content (see Table 6). Although we need more experimental data at various water content, in fovor of our criteria, it can be said that our criteria are very simple and are trustworthy compared to the ones proposed so far.

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Effects of Hydration and Metal Ions on the Conformation of Daunomycin

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Daunomycin, an anthracycline antibiotic, has been found to inhibit virus multiplication and shows considerable activity against tumors. Its activity may be varied by conformational changes of daunomycin. The conformational changes are come from the pucker of D-ring and variation of envirbnments. We have carried out conformational analyses by using empirical potential function. We found that when daunomycin is hydrated or bound to Mg^{2+} ion, the minimum conformer of each state is altered from α conformer to β conformer through the pathway having four local minima. Our calculated results are in good agreements with those of X-ray crystallography and biological experiments, in which metal ion inhibits the binding of daunomycin to DNA.

Introduction

The daunomycin is the anthracycline antibiotic with an amino sugar group. It is thought that the interference with DNA synthesis and function occurs via an intercalation of the anthracycline chromophore between base pairs in duplex DNA[1,2]. Since small changes in the drug structure may lead to marked changes in the biological activity[2,4], the conformation study around the ether linkage connecting the chromophore and amino sugar is found to be very important[5-7].

Several workers have studied the conformation of daunomycin according to potential energy calculations[5-7] as well as the X-ray crystallography[8-10]. However, previous reports have shown conflicting results in comparing the conformation of minimum energy with those of the crystals[5,6,11]. The conformation of daunomycin bound to DNA may be nearly identified with the crystal structure of daunomycin itself[4,5,10]. However NMR data in aqueous solution indicate that the conformation of daunomycin may differ from the preferred conformation found in the crystal form[11].

Several different conformations come from the pucker of cyclohexene ring (D-ring) within daunomycin. Prominent differences are that there is a hydrogen bond between C7 oxygen and C9 hydroxyl group (α conformer) or the amino sugar becomes oriented equatorially rather than axially (β form)[4,5,11].

The pucker of D-ring is occurred via the variation of environments around the daunomycin. That is, the internal hydrogen bond which may stabilize α conformer can be broken by water molecules in aqueous solution[5].

The conformational analysis of daunomycin could be performed by examining relative dispositions of the sugar group, anthracycline chromophore, and functional groups bound to the phosphate backbone of DNA in various environments. Previous conformational analyses have centered on $\varnothing_3$ and $\varnothing_4$

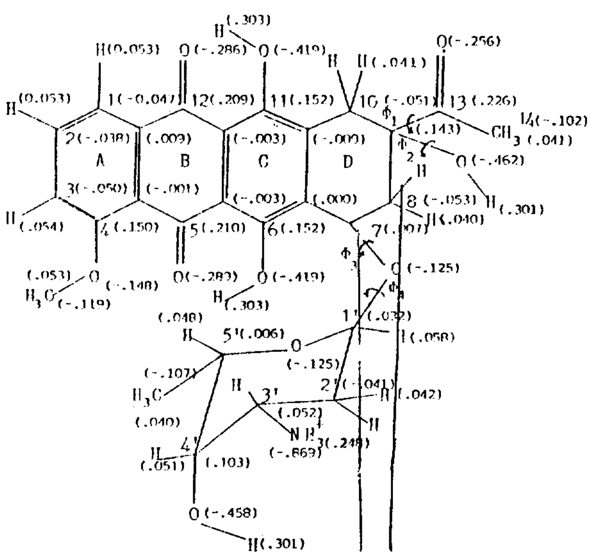

Figure 1. The notation for torsion angles of daunomycin. The values in a parenthesis represent the atomic partial charges.

shown in Figure 1 through maintaining the rest of the molecular structure in the crystal geometry[5,6].

We have carried out conformational analysis by varying four bond torsion angles $\varnothing_1$, $\varnothing_2$, $\varnothing_3$, and $\varnothing_4$ for various types of D-ring pucker. We have systematically investigated the conformation of daunomycin in free (vacuum), hydrated, and metal ion binding states using the empirical potential function and then compared our results with those of previous reports[4-11].

Potentials

Conformational Potential Function. The total conformational energy E_{tot} was calculated as the sum of terms representing electrostatic (E_{el}), polarization(E_{pol}), nonbonded (E_{nb}), torsional (E_{tor}), and hydrogen bond energies (E_{hb});[12,13]

$$E_{tot}=\sum_{nb\ pairs}(E_{el}+E_{pol}+E_{nb})+\sum_{torsions}E_{tor}+\sum_{hb\ pairs}E_{hb} \quad (1)$$

Explicit functional forms of each energy are described in the Appendix. In the case of the free state, a dielectric constant of 1 was used. For the hydrated state, a distance-dependent dielectric constant was used[13].

