= 1, 8 Hz, C_4H), 8.19 (1 H, d, J = 8 Hz, C_3H), 8.22 (1 H, d, J = 8 Hz, C_8H), 8.56 (1 H, d, J = 1 Hz, C_1H); MS m/z 522 (MH^+); HRMS found 522.2220, calcd for $C_{28}H_{31}N_3O_7 \cdot H^+$ 522.2240.

3k: pale yellow solids; mp 184–186 °C; UV λ_{max} 238, 280 (sh) nm; fluorescence λ_{max} 408, 438, 473, 513 nm; IR ν_{max} 3498 (OH), 3318 (NH), 1726, 1674, 1651 cm^{-1} (C=O); ^1H NMR δ 1.23 (3 H, s), 1.25 (3 H, s), 1.30–1.50 (4 H, m), 1.75 (2 H, m), 2.16 (3 H, s), 2.77 (1 H, m), 3.60 (1 H, m), 4.18 (1 H, m), 4.77 (1 H, d, J = 7 Hz, $GlyC_\alpha H$), 4.88 (1 H, m), 5.19 (1 H, m), 6.05 (1 H, s), 6.46 (1 H, d, J = 7 Hz), 6.91 (1 H, s), 7.55 (1 H, dt, J = 4, 9 Hz, C_7H), 7.69 (1 H, dt, J = 1, 7 Hz, C_6H), 7.78 (1 H, d, J = 7 Hz, C_5H), 8.23 (1 H, d, J = 7 Hz, C_9H), 8.33 (1 H, d, J = 8 Hz, C_4H), 8.55 (1 H, d, J = 2, 8 Hz, C_3H), 8.80 (1 H, d, J = 1.5 Hz, C_1H); MS m/z 522 (MH^+). Anal. Calcd for $C_{28}H_{31}N_3O_7 \cdot {}^1/_2H_2O$: C, 63.39; H, 6.08; N, 7.92. Found: C, 63.22; H, 5.81; N, 7.74.

3m: pale yellow solids; mp 149–151 °C; UV λ_{max} 229, 275 (sh) nm; fluorescence λ_{max} 404, 443, 446, 504 and 419, 452, 488, 530 nm; IR ν_{max} 3388 (OH, NH), 1724, 1662 cm^{-1} (C=O); ^1H NMR δ 1.47 (6 H, s), 1.56 (4 H, m), 1.58 (6 H, s), 1.85 (2 H, m), 1.90 (3 H, s), 3.23 (2 H, m), 4.38 (1 H, m), 4.58 (1 H, m), 4.89 (1 H, d, J = 8.5 Hz, $GlyC_\alpha H$), 6.01 (1 H, s), 6.16 (1 H, d, J = 8.5 Hz), 6.34 (1 H, s), 6.38 (1 H, t, J = 5.5 Hz), 6.73 (1 H, s), 7.53 (1 H, dt, J = 1, 7.5 Hz, C_7H), 7.70 (1 H, dt, J = 1, 8 Hz, C_6H), 7.99 (1 H, d, J = 8 Hz, C_5H), 8.10 (1 H, d, J = 8 Hz, C_4H), 8.18 (1 H, dd, J = 1, 8 Hz, C_9H), 8.32 (1 H, dd, J = 2, 8 Hz, C_3H), 8.88 (1 H, d, J = 2 Hz, C_1H); MS m/z 607 (MH^+); HRMS found 607.2788, calcd for $C_{32}H_{38}N_4O_8 \cdot H^+$ 607.2768.

Measurement of Quantum Yield of Disappearance of Starting Anthraquinones 1. A 300-W high-pressure mercury projector lamp was used as a light source. A combination of Corning 7-54 glass filter and an aqueous K_2CrO_4 solution filter was used for isolation of 313-nm light.[20] Light intensity was determined by potassium ferrioxalate actiometry. An CH_3CN solution (1 mM) of starting anthraquinones 1 in a quartz cell under Ar was irradiated for 40 s; the conversion of 1 was less than 10%. The amount of 1 that disappeared was determined by measuring the amount of 1 before and after irradiation by HPLC (Cosmosil $5C_{18}$ column) with 40% H_2O and 60% MeOH as eluents. Methyl benzoate was used for a standard. Each quantum yield was an average value of more than two experiments.

