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MOLECULAR SHAPE ANALYSIS AND ACTIVITY OF TAUTOMYCIN, A PROTEIN PHOSPHATASE INHIBITOR

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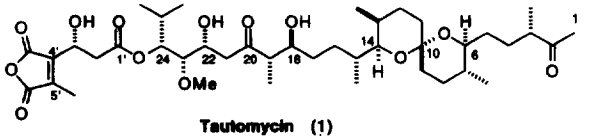
Abstract. Tautomycin, a well-known protein phosphatase inhibitor, exists in two forms (acid anhydride and diacid). We successfully isolated them and proved that diacid is the real active form of tautomycin. The molecular shape-activity relationship of tautomycin and its conformational analysis are also described.

Protein phosphatases type 1 (PP1) and type 2A (PP2A) are two of the four major enzymes that dephosphorylate serine/threonine residues of proteins in eukaryotic cells.[1] These enzymes play an important role as the regulators of several cellular events, e.g., metabolism, gene expression and cell division. It has been reported that these enzymes are inhibited by natural toxins,[2] such as okadaic acid, calyculin A, microcystin-LR and tautomycin (**1**).[3] We were interested in **1**, because **1** equally inhibits PP1 and PP2A,[4] whereas okadaic acid, calyculin A and microcystin-LR inhibit PP2A more strongly than PP1.[2] In this paper, we describe the molecular shape-activity relationship of tautomycin (**1**) and its conformational analysis.



Tautomycin (1)

Protein phosphatase inhibitory activity was measured by our newly developed method,[5] in which activity was monitored by the amount of light emitted by firefly luciferin-luciferase reaction (Figure 1). This method is applicable at the pH 6.5 to 8.4 region.[6]

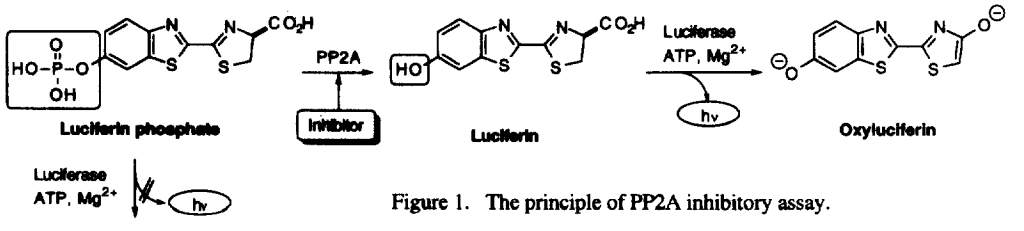


Figure 1. The principle of PP2A inhibitory assay.

Tautomycin exists in two forms as equilibrium, i.e., **1a** [acid anhydride, FABMS *m/z* 767 ($C_{41}H_{67}O_{13}$, MH^+)] and **1b** {diacid, FABMS (positive mode) *m/z* 785 ($C_{41}H_{69}O_{14}$, MH^+), 807 ($[M+Na]^+$), 767 ($[MH-H_2O]^+$), 749 ($[MH-2H_2O]^+$), FABMS (negative mode) *m/z* 783 ($C_{41}H_{67}O_{14}$, $[M-H]^-$)}, which were successfully isolated by ODS-HPLC using CH_3CN-H_2O (Figure 2). Equilibrium did not occur in buffer solution (HEPES or Tris buffer, pH 6.0 - 8.4) of **1b** for 15 min (Figure 3) and was not observed in CH_3CN-H_2O solution or solid state of **1b** for several days at neutral condition. On the contrary, **1a** became an equilibrium mixture under basic condition, e.g., **1a**:**1b** = 19:81 at pH 8.4 after 15 min incubation (Figure 3). However, only 10% of **1b** was formed from **1a** at weakly acidic condition, e.g., **1a**:**1b** = 90:10 at pH 6.5. Thus PP2A inhibitory activity assay, which takes about 15 min, was done under two conditions at pH 6.5 and pH 8.4 (Figure 4). At both pH, **1b** showed strong activity (IC_{50} 8 nM). The IC_{50} values of **1a** were 356 nM and 63 nM at pH 6.5 and 8.4, respectively, which reflect the amount of **1b** formed by equilibrium. These results indicate that **1b** is the real active form of tautomycin.

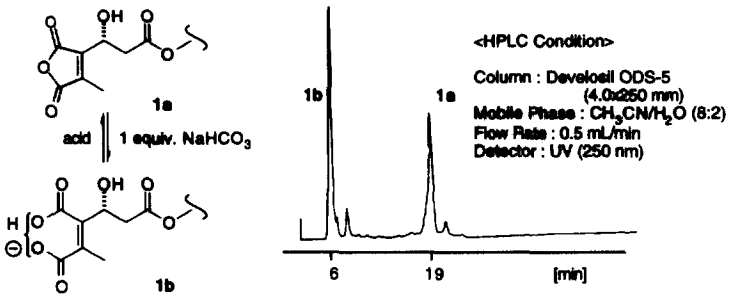


Figure 2. Hydrolysis of maleic anhydride moiety of tautomycin (**1**).

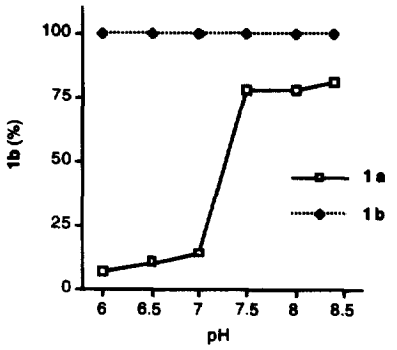


Figure 3. Population of **1b*** in buffer solution of **1a** or **1b** after 15 min incubation at different pH.
(* Determined by ODS-HPLC analysis.)

