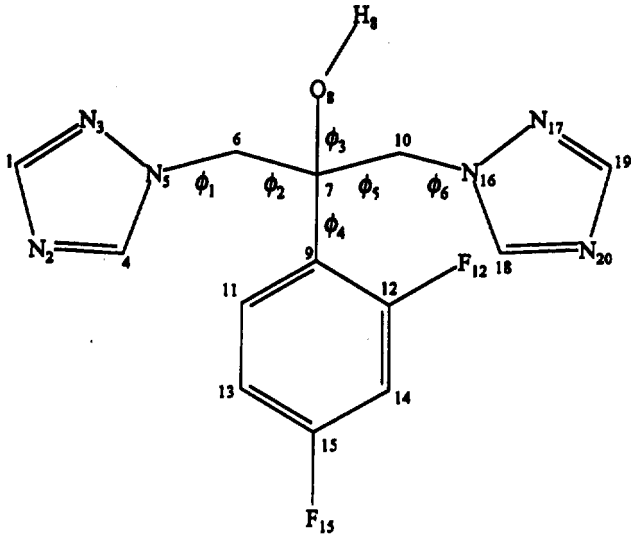


**Figure 1.** The chemical structure and torsion angles of fluconazole; all hydrogen atoms are not shown for clarity.

in understanding its pharmacological properties.

## Methods

The chemical structure and torsion angles of fluconazole are shown in Figure 1. Because no X-ray crystallographic nor spectroscopic structure of fluconazole is available, the conformations of its two fragments, (1*H*-1,2,4-triazole-1-yl) ethane (TAZE) and 2-(2,4-difluorophenyl)propan-2-ol (DFPP), were studied first. The initial structures for TAZE and DFPP were taken from the X-ray crystal structure of triazole[3] and from the electron diffraction results of isopropanol[4] and 1,3-difluorobenzene,[5] respectively. Using the molecular mechanics program MMPMI,[6] conformational energies of TAZE and DFPP were fully minimized varying (30° and 60° increments, respectively) all torsion angles. The structure of fluconazole was optimized by the AM1 method in the AMPAC program (version 2.1),[7] in which the initial structure was obtained from the combination of the lowest energy conformations of TAZE and DFPP. The geometry (*i.e.*, a set of bond lengths and bond angles) and partial atomic charges used for the energy calculations were obtained from that of the optimized structure.

In the conformational energy calculation, bond lengths and bond angles were fixed and only the torsion angles for internal rotation were taken as the variables. The definition of torsion angles is listed in Table 1. The conformational energy computations were carried out with the CONBIO program of Kang,[8] the potential parameters for which were taken from the program ECEPP/2.[9] The potential parameters for F atom were separately determined in ref 8c, as done before for ECEPP/2. The total conformational energy is the sum of the electrostatic energy, the nonbonded energy, and the torsional energy. The hydrogen-bond energy is included in the nonbonded energy component. The hydration shell model improved recently[10] was used to compute the hydration free energy of each conformation of fluconazole in the hydrated state, where the hydration free energy was obtained as the sum of two contributions from water-accessible volume and

**Table 1.** Definition of Torsion Angles of Fluconazole[a]

| Torsion angle | Sequence of atoms |
|---|---|
| $\Phi_1$ | $N_3-N_5-C_6-C_7$ |
| $\Phi_2$ | $N_5-C_6-C_7-C_{10}$ |
| $\Phi_3$ | $C_6-C_7-O_8-H_8$ |
| $\Phi_4$ | $C_6-C_7-C_9-C_{12}$ |
| $\Phi_5$ | $C_6-C_7-C_{10}-N_{16}$ |
| $\Phi_6$ | $C_7-C_{10}-N_{16}-N_{17}$ |

[a] See Figure 1.

polarization. The van der Waals radius, hydration shell radius, and free energy density of hydration for F atom were taken from ref 8c. A quasi-Newton algorithm SUMSL (Secant-type Unconstrained Minimization problem SoLver)[11] was used for energy and free energy minimization.

The 729 conformations for fluconazole were selected as starting points for energy minimization in the unhydrated state from the combination of six torsion angles defined in Figure 1 and Table 1. For the initial torsion angles, the values of ± 60° and 180° were selected. These six torsion angles were allowed to vary during energy and free energy (*i.e.*, the sum of conformational energy and hydration free energy) minimization in the unhydrated and hydrated states, respectively. Each conformation obtained by the minimization of the unhydrated molecule was used as a starting conformation for free energy minimization in the hydrated state.