The equilibrium distance in nonbonded energy component was assigned to be a distance approximately 0.2 Å greater than the sum of the van der Waals radii of the interacting atoms.[13] Atomic partial charges were obtained from the so-called σ-π method[14]. Atomic partial charges on the atoms of daunomycin are represented in Figure 1.

Throughout our calculation, we have employed fixed bond distance and angle geometry, since the several precise crystallographic studies indicate that the bonding geometry does not vary in diverse crystallographic environments[15].

Details of the nature of potential energy functions and parameters are well described in the previously noted references.[12,13]

Strain Potential Energy. The strain energy (E_{st}) of D-ring of daunomycin is the sum of terms representing bond stretching (E_{bs}), bond bending (E_{bb}), and torsional energies (E_{tor})[16,17].

$$E_{st}=E_{bs}+E_{bb}+E_{tor} \quad (2)$$

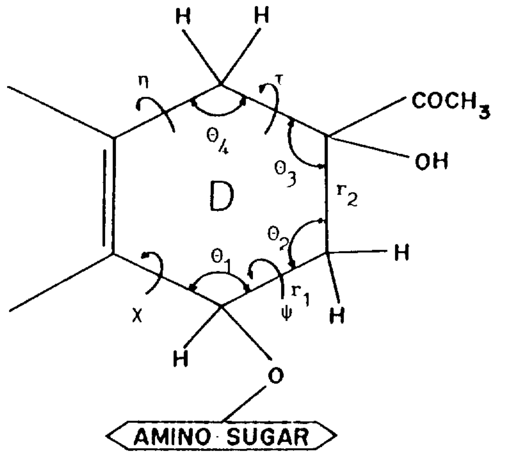

Figure 2. Structural parameters to constrain the D-ring pucker in daunomycin.

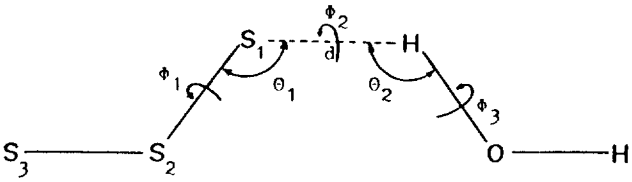

Figure 3. Geometrical parameters for water molecule.

Table 1. Properties[a], Calculated Ion-oxygen Distances, and Hydration Energies of Ions

Ion	α_i(Å^3)	I_i(eV)	R_w(Å)	$R_{M\text{-}O}$(Å)	R_{ab}(Å)[b]	$-\Delta E_{hyd}$[c]	$-\Delta E_{ab}$[b]
Mg^{2+}	0.12	80.14	2.41	2.03	2.07	395.9	396.5
Ca^{2+}	0.90	50.91	1.90	2.30	2.40	303.4	304.0
Cu^{2+}	1.00	36.83	1.32	2.04	2.00	411.2	408.7
Zn^{2+}	0.94	39.72	1.36	2.05	2.04	405.1	403.4

[a] α_i, I_i, and R_w are the polarizability, ionization potential, and van der Waals radius of ion, respectively. [b] The ion-oxygen distances and the hexahydrate energies for cations according to ab initio calculation[22]. [c] Values for energies in kcal/mole.

where

$$E_{bs}=\sum_{i=1}^{3}kr\,(\gamma_i-\gamma_0)^2$$
$$E_{bb}=\sum_{i=1}^{3}k\theta\,(\theta_i-\theta_0)^2$$
$$E_{tor}=\sum_{i=1}^{5}(V_i/2)\,(1+\cos n_i\chi_i)$$

The bond length r_i bond angle θ_i and torsional angles (χ, ψ) are defined in Figure 2. The parameters of function evaluated by Brant was used[17]. Since the rest of variables in Figure 2 can be represented by r_1, θ_1, r_2, and θ_2, the strain energy is the function of four variables in a given point (χ, ψ) and the gradient of strain energy for each variable can be obtained. The potential map of $E_{st}(\chi, \psi)$ can be obtained by using the conjugate gradient method in 500 (χ, ψ) values[18].

Computations

The coordinates of a water molecule bound to biomolecule can be expressed with six external variables (one distance, two bond angles, and three torsion angles) if the coordinates of three atoms to which a rigid water molecule is attached are known. The external variables are defined in Figure 3[12,13].