Molecular Dynamics (MD) Calculations. All model building and calculations were performed on either a Silicon Graphics 4D/35 or a Cray YMP2E, using the program Discover (CVFF was employed as molecular mechanics force field and the dielectric constant = 1). Molecular structures of 1 were constructed by use of Builder module of Insight II, energy minimized and used as an initial structure for MD calculations. At 1000 K, MD were performed for 100 ps (1 fs/step). Conformational sampling was done every 1000 steps (1 ps), and the structure was energy minimized. On the 100 conformers, the distances C_9–C_α and C_{10}–C_α were estimated.

Abbreviations

Gly = glycine, Ala = alanine, Ser = serine, Aib = α-aminoisobutyric acid, DCU = dicyclohexylurea, HONSu = *N*-hydroxysuccinimide, HOBt = 1-hydroxybenzotriazole, AQ– = 2-anthraquinonyl.

Acknowledgment. We thank Mr. Kazunori Toma and Ms. Mizuka Tagashira of Asahi Chemical Industry Co., Ltd., for MD calculations and Mr. Kimiatsu Nomura of Kyoto University for the measurement of HRMS.

Supplementary Material Available: IR and ^1H NMR spectral data of Boc-protected compounds, ^1H NMR spectra for photoproducts 2 and 3, NOESY spectra of **2j** and **2k**, and the fluorescence spectrum of **3k** (18 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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Prediction of Ring Conformations of Indolactams. Crystal and Solution Structures

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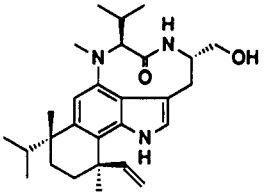
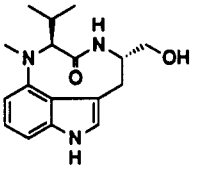
The preferred ring conformations of the nine-membered lactam rings of indolactams, which are extremely sensitive to substituent groups on the ring, were predicted by a high-temperature molecular dynamics calculation method. Two new ring conformations were predicted as preferred ones in two congeners, indolactam G and epiindolactam V. The predicted structures were validated by analyses of the crystal and solution structures by X-ray crystallography and NMR spectroscopy.

Introduction

Indolactam-V,[1-3] which has a partial structure of a potent tumor promoter teleocidin,[4] is known to be the minimum-sized tumor promoter. Both teleocidin (1) and indolactam-V (2) exist in an equilibrium of two conformational states of the nine-membered lactam ring, the twist and sofa forms, as shown in Figure 1, with a ratio of about 2:1 in methanol solution.[5] On the other hand, in the crystalline state, either of the conformations was alternatively found in several telecidin congeners,[6-8] although indolactam-V itself did not afford crystals suitable for crystal analysis.



teleocidin B-4 (1)



indolactam-V (2)

The twist form has a cis amide bond, whereas the sofa form has a trans amide. Since these two conformations

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(1) Endo, Y.; Shudo, K.; Okamoto, T. *Chem. Pharm. Bull.* **1982**, *30*, 3457–3460.

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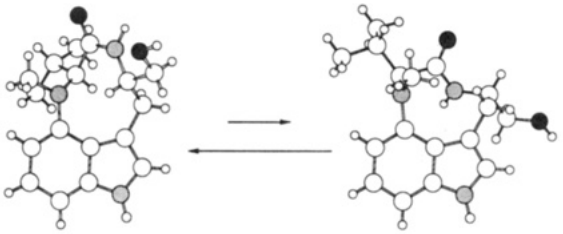
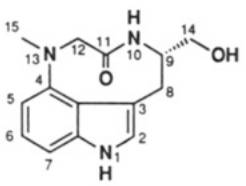
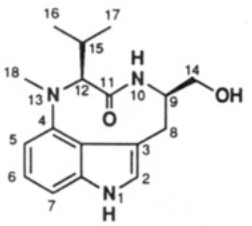


Figure 1. Equilibrium between the twist and sofa forms in indolactam-V.

are quite different, only one of them should be the active conformation in which teleocidins bind to the receptor protein. It is very important to determine the active ring conformation of teleocidins in order to explain the relationships between the structures and activities of several naturally-occurring potent tumor promoter compounds with various skeletal structures.[9] For investigation of the active conformation of teleocidin, a number of indolactam congeners were synthesized and their activity was tested.[10-12] It was revealed that all these congeners exist in different equilibria of at least two conformers, including unknown conformers, from the ^{1}H-NMR spectra. This implies that there are a number of possible ring conformers and that their relative stabilities greatly vary depending on the size of the substituent groups on the lactam ring. Among the congeners, two indolactams, indolactam-G (**3**)[1] and epi-indolactam-V (**4**),[1] were proved to exist in solution as major conformers with unknown structures, which were neither the twist nor the sofa form.



indolactam-G (3)

