A Pharmacophore Model of Tautomycin, an Inhibitor of Protein Phosphatases 1 and 2A

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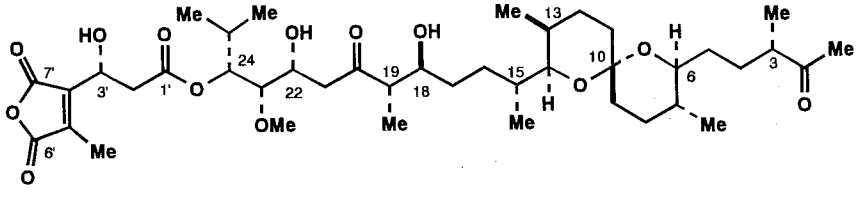
Over the last decade there has been a growing realization that phosphatases are extremely important in cellular and organismal functions[1]. Tautomycin, an inhibitor of protein phosphatases (PP) 1 and 2A, was isolated from a culture broth of *Streptomyces spiroverticillatus* in 1987[2]. The structure that we established in 1990[3] showed some similarities with that of okadaic acid, and the target protein of okadaic acid had already been found to be protein phosphatase in 1988[4]. Indeed, it was later found that the target proteins of tautomycin were also protein phosphatases[5,6]. However, this molecule is not a tumor promoter on mouse skin and in rat glandular stomach[7]. These differences in biological activities between tautomycin and the okadaic acid class tumor promoters are interesting topics in structural biology. The three-dimensional structures of the related compound acanthifolicin[8] and the *o*-bromobenzyl ester derivative of okadaic acid[9] were determined by X-ray crystallography, and the solution-state conformation of okadaic acid was proposed by molecular modelling[10] and NMR[11]. Since tautomycin inhibits specific [^{3}H]okadaic acid binding to PP-1 and PP-2A[5], it has been assumed to have similar conformation to that of okadaic acid[7,12]. In order to define homology in the 3-D structures of both inhibitors, and to provide a pharmacophore model of tautomycin against PP-1 and PP-2A, we performed the conformational analysis of tautomycin in organic solvents, starting from the established absolute configuration of tautomycin[12] (Fig. 1).

The proton resonances of tautomycin in $CDCl_3$ and C_6D_6 have been assigned by ^{1}H-^{1}H COSY, *J*-resolved 2D NMR, E. COSY[13], ^{1}H-^{13}C COSY, HMQC and HMBC. The coupling constant data and the NOE data obtained from decoupling experiments, *J*-resolved 2D NMR, differential nuclear Overhauser effect (DIF-NOE) experiments, NOESY and ROESY[14] have been used for elucidation of the molecular conformation of tautomycin in solution. All the proton resonances of tautomycin in $CDCl_3$ were assigned through a combined use of 600-MHz ^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY and HMBC. By using the carbon resonance assignments of tautomycin determined by biosynthetic study[15], all the chemical shifts of the proton resonances were traced from a combination of ^{1}H-^{13}C COSY and HMBC spectra. Since

Fig. 1. Absolute configuration of tautomycin.



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severe overlapping was observed in the methylene region of the ^1H NMR spectrum, normal 1D-selective PFG-TOCSY experiments and 1D selective PFG-TOCSY with homo-decoupling were mainly used for the assignments of the coupling constants of some methylene and methine units. The ^1H NMR spectrum of tautomycin in C_6D_6 was measured to determine the coupling constants that had been difficult to obtain from the data[16] in $CDCl_3$.