In order to study the effect of a divalent cation on the daunomycin we must obtain parameters used to calculate the binding energy of cations. The polarizabilities[19], ionization potentials[20], and van der Waals radii of ions are summarized in Table 1. The van der Waals radii of cations in this work were optimally obtained by fitting the ion-oxygen distance[21] and the hexahydrated energy for cation to the result of ab initio computation[22]. The charge of all ions in the calculation is fixed as +2:

The notation for torsion angles of daunomycin is illustrated in Figure 1. Torsion angles of daunomycin for each local energy minimum of D-ring were freely rotated during minimization by using quasi-Newton method by Fletcher[23]. The starting points for minimization of the total energy of each conformation were determined by the refinement of those geometries varying each torsion angle of the backbones one after another[12].

In order to see the conformational change in a hydrated state at each local minimum of D-ring, water molecules were systematically bound to all hydrophilic sites of daunomycin. The hydration schemes were initially taken from the coordinates for the bound water which were obtained from our previous researches for the hydration sites of various biomolecule[12,13]. We achieved the hydration according to the optimization of each water molecule around the daunomycin. Finally, the total interaction energy of each conformer was minimized by varying all torsion angles of daunomycin to which water molecules are bound.

In order to search the binding sites of cation on the daunomycin, the cation is initially scanned around daunomycin by r, θ, and $\varnothing$ of polar coordinate and then the binding energy map of metal ion was obtained by varying θ, $\varnothing$ at several r values. The binding energy in the map was calculated by a distance-dependent dielectric constant. Next, the metal ion at each local minimum was optimized by varying three variables by Fletcher procedure[23]. The water molecules bound to metal ion are initially optimized from the octahedral structure. And the optimization positions of water molecules in a hydrated state are chosen as starting positions in optimizing the rest of water molecules.

All energy minimizations in this work are carried out using the quasi-Newton method developed by Fletcher[23] with a convergence criterion of 0.0001 kcal/mol. The increment step lengths were 1×10^{-5} Å for distance and 0.001° for all angles. The maximum number of iteration was 300 cycles.

Results and Discussions

The potential map for the pucker of D-ring is shown in Figure 4 and conformational geometries and strain energies of D-ring at local energy minima are represented in Table 2. Four variables of pucker in all conformers are similar but two torsion angles χ and ψ are very different. Specially, since the strain energy of each local minimum is low, the interconversion in D-ring is easily occurred. As shown in Figure 4, α conformer is the most stable and boat forms B and B′ are obtained near α and β conformers.

Recently, Kollman has proposed that the interconversion of D-ring is occurred through one intermediate minimum[5]. But we found that there are four local minima in the pathway for interconversion in D-ring of daunomycin.

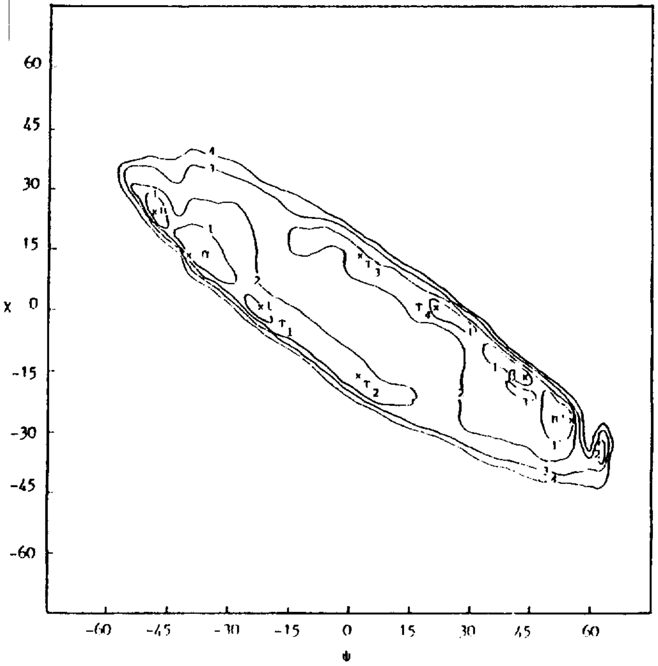

Figure 4. The potential map for pucker of D-ring in daunomycin.