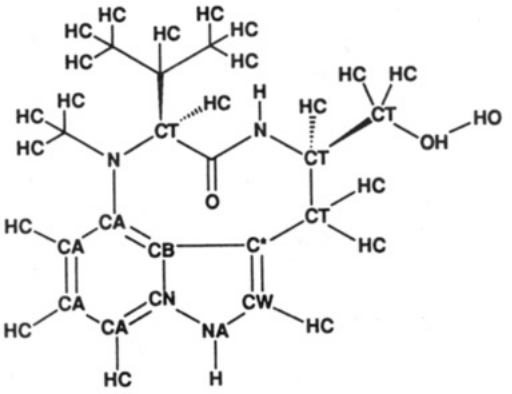


epi-indolactam-V (4)

For the purpose of predicting or analyzing complicated conformational features of this series of compounds, it is necessary to use a computational method which can give us a result independent of the starting structure. As it is very difficult to cover all the possible ring structures including conformational freedoms in substituent groups on the ring, molecular mechanics calculation is not efficient in this molecular system. The molecular dynamics (MD) calculation is much better in overcoming the local minima problem. However, it is still impossible to cover all possible conformers partitioned off by various energy barriers by MD calculation at 300 K. We have already reported that the existence ratio of the sofa form in four indolactam congeners as found by high-temperature MD (HTMD) calculation[13-15] has a good correlation with their biological activity.[16] In the previous paper, we showed that 10 different ring conformers can exist within 7.0 kcal/mol from the global minimum structure in indolactam-G, and the NOE observed in a signal of the major conformer can be well explained by the most stable calculated structure, which was new and named the fold form.

Here, we describe the further validation of the prediction by examination of NMR coupling constants and by X-ray crystal analysis for indolactam G, and the prediction of the preferred conformations, which were also validated by NMR studies and X-ray crystallography, for epi-indolactam-V.

Chart I



Methods

Model Building. Three-dimensional models of (−)-indolactam-G and (−)-epi-indolactam-V were prepared as the sofa form based on the crystal structure of olivoretin B (O-methylated teleocidin B-4).[7] All hydrogen atoms were relocated at the geometrically expected positions. The structures were optimized by the MNDO method in the MOPAC program version 5.0.[17] The formal atomic charges calculated from the optimized structures were used for the molecular dynamics (MD) calculations and subsequent molecular mechanics (MM) calculations.

Procedures of MD and MM Calculations. All the MD calculations were performed using AMBER program version 3.0 revision A.[18] All-atom type calculation was performed, assigning the atom-type to each atom as shown in the chemical structure in Chart I. The force field parameters used in the calculations are described later.

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The dielectric constant (ϵ) was assumed to be proportional to interatomic distances (r) as $\epsilon = r$.

First, each starting structure was subjected to energy minimization. After equilibration at 300 K for 10 ps, the temperature was gradually raised to 3000 K. This temperature was chosen on the basis of several trial-and-error tests, judging from the arrival at equilibrium between possible conformers. The MD calculations were performed at 3000 K with a time step of 1.0 fs. Solvent molecules were not included in the calculations. The frame data were stored every 50 steps, to result in 2000 frames in 100-ps simulation.

All the snapshot structures in the stored frames were energy-minimized by the conjugate gradient method with a convergence criterion of 0.01 kcal/mol. The double-precision energy-minimization program in the AMBER was modified so as to minimize all snapshots successively.

Classification of Energy-Minimized Structures. The energy-minimized structures with energy values within 7.0 kcal/mol from the global minimum were classified into the typical ring conformations. Classification was performed by comparing the conformational similarity in terms of torsion angles along the nine-membered ring with the template structures successively. Structures, between which all the differences in corresponding torsion angles were within a tolerance value of 30°, were regarded as the same ring conformation. Structures which were judged to be different from all of the existing template structures are added into the list of template structures, which contained only the first frame structure at the beginning of the classification. After that, the lowest energy structure was chosen as the representative one for each ring conformation. The existence ratio of each ring conformation at 300 K was calculated by means of the following equation, assuming that each conformation exists independently and the entropy differences among them are negligible:

$$K = \exp(-\Delta H/RT) \quad (1)$$

where K is the existence ratio, ΔH is the energy difference (kcal/mol), R is the gas constant (1.987×10^{-3} kcal mol^{-1} K^{-1},) and T is the temperature (300 K).

