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Ab Initio Molecular Orbital Study on Molecular and Hydration Structures of Ectoine

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The molecular and hydration structures of ectoine (2-methyl-4-carboxy-3,4,5,6-tetrahydropyrimidine), which is one of the most important compatible solutes in halophilic and halotolerant microorganisms, were investigated for the first time via ab initio molecular orbital (MO) calculations. The electronic structure of ectoine was assumed to be a zwitterionic form, and possible stable molecular structures in the gas phase were searched by geometry optimizations. Two stable structures were obtained at the MP2/6-31G* level, and their stability were almost the same. One has the geometry in which the COO group is in axial position and the other in equatorial position. The transition state (TS) of proton transfer between the zwitterionic ectoine where the COO group is in equatorial position and neutral ectoine was obtained at the MP2/6-31G* level. However, the energy barrier of the proton transfer was sensitive to the basis sets used and disappeared at the MP2/6-31G**//MP2/6-31G* level, indicating that the zwitterionic ectoine should not be a stable structure in the gas phase. In addition, the SCRF treatments at the MP2/6-31G* level showed that, in aqueous solution, the energy barrier is sensitive to the cavity radii. The ectoine−water 1:1 complexes were also calculated, and 10 stable structures were found. In the most stable complex, ectoine is hydrogen bonding to one water molecule both at the oxygen atom of the COO group and at the hydrogen atom of the NH group. The calculation of the ectoine−water 1:4 complex showed that four types of hydration occur simultaneously. However, the hydrogen-bonding pattern found in the four types of hydration showed that more than four water molecules could solvate to ectoine.

I. Introduction

The production and/or accumulation of organic solutes, called compatible solutes (CSs), are/is the typical characteristic of many halophilic and halotolerant microorganisms.[1,2] CSs not only serve to compensate for osmotic pressure but also protect and stabilize the protein conformation.[1,2] Although such various types of CS as betaines, ectoines, proline, N-acetylated diamino acids, and N-derivatized carboxamides of glutamine are known in nature, they have some common molecular characteristics; CSs are highly soluble, polar, and neutral molecules.[1,2]

Ectoine is a common and abundant CS in halophilic and halotolerant bacterium and has been extensively studied since it was first discovered in 1985[1−3] (Figure 1). Ectoine has remained undetected for a long time because it does not react with common amino acid reagents. Recently, it was found that ectoine becomes a predominant compatible solute at high external salt concentration (i.e. >1.5 M NaCl).[4]

Schuh et al. reported the crystal structure of ectoine−methanol 1:1 complex.[5] Also, Inbar et al. determined the structures of ectoine and hydroxyectoine in both aqueous solution and crystal by NMR and X-ray crystallography, respectively.[6] They concluded that in both phases ectoine exists in the zwitterionic form with half-chair conformation and the COO group occupies an axial position.

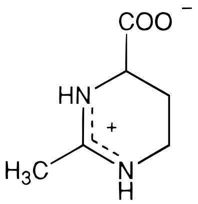

Figure 1. Structural formula of ectoine.

Hydration of CSs has been also considered quite important in conjunction with the stabilization mechanism of protein conformation.[1,7] A number of models, such as the water replacement,[8] the hydrophobic interaction,[9] and the preferential exclusion model,[10] have been proposed. Galinski et al. have been focusing on the preferential exclusion model, where CSs are literally preferentially excluded from the hydration sphere of proteins.[11] They considered the reason CSs are so effectively excluded from the hydration of proteins and paid attention to the water−CS interaction.[1,7] Near-infrared spectroscopy was used to examine the overtones of O−H vibrations of solvent water molecules interacting with CSs (i.e., betaine, proline, and ectoine). They indicated that these CSs involve two types of hydrogen bond with water, i.e. strong and weak hydrogen bond

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types. The strong hydrogen bond is similar to the one found in ice; namely, it is an ideally straight hydrogen bonding. On the contrary, the weak one was explained by more weakly bound solvent water molecules, for example, those in a layer of relative disorientation at the interface with bulk water. Further, they obtained the hydration numbers for CSs from the difference spectroscopy. For example, the estimated value for ectoine was 3.8.[7]

Under the circumstances, it is of primary importance to study the conformational and electronic properties of ectoine as the basis of understanding its biological activity. For this purpose, the quantum mechanical calculation would be appropriate since this can give information of molecular level such as stable structure and charge distribution. Therefore, we report for the first time our investigation on ectoine by ab initio MO calculations. Because CSs are acting in aqueous solution, it is also important to consider solvent water molecules as well as ectoine. In this article, the effect of solvent water on ectoine is considered by the cluster model and the SCRF method.[12,13]

First, stable structures of the zwitterionic form of ectoine in the gas phase were examined with the geometry optimization technique. Then the optimized structures were compared with the experimental one. The total energies and the charge distributions were discussed. The rotational barriers of the COO group and the conversion barrier between the zwitterionic and neutral ectoines were also calculated and discussed. Second, in order to understand the solvent effect of water, the cluster consisting of an ectoine and a water molecule was considered and then the stable structures were searched. The structure of hydrogen bonds, their strengths, and the solvent effect of one water molecule on the structures of ectoine were discussed. Finally, four water molecules were included in the cluster at the same time to examine the many-body effect on the ectoine−water interaction and its structure.