Table 2. Conformational Geometries and Strain Energies of D-ring in Daunomycin at Local Energy Minima[a]

Conformation	χ	ψ	r_1	θ_1	r_2	θ_2	E_{st}[b]
B	24	-50	1.52	105.2	1.53	105.2	0.8
α	14	-40	1.52	106.6	1.53	112.2	0.0
T_1	0	-24	1.52	108.0	1.53	115.0	0.5
T_2	-15	-1	1.52	108.3	1.53	117.0	1.4
T_3	15	1	1.52	108.3	1.53	117.0	1.4
T_4	0	24	1.52	108.2	1.53	114.5	0.5
β	-16	44	1.52	105.0	1.53	112.7	0.0
B′	-26	55	1.52	102.0	1.53	108.0	0.5

[a] Values for angles in degree, ones for distance in Å, and ones for energies in kcal/mole. [b] E_{st} is the relative strain energy of D-ring in daunomycin.

Table 3. Conformational Energies at Each Local Minimum in Free State

Conformation	$\varnothing_1$	$\varnothing_2$	$\varnothing_3$	$\varnothing_4$	E_{tot}
B	143	175	-136	-70	10.8
α	143	38	-140	-74	-3.8
T_1	144	27	-151	-79	-3.2
T_2	91	49	-167	-82	6.4
T_3	145	71	-166	-83	6.5
T_4	148	62	-171	-82	8.2
β	85	62	-176	-78	5.5
B′	78	61	-177	-75	10.1

Values for angles in degree, and ones for E_{tot} in kcal/mole.

Conformational energies at each local minimum in a free state are represented in Table 3. Although it is difficult to decide the order in intermediate minima, we suggested that

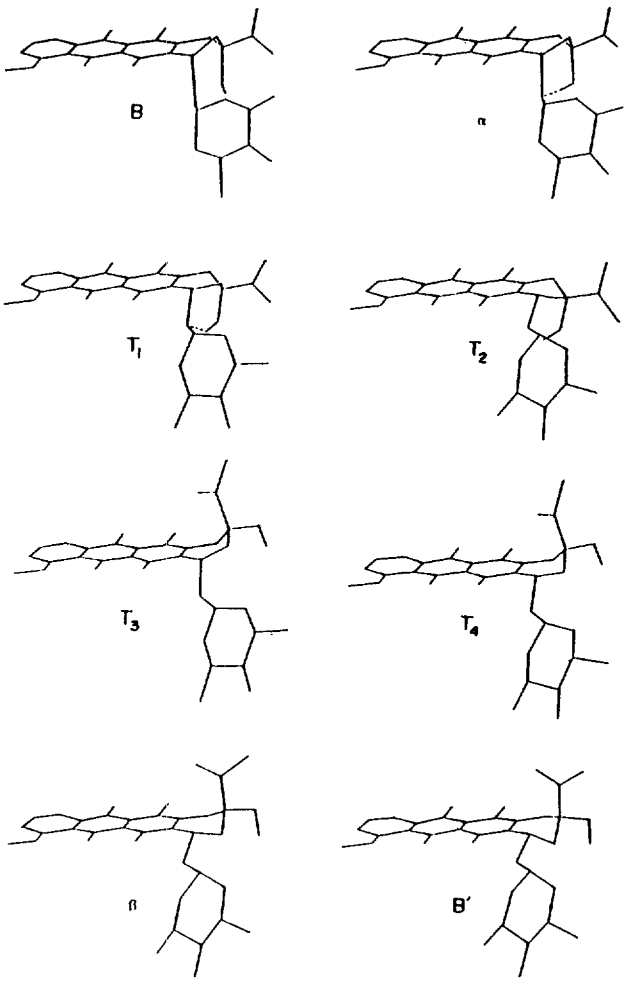

Figure 5. The conformations of daunomycin at each local minimum of D-ring in the free state.

Table 4. Torsion Angles and Energy Components of Each Conformer of Daunomycin in the Hydrated State[a]

Conformation	$\varnothing_1$	$\varnothing_2$	$\varnothing_3$	$\varnothing_4$	Dau[b]	Dau+W[c]	W+W[d]	E_{tot}
B	139	174	-139	-69	10.9	-71.1	-1.4	-61.6
α	142	38	-142	-74	-3.3	-71.2	-2.4	-76.9
T_1	144	27	-152	-77	-4.0	-70.0	-3.7	-77.7
T_2	95	50	-166	-80	5.8	-81.9	-6.9	-83.0
T_3	147	69	-163	-83	5.7	-89.9	-1.9	-86.1
T_4	154	61	-168	-81	8.7	-90.3	-0.7	-82.3
β	68	62	-177	-84	7.6	-90.1	-3.7	-86.2
B'	75	62	-177	-77	11.0	-72.5	-2.1	-63.6

[a] Values for angles in degree and ones for energies in kcal/mole. [b] Dau represents the intramolecular interaction of daunomycin. [c] Dau+W represents the interaction energy between daunomycin and water molecules. [d] W+W represents the interaction energy between water molecules around daunomycin.