At each energy minimum in the unhydrated and hydrated states, the conformational entropy was computed using a harmonic method.[12] The elements of a hessian matrix of second derivatives at each minimum were numerically calculated.[8b,8e,13] The relative conformational energy is given by $\Delta E = E - E^\circ$, where $E^\circ$ is the conformational energy of the lowest free energy conformation. The relative entropic contribution to the relative free energy ($\Delta G$) is given by $-T\Delta S$ at 298 K. The relative total free energy ($\Delta G_{tot}$) in the hydrated state is given by the sum of $\Delta G$ and $\Delta\Delta G_{hyd}$, where $\Delta\Delta G_{hyd}$ is the hydration free energy relative to the lowest free energy state.

## Results and Discussion

Torsion angles and energetics of low free energy conformations (*i.e.*, relative free energy $\Delta G$ or $\Delta G_{tot}$<1 kcal/mol) of fluconazole in the unhydrated and hydrated states are listed in Tables 2 and 3, respectively. For each conformation, the Tables contain (i) the conformational letter code, (ii) the relative free energies $\Delta G$ and $\Delta G_{tot}$, (iii) the normalized statistical weight ω, (iv) the relative conformational energy $\Delta E$, (v) the relative entropic contribution to conformational free energy $-T\Delta S$, and (vi) the relative hydration free energy $\Delta\Delta G_{hyd}$. For each conformation, a six-letter conformational code is used for six torsion angles of fluconazole defined in Figure 1 and Table 1 (see footnote b of Table 2 for detailed codes).

**Unhydrated Fluconazole.** From the 729 starting conformations of fluconazole in the unhydrated state, we obtained the 145 different conformations after minimization, which have the relative conformational free energy less than 5

**Table 2.** Torsion Angles and Energetics of Low Energy Conformations of Unhydrated Fluconazole[a]

| No. | Conf.[b] | $\Delta G$[c] | $\omega$[d] | $\Delta E$[e] | $-T\Delta S$[f] | Torsion angles[g] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | $\phi_1$ | $\phi_2$ | $\phi_3$ | $\phi_4$ | $\phi_5$ | $\phi_6$ |
| 1 | $p^+ttg^-tg^+$ | 0.00 | 0.081 | 0.00 | 0.00 | 112 | −163 | 163 | −59 | 174 | 62 |
| 2 | $g^-tg^-p^+tp^-$ | 0.04 | 0.076 | −0.13 | 0.17 | −48 | −168 | −53 | 134 | 175 | −106 |
| 3 | $p^+ttp^+tg^+$ | 0.10 | 0.068 | 0.49 | −0.39 | 110 | −172 | 157 | 119 | 176 | 66 |
| 4 | $g^-tg^-g^-tp^-$ | 0.14 | 0.063 | −0.21 | 0.35 | −56 | −164 | −50 | −57 | 172 | −104 |
| 5 | $g^+g^+g^+g^-g^-g^-$ | 0.30 | 0.049 | −0.49 | 0.78 | 69 | 74 | 60 | −57 | −61 | −57 |
| 6 | $g^+g^+g^+p^+g^-g^-$ | 0.31 | 0.048 | −0.10 | 0.41 | 65 | 67 | 63 | 125 | −61 | −60 |
| 7 | $g^-ttg^-tg^+$ | 0.33 | 0.046 | −0.01 | 0.34 | −59 | −161 | 162 | −58 | 175 | 64 |
| 8 | $g^-tg^-g^-tg^+$ | 0.51 | 0.034 | 0.28 | 0.23 | −57 | −165 | −49 | −58 | 170 | 73 |
| 9 | $g^+g^+g^+p^+tp^-$ | 0.55 | 0.032 | 0.91 | −0.36 | 71 | 65 | 52 | 124 | 173 | −109 |
| 10 | $g^+g^+g^+g^-tp^-$ | 0.73 | 0.023 | 0.63 | 0.10 | 74 | 68 | 50 | −61 | 167 | −109 |
| 11 | $p^+tg^+p^+g^-g^-$ | 0.75 | 0.023 | 0.97 | −0.22 | 114 | −168 | 78 | 128 | −61 | −69 |
| 12 | $g^-ttp^+tg^-$ | 0.81 | 0.020 | 1.10 | −0.29 | −77 | −174 | 159 | 116 | 174 | 62 |
| 13 | $p^+tg^+g^-g^-g^-$ | 0.97 | 0.016 | 0.73 | 0.24 | 116 | −162 | 79 | −57 | −64 | −72 |
| 14 | $p^+tg^+p^-tp^-$ | 0.97 | 0.016 | 1.45 | −0.48 | 113 | −169 | 69 | 128 | 177 | −104 |