Force Field Parameters. The force field parameters related to the anilide nitrogen in the nine-membered ring, which were not provided in the AMBER program, were settled in the following way. At first, the parameters were determined so the various geometries of the nine-membered rings found in teleocidin crystals would be reproduced by the MM calculation of the sofa and twist forms of indolactam-V, and at the same time, the energy difference between the twist and sofa forms of 0.5 kcal/mol (which was estimated from the existence ratio of 2:1 in the ^{1}H-NMR spectrum of indolactam-V[5]) would be reproduced also. Iterative improvements of the parameters were performed between the preliminary MD calculations and subsequent MM calculations. The final force field parameters are listed in Table I; they satisfy the two requirements described above. The torsion angle differences in the nine-membered ring between the reproduced and the crystal structures were within 7.4 deg and the energy difference between the twist and sofa forms was 0.46 kcal/mol.

Prediction of Coupling Constants. The coupling constants between C-8 and C-9 hydrogens were calculated from dihedral angles of H8 (or H8′)–C8–C9–H9 by using an electronegativity sensitive Karplus equation as follows:[19]

$$^3J_{HH} = 13.86\cos^2(\phi) - 0.81\cos(\phi) + \sum\Delta\chi_i\{0.56 - 2.32\cos^2(\xi_i\phi + 17.9|\Delta\chi_i|)\} \quad (2)$$

Table I. Additional Force Field Parameters

dihedral angle	multiplicity	$V_n/2$ (kcal/mol)	γ (deg)	n
X–CA–NT–X	4	3.6	180.0	2
X–NT–CT–X	6	2.0	0.0	3
CA–NT–CT–CT	6	6.0	0.0	2
	6	4.8	0.0	1
CA–NT–CT–C	6	6.0	0.0	2
	6	4.8	0.0	1
H–N–C–O	1	2.5	180.0	2
	1	0.19	0.0	1

bond	K_r (kcal/mol·Å^2)	r_{eq} (Å)
CA–NT	427.0	1.392
CT–NT	337.0	1.463

angle	K_θ (kcal/mol·rad^2)	θ_{eq} (deg)
C–CT–NT	80.0	111.2
HC–CT–NT	35.0	109.5
CA–CA–NT	85.0	120.0
CB–CA–NT	70.0	123.5
CT–NT–CT	50.0	113.0
CA–NT–CT	50.0	113.0
CT–CT–NT	80.0	111.2

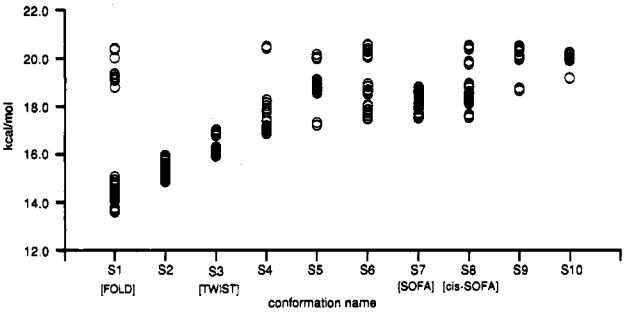

Figure 2. Energy dispersion map of indolactam-G.

where ϕ is the torsion angle, $\Delta\chi_i$ is the difference in electronegativity between the substituents of the H–C–C–H fragment under study and hydrogen, and ξ_i stands for +1 or −1 according to the orientation of the substituent. $\Delta\chi_i$ values used here were 0.4 for C-substituents and 0.9 for N-substituents.

Results and Discussion

Prediction of the Preferred Conformation in Indolactam-G. The results of HTMD calculation for indolactam-G can be summarized as follows.[20] The 2000 energy-minimized structures are classified into typical ring conformations as shown in Figure 2, which we named the "energy dispersion map".[16] As indolactam-G has two rotatable groups on the nine-membered lactam ring, a methyl group at N-13 and a hydroxymethyl group at C-9, there can be a large number of local minimum states due to conformational variation even for one ring conformation. The map shows how many local minimum structures belong to each ring conformation and how stable they are. In indolactam-G, 10 different ring conformations, S1–S10,

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(20) It was found that our results of HTMD conformational analyses were affected significantly by the precision of the energy minimization, although the changes were very small. The standard conditions of energy minimization in the AMBER program (single precision with a convergence criterion of 0.1 kcal/mol for a gradient norm) using the combined steepest descent and conjugate gradient seemed to be rather inaccurate for small molecules. In this paper, energy-minimization processes for indolactam-G and epi-indolactam-V were calculated by double precision with a convergence criterion of 0.01 kcal/mol using the same method. Consequently, the aspect of the energy dispersion map of indolactam-G is a little different from that in the previous paper.[16]