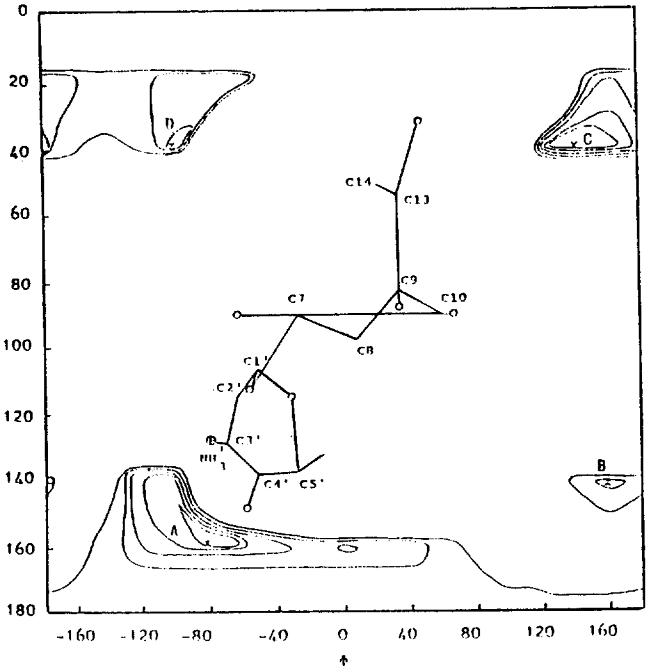

Figure 6. The binding energy map of Mg^{2+} ion bound to daunomycin.

the conformational change of α ↔ β can be occurred through the pathway for $T_1 \leftrightarrow T_2 \leftrightarrow T_3 \leftrightarrow T_4$ as shown in Figurs 4 and 5. From the result, α and T_1 conformers having intrahydrogen bond between H9 and 07 are more stable than other conformers. Comparing the torsion angles $\varnothing_3$ and $\varnothing_4$ with those of X-ray crystallography, our result of α conformer is in good agreement with that of Quigley et al.[10] showing that angles of $\varnothing_3$ and $\varnothing_4$ are -148° and -78°, respectively. In α conformer, C8 is the most out of plane atom and there is a hydrogen bond between 07 oxygen and C9 hydroxy group. On the other hand, in β conformer, C8 lies behind rather than in front of the plane of the ring system and then the amino-sugar becomes oriented equatorially rather than axially.

Since the hydration effect of biomolecule exclusively arise from the first hydration shell water molecules around the biomolecule, the hydration of daunomycin can be restrictedly performed in the region of the first hydration shell[12,13].

Torsion angles and energy components of each conformer are shown in Table 4. Comparing these results with those of free state, we found that the conformer having a minimum energy changes from α state to β state. The energy loss in terms of a broken intra-hydrogen bond is compensated by the hydrogen bond energy of water molecule bound to 07 oxygen. In Table 4, when the daunomycin is hydrated, the number of water molecule for each conformer is six for B, α, T_1 and B' and seven for T_2, T_3, T_4 and β. This means that the intra-hydrogen bond and H8 hydrogen inhibit water molecules bound to 07 oxygen of daunomycin.

Although the torsion angle $\varnothing_1$ of β conformer is greatly varied, torsion angles of other conformers are not nearly varied according to the hydration. The finding of stable β conformer in a hydration state can be the strong evidence to the existence of β conformer by NMR measurements in aqueous solution[4,5].

It is interesting to see the conformational change in electrolyte solution, because physicochemical properties of daunomycin are varied by metal ions[24-26]. In this work, the binding energy map of Mg^{2+} ion in β conformer is shown in Figure 6. We found that the order of decreasing binding energy to four binding sites is A > C > B > D regardless of the pucker of D-ring and metal ion binding to daunomycin occurs primarily at the anthracycline chomophore and little or no binding occurs at the amino group. We calculated the conformational change of daunomycin having Mg^{2+} ion bound

Table 5. Torsion Angles and Energy Components of each Conformer of Daunomycin in Mg^{2+} Binding State[a]