Section II explains the method of the ab initio MO calculations. In section III, results and discussion are presented. Finally, the conclusions are drawn in section IV.

II. Method of Calculation

A. Potential Energy Surface of Ectoine. The potential energy surface including stable structures, rotational barriers of the COO group, and a TS between the neutral and zwitterionic form of ectoine was examined by ab initio MO calculations using the GAUSSIAN92 and GAUSSIAN94 program packages.[14,15]

Ectoine was assumed to be a zwitterionic form which has been found in aqueous solution and crystal.[5,6] At first, geometry optimizations were done at the HF/6-31G* level, and optimized structures were checked whether or not those were stable structures by the frequency analysis. Consequently, two stable structures and two rotational TSs were found. It is noted that any other local minima were not found at the HF/6-31+G* level. Using these optimized structures as starting points, geometry optimizations at the MP2/6-31G* level were performed. These structures were found to converge successfully to stable or transition structures without any drastic geometric changes. For these optimized structures, single-point energy calculations were performed at the MP2/6-31G**, MP2/6-31+G*, and MP2/6-31++G** level. Further, the energy for the X-ray structure reported by Schuh et al.[5] was also calculated at these four MP2 levels.

Second, the proton transfer between a neutral form and one of the zwitterionic forms obtained was considered. First, geometry optimizations were done at the HF/6-31G* level. A neutral stable structure and a TS corresponding to the proton transfer were found. For these stationary points as initial structures, geometry optimizations were performed at the MP2/6-31G* level. These were thus found to converge to stationary points which were similar to those at HF/6-31G* level. For these points, single-point energy calculations were performed at the MP2/6-31G**, MP2/6-31+G*, and MP2/6-31++G** level. Further, the effect of solvent water on the proton transfer was examined with the SCRF method[12,13] implemented in the GAUSSIAN94 program. Namely, single-point energy calculations were performed at the SCRF/MP2/6-31G* level.

B. Ectoine−Water Complex. First, stable structures of the ectoine−water 1:1 complex were searched at the HF/6-31G* level. One water molecule was placed at some probable positions around an ectoine molecule to generate initial structures, and then geometry optimizations were started. Consequently, 10 stable hydrogen bond complexes were obtained. For these stable structures, single-point energy calculations were performed at the HF/6-31+G*, HF/6-31++G**, and MP2/6-31G* level. Using the HF/6-31G* optimized structures, the counterpoise-corrected interaction energies ΔE between ectoine and water were estimated as follows:[16]

$$\Delta E = E_{\mathrm{complex}} - E_{\mathrm{ectoine}} - E_{\mathrm{water}} \tag{1}$$

where E_{complex} is the total energy of the ectoine−water complex, E_{ectoine} is the total energy of ectoine at the geometry of the ectoine−water complex including the basis sets of water, and E_{water} is the total energy of water at the geometry of the ectoine−water complex including the basis sets of ectoine.

Second, four water molecules were included in the cluster at the same time to examine the many-body effect on the ectoine−water interaction and its structure since the experiment suggested that at least four water molecules can solvate to ectoine.[7] A ectoine−4H_2O complex was optimized at the HF/6-31G* level and found to be a stable structure.

III. Results and Discussion

A. Molecular Structure and Energy of Ectoine. Figure 2 shows the two optimized structures of ectoine at the MP2/6-31G* level and the X-ray structure obtained by Schuh et al.,[5] which are referred to as ECA, ECB, and ECX, respectively. Bond lengths and bond angles concerned with non-hydrogen atoms are also shown in Figure 2, and several important dihedral angles are listed in Table 1. Total energies and relative energies are shown in Table 2.

Two research groups reported the crystal structure of ectoine (i.e., Schuh et al. and Lapidot et al.).[5,6] The differences between the two reports exist in the solvent from which ectoine was crystallized, the crystal packing, and the hydrogen-bonding pattern. However, since Lapidot et al. pointed out that the two molecular structures of ectoine are similar, we will only refer to the crystal structure ECX by Schuh et al.[5] In ECX, ectoine forms a zwitterionic molecule with the half-chair conformation, keeping the carboxyl group in an axial position. ECA seems to be similar to ECX in conformation. For example, the dihedral angles including the C2 atom have the same signs and similar values (i.e., ϕ(C1−C2−C3−N4), ϕ(C2−C3−N4−C5), ϕ(C5−N6−C1−C2), and ϕ(N6−C1−C2−C3)). On the contrary, the corresponding dihedral angles of ECB have opposite signs of those of ECA and ECX. The consideration of the position of the COO group also leads to the similarity between ECA and ECX since ϕ(C5−N6−C1−C7) of ECA and ECX are 64.7° and 94.2°, respectively, but that of ECB is 145.2°. However,