Table II. Predicted Existence Ratios and Geometries for the 10 Ring Conformers in Indolactam-G

	conformer (conformer name)									
	S1 (fold)	S2	S3 (twist)	S4	S5	S6	S7 (sofa)	S8 (cis-sofa)	S9	S10
energy (kcal/mol)	13.60	14.85	15.92	16.85	17.20	17.47	17.50	17.53	18.65	19.15
existence ratio at 300 K (%)	86.61	10.64	1.78	0.37	0.21	0.13	0.12	0.12	0.02	0.01
torsion angle (deg)										
ϕ(H8–C8–C9–H9)	−144.9	123.5	127.9	166.9	−48.8	−140.1	−61.5	−19.6	178.0	−19.3
ϕ(H8′–C8–C9–H9)	−29.7	−120.1	−117.4	−77.3	66.4	−24.8	54.8	94.9	−65.0	92.7
predicted coupling constant (Hz)										
J(H8,H9)	8.8	3.8	4.6	11.1	4.1	8.0	2.3	8.8	11.8	8.8
J(H8′,H9)	7.5	4.0	3.6	0.9	2.3	8.3	4.0	0.9	2.0	0.8
distance (Å)										
H9···H12	3.67	3.73	3.31	4.71	5.34	4.20	4.60	3.91	5.32	5.11
H9···H12′	2.39	2.15	4.06	4.58	4.52	5.19	4.67	5.10	4.53	4.05

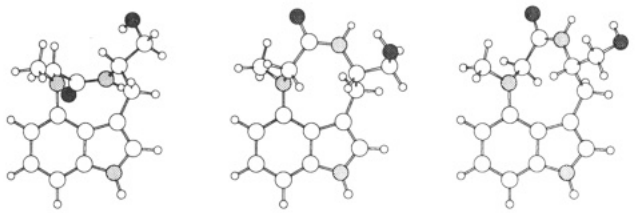

Figure 3. Structures of the three most stable conformations of indolactam-G.

were found within 7.0 kcal/mol from the most stable one. It can be seen from the map that energy values are dispersed in a wide range in each ring conformation. The lowest energy structure was taken as the representative structure of the ring conformer, and its energy value was assumed to show the global stability of the ring conformer in this study.

The most stable conformer S1 (named the fold form) is shown in Figure 3, together with the second most stable S2 and the third most stable S3 conformers. All of them have cis amide bonds. The twist and sofa forms, corresponding to S3 and S7, respectively, were not so stable in indolactam-G. The predicted existence ratios for the 10 ring conformers calculated from the energy differences between the conformers are listed in Table II. Their existence ratios at 300 K were S1 (fold form; 86.6%), S2 (10.6%), and S3 (twist form; 1.8%).

The ^{1}H-NMR experiments on indolactam G at −30 °C clearly revealed the coexistence of two conformers in CD_3OD. The ratio was observed as 1:0.2 from the peak areas of the two lower signals of doublet C-12 hydrogens at δ 3.94 for major conformer and δ 4.18 for minor conformer, respectively.[16] In the signals of the major conformer, the signals of C-8 hydrogens were split by the C-9 hydrogen with the coupling constants of 8.3 and 6.9 Hz, and an NOE was observed between hydrogen at C-9 and one of the C-12 hydrogens. The corresponding NMR data were calculated using the three-dimensional structures of the 10 ring conformers for comparison with the observed ones. The vicinal coupling constants (J(H8, H9) and J(H8′, H9)) were predicted from the torsion angles (H8–C8–C9–H9 and H8′–C8–C9–H9) and the two interatomic distances (H9···H12 and H9···H12′) were calculated, for each ring conformer, as also listed in Table II. Among the 10 ring confomers, only S1 and S2 structures have the interatomic distances (2.39 and 2.15 Å) satisfying the observed NOE. Furthermore, only S1 structure has coupling constants (8.8 and 7.5 Hz) which are consistent with the observed ones. Thus, the major conformer of indolactam G in solution was concluded to be the fold form, which was predicted to be the most stable conformer. The minor conformer in solution was supposed to be S2 conformer. S2 conformer is also a new conformer. The chemical shifts of observed signals of the minor conformer in the NMR spectra did not coincide with those of any conformers known as far. Although the structure determination of the minor conformer is not easy, the NMR data are consistent with the hypothesis that S2 is the minor conformer.

