Active Conformation of a Tumor Promoter, Teleocidin. A Molecular Dynamics Study

Takatoshi Kawai, Tazuko Ichinose, Yasuyuki Endo, Koichi Shudo, and Akiko Itai*

Faculty of Pharmaceutical Sciences, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113. *Received January 13, 1992*

Teleocidins are potent tumor promoters, having a nine-membered lactam structure. Teleocidins and their small-molecular-sized active congeners (indolactams) are known to exist in an equilibrium between at least two conformational states, the twist and the sofa form. Molecular dynamics (MD) calculations were performed on four indolactams, in order to examine the relationships between preferred ring conformations and the biological activities. It was shown that the tumor-promoting activities are closely related with the existence ratio of the sofa form among 10 possible conformations. This implies that the sofa form is the active ring conformation, which is compatible with the previous result obtained independently from the superposition of teleocidin and phorbol ester. The predicted ratios of conformers for each indolactam were in good agreement with those observed by NMR spectral analysis. The high-temperature MD method proved to be very useful for predicting the preferred structures of these cyclic compounds, in which the overall stabilities are strongly influenced by the conformations of substituent groups on the ring.

Introduction

Since the discovery of the tumor-promoting activity of a 28,29-dihydro derivative of teleocidin B-4 (1, Chart I),[1] a number of investigations have shown that other members of the teleocidin family exhibit the same activity.[2] The structure of dihydroteleocidin B-4 was determined by Hirata and co-workers by X-ray crystallography.[3] The absolute stereochemistry was determined by comparison of the CD spectrum of teleocidin B-4 with that of (−)-indolactam-V (2), whose absolute stereochemistry was synthetically determined.[4] The ^{1}H NMR spectra of (−)-indolactam-V revealed that the molecule exists in two conformational states, "twist" and "sofa" forms.[5] The twist form has a *cis*-amide bond, whereas the sofa form has a *trans*-amide bond. Furthermore, all teleocidins and olivoretins (*O*-methyl derivative of teleocidin: inactive) were shown to equilibrate rapidly between the sofa and the twist forms in solution,[6,7] although a single crystal contains molecules in only one of the two conformations in the crystalline state.[7,8] The low-energy barrier between the two conformers (the observed activation free energy was $G^{\ddagger}$ = 19.2 kcal/mol),[5] and the short half-life of interconversion (estimated to be 1.2 s at 37 °C)[5] raised the question as to which is the important conformation for the biological activity.

The activity of (−)-indolactam-V is 0.1–0.01 of that of teleocidin B-4 in several kinds of bioassays, including skin tumor promotion.[9,10] The reduced but significant activity

Chart I

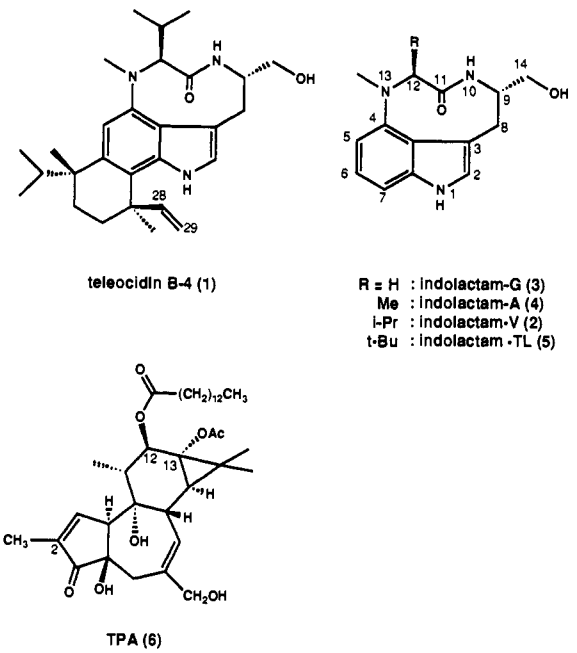

Table I. Biological Activities of Four Indolactams and Teleocidin B-4

compound	alkyl group at C-12	induction of HL-60 cell adhesion ED_{50} (mol/L)
(±)-indolactam-G	H	3.9×10^{-5}
(±)-indolactam-A	CH_3	3.7×10^{-6}
(±)-indolactam-V	C_3H_7	5.3×10^{-7}
(±)-indolactam-TL	C_4H_9	1.1×10^{-7}
teleocidin B-4		1.2×10^{-8}