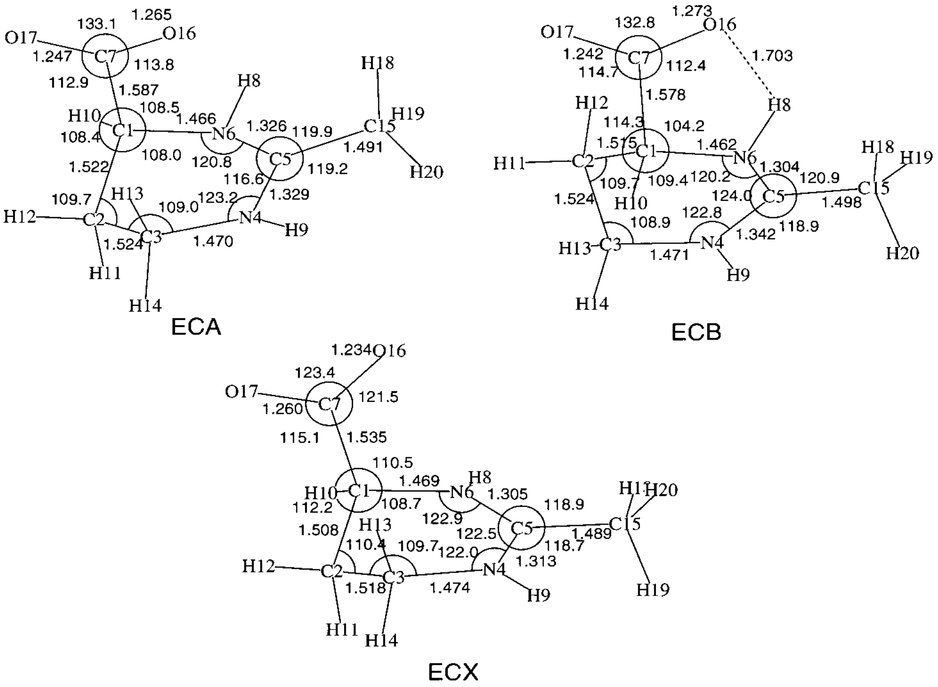


Figure 2. Structural parameters of ectoine. ECA and ECB are the stable structures at the MP2/6-31G* level, and ECX is the X-ray structure reported by Schuh et al.[4] Bond distances in angstroms and bond angles in degrees.

TABLE 1: Several Dihedral Angles (in deg) of ECA, ECB, and ECX

atom	ECA	ECB	ECX
C1−C2−C3−N4	−47.0	52.3	−50.7
C2−C3−N4−C5	27.7	−23.9	25.1
C3−N4−C5−N6	−19.7	−7.2	−0.1
N4−C5−N6−C1	32.3	8.1	2.4
C5−N6−C1−C2	−52.5	22.6	−29.3
N6−C1−C2−C3	59.5	−51.9	52.3
C5−N6−C1−C7	64.7	145.2	94.2
C1−N6−C5−C15	−151.0	−170.6	−178.4

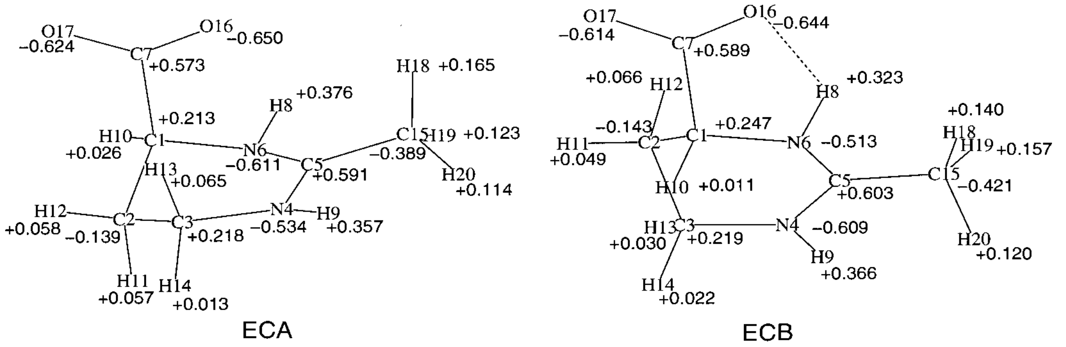
there are some large differences between ECA and ECX. For example, the atoms C3, N4, C5, N6, and C1 are almost on a plane (ϕ(C3−N4−C5−N6) = −0.1°, ϕ(N4−C5−N6−C1) = 2.4°) in ECX, but are not in ECA (ϕ(C3−N4−C5−N6) = −19.7°, ϕ(N4−C5−N6−C1) = 32.3°). This large deviation from a plane in ECA may come from the limitation of the MO calculation in which the zwitterionic form of the ectoine molecule is inevitably placed in a vacuum. On the contrary, it is interesting that these atoms are almost on the same plane in ECB (ϕ(C3−N4−C5−N6) = −7.2°, ϕ(N4−C5−N6−C1) = 8.1°). In ECB, there exists an intramolecular hydrogen bond between H8 and O16 (the bond distance is 1.703 Å) which seems to contribute to keep the planar structure. As is shown in Table 2, the energy differences between ECA and ECB are quite small. For example, the relative energy of ECB to ECA at the MP2/6-31++G**//MP2/6-31G* level is −1.4 kcal/mol. This suggests that the ECB type is also stable enough to exist in aqueous solution as well as the ECA type. The calculated dipole moments support this consideration since the dipole moments of ECA and ECB at the MP2/6-31G* level are 9.6 and 12.7 D, respectively, and then ECB could be more stabilized than ECA in aqueous medium. On the other hand, the large energy difference (28.2 kcal/mol at the MP2/6-31++G**//MP2/6-31G* level) between ECA and ECX is found despite the similarity of their structures. This may be attributed to the difference in several bond lengths rather than the dihedral angles described above. For example, the large difference of 0.052 Å is found in the bond length C1−C7. Further, in ECA, the distance between C5 and O16 is much shorter than that in ECX, showing the strong attractive interaction. However, O16 in ECX interacts with hydrogen atoms of other molecules in the crystal.[5]