As a result of crystallographic analysis, it was proved that the crystal structure of indolactam-G was also the fold form, as shown in Figure 4 by an ORTEP drawing. The most stable predicted structure was quite similar to the

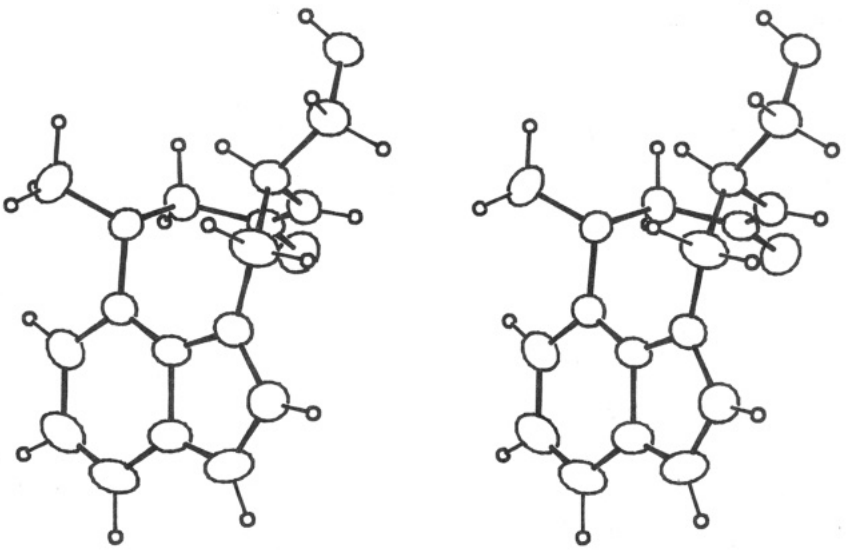

Figure 4. Stereoscopic ORTEP drawing of the crystal structure of indolactam-G.

Table III. Predicted Existence Ratios and Geometries for the 10 Ring Conformers of Epi-indolactam-V

	conformer (conformer name)									
	S1 (fold)	S2	S3 (twist)	S4	S5	S6	S7 (sofa)	S8 (cis-sofa)	S9	S10
energy (kcal/mol)			21.69	23.04	22.97	20.68	19.93	17.12	22.25	
existence ratio at 300 K (%)			0.05	0.00	0.01	0.25	0.88	98.79	0.02	
torsion angle (deg)										
ϕ(H8–C8–C9–H9)			−90.2	−65.3	76.8	−27.4	64.3	116.3	−54.5	
ϕ(H8′–C8–C9–H9)			21.5	49.9	−167.1	87.7	179.4	−127.7	59.7	
predicted coupling constant (Hz)										
J(H8,H9)			0.8	2.5	1.0	8.4	2.1	3.4	4.1	
J(H8′,H9)			8.5	4.0	11.1	0.7	11.9	4.6	2.5	
distance (Å)										
H9···H12			3.95	4.36	4.59	2.23	4.33	2.13	4.72	

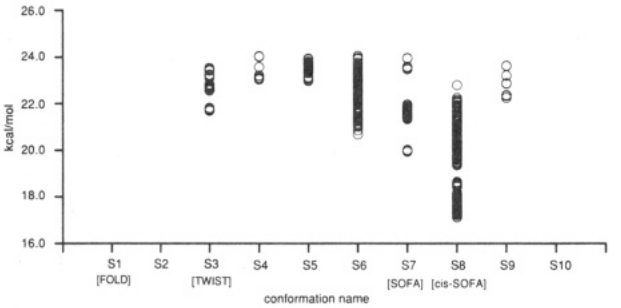

Figure 5. Energy dispersion map of epi-indolactam-V.

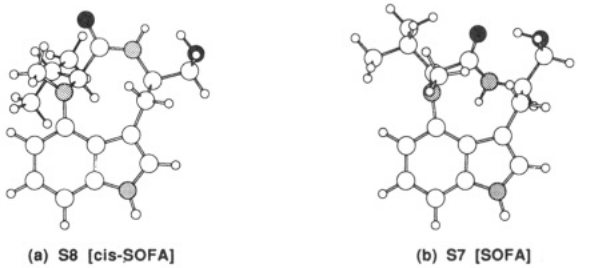

Figure 6. Structures of the two most stable conformations of epi-indolactam-V.

crystal structure, including the conformation of hydroxymethyl group at C-9, and the differences in the corresponding nine torsion angles along lactam ring between the predicted and the crystal structure were within 10.6°. Thus, it was confirmed that the predicted preferred structure of indolactam-G, the fold form, does exist both in solution and in the crystal.

Prediction of the Preferred Conformation in Epi-indolactam-V. The results of HTMD analysis of epi-indolactam-V are summarized in the same way as for indolactam-G, in Figure 5, which shows the energy dispersion map for epi-indolactam-V, arranging the ring conformers S1–S10 in the same order as in the map of indolactam-G.