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(8) Sakai, S.; Aimi, N.; Yamaguchi, K.; Hitotsuyanagi, Y.; Watanabe, C.; Yokose, K.; Koyama, Y.; Shudo, K.; Itai, A. Elucidation of the Structure of Olivoretin A and D (Teleocidin B). *Chem. Pharm. Bull.* **1984**, *32*, 354–357.

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clearly shows that the structure of (−)-indolactam-V satisfies the minimum requirement for a tumor promoter. The terpenoid side chain, which can be substituted by simpler alkyl groups without loss of activity, appears to be intimately involved in increasing the biological potency.[11] Methylation at N-1 diminished the activity, but N-prenylation or N-geranylation at N-1 increased the activity.[10] The methylation of the 9-CH_2OH group completely abolished the activity.[10] A study of stereoisomers of (−)-indolactam-V revealed that a single stereochemical inversion at C-9 or C-12 results in loss of activity.[9]

Several congeners of (−)-indolactam-V, which have various alkyl groups at C-12, were synthesized[12] and their biological activities were tested, in order to get information on the structure–activity relationships. Values (ED_{50}) of an important biological activity, induction of HL-60 cell adhesion, which is related to skin tumor promotion, for four racemic indolactams are compared in Table I.[13] The potencies increase in the order of (±)-indolactam-G (3), (±)-indolactam-A (4), (±)-indolactam-V (2), and (±)-indolactam-TL (5), and then teleocidin B-4 (1). Although the activities appear superficially to be correlated to the bulkiness of the substituent groups, there is an important stereochemical problem which should be considered. The ^1H NMR spectra showed that these four indolactams have different conformational characteristics in solution. Indolactam-V and -TL are in equilibria between the sofa and twist forms, although in different ratios;[12] indolactam-A is in the twist form only;[12] indolactam-G is in so far unidentified conformations different from the twist and the sofa forms.[14]

All the hydrophilic functional groups essential for the biological activity in indolactams are involved in the lactam ring as described above. The relative positions and orientations of the functional groups, which are the most important for specific binding to the target receptor, greatly vary depending on the ring conformation. So, the active ring conformation must first be identified, in order to interpret the structure–activity relationships and make clear the structural requirements for the tumor-promoting activity of the indolactams. This knowledge will help us to understand the essential features of all phorbol–teleocidin type tumor-promoting compounds.

Among various methods for computer-searching for possible conformations, the molecular dynamics (MD) calculation method is known to be useful for cyclic systems.[15-17] The high-temperature MD (HTMD) method

Chart II

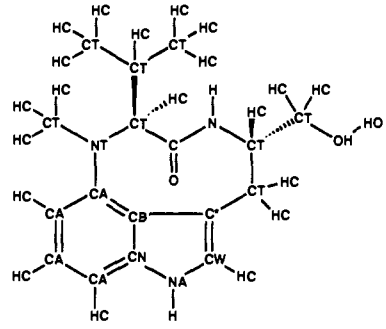

Table II. Additional Force Field Parameters

bond	Kr (kcal/mol·Å²)	r_{eq} (Å)
CA–NT	427.0	1.392
CT–NT	337.0	1.463

angle	Kθ (kcal/mol·rad²)	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

dihedral	multiplicity	Vn/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
CA–NT–CT–CT	6	4.8	0.0	1
CA–NT–CT–C	6	6.0	0.0	−2
CA–NT–CT–C	6	4.8	0.0	1

was chosen for the present purpose because of its efficiency for conformation searching in a conformational space with rather high energy barriers. The calculation provides us with energetically possible structures and their stabilities. Although it cannot afford the structure of the active conformation directly, the active conformation should be included within the possible structures for each compound, and the relative energy values will indicate how easily the molecule can adopt a given conformation. The active skeletal conformation should be identifiable by comparison of the conformational aspects of plural active compounds.