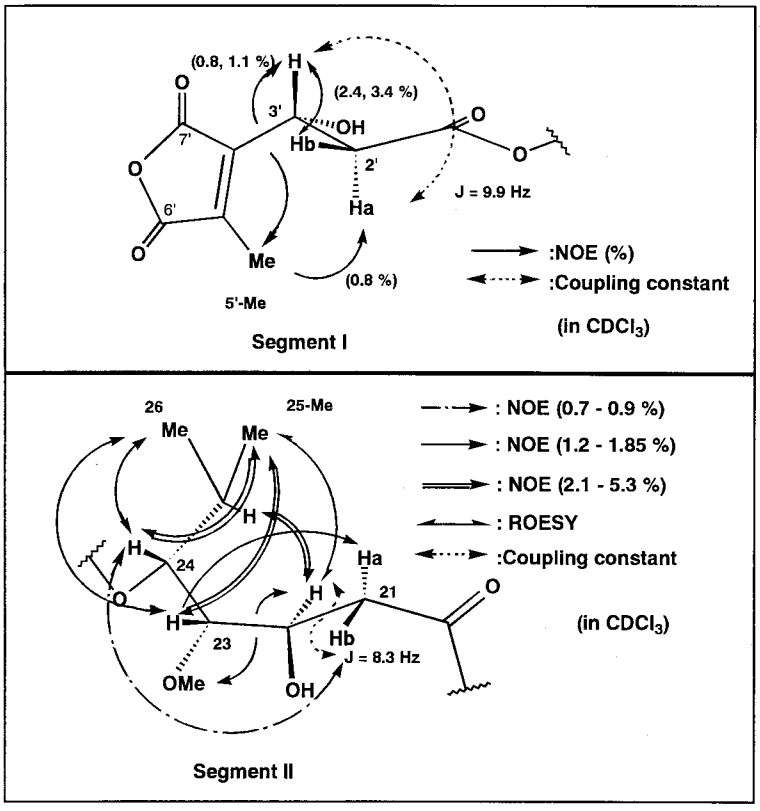
Table 1. Selected ^1H NMR data of tautomycin in C_6D_6.

Position	δ (ppm)	J (Hz)
14	3.49, dd	$J_{13,14}=2.2$, $J_{14,15}=9.8$
15	1.69, dddq	$J_{14,15}=9.9$, $J_{15,16a}=8.5$, $J_{15,16b}=3.3$, $J_{15,15Me}=6.6$
15Me	1.18, d	$J_{15,15Me}=6.6$
16a	1.06, dddd	$J_{16a,16b}=12.6$, $J_{16a,17a}=12.6$, $J_{15,16a}=8.5$, $J_{16a,17b}=3.0$
16b	1.56, dddd	$J_{16a,16b}=12.6$, $J_{16b,17a}=12.6$, $J_{15,16b}=3.3$, $J_{16b,17a}=3.0$
17a	1.20, dddd	$J_{17a,17b}=12.6$, $J_{16a,17a}=12.6$, $J_{17a,18}=8.0$, $J_{16b,17a}=3.0$
17b	1.53, dddd	$J_{17a,17b}=12.6$, $J_{16b,17b}=12.6$, $J_{16a,17b}=3.0$, $J_{17b,18}=2.7$
18	3.57, ddd	$J_{17a,18}=8.0$, $J_{18,19}=8.0$, $J_{18,17b}=2.7$

The selected ^1H NMR data of tautomycin in C_6D_6 are shown in Table 1.