[a]Energies are in kcal/mol, and free energies and entropic contributions are calculated at 298 K. Only the conformations with the relative total free energy to that of the lowest free energy, *i.e.*, conformation **1** ($\Delta G<1$ kcal/mol) are listed. [b]Each conformation is defined by conformational letter codes of six torsion angles defined in Figure 1 and Table 1, *i.e.*, $30°\le g^+\le 90°$, $90°<p^+<150°$, $150°\le t\le 210°$, or $-210°\le t\le -150°$, $-150°<p^-<-90°$, and $-90°\le g^-\le -30°$. [c]The total free energy of each conformation. [d]Normalized statistical weight. [e]Intramolecular interaction energy change. [f]Conformational entropic contribution. [g]See Figure 1 and Table 1 for the definition.

**Table 3.** Torsion Angles and Energetics of Low Energy Conformations of Hydrated Fluconazole[a]

| No. | Conf. | $\Delta G$ | $\omega$ | $\Delta E$ | $-T\Delta S$ | $\Delta\Delta G_{hyd}$[b] | Torsion angles | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | $\phi_1$ | $\phi_2$ | $\phi_3$ | $\phi_4$ | $\phi_5$ | $\phi_6$ |
| 1 | $g^-tg^-g^-tg^+$ | 0.00 | 0.177 | 0.00 | 0.00 | 0.00 | −62 | −170 | −47 | −60 | 168 | 75 |
| 2 | $g^-g^+g^+g^-tp^-$ | 0.26 | 0.115 | 0.55 | 0.03 | −0.33 | −46 | 84 | 52 | −55 | 173 | −106 |
| 3 | $p^+ttg^-tg^+$ | 0.41 | 0.089 | −0.33 | 0.12 | 0.61 | 114 | −164 | 162 | −59 | 174 | 67 |
| 4 | $g^+g^+g^+p^+tp^-$ | 0.73 | 0.052 | 0.61 | −0.21 | 0.33 | 72 | 65 | 49 | 123 | 171 | −115 |
| 5 | $g^-tg^-g^-g^-p^+$ | 0.79 | 0.047 | 0.56 | 0.48 | −0.25 | −54 | −164 | −50 | −56 | −60 | 100 |
| 6 | $g^+g^+g^+g^-tp^-$ | 0.79 | 0.046 | 0.32 | 1.21 | −0.74 | 73 | 66 | 48 | −63 | 164 | −111 |
| 7 | $g^-tg^-g^-tp^-$ | 0.86 | 0.041 | −0.49 | 1.23 | 0.12 | −58 | −168 | −48 | −60 | 170 | −105 |
| 8 | $g^+g^+g^+p^+g^+p^-$ | 0.87 | 0.041 | 1.06 | −0.24 | 0.45 | 76 | 75 | 50 | 147 | 68 | −91 |
| 9 | $g^-ttg^-tg^+$ | 0.94 | 0.035 | −0.31 | 0.50 | 0.77 | −65 | −164 | 162 | −59 | 174 | 67 |

[a]See footnotes of Table 2. [b]Relative hydration free energy.

kcal/mol. Only the first 14 low free energy conformations with $\Delta G<1$ kcal/mol are shown in Table 2. The computed low free energy conformations **1** and **2** seem to be the most probable conformations of fluconazole.

From the analysis of total free energies of the conformations, the conformational energy and entropy are both the major contributions to the total free energy. Although the conformations **2**, **4**, **5**, **6**, and **7** have lower conformational energy than that of the conformation **1**, the latter has lower conformational free energy due to the more negative contribution of $-T\Delta S$ to $\Delta G$. This corresponds to the increasing conformational entropy and indicates that there is a deeper potential surface around the energy minimum of the conformation **1** than those of the other conformations. The computed torsion angles indicate that torsion angles $\phi_2$ and $\phi_5$ of stable conformations are *trans*.