Here, we have performed MD calculation for the four active indolactams, in order to extract the common ring conformation which can explain the biological activity of teleocidins well.

Methods and Materials

All the MD calculations were performed using the AMBER program (version 3.0, revision A).[18] For the energy min-

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(18) Weiner, S. J.; Kollman, P. A.; Nguyen, D. T.; Case, D. A. An All Atom Force Field for Simulations of Proteins and Nucleic Acids. *J. Comput. Chem.* 1986, *7*, 230–252.

imization of the stored snapshot structures from the trajectory, a program in AMBER was modified, so as to perform calculations continuously. For the classification of the energy-minimized structures into the typical ring conformations, we have written a program to judge the conformational similarity for all energy-minimized structures according to torsion angles continuously. The atom types are shown in Chart II. The force field parameters used are those in the AMBER program, except for those listed in Table II. The dielectric constant was assumed to be proportional to distance: $\epsilon = r$.

Three-dimensional models of all indolactams (indolactam-G, -A, -V and -TL) were prepared on the basis of the crystal structure of olivoretin B.[7] All hydrogen atoms in the molecules were relocated at the geometrically expected positions. The structures were optimized by the MNDO method in the MOPAC program (version 5.0).[19] The formal atomic charges used for the MD and subsequent molecular mechanics calculations were obtained from those of the optimized structures.

First, the starting structures were energy-minimized using the same force field parameters as for the MD calculations. Then, molecular dynamics calculations were performed for equilibration at 300 K for 10 ps. The high-temperature MD calculations were carried out at 3000 K. The simulations were made for 100 ps with the time step of 1.0 fs. Solvent molecules were not included in the calculations. The frame data were stored every 50 steps, giving 2000 frames after a 100-ps simulation.

All the snapshot structures in the stored frame were subjected to energy minimization continuously. Then, the energy-minimized structures were classified into the typical ring conformations one by one according to the set of nine torsion angles of the nine-membered lactam ring. At the beginning of a classification, the template list of referential conformations contained the first frame structure only. Structures whose difference in every corresponding torsion angle was within a tolerance value of 30° were classified into the same ring conformation. If some of the differences were outside the given tolerance values, the structure was added to the template structure list as a new referential conformation. The classification was applied only to the structures whose energies were within 7.0 kcal/mol of the lowest energy. After the classification, we selected the lowest energy structures of each conformation set as the representative one. The existence ratio of each conformation at 300 K was calculated from the following equation, assuming that each conformation exists independently and entropy differences among them are negligible:

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

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

Results

The molecular structures of teleocidins and olivoretins in crystals were unusual in the geometry of the anilide nitrogen, showing intermediate hybridization between sp^2 and sp^3, and the lack of coplanarity of the two planes of the tertiary amine and benzene ring. These features could not be reproduced by molecular mechanics calculation using the parameters provided in the AMBER program. Then, for the anilide nitrogen, special force field parameters were set so that the bond angles and torsion angles

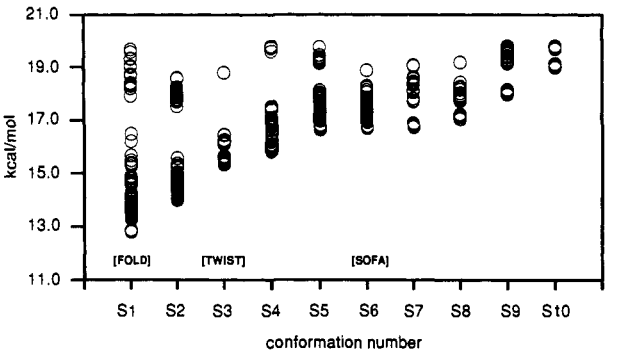

Figure 1. Energy-dispersion map of indolactam-G calculated by high-temperature molecular dynamics (HTMD) at 3000 K. The conformation numbers correspond to those in Table III. The energy dispersions of S1, S2, and S5 are clearly split into two bands. The conformations with an outer *N*-methyl group are distributed in the lower energy band, and those with an inner *N*-methyl group are in the higher one.