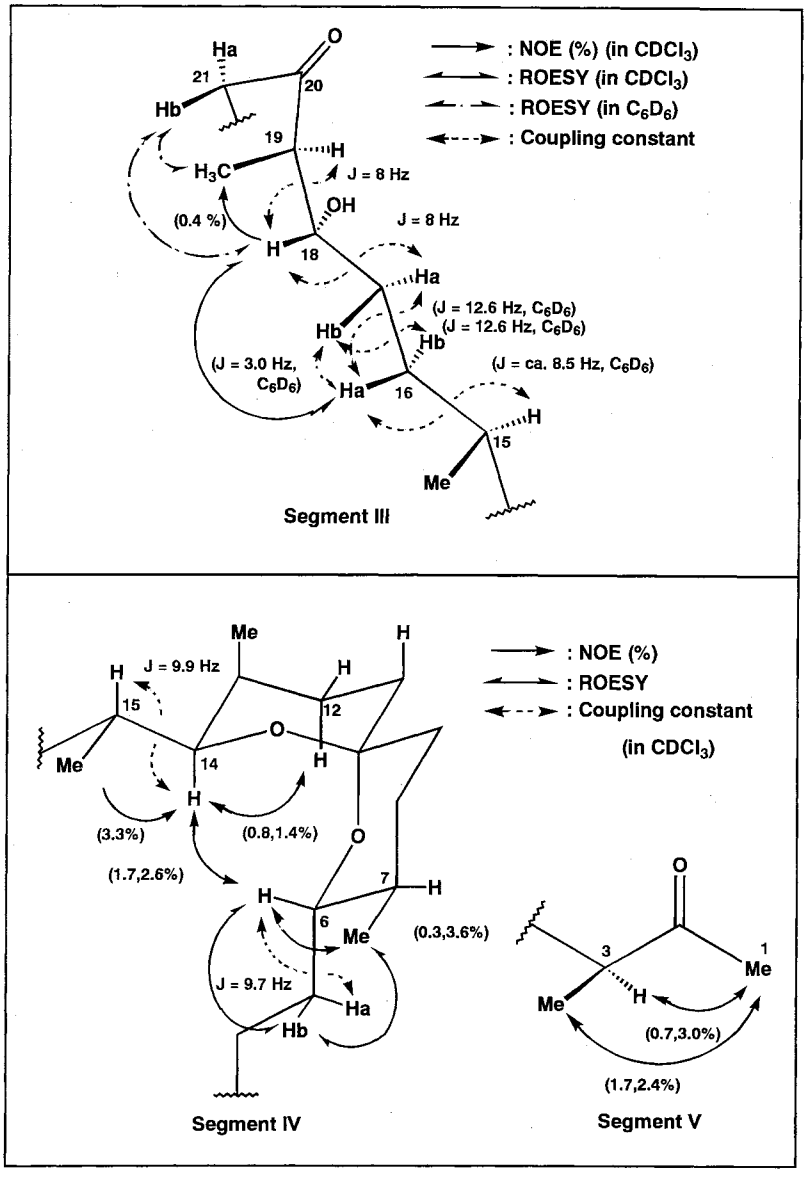
To estimate interproton distances, we recorded a series of DIF-NOE, NOESY (mixing times, 150, 300 msec) and ROESY (mixing times, 150, 300, 400, 500 msec) data. Figure 2 shows the NOE networks and coupling constants of segments I, II, III, IV and V of tautomycin. The NOE networks and the coupling constants of this molecule indicated the prochiralities of methylene protons, H-16a and H-16b, H-17a and H-17b (Fig. 2, Segment III), H-21a and H-21b, and H-2′a and H-2′b (Fig. 2, Segments I and II). Phase-sensitive ROESY spectra (mixing time, 400 msec) showed strong ROE between H-21a and H-23, moderate ROEs between H-18 and 19-Me, and weak ROEs between 19-Me and H-21b, and H-18 and H-21b (Fig. 2, Segments II and III). The selected NOE values determined by DIF-NOE experiments are shown as percentages in Fig. 2. In order to obtain convergent conformations, we needed to use the angle constraints as well as interproton distance constraints in the calculations. Thus, the geometry of the dihedral angle between protons having a large coupling constant was fixed to *trans*: $J_{5Ha\text{-}6H}=9.7$ Hz, $J_{14H\text{-}15H}=$

Fig. 2-1. NMR data segments I and II of tautomycin.



NMR Experiments: A 26 mM solution was prepared by dissolving the antibiotic in $CDCl_3$ and placed in a 5 mm tube. To detect some coupling constants and ROEs around C-15 to C-21, C_6D_6 was used instead of $CDCl_3$.

Fig. 2-2. NMR data of segments III, IV and V of tautomycin.