Figure 3 shows the CHELPG charge distributions of ECA and ECB using the MP2/6-31G* level.[17] In each structure, O16 and O17 have large negative charges, e.g., −0.624 and −0.650 in ECA. On the contrary, H8 and H9 have large positive charges, e.g., +0.376 and +0.357 in ECA. These four atoms are expected to form strong hydrogen bonds with solvent water molecules. Large positive or negative charges are also found on C5(+0.591), N4(−0.611), and N6(−0.534). However, it is not clear whether these atoms could directly interact with water or not. The negative charge on O16 and the positive charge

TABLE 2: Total Energies (au) and Relative Energies (kcal/mol in Parentheses) of ECA, ECB, ECX, ECA-ROT, ECB-ROT, TS-NE, and ECB-NE

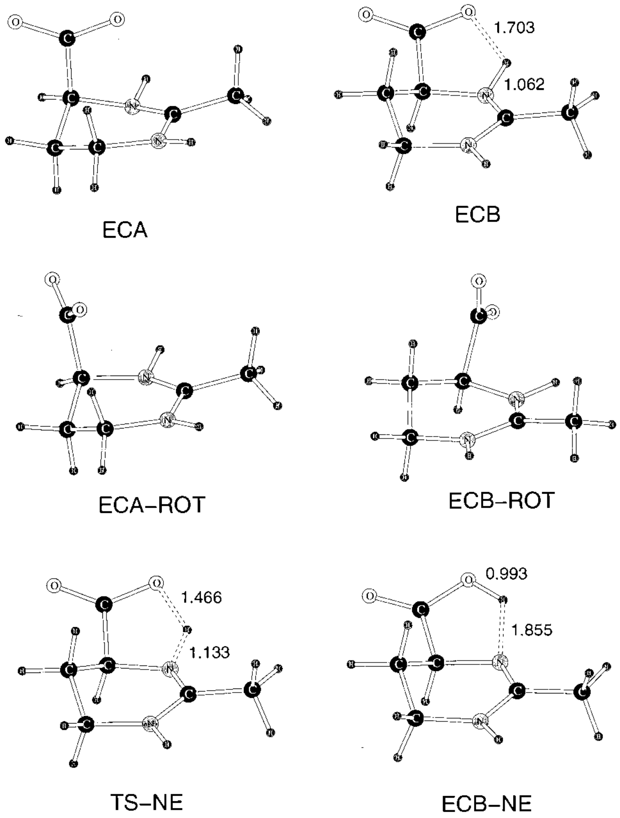
structure	MP2/6-31G*[a]	MP2/6-31G**[b]	MP2/6-31+G*[b]	MP2/6-31++G**[b]
ECA	−493.094 749 (0.0)	−493.176 250 (0.0)	−493.135 294 (0.0)	−493.216 862 (0.0)
ECB	−493.098 610 (−2.4)	−493.180 547 (−2.7)	−493.137 441 (−1.3)	−493.219 152 (−1.4)
ECX	−493.043 569 (32.1)	−493.127 354 (30.7)	−493.088 068 (29.6)	−493.171 875 (28.2)
ECA-ROT	−493.088 196 (4.1)	−493.169 839 (4.0)	−493.129 039 (3.9)	−493.210 538 (4.0)
ECB-ROT	−493.080 152 (9.2)	−493.161 632 (9.2)	−493.123 060 (7.7)	−493.204 431 (7.8)
TS-NE	−493.098 132 (−2.1)	−493.181 451 (−3.3)	−493.135 731 (−0.3)	−493.218 909 (−1.3)
ECB-NE	−493.120 787 (−16.3)	−493.204 818 (−17.9)	−493.155 060 (−12.4)	−493.238 998 (−13.9)

[a] Geometry optimization was performed except for ECX. [b] Single-point energy calculation using the geometry optimized at the MP2/6-31G* level.