related to the nitrogen atom could be reproduced by molecular mechanics calculation. The validity of the parameters was further confirmed by the reproduction of the energy difference between the twist and the sofa forms of indolactam-V. From the existence ratio of the twist and the sofa forms (2:1) in solution of indolactam-V, the energy difference was estimated to be 0.5 kcal/mol.[5] Although the structures of the twist and the sofa forms of indolactam-V, modeled on the basis of the crystal structures of teleocidins and olivoretins, are the most stable ones in the crystal packing, they are not the most stable ones as a single molecule, because the internal rotations of the substituent groups (NCH_3, CH_2OH and isopropyl group) greatly affect the overall stability. Thus, the most stable structures for both forms were searched by preliminary MD calculation and subsequent analyses. The parameters, which are listed in Table II, could reproduce not only the unusual geometry related to the anilide nitrogen but also the energy difference between the twist and the sofa form structures, which were the most stable ones found in a preliminary MD run. As regards the temperature for the MD calculation, several temperatures were tested in preliminary calculations. Finally, the calculations were performed at 3000 K, so as to sufficiently overcome the energy barrier (19.2 kcal/mol) between *cis*-amide (the twist form) and *trans*-amide (the sofa form) of indolactam-V and -TL.

First, we made calculations on the simplest indolactam, indolactam-G, in order to cover all the possible ring conformations. The 2000 structures obtained by energy minimization of the MD trajectory were classified on the basis of similarity of the nine torsion angles along the lactam ring. They were classified into 10 independent ring conformations. Among the 10 ring conformations, six structures had a *cis*-amide bond and four structures had a *trans*-amide bond. Each ring conformation proved to include many secondary conformational states due to the orientations of the methyl group at N-13, based on nitrogen inversion, and the rotations of the CH_2OH group. Since the overall stability of a molecule depends not only on ring conformation but also on substituent conformation, the overall energy values in each ring conformation are dispersed over a wide range, as shown in Figure 1. The figure, which we call an "energy dispersion map", clearly shows that energy deviations within a ring conformation are much larger than those between ring conformations. The structure and energy of the minimum-energy conformational state in each ring conformation were taken as representative of the family. Thus, the stability of each ring conformation was expressed by a concrete energy

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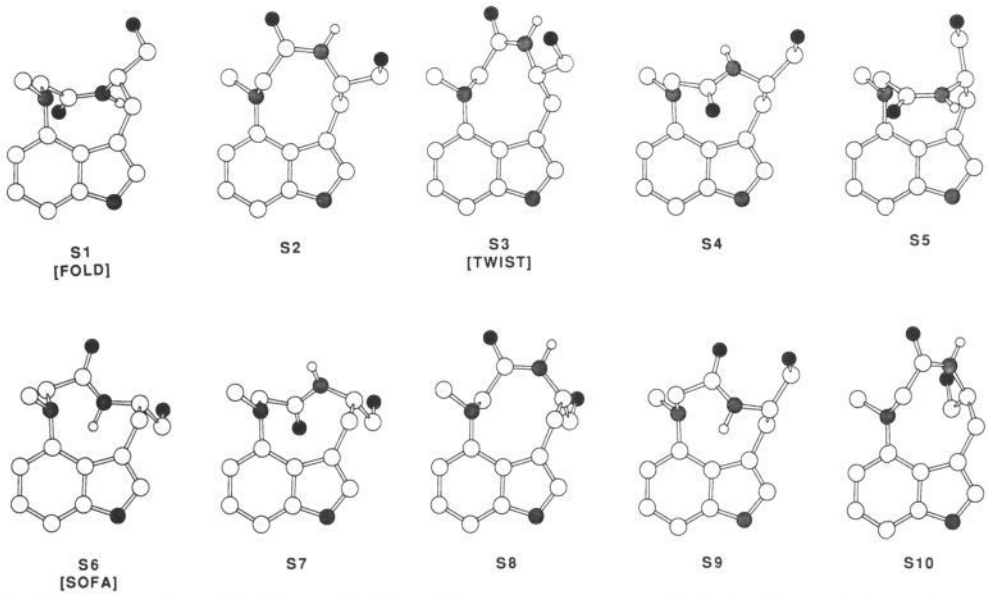

Figure 2. The 10 ring conformations of indolactam-G. The minimum-energy structure is depicted for each ring conformation. Hydrogen atoms except for amide hydrogen are not drawn for clarity.