NMR Experiments: A 26 mM solution was prepared by dissolving the antibiotic in $CDCl_3$ and placed in a 5 mm tube. To detect some coupling constants and ROEs around C-15 to C-21, C_6D_6 was used instead of $CDCl_3$.

9.9 Hz, $J_{15H\text{-}16Ha}$ = 8.5 Hz, $J_{16Ha\text{-}17Ha}$ = 12.6 Hz, $J_{17Ha\text{-}18H}$ = 8.0 Hz, $J_{18H\text{-}19H}$ = 8.0 Hz, and $J_{21Hb\text{-}22H}$ = 8.3 Hz (Fig. 2, Segments II, III and IV). J values between 8.0 and 8.5 Hz probably indicate the fluctuation of these dihedral angles. The ^{13}C spin-lattice relaxation time data of tautomycin show the backbone dynamics in these parts as well as the dialkylmaleic anhydride moiety (data not shown). A proton-oxygen distance constraint between H-24 and C-1′ carbonyl oxygen was also defined, because the ester carbonyl is generally staggered 30° to either side of the carbinyl proton[12,17]. The distance constraints and angle constraints are listed in Table 2.

Search for tautomycin conformation was executed with the 27 distance constraints and the 7 angle constraints using the DADAS90 program[18,19] on an SGI Indy computer. Starting with 1000 randomly generated initial structures, 19 final structures with an allowable error in the target function values, pseudo-energy values which are regarded as variations of the conformational-energy function values, were obtained as a result of a series of optimizations of the function. None of the 3-D structures have NOE violations over 0.56 Å or van der Waals distance violations larger than 0.43 Å.

The conformation in the dialkylmaleic anhydride moiety was disordered because of the molecular flexibility and the lack of distance constraints in the vicinity of the

Table 2. Distance and angle constraints for DADAS90 calculations.

1) Distance constraints

Atom pair		Upper distance constraint	Atom pair		Upper distance constraint
Atom 1	Atom 2	(Å)	Atom 1	Atom 2	(Å)
H-2'a	5-Me	5.00	H-23	H-21a	2.50
H-3'	5-Me	4.20	H-23	H-22	2.60
H-3'	H-2'b	2.40	H-22	23-OMe	5.00
H-24	H-26	4.00	H-22	H-25	2.50
H-24	25-Me	3.30	H-22	H-21a	2.50
H-24	H-25	3.50	H-21b	19-Me	2.50
H-24	H-23	4.00	H-18	19-Me	3.00
H-24	23-OMe	4.00	H-18	H-21b	2.50
H-24	H-21b	5.50	H-3	H-1	3.50
H-23	25-Me	2.90	3-Me	H-1	3.50
H-23	H-26	5.00	H-24	1-CO	2.27
H-23	23-OMe	6.00			

2) Angle constraints

Dihedral angle position	Angle constraint (deg)	Dihedral angle position	Angle constraint (deg)
H-5a-CC-H-6	180 fixed	H-17a-CC-H-18	180 fixed
H-14-CC-H-15	180 fixed	H-18-CC-H-19	180 fixed
H-15-CC-H-16a	180 fixed	H-21b-CC-H-22	180 fixed
H-16a-CC-H-17a	180 fixed		

Conformational search was executed by minimizing the target function values in DADAS 90 calculation. The target function was defined as the following pseudo-energy (V_O). $V_O = W_D \cdot V_{NOE} + W_V \cdot V_{VDW} + W_J \cdot V_{ANG}$. V_{NOE}: a sum of squares of differences between a distance constraint and a distance between the same pair of atoms in a computer-generated conformation, V_{VDW}: an interatomic distance derived from van der Waals radius was used instead of the above distance constraint, V_{ANG}: an angle constraint and the corresponding dihedral angle in a computer-generated conformation were adopted instead of the distance constraint and the interatomic distance in the above definition (V_{NOE}). W_D: weight for V_{NOE}, W_V: weight for V_{VDW}, W_J: weight for V_{ANG}.