Conformation	$\varnothing_1$	$\varnothing_2$	$\varnothing_3$	$\varnothing_4$	Dau	Dau + Ion + W[b]	Ion + W[c]	W + W	E_{tot}
B	120	156	-132	-76	9.7	-147.2	-233.7	4.2	-367.0
α	144	37	-139	-80	-4.5	-121.7	-277.8	7.7	-396.3
T_1	145	16	-149	-80	-4.9	-127.0	-272.9	8.2	-396.6
T_2	95	48	-182	-82	6.5	-157.2	-246.5	5.5	-391.7
T_3	146	71	-158	-87	5.2	-147.6	-260.5	2.9	-400.0
T_4	149	62	-172	-81	8.4	-153.9	-254.0	9.7	-389.8
β	85	62	-176	-78	6.6	-167.4	-277.8	13.6	-425.0
B′	78	80	-177	-79	10.6	-137.5	-280.2	12.4	-394.7

[a] Values for angles in degree and ones for energies in kcal/mole. [b] Dau + Ion + W represents the interaction energy between daunomycin, ion and water molecules. [c] Ion + W represents the interaction energy between ion and water molecules.

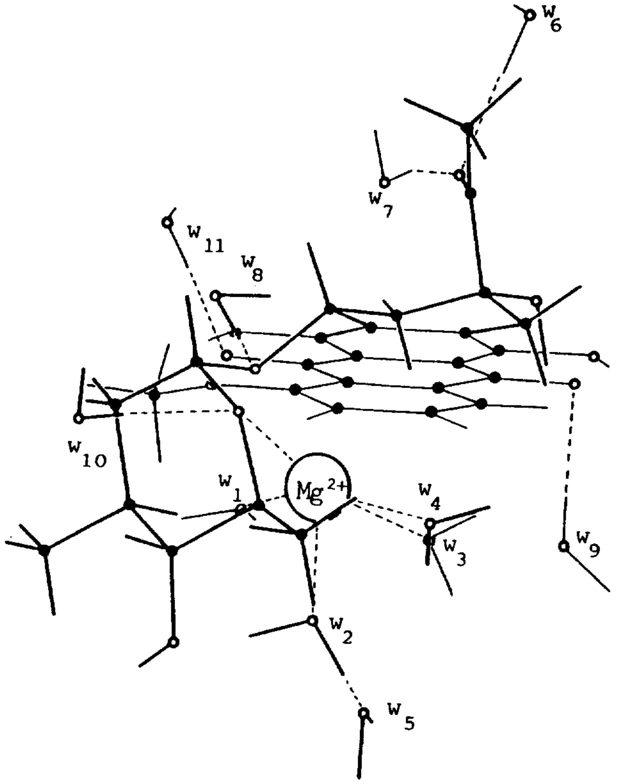

Figure 7. The site of Mg^{2+} ion bound to β conformer of daunomycin.

Table 6. Torsion Angles and Energy Components of β Conformer of Daunomycin in four Ions Binding States[a]

Ion	$\varnothing_1$	$\varnothing_2$	$\varnothing_3$	$\varnothing_4$	Dau	Dau + Ion + W	Ion + W	W + W	E_{tot}
Ca^{2+}	85	64	-180	-79	6.7	-184.8	-200.4	14.9	-363.6
Mg^{2+}	85	62	-176	-78	6.6	-167.4	-277.8	13.6	-425.0
Zn^{2+}	85	62	-176	-78	6.6	-187.5	-264.3	14.5	-430.7
Cu^{2+}	85	62	-176	-78	6.6	-178.2	-278.0	10.2	-439.4

[a] Values for angles in degree and ones for energies in kcal/mole.