Table III. Relative Stabilities (kcal/mol) of the 10 Ring Conformations

conformation	indolactam G	A	V	TL
S1 (fold)	0.000 (86)	0.576 (26)	2.570	3.724
S2	1.197 (12)	6.205	6.236	
S3 (twist)	2.537 (1.2)	0.000 (67)	0.000 (62)	0.045 (45)
S4	3.023 (0.5)	2.028 (2.0)	3.688	3.123
S5	3.833 (0.2)	2.818	3.824	2.716
S6 (sofa)	3.881 (0.08)	2.453 (1.3)	0.486 (27)	0.000 (49)
S7	3.931	3.992	4.902	4.826
S8	4.228	1.815 (3.4)	1.085 (10)	1.311 (5.3)
S9	5.159	4.774	4.865	4.208
S10	6.186			

[a] Estimated existence ratios at 300 K (%) are shown in parentheses.

value. The relative stability of these structures is summarized in Table III, together with those of the other three indolactams. The minimum-energy structures for the 10 independent ring conformations of indolactam-G are shown in Figure 2.

As a result, the preferred ring conformation of indolactam-G is predicted to be the most stable S1 form, which was named the "fold" form. The twist and the sofa forms, which were preferred by indolactam-V, were the third and the sixth most stable ring conformations, respectively, in indolactam-G. The existence ratio of the fold (S1), the second most stable (S2), the twist (S3), and the sofa (S6) forms at 300 K would be 86%, 12%, 1.2%, and 0.08%, respectively. The ^{1}H NMR spectra of indolactam-G at −30 °C clearly showed the coexistence of two conformations, although very broad signals were observed at room temperature, suggesting a rapid equilibrium between conformers. The ratio of the two conformers was 5:1 in CD_3OD. The signals of the major conformer were well-interpreted as being due to the fold form structure, from the NOE between H-9 and one of the C-12 protons and between the same H-12 and the *N*-methyl protons. As for the minor conformer, the signals of the *N*-methyl protons, two geminal protons at C-12, and the aromatic proton at C-5 could be assigned. The large high-field shift of one of the geminal protons at C-12 is consistent with the S2 form structure. Thus, the predicted structures and stability were in good agreement with those found in solution,

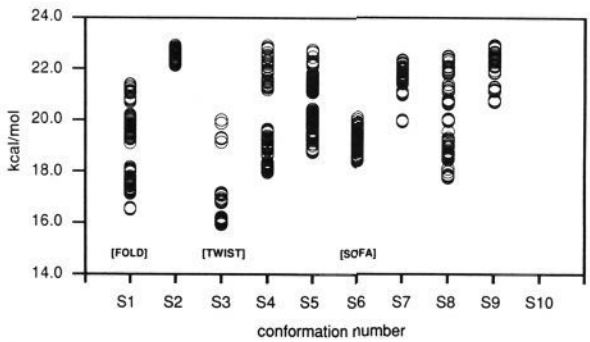

Figure 3. Energy-dispersion map of indolactam-A calculated by HTMD at 3000 K. The S10 conformer did not appear. The order of stability of conformations is very different from that of indolactam-G. The twist form is the most stable conformation.

and it was confirmed that the predictions made by the MD calculation were plausible.

Then, calculations were conducted for indolactam-A (**4**), -V (**2**), and -TL (**5**) under the same conditions. Figure 3 shows the energy-dispersion map for indolactam-A (**4**). The minimum-energy values for the 10 ring conformations are listed in Table III. The most stable ring conformation was the twist form, and the second and the third most stable ones were the fold and the S8 forms. The sofa form was the fifth most stable with the energy difference of 2.5 kcal/mol from the twist form. The existence ratio of the twist (S3), the fold (S1), the S8, and the sofa (S6) forms at 300 K is 67%, 26%, 3.4%, and 1.3%, respectively. These results suggest that the preferred conformation for indolactam-A is the twist form. From the ^{1}H NMR spectra, it was confirmed that molecules of indolactam-A mainly adopt the twist form conformation in solution.