Fig. 3. Superposition of nineteen final structures of tautomycin obtained from DADAS90 calculation.

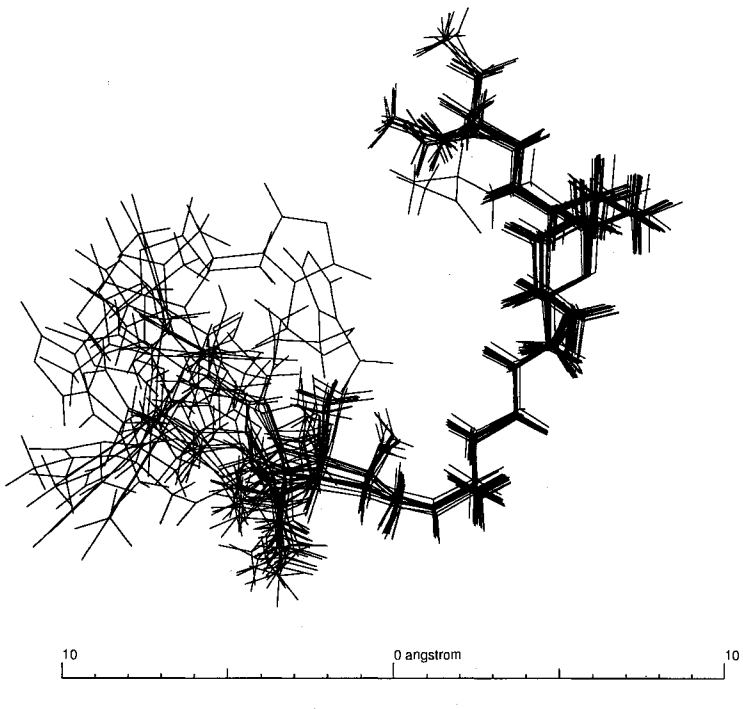


Fig. 4. A stable solution conformation of tautomycin, comparison with the okadaic acid molecule.

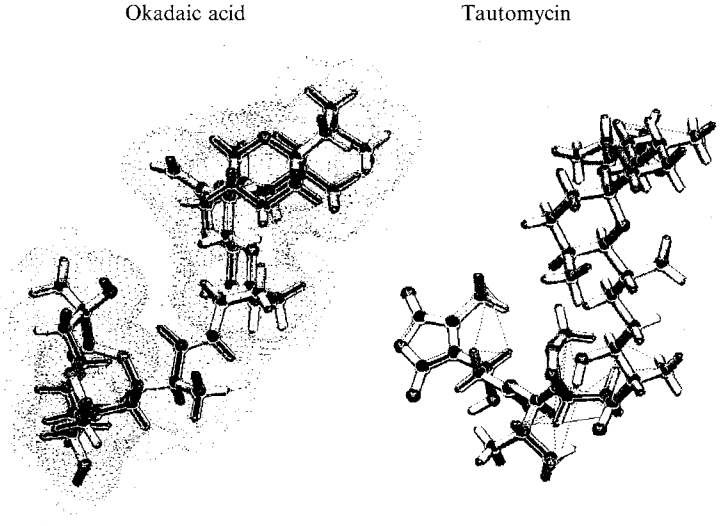
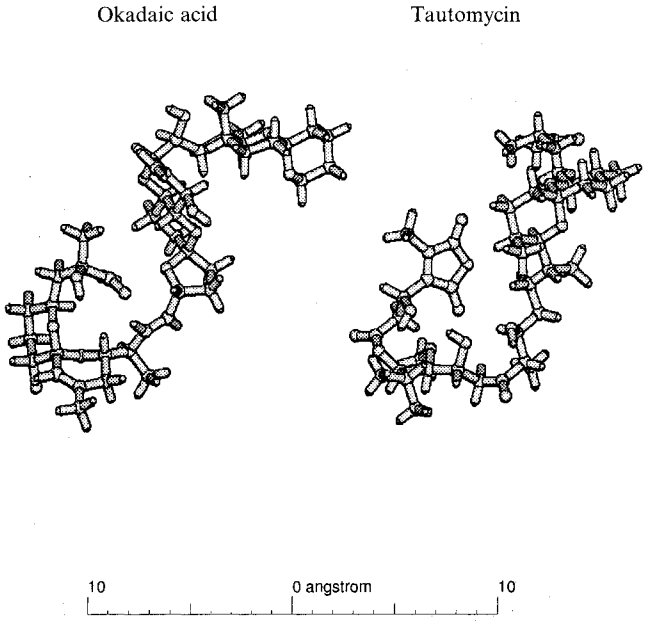