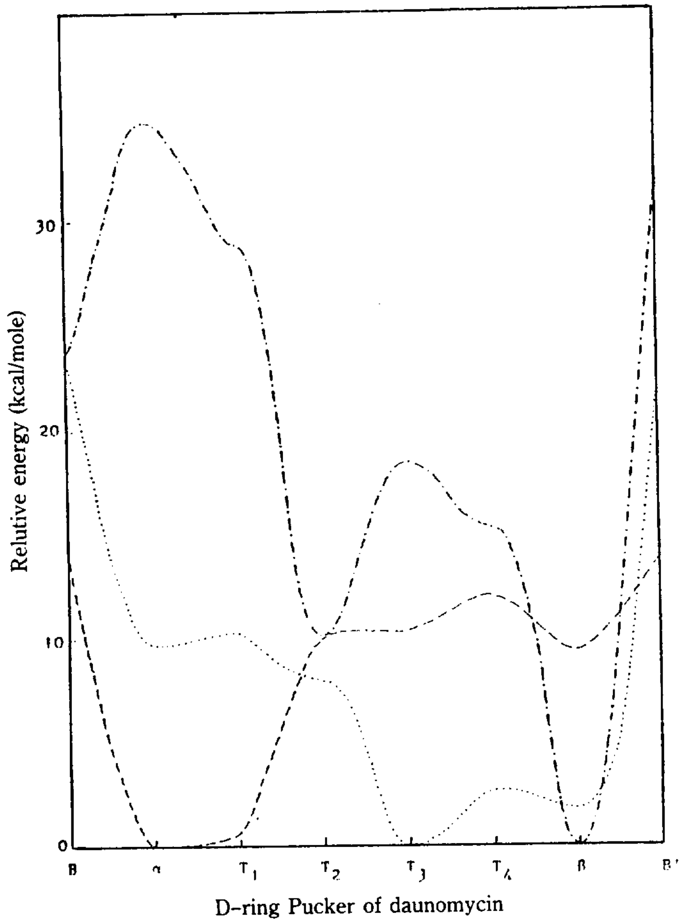

Figure 8. The relative conformational energies of daunomycin at each local minimum of D-ring in free (-----), hydrated (--------), and Mg^{2+} binding state (-·-·-).

to site A. Torsion angles and energy components are shown in Table 5. Compared with other states, the torsion angle of each conformer of daunomycin in Mg^{2+} binding state are not varied. The optimum conformation of daunomycin in Mg^{2+} binding state is found to be β conformer.

The number of water molecules for each conformer is ten for B, α, T_1, and B′ and eleven for T_2, T_3, T_4, and β. After the optimization, Mg^{2+} has four bound water molecules and makes one direct contact to 07 oxygen of ether linkage connecting the chromophore and amino sugar.

As shown in Figure 7, since the hydrated Mg^{2+} ion performes a complex in the empty site between the chromophore and sugar group, the stability of β conformer of daunomycin is more increased.

In order to compare Mg^{2+} ion with other ions, we chosen Cu^{2+}, Zn^{2+}, and Ca^{2+} ions which have a important role in biological system. Since these cations are also bound to same

Table 7. Torsional Potential Barriers

Bond Type	Barriers(kcal/mole)
C — O	0.86[a]
C — C	0.80[b]
C - O	0.28[c]

[a]Value for C — O bond of nucleic acid backbone. [b]6 — fold symmetry. [c]Value for C — O bond of peptide.

site of daunomycin according to potential maps, conformational change of daunomycin and their binding energies can be calculated in β conformer which is the most stable in Mg^{2+} ion binding state. Torsion angles of daunomycin and energy components for each ion are represented in Table 6. The order of decreasing total energy in cation binding state is given by $Cu^{2+} > Zn^{2+} > Mg^{2+} > Ca^{2+}$, which is in good agreement with the order of the experimental stability constant[26].

Since all ions form strong complex with the oxygen of the aglycone portion in the daunomycin and the sugar moiety, the cations can cause the release of the antibiotic into solution as a simple metal ion-anthracycline complex[25]. One can see that the stronger the metal ion-drug complex is, the larger the possibility of separation of the antibiotic from DNA is.

Conclusion

Considering the effect of binding on the physical properties of the drug bound to DNA, daunomycin can not be reduced polarographically and does not give a bathochromic shift on basification, showing that drug molecules are removed from the aqueous environment[27]. Similarly, our results show that the optimum conformation of daunomycin is altered from β conformer to α conformer via the dehydration.

Since the hydration energy of the excluded water molecule from the first hydration shell by the conformational change of daunomycin is not so large, it is possible to compare the hydration energy of daunomycin regardless of the difference of the number of water molecules in the first hydration shell. Figure 8 represents the relative conformational energies of daunomycin at each local minimum of D-ring in three states. In the free state which corresponds to X-ray structure, α conformer is the most stable, while in the hydrated and Mg^{2+} binding states, T_3, β conformer are the most stable, respectively. Our results are the strong evidence for the existence of β conformer by NMR measurement in solution and can explain the role of metal ions in the binding between daunomycin and DNA.

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Appendix

Potential Function

(a) The electrostatic energy is calculated by using Eq. Al.

$$E_{el}=q_i q_j/\gamma_{ij} D \qquad \text{(A1)}$$

where r_{ij} is the distance between atoms i and j, q_i and q_j are the partial charges on atoms i and j, respectively.