The relative stabilities among the conformers in this molecule are quite different from those of indolactam-G and indolactam-V. The S1, S2, and S10 did not appear within 7.0 kcal/mol from the most stable structures. The global minimum structure, S8, is a new conformation different from the twist or sofa or fold forms. The structure, which was named the cis-sofa form, is shown in Figure 6, together with the second most stable conformer S7 (sofa form). The existence ratios at 300 K, which were calculated from the energy differences between conformers, were 98.8% cis-sofa form and 0.9% sofa form, as listed in Table III.

The ^{1}H-NMR experiments on epi-indolactam-V indicated the coexistence of two conformers in CD_3OD. The existence ratio was determined as 1:0.13 from the peak areas for methyl groups of the i-Pr substituent at C-12. The peak areas of respective methyl groups of i-Pr substituent showed their existence ratio as 1:0.13. In the signals of the major conformer, the coupling constants of the two C-8 hydrogens with the C-9 hydrogen were 2.2 and 3.7 Hz, and there was an NOE between the C-9 hydrogen and the C-12 hydrogen. The corresponding NMR data were predicted on the basis of the atomic coordinates of the seven ring conformers, as also listed in Table III. Conformers that satisfy the observed NOE are S6 and S8 structures (the interatomic distances are 2.23 and 2.13 Å). Of the two conformers, the observed coupling constants between the C-9 hydrogen and C-8 hydrogens can be explained only by S8 conformer (3.4 and 4.6 Hz). Thus, the major solution structure of epi-indolactam-V was concluded to be the cis-sofa form. The structure of the minor conformer can be presumed to be the second most stable, the sofa form. Although the deviation between the observed and predicted ratio of the minor conformer was rather large, there is no contradiction between the signals of the minor conformer and the known signals of indolactam-V.

From the crystallographic study, it was proved that molecules of epi-indolactam-V adopt the cis-sofa form in the crystal. The ORTEP drawing of the molecule is shown in Figure 7. As regards conformations of the two substituent groups, the i-Pr group at C-12 was in the similar conformation. The hydroxymethyl group at C-9 adopted a –gauche conformation in the predicted minimum energy structure, whereas a +gauche in the crystal structure. The –gauche conformation was more stable than +gauche conformation by 0.16 kcal/mol. The differences in the corresponding torsion angles along nine-membered lactam ring were less than 8.4° between predicted and crystal structures. Thus, it was confirmed that the new ring conformation predicted to be preferred in epi-indolactam-V, cis-sofa form, does exist both in solution and in the crystal.

Two new ring conformers were predicted as the most preferred ones for two indolactam congeners by HTMD calculation and subsequent analyses. The predictions were confirmed experimentally. The validity of the relative stabilities between conformers was supported by the NMR spectra and the three-dimensional structures of the most preferred conformers were demonstrated by X-ray crystal analyses. These results prove that the structures and relative stabilities of unknown, unexpected conformers can be predicted without any assumption about the probable conformers by the HTMD method. Usual computational search methods for covering possible ring conformations were less useful for this highly-strained cyclic system. As can be easily seen from both energy dispersion maps, there are a number of local minima structures in each ring conformer due to the conformational freedoms of the substituent groups on the ring. In addition, the relative stabilities between conformers are very sensitive to the size

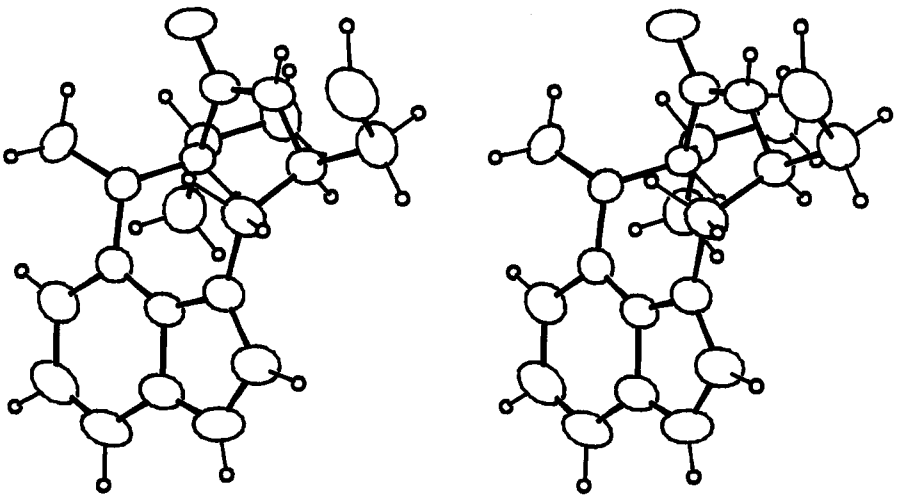

Figure 7. Stereoscopic ORTEP drawing of the crystal structure of epi-indolactam-V.