ECA ECB

Figure 3. Charge distributions for ECA and ECB at the MP2/6-31G* level.



ECA ECB

ECA–ROT ECB–ROT

TS–NE ECB–NE

Figure 4. Optimized structures of ectoine at the MP2/6-31G* level. Bond distances in angstroms.

on H8 in ECB (−0.644 and 0.323) are smaller than the corresponding ones in ECA (−0.650 and 0.376), indicating that the intramolecular hydrogen bond between H8 and O16 induces the charge transfer, as is shown by a dotted line in Figure 3.

B. Rotation of the Carboxylic Group. The rotational barrier of the COO group was also examined. For ECA and ECB, there are rotational TSs, abbreviated as ECA-ROT and ECB-ROT, respectively, as shown in Figure 4. Since the COO group contains two equivalent oxygen atoms, the rotational paths are ECA → ECA-ROT → ECA′ → ECA-ROT′ → ECA and ECB → ECB-ROT → ECB′ → ECB-ROT′ → ECB, where ECA′, etc., mean that the geometries are the same as ECA, etc., but whose oxygen atoms are exchanged. The barrier heights at the MP2/6-31G* level are 4.1 and 11.6 kcal/mol for ECA and ECB, respectively. Namely, the barrier height of ECB is 7.5 kcal/mol higher than that of ECA. This is due to the stabilization of ECB by the intramolecular hydrogen bond. The energies with larger basis sets show that the barrier height of ECA is not so influenced by the method, whereas that of ECB became smaller. Namely, the values at the MP2/6-31G**//MP2/6-31G*, MP2/6-31+G*//MP2/6-31G*, and MP2/6-31++G**//MP2/6-31G* levels are 4.0, 3.9, and 4.0 kcal/mol, respectively, for ECA and 11.9, 9.0, and 9.1 kcal/mol, respectively, for ECB.

C. Proton Transfer between Zwitterionic and Neutral Form. As discussed in subsection A, there is the possibility that the ECB type is stable enough to exist in aqueous solution as well as the ECA type since ECB has almost the same energy as ECA and would be more stabilized by solvent water than ECA. Since the positions of H8 and O16 in ECB are very close to each other due to the intramolecular hydrogen bond, the proton transfer between them could occur with a low energy barrier. Therefore it is important to examine the potential energy profile of the proton transfer between ECB and its neutral form. Optimized geometries of a neutral form of ectoine (ECB-NE) and a transition state (TS-NE) of proton transfer between ECB-NE and ECB are shown in Figure 4. The total energies and relative energies of ECB-NE and TS-NE are shown in Table 2. At the MP2/6-31G* level, ECB-NE is 13.9 kcal/mol lower than ECB and TS-NE is located 0.3 kcal/mol higher than ECB.

The energies with larger basis sets may be important since, for example, in the case of glycine, a TS of proton transfer between a neutral and a zwitterionic form disappeared when the basis sets including p-type polarization functions on the migrating hydrogen atom (e.g., 6-31G**) were used.[18−21] The barrier height is found to be sensitive to the basis sets used for the calculations. Whereas there is quite a low energy barrier at the MP2/6-31G*//MP2/6-31G*, MP2/6-31+G*//MP2/6-31G*, or MP2/6-31++G**//MP2/6-31G** level, it disappears at the MP2/6-31G**//MP2/6-31G* level, as shown in Table 2. These indicate that ECB could easily converge to ECB-NE in the gas phase.

However, the zwitterionic form is of primary importance in both solution and crystal, as mentioned in the previous section. Therefore the medium effect was included by the SCRF method implemented in the GAUSSIAN94 program.[12−14] In the SCRF formalism, the solute is placed in a spherical cavity immersed in a continuous medium with a dielectric constant ϵ. A dipole in the solution induces a dipole in the medium, and the electric field applied to the solute by the solvent dipole in turn interacts with the solute dipole to result in stabilization. Using the optimized geometries at the MP2/6-31G* level and the VOLUME keyword, the cavity radii were determined to be 4.51, 4.1, and 4.24 Å for ECB, TS-NE, and ECB-NE, respectively. Then, we have performed successive single-point calculations using the SCRF/MP2/6-31G* level. The dielectric constant ϵ was assumed to be 78.5 for the bulk water. The total energies and the relative energies are shown in Table 3. The energies of TS-NE and ECB-NE relative to ECB become −2.6 and −5.4 kcal/mol, respectively. These results indicate that ECB cannot exist as a relatively stable species in the aqueous solution and converts to a neutral form of ectoine. However, the disappear-

TABLE 3: Total Energies (au), Relative Energies (kcal/mol in Parentheses), and Cavity Radii (Å) of ECB, TS-NE, and ECB-NE

structure	total energy	cavity radius
ECB	−493.121 910 (0.0)	4.51
TS-NE	−493.126 065 (−2.6)	4.10
ECB-NE	−493.130 554 (−5.4)	4.24
TS-NE	−493.117 890 (2.5)	4.51
ECB-NE	−493.128 626 (−4.2)	4.51

ance of the transition states is due to the difference of the cavity radius. When the same cavity radius was used for the three structures (i.e., 4.51 Å), different results were obtained, as shown in Table 3. In this case, the energy barrier exists, although it is still small (2.5 kcal/mol). The small energy difference between ECB and TS-NE arises from the similarity of those structures. TS-NE is a very reactant-like TS since the bond distance of N−H is 1.133 Å, which is close to that of ECB (1.062 Å), as shown in Figure 4. However, in aqueous solution, the structure of the TS might be drastically changed. Thus, further investigation would be needed from the viewpoint of the shape and size of the cavity and the change of the transition state in aqueous solution.