The energy-dispersion map for indolactam-V (**2**) is shown in Figure 4. The minimum-energy values are compared in Table III. The energy difference between the most stable twist form and the second most stable sofa form was 0.49 kcal/mol, as expected. The third most stable conformation was the S8 form, which has an energy difference of 1.09 kcal/mol from the twist form. The existence ratio of the twist, the sofa, and the S8 forms at 300 K was estimated to be 62%, 27%, and 10%. In the ^{1}H NMR spectra, signals due to the twist and the sofa forms

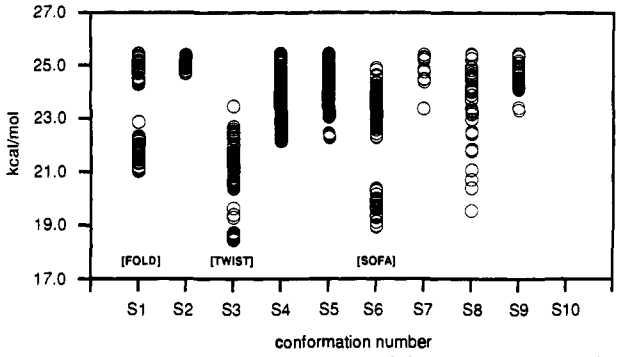

Figure 4. Energy-dispersion map of indolactam-V calculated by HTMD at 3000 K. The S10 conformation did not appear. The energy band splits seen in S1, S3, and S6 are mainly caused by internal rotation of the isopropyl substituent at C-12 instead of by nitrogen inversion as observed in indolactam-G.

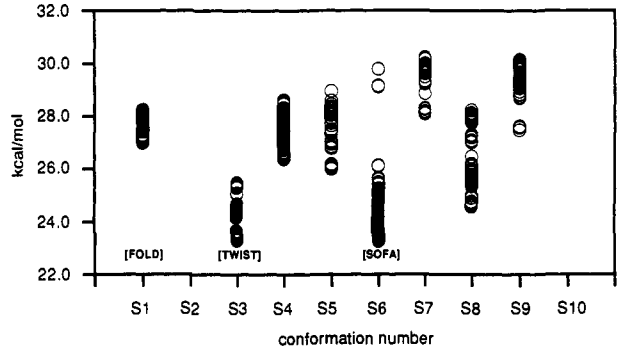

Figure 5. Energy dispersion map of indolactam-TL. The S2 and the S10 conformations did not appear. The energy dispersion is quite small compared with those of the other indolactams. This is due to the symmetric structure of the *tert*-butyl group at C-12. The most stable conformation is the sofa form, and the second most stable one is the twist form. The other conformations are rather unstable.

were observed in the ratio of 2:1 in CD_3OD. No significant signals due to the S8 form were observed in the spectra.

The energy-dispersion map for indolactam-TL (**5**) is shown in Figure 5. In this molecule, the stability of the sofa form was remarkably increased compared with that of indolactam-V. The most stable ring conformation was the sofa form, and the second most stable one was the twist form with the energy difference of 0.05 kcal/mol. The third most stable conformation was the S8 form with the energy difference of 1.31 kcal/mol from the sofa form. The existence ratio of the sofa, the twist, and the S8 forms at 300 K was estimated to be 49%, 45%, and 5.3%, respectively. The ^1H NMR spectra of this compound distinctly showed the coexistence of the twist and the sofa conformations, in a ratio of 1:1 in CD_3OD, which is in good agreement with the calculations. No significant signals due to the S8 form were observed in the spectra.

Discussion

TPA (12-*O*-tetradecanoylphorbol 13-acetate (**6**) is also a potent tumor promoter,[20] which is presumed to interact with the same target receptor as teleocidins on the basis of the results of various biological assays.[21] At the binding site on the receptor, they should take a definite three-dimensional ring structure, namely the active conformation. It goes without saying that the active conformation of a ligand is not necessarily the global minimum structure itself. A less stable conformation can be the active conformation, if it satisfies the three-dimensional structural requirements for interacting strongly with the target receptor. It is expected that the more stable the active conformation is, the larger the activity of the molecule. The existence ratios are considered to be better indexes than the energy differences from the global minimum energy to compare the relative stability of possible conformations among congeners.