The lowest free energy conformation **1** in the unhydrated state is drawn in Figure 2a, which has a weak intramolecular hydrogen bond (HB) between $H_8$ of isopropyl hydroxyl group and $N_{17}$ of triazole ring with the distance 2.27 Å. The conformations **2** and **4** form HBs between $N_3$ of the second triazole ring and $H_8$ with the distances 2.25 Å and 2.34 Å, respectively. Although the conformations **5** and **6** have these two HBs ($R(H_8\cdots N_{17})=2.29$ Å and 2.23 Å, $R(N_3\cdots H_8)=2.30$ Å and 2.26 Å, respectively), they are less stable than the conformations **1**, **2**, and **4**. This indicates that the overall conformations of fluconazole in the unhydrated state are governed by HBs, but the structural flexibility is more important in stabilizing the molecule.

**Hydrated Fluconazole.** In the hydrated state, the 113

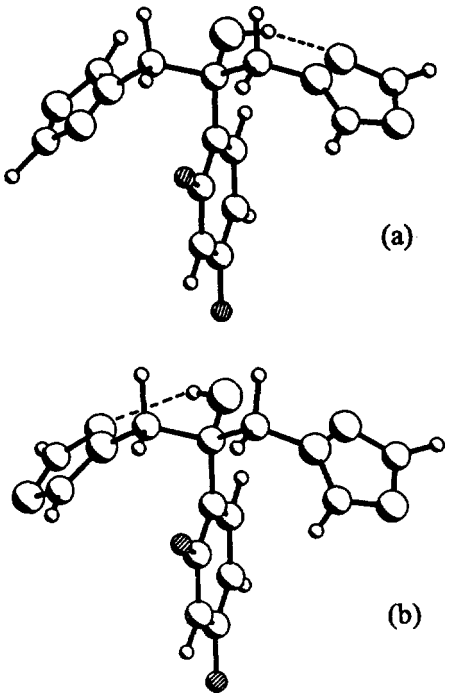


**Figure 2.** The lowest free energy conformations of fluconazole in the unhydrated (a) and hydrated (b) states; F atoms are indicated by shading and hydrogen bonds are represented by dotted lines.

low free energy conformations with $\Delta G_{tot}$<5 kcal/mol were obtained after minimization from the 145 minima in the unhydrated state. Table 3 lists energetics and torsion angles of the first nine low free energy conformations with the relative total free energy $\Delta G_{tot}$<1 kcal/mol.

From the analysis of total free energies of the conformations, the hydration free energy, conformational energy, and conformational entropy are found to be the major contributions to the total free energy. Especially, in the conformations **3**, **6**, and **9**, the hydration is most significant, and conformational entropy contributes dominantly in stabilizing the conformations **6** and **7**. It is found that the hydration does not directly affect each conformation, but contributes in altering the potential surface around each free energy minimum. The first two low free energy conformations **1** and **2** have relatively larger statistical weights than those in the unhydrated state. The conformations with torsion angles $\phi_4$ and $\phi_5$ to be *gauche-* and *trans*, respectively, were found to be stable.

The lowest free energy conformation **1** in the hydrated state is drawn in Figure 2b, which forms a weak HB between $N_3$ and $H_8$ with the distance 2.26 Å, similar to that of $H_8$ and $N_{17}$ of the lowest free energy conformation in the unhydrated state. The conformations **3** and **9** have HBs between $H_8$ and $N_{17}$ with the distance 2.31 Å, and the other conformations form HBs between $N_3$ and $H_8$ ($R(N_3 \cdots H_8)$=2.21-2.33 Å). The hydrated conformations do not have any bridged HBs of $H_8$ with $N_3$ and $N_{17}$, different from the conformations in the unhydrated state. This indicates that HBs are still of significant importance in stabilizing the overall conformations of fluconazole in the hydrated state but the hydration may inhibit these bridged HBs. The computed hydration free energies of the groups of the molecule indicate that the unfavorable hydration of atoms $N_3$, $N_5$ and $H_8$ forces the lowest energy conformation **3** to be less stable.

In summary, the intramolecular HBs of isopropyl hydroxyl hydrogen and triazole nitrogens and the structural flexibility are of significant importance in stabilizing the conformations of fluconazole in both states. Hydration is proved to be one of the essential factors in stabilizing the overall conformation in aqueous solution. Two F atoms of phenyl ring are not identified as an essential key in determining the stable conformations and may be responsible for the interaction with the receptor of fluconazole. Further conformational study on various fluconazole analogues may be of use in guiding the design of triazole antifungal agents.

**Acknowledgement.** U.T.C. thanks the Korea Science and Engineering Foundation (Grant No. KOSEF 911-0303-030-1) for support.

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