D. Ectoine−H_2O 1:1 Complex. Figures 5 and 6 show the optimized geometries for the ECA−H_2O and ECB−H_2O complexes, respectively. Table 4 shows the total energies and the interaction energies.

Five optimized structures were found for the ECA−H_2O complex. The most stable complex is ECA−W2, where the interaction energy is −17.4 kcal/mol at the HF/6-31G* level and ectoine is hydrogen bonded to water both at either of the two oxygen atoms of the COO group and at the hydrogen atom of the NH group. The hydrogen bond distances of the water

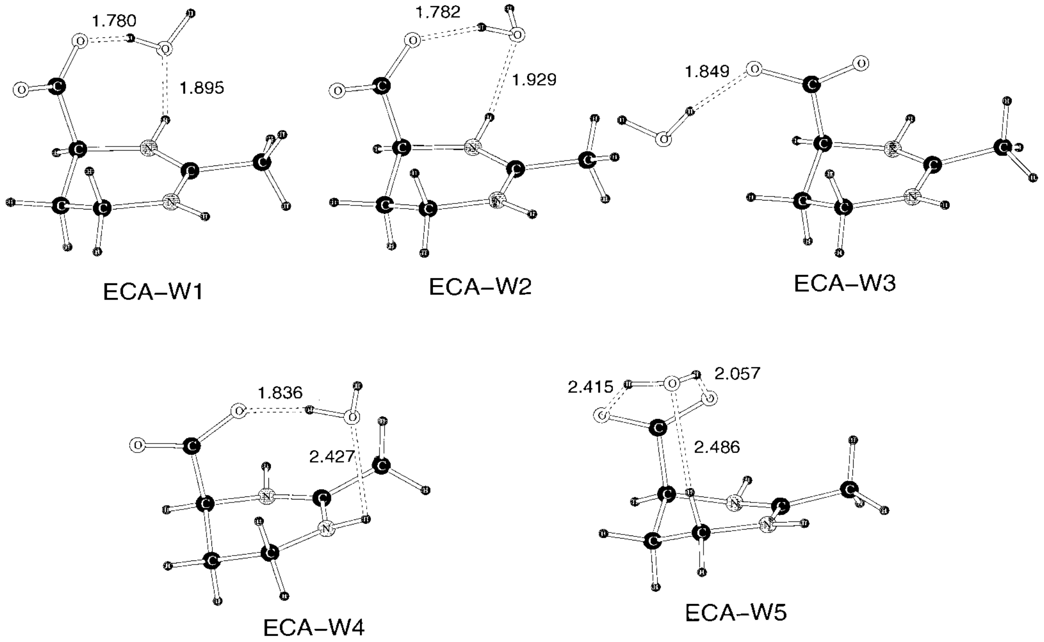


Figure 5. Optimized structures of ectoine−water 1:1 complexes for ECA at the HF/6-31G* level. Bond distances in angstroms.