Relative stability of the 10 typical ring conformations and the existence ratios of selected conformations for the four indolactams are summarized in Table III. It is clear that the relative stability between conformations and even the order of stability greatly vary depending on the substituent group at C-12. The predicted predominant conformations for each compound were in good agreement with the conformations found in solution by NMR examination. This fact shows that the calculation is very useful for reproducing stable structures and their relative stability, and the results of the calculations appear to be sufficiently reliable to be used for detailed energetic discussions on the four indolactams.

As for the active conformation, the following three possibilities may be considered. The first possibility is that the twist form is the active conformation. The relative potencies of the four indolactams, indolactam-G, -A, -V, and -TL, appeared superficially to have a good correlation to the bulkiness of the substituent groups, H, CH_3, C_3H_7, and C_4H_9. But, when the adaptabilities to the twist form are taken into account, the correlation becomes poor. The existence ratios of the twist form in the four indolactams were predicted as 1.2%, 67%, 62%, and 45%, respectively. This possibility cannot be accepted, unless we assume that the contribution of the substituent bulkiness to the activity greatly exceeds the conformational disadvantage.

The second possibility is that the sofa form is the active conformation. This hypothesis appears to explain the activities of the four indolactams well. The existence ratios of the sofa form conformation in the four indolactams were estimated as 0.08%, 1.3%, 27%, and 49%. The greater the existence ratio of the sofa form is, the more potent the biological activity is. If we assume that increases of both the existence ratio of the active conformation and the substituent bulkiness cause an increase of the activity, the relative potencies of the four indolactams can be better understood.

The third possibility is that the S8 form or some other conformation is the active conformation. Although we have no positive data to support or eliminate this possibility, it can be judged to be less likely in comparison with the second one. Thus, the second possibility is better supported by the results of the MD calculations that the other two possibilities. It is strongly indicated that the sofa form is the active ring conformation of teleocidins and indolactams.

There is a marked structural dissimilarity between TPA and teleocidin, although they bind to the same receptor. In order to clarify structural features essential for their tumor-promoting activity, several groups have attempted

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to superpose the three-dimensional structures of teleocidin and TPA using computers.[22,23] We have also superposed them by a new rational method which is designed to superpose molecules in terms of physical and chemical properties related to receptor binding, but not in terms of superficial chemical structures or positions of heteroatoms.[24] In our study, the superpositions of both the twist and the sofa form conformers of teleocidin onto the TPA molecule were examined independently, whereas the other groups examined the superposition only for the twist conformation. The results showed that the TPA molecule and the teleocidin sofa form could interact with the common receptor through three hydrogen bonds, and the sofa form could be superposed onto TPA much better than the twist form. In the superposed structures, the spatial position of the alkyl group at C-12 in teleocidin corresponded to that of the methyl group at C-2 in the TPA molecule.

In this study, the biological activity of the four indolactams can be reasonably interpreted in terms of the existence ratio of the sofa form. This finding is consistent with the results from the superposition of teleocidin and TPA molecules. The coincidence of the results from two independent approaches favors the hypothesis that the sofa form is very close to the active conformation for tumor-promoting activity of teleocidins.

This work has also shown that MD calculations are very useful for searching for the stable conformations in highly strained cyclic compounds. They are especially useful in molecules whose stability is strongly influenced by the conformations of the substituent groups on them.

Conclusion

The importance of the sofa form for the tumor-promoting activity of teleocidins and indolactams was indicated by conformation analyses of four indolactam congeners using high-temperature MD calculations.