Fig. 5. The pharmacophore model of tautomycin, comparison with the okadaic acid molecule.



ester linkage. The 19 structures were superimposed for the best fit of backbone atoms of the right chain of the molecule (Fig. 3). A survey of these structures revealed that one of the conformations is similar in molecular size and shape to the proposed 3-D structure of okadaic acid[11] (Fig. 4). Therefore, we have generated a pharmacophore model, which mimics the okadaic acid molecule, using this conformer. The best pharmacophore model was easily found by a slight movement of the flaccid moiety (C-2′ to C-24) in the tautomycin molecule. Only the C-7′ carbonyl group of tautomycin could be superimposed on C-1 carboxylic acid of okadaic acid and the pharmacophore model has a striking resemblance to okadaic acid in topology and architecture (Fig. 5). Thus, C-7′ carboxylic acid of the diacid form (active form) of tautomycin[12,20] may be participating in the hydrogen-bonding network in the active site of PP-1 and PP-2A. Although the role of C-6′ carboxylic acid could not be evaluated from the topological similarities with the conformation of okadaic acid, this model could be fitted in the microcystin-LR binding site of the catalytic subunit of PP-1[21]. Since both carboxylic acids (β-linked D-*erythro*-β-methylaspartic acid and γ-linked D-glutamic acid) in the microcystin-LR molecule form hydrogen bonds with ligands in the active site of PP-1, C-6′ carboxylic acid of tautomycin may also play an important role in the binding to the enzyme.

Tautomycin did not induce tumor promotion in a two-stage carcinogenesis experiment on mouse skin, and neither enhanced TNF-α, an endogenous tumor promoter, mRNA expression in mouse skin nor induced TNF-α release in human stomach cancer cell line, whereas okadaic acid did both[7]. Okadaic acid that is a specific inhibitor of PP-2A, has a rigid structure, whereas tautomycin that inhibits PP-1 more strongly than PP-2A[6,22], has a flexible structure. Most of the 3-D structures of the okadaic acid class inhibitors have been estimated. A similar conformer of okadaic acid to the solid 3-D structure of the derivative, acanthifolicin was easily obtained even by a simple COSMIC force-field calculation using a Nemesis program[12,23,24] (data not shown). However, the determination of the solution

conformation of tautomycin has been extremely difficult, because of its flexibility. The aim of our present study is to find the major conformer of the flexible molecule, tautomycin and to provide a pharmacophore model deduced from the result. Therefore, we tried to estimate its solution conformation using DADAS90 calculation. As mentioned above, this molecule must be an ensemble of many states in solution. However, the convergent conformations of the right-hand part of the tautomycin molecule given in Fig. 3 represent a significant subset in the ensemble of many states. The proposed pharmacophore model and the differences between the structural features of tautomycin and those of the okadaic acid class tumor promoters, okadaic acid[9], microcystin[21] and calyculin A[25], will provide insight into the development of fungicide[2] and medicine such as type 2 helper T cell selective immunosuppressant[26] as well as biological probes[27~32].

Acknowledgments

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