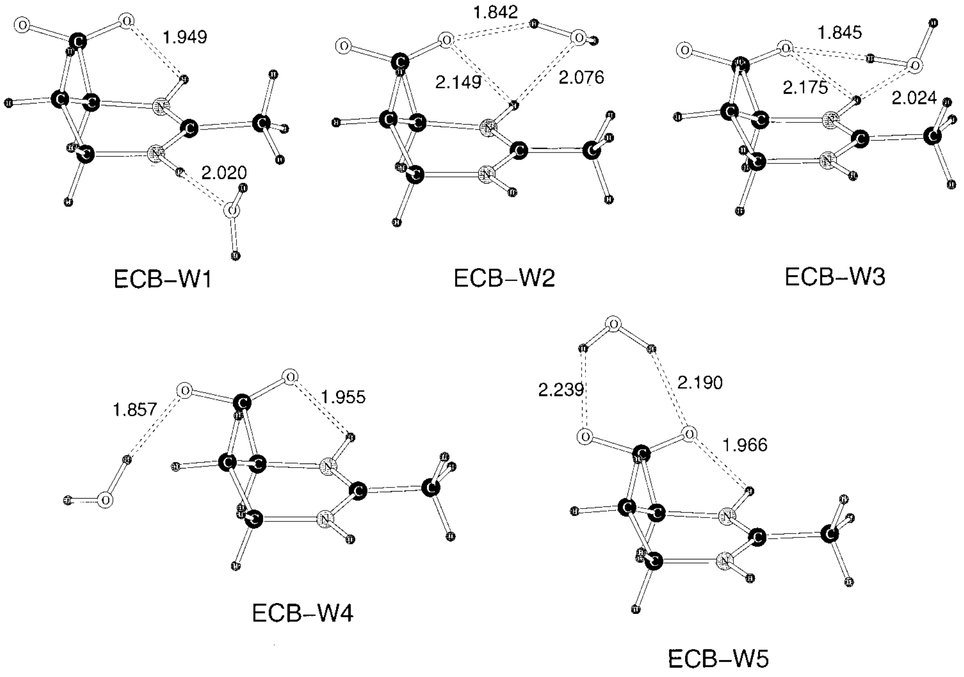


Figure 6. Optimized structures of ectoine−water 1:1 complexes for ECB at the HF/6-31G* level. Bond distances in angstroms.

TABLE 4: Total Energies (au) and Interaction Energies (ΔE) (kcal/mol in Parentheses) of Ectoine−Water 1:1 Complexes. All the Interaction Energies Were Corrected with the Counterpoise Method

structure	HF/6-31G*[a]	HF/6-31+G*[b]	HF/6-31++G**[b]	MP2/6-31G*[b]
ECA−W1	−567.685 112 (−16.5)	−567.706 408 (−15.6)	−567.739 673 (−15.7)	−569.318 855 (−17.6)
ECA−W2	−567.686 673 (−17.4)	−567.708 131 (−16.4)	−567.741 440 (−16.5)	−569.320 229 (−18.5)
ECA−W3	−567.678 228 (−10.3)	−567.700 845 (−10.5)	−567.734 359 (−10.5)	−569.309 075 (−10.7)
ECA−W4	−567.684 917 (−14.7)	567.707 171 (−14.5)	−567.740 194 (−14.3)	−569.317 411 (−15.7)
ECA−W5	−567.681 814 (−11.5)	−567.702 105 (−11.1)	−567.735 289 (−11.1)	−569.314 523 (−12.2)
ECB−W1	−567.675 484 (−7.9)	−567.698 425 (−7.1)	−567.732 084 (−7.1)	−569.305 420 (−8.9)
ECB−W2	−567.686 511 (−14.3)	−567.707 762 (−13.6)	−567.741 009 (−13.5)	−569.317 598 (−14.7)
ECB−W3	−567.685 238 (−13.7)	−567.706 666 (−13.1)	−567.739 752 (−13.0)	−569.316 159 (−14.2)
ECB−W4	−567.681 932 (−10.9)	−567.704 227 (−11.1)	−567.737 828 (−11.1)	−569.313 033 (−11.4)
ECB−W5	−567.681 188 (−10.3)	−567.703 103 (−10.4)	−567.736 577 (−10.4)	−569.310 926 (−10.0)

[a] Geometry optimization was performed. [b] Single-point energy calculation using the geometry optimized at the HF/6-31G* level.

hydrogen−COO oxygen and the water oxygen−NH hydrogen are 1.782 and 1.929 Å, respectively. This type of hydrogen bond is also found in the glycine−H_2O complex.[19,20] In ECA−W1, there are the same types of hydrogen bond as those in ECA−W2, and the interaction energy is almost the same (−16.5 kcal/mol). In ECA−W3, a hydrogen bond exists between the carboxyl oxygen and a water hydrogen. The interaction energy is −10.3 kcal/mol, which shows the weakest interaction among the five complexes. The geometries of ECA−W4 and ECA−W5 are more complicated. In ECA−W4, the water hydrogen atom is hydrogen bonding to one of the two oxygen atoms of the COO group and the water oxygen atom is hydrogen bonding to the hydrogen atom of the NH group, which is really the one farther from the COO group. Although the interaction energy of ECA−W4 is large (i.e., −14.7 kcal/mol), ECA−W4 does not seem to exist in the aqueous solution, because the distance between the water oxygen atom and the hydrogen atom of the NH group is much larger than that in ECA−W2, and the connection of atoms N−H---O(water) deviates from a straight line, which is unfavorable for the hydrogen bond. In the aqueous solution, the two groups in ectoine should be solvated by water molecules separately. In ECA−W5, the two water hydrogens are separately hydrogen bonding to the two oxygen atoms of the COO group and the water oxygen atom is close to the hydrogen atom of the CH_2 group (2.486 Å). However, this structure should not exist in the aqueous solution because the close contact of the water oxygen atom to the hydrogen atom of the CH_2 group does not seem to be plausible in the aqueous solution. In all five complexes, the structure of ECA is strongly influenced by one water molecule. For example, in ECA−W2, the dihedral angles of ϕ(C3−N4−C5−N6) and ϕ(N4−C5−N6−C1) become −5.5° and 9.5°, respectively. These values are closer to those of ECB and ECX. Namely, the atoms C3, N4, C5, N6, and C1 are located almost on a plane. A similar change is found for ECA−W1: ϕ(C3−N4−C5−N6) = −6.0° and ϕ(N4−C5−N6−C1) = 6.2°.