and orientation of substituent groups in indolactam congeners. In such molecular systems, the HTMD method seems to be very effective to predict preferred structures correctly. Needless to say, force field parameters should be examined in advance for reliable prediction. In this work, the two known conformers of indolactam-V were used for examining the validity of the parameters, structurally and energetically.

As for the use of high temperature, which is abnormal in the chemical sense, it is just for convenience in covering all possible conformers by overcoming the energy barriers. In the case of indolactam-V, the energy barrier between the sofa and twist forms, which is ascribed to cis–trans amide conversion, was estimated to be about 18 kcal/mol by variable-temperature NMR experiments.[5] It was confirmed that the interconversion occurred freely at 3000 K in 100 ps MD calculation, but scarcely occurred at 2000 K in indolactam-V. The interconversion between fold and twist forms in indolactam-G easily occurred at lower temperatures such as 1500 K or 2000 K, since the energy barrier between the two cis conformers is not so high. Rather high temperature would be favorable for covering all possible conformers, including unknown ones, in a short calculation time. Although deformations in geometries in the trajectorial structures at the molecular dynamics process were large, in proportion to the temperature, they could be corrected by the subsequent energy minimization (structure optimization) process.

It is not easy to determine structures only from NMR spectra of such molecules as indolactams, which have a small number of hydrogens and a great many allowed conformations. This paper shows that the HTMD method is useful for determining reliably the three-dimensional structure of the major conformer, to say nothing of the minor conformers. The NMR information (such as coupling constants, NOE distance, and benzene ring anisotropy as the case may be), which can be calculated on the basis of atomic coordinates of all possible conformers produced from HTMD calculations, will assist structure determination through comparison with the observed values.

It was shown that the HTMD method is effective for predicting preferred structures in indolactam congeners. The method should be useful in general for analyzing or predicting preferred conformations of cyclic compounds. Using this method we are attempting to design indolactams which can predominantly adopt a targetted ring conformation in order to investigate the active conformation for tumor-promoting activity of teleocidins.

Experimental Section

X-ray Crystal Structure Analyses. Crystal Data. (±)-Indolactam G: $C_{14}H_{17}N_3O_2$, M_r = 259.31, recrystallized from a mixed solution of methanol and acetone, monoclinic $P2_1/c$, cell parameters of a = 16.48 (2) Å, b = 6.399 (3) Å, c = 13.88 (1) Å, β = 113.50 (8)°, V = 1342.4 Å^3, Z = 4. The final R = 0.054 for 1938 observed reflections. (±)-Epi-indolactam-V: $C_{17}H_{23}N_3O_2$, M_r = 301.39, recrystallized from a mixed solution of methanol and ethyl acetate, monoclinic, $P2_1/n$, cell parameters of a = 15.248 (5) Å, b = 17.578 (5) Å, c = 6.010 (1) Å, β = 92.01 (2)°, V = 1609.7 Å^3, Z = 4. The final R = 0.054 for 2449 observed reflections.

The intensity data for both crystals were collected on a four-circle diffractometer using Cu Kα radiation monochromated by a graphite plate. The data were corrected for Lorentz and polarization effects but not for absorption effect. Both structures were solved by the direct method using the MULTAN program and refined by full-matrix least-squares calculation assuming anisotropic thermal motions for non-hydrogen atoms and isotropic ones for hydrogen atoms. The crystallographic results will be published in a specialized journal,[21] for discussion of the structures in comparison with those of teleocidins and olivoretins.

NMR Spectroscopy. The ^{1}H-NMR spectrum of (±)-epi-indolactam-V was published before.[5] Although the signals in the ^{1}H-NMR spectrum of (±)-indolactam-G at room temperature were so broad that they could not be interpreted, those in the spectrum measured at −30 °C were much sharper. The spectrum data have already been published.[1,16]

Supplementary Material Available: The Cartesian coordinate sets of the crystal structures and the minimum-energy structures of each conformer of indolactam-G and epi-indolactam-V (8 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(21) Kawai, T.; Endo, Y.; Yamaguchi, K.; Shudo, K.; Itai, A. *Acta Crystallogr., Sect. B*, submitted.