(b) The polarization energy is given by

$$E_{pol}=-\frac{1}{2}\bar{\alpha}_i(\vec{\varepsilon}_i \cdot \vec{\varepsilon}_i) \qquad \text{(A2)}$$

where $\bar{\alpha}_i$ is the atomic static polarizability of the atom i[28] and $\bar{\varepsilon}_i$ is the electric field created at the atom i by all surrounding atoms in the interacting molecule, which is given by the following expression.

$$\vec{\varepsilon}_i=\sum_i q_j \vec{r}_{ij}/r_{ij}^3 \qquad \text{(A3)}$$

where $\bar{r}_{ij}$ is the vector joining the atoms i and j.

(c) The nonbonded energy is given by Eq. A4

$$E_{nd}=-K_6(i,j)/Z_{ij}^6(1-0.5/Z_{ij}^6) \qquad \text{(A4)}$$

where $K_6(i,j) = C(i,j)/r_{ij}^{o6}$ and $Z_{ij} = r_{ij}/r_{ij}^o$, r_{ij}^o is the sum of the van der Waals radii of the interacting molecules and C(i, j) is calculated by using London–Margenau formulas[28].

(d) Torsional energy is assumed to have n–fold periodicity such as

$$E_{tor}=\frac{V_k^\circ}{2}(1+\cos n\theta_k) \qquad \text{(A5)}$$

where V_k° is the rotational barrier of the kth torsion angle θ_k, which is obtained from the experimental data[29] and is shown in Table 7.

(e) Hydrogen bond energy is given by[30]

$$E_{hb}(r,\phi)=E_{hb}^\circ(r)\cdot A(\phi) \qquad \text{(A6)}$$

and $E_{hb}^\circ(r)=De(1-(r/r_e)^n \exp(-a(r/r_e-1)))^2-De$

$$A(\phi)=b(\cos^2\phi-1)+1$$

where D_e is the dissociation energy, r is the internuclear distance between atoms directly involved in a hydrogen bond, and r_e is its equilibrium distance. Ø is the bent angle of hydrogen bond and b is a cnstant whose value was taken as 0.456 except for hydrogen bond between water molecules as 0.027. When the angle Ø is less than 120° or hydrogen bond distance is longer than the critical distance r_c, n and a satisfy the following relationship.

$$a r_e=n+\Delta^{1/2} \qquad \text{(A7)}$$

where Δ is Sutherland parameter[31]

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Liquid Chromatographic Resolution of Racemic Drugs on Various α-Arylalkylamine Derived Chiral Stationary Phases

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After conversion to their 3,5-dinitrobenzoyl or 3,5-dinitroanilide derivatives, the enantiomers of a number of drugs may be chromatographically separated on various α-arylalkylamine-derived chiral stationary phases (CSPs). While each CSP used in this study is useful, CSP **1** is best able to resolve the 3,5-dinitroanilide derivative of Ibuprofen while CSP **9** generally gives rather large α values for the resolution of 3,5-dinitrobenzoyl derivatives of the enantiomers of β-adrenergic blocking drugs.

Introduction

Direct chromatographic resolution of enantiomers on chiral stationary phases (CSP's) is now understood as the most rapid, sensitive, accurate and convenient means for determining enantiomeric purity and absolute configuration. In addition, this technique provides the preparative means for obtaining enantiomerically pure samples which may be needed for a variety of purposes. Owing to their potential utility, the development of effective CSPs has challenged a great many workers[1].

In recent years, our efforts have been focused on the development of new CSPs and the application of these CSPs to the resolution of various racemates. In the course of this work, we prepared α-arylalkylamine derived CSPs **1-9** which prove to be able to resolve the enatiomers of a wide variety of racemates[2-5]. As a general rule, the enantiomers separated by these CSPs must initially be derivatized with an appropriate achiral π-acid reagent owing to the chiral recognition requirements of these CSP.

Among various racemates which are resolvable upon CSP **1-9**, are a number of interesting drugs. It is well known that enantiomers of drugs frequently show quantitatively different pharmacological properties, possibly because one enantiomer better fits the receptor site that does the other[6]. For the study of pharmacokinetics of drug enantiomers, chromatographic determinations of enantiomer concentrations on chiral columns have been suggested as a superior method[7].

We have briefly reported the resolution of several racemic β-adrenergic blocking drugs on CSP **7**[3]. Since each of CSPs **1-9** shows somewhat different abilities to separate drug enantiomers, we now compare the ability of the various α-arylalkylamine-derived CSPs to separate the enantiomers of selected drug derivatives.