In ECB−W2, there are two hydrogen bonds similar to those in ECA−W2, and the interaction energy is −14.3 kcal/mol, which is the most stable among the five complexes of ECB. In ECB−W3, the same types of hydrogen bond as ECB−W2 are found, and the interaction energy is similarly −13.7 kcal/mol. In addition, there are three different types of complex, ECB−W1, ECB−W4, and ECB−W5. In ECB−W1, there is a hydrogen bond between the hydrogen atom of the NH group and the water oxygen atom. The interaction energy is −7.9 kcal/mol, which shows the weakest interaction among the five complexes. In ECB−W4, there is a hydrogen bond between one of the two oxygen atoms of the COO group and the water hydrogen atom with an interaction energy of −10.9 kcal/mol. In ECB−W5, those water hydrogens are hydrogen bonding to two oxygen atoms of the COO group separately, and the interaction energy is −10.3 kcal/mol. In contrast to the ECA−H_2O complexes, one water molecule does not change so much the geometry of ECB itself because the intramolecular hydrogen bond is strong enough to still be held in the complexes.

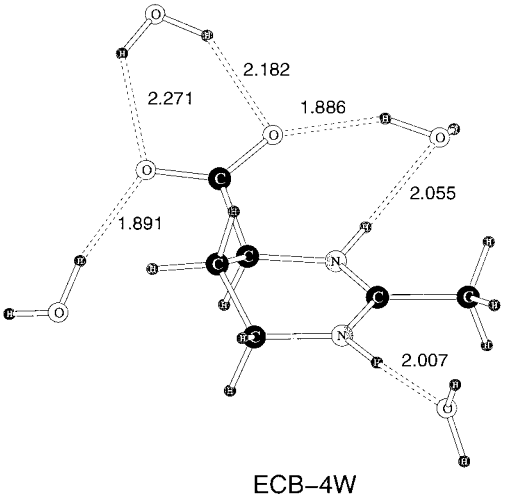


Figure 7. Optimized structure of ectoine−water 1:4 complex for HF/6-31G* level. Bond distances in angstroms.

Table 4 also shows the total energies and the interaction energies at higher levels. It is found that each complex has similar interaction energies at the four levels. Further, the relative stability of each complex among the ECA−H_2O or ECB−H_2O complexes is the same at the four levels. The total energies of ECA−W2 and ECB−W2, which are the most stable among the ECA−H_2O and ECB−H_2O complexes, respectively, are compared. The energy of ECB−W2 relative to ECA−W2 at the MP2/6-31G*//HF/6-31G* level is 1.7 kcal/mol and shows that their stability is similar. As in the case of ectoine itself, the results of the ectoine−water 1:1 complex also support that two types of structure can exist in the aqueous solution.

E. The Hydration Structure: Ectoine−H_2O 1:4 Complex. Ectoine is suggested to be solvated about four water molecules in aqueous solution from the experimental study.[7] Therefore, it is interesting to examine the hydration structure where ectoine is solvated by a number of water molecules. In this study, the cluster that includes the ECB type of ectoine and four water molecules was considered since four hydration patterns were clearly derived from the ECB−H_2O 1:1 complexes. Figure 7 shows an optimized geometry of the complex (ECB−4W) at the HF/6-31G* level. In ECB−4W, the hydrogen bonds are the same as those found in ectoine−H_2O 1:1 complexes. This means that ECB can interact directly with at least four water molecules. In addition, it is shown that ectoine can hydrogen bond with more than four water molecules since two carboxyl oxygen atoms seem to be hydrogen bonding to more than one water molecule in aqueous solution. That situation is also expected for the ECA−H_2O complex. However, in the present MO calculation, the number of water molecules in the cluster might have been restricted due to the computational incompleteness. Therefore, we are performing a Monte Carlo study to take into consideration the influence of hundreds of water molecules.[22]

IV. Conclusions

Ab initio MO calculation of the zwitterionic form of ectoine in the gas phase showed that there are two stable structures at the MP2/6-31G* level with almost the same stability. In addition, the rotational barriers of the COO group were obtained. The TS for the proton transfer between the zwitterionic (ECB) and the neutral ectoine (ECB-NE) was obtained at the MP2/6-31G* level. However, the energy barrier barrier of the TS is sensitive to the basis sets used for computation. For example, at the MP2/6-31G**//MP2/6-31G* level, the energy barrier disappeared. The SCRF treatments at the MP2/6-31G* level showed that the energy barrier is sensitive to the cavity radii used for the calculations. When the same cavity radius was used for ECA, TS-NE, and ECB-NE, the energy barrier of TS appeared. The calculations for the ectoine−water 1:1 complex show that there are four types of hydration structure and, in the most stable structure, ectoine is hydrogen bonding to one water molecule both at the oxygen atom of the COO group and at the hydrogen atom of the NH group. The four types of hydration were found to occur simultaneously, suggesting that ectoine can interact with at least four water molecules. However, their hydrogen bond pattern showed that more than four water molecules could solvate to ectoine.

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