Experimental Section

(±)-Indolactam-G (3). The preparation method, combustion elemental analysis, and ^{1}H NMR at room temperature have been published.[14] The 400-MHz ^{1}H NMR spectrum of indolactam-G at −30 °C was measured with a JEOL GX400 spectrometer. The chemical shifts at −30 °C are as follows: The fold conformer, 2.78 (dd, 1 H, J = 15.4, 8.3, 8-CH$_2$), 2.86 (s, 3 H, NCH$_3$), 3.17 (dd, 1 H, J = 15.4, 6.9, 8-CH$_2$), 3.51 (d, 1 H, J = 13.5, 12-CH$_2$), 3.58 (dd, 1 H, J = 11.3, 8.2, 14-CH$_2$), 3.69 (dd, 1 H, J = 11.3, 4.1, 14-CH$_2$), 3.94 (d, 1 H, J = 13.5, 12-CH$_2$), 5.05 (m, 1 H, 9-CH), 6.91 (d, 1 H, J = 7.6, 5-CH), 6.94 (s, 1 H, 2-CH), 7.03 (t, 1 H, J = 7.6, 6-CH), 7.09 (d, 1 H, J = 7.6, 7-CH); The S8 conformer, 2.94 (s, 3 H, NCH$_3$), 3.06 (d, 1 H, J = 13.8, 12-CH$_2$), 4.18 (d, 1 H, J = 13.8, 12-CH$_2$), 6.78 (d, 1 H, J = 7.6, 5-CH); other peaks could not be assigned since they overlapped with the peaks of the fold form. The signal ratio of these two conformers is 1:0.2.

Registry No. 1, 11032-05-6; 2, 90365-57-4; 3, 84590-50-1; 4, 110073-31-9; 5, 110073-28-4.

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Nucleosides and Nucleotides. 107. 2-(Cycloalkylalkynyl)adenosines: Adenosine A$_2$ Receptor Agonists with Potent Antihypertensive Effects[1]

Toichi Abiru,*,† Takanori Miyashita,† Yohko Watanabe,† Toyofumi Yamaguchi,†,§ Haruhiko Machida,† and Akira Matsuda‡

Biology Laboratory, R & D Division, Yamasa Shoyu Co., Ltd., Araoicho 2-10-1, Choshi, Chiba 288, Japan, and Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060, Japan. *Received October 4, 1991*

Adenosine receptor-binding profiles in rat brain tissues and antihypertensive effects in spontaneously hypertensive rats (SHR) of a series of 2-(cycloalkylalkynyl)adenosines (2-CAAs) and their congeners are described. The structure–activity relationship of this series of compounds is discussed, focusing on the length of the alkynyl side chain and bulkiness of the terminal cycloalkyl substituents in terms of binding activity and cardiovascular effects. All the 2-CAAs had a preferential affinity for A$_2$ receptors. Of these derivatives, 2-(3-cyclopentyl-1-propyn-1-yl)adenosine (**10b**) exhibited the most selective affinity for A$_2$ receptors (K_i ratio: A$_1$/A$_2$ = 70) on the basis of receptor binding. In the C-2 binding region of adenosine, compounds often have potent and/or selective A$_2$ activity from introduction of an acetylenic group at the C-2 position followed by one methylene residue further followed by a hydrophobic substituent such as a cycloalkyl ring at the terminal position of the alkynyl side chain. Intravenous injection of **10b** up to 100 μg/kg had a potent hypotensive effect without a marked decrease in heart rate in anesthetized SHR. Compounds **10j–s**, with a hydroxyl group in the C-3″ position of the alkynyl side chain, had a potent affinity for both A$_1$ and A$_2$ receptors, but they were not highly selective for A$_2$ receptors. These compounds caused a marked bradycardia upon intravenous administration in anesthetized SHR. Oral administration of **10b** (0.1–1 mg/kg) had a potent and long-lasting antihypertensive effect in conscious SHR.

Adenosine receptors in cell membranes have been classified into A$_1$ and A$_2$ receptors on the basis of receptor-mediated inhibition (A$_1$ receptors) or stimulation (A$_2$ receptors) of adenylate cyclase.[2] Some adenosine ana-

† Yamasa Shoyu Co., Ltd.
‡ Hokkaido University.
* Author to whom correspondence should be addressed.
§ Present address: Department of Biological Sciences, The Nishi Tokyo University, Uenohara, Yamanashi 409-01, Japan.

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