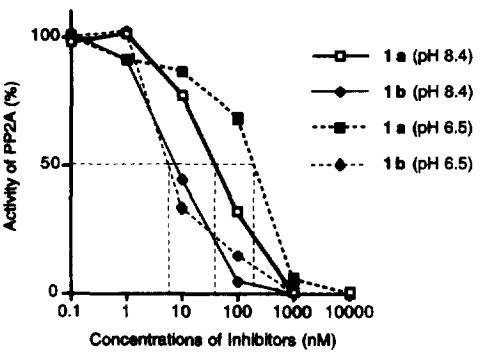
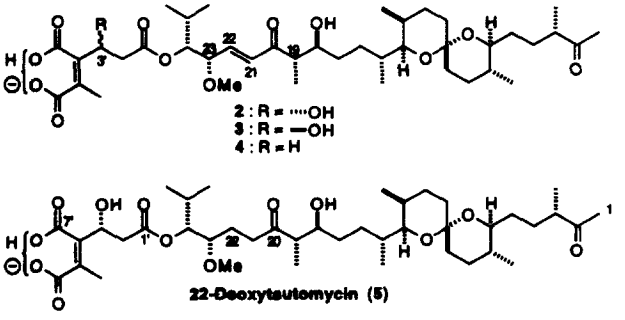


Figure 4. PP2A Inhibitory activities of tautomycin (**1a**) and diacid (**1b**).*
(* 5 nM of PP2A was used for assay.)

The enone derivatives (**2**-**4**),[7] which have rigid structure around the C19 to the C23, were prepared by a similar manner as reported for the total synthesis of **1**.[3C] None of them showed PP2A inhibitory activity, therefore the molecular shape around the C19 to the C23 or a polar function at the C22 position may be important for activity. Derivative **5**, 22-deoxy-**1**, was then prepared.[8] Since the ^1H NMR (Figure 5) and ^{13}C

NMR data for the C1 through the C20 and the C1' through the C7' of **5** are similar to those of **1b**, **5** might have similar conformation to **1b** in solution. In spite of its flexibility around the C19 to the C23 and conformational similarity to **1b**, **5** didn't show activity in our assay system. This suggested that hydroxyl group or minus charge at the C22 is required for activity.



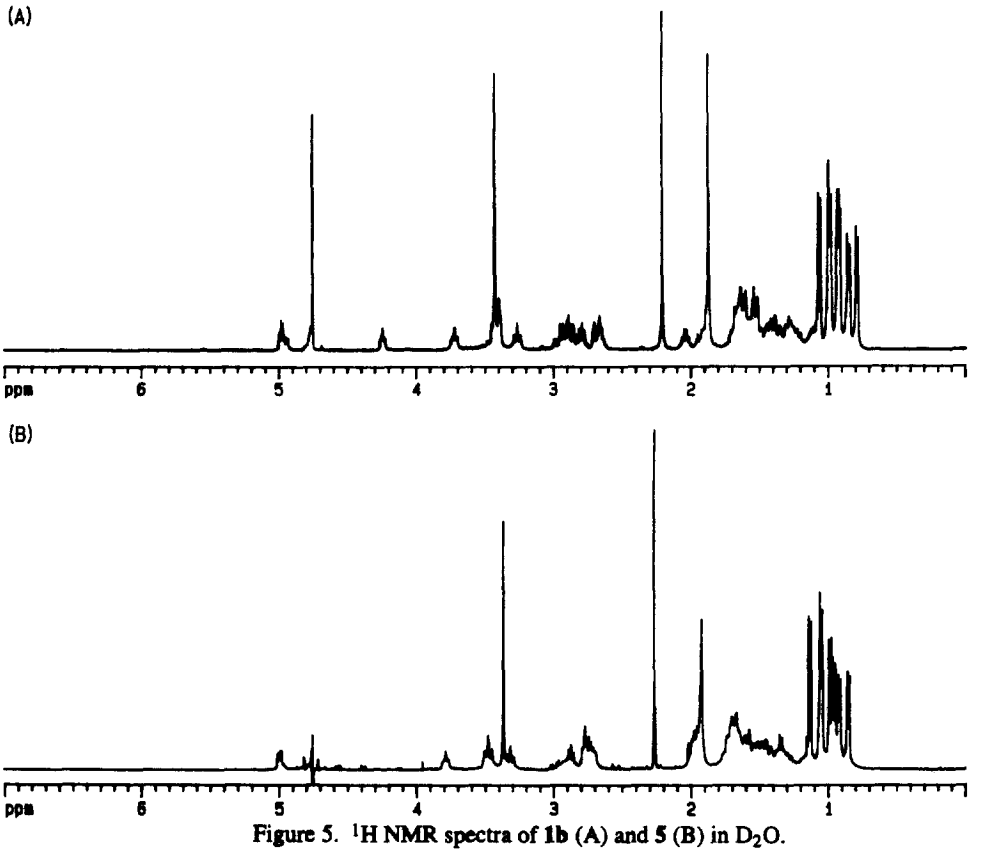


Figure 5. ^{1}H NMR spectra of **1b** (A) and **5** (B) in D_2O.

We found different chemical nature between **1** and **5** that provided another information about the conformation of **1**. Treatment of **1** with alkali (pH 9) could render migration of the ester group at the C24

hydroxyl group into the C22 to result in β-elimination and formation of **7** (Figure 6). This mechanism was supported by extreme stability of **5** (22-deoxy-**1**) under the same condition (Figure 7).[9] It was suggested that oxygen atom of OH at the C22 could situate near to the ester carbon of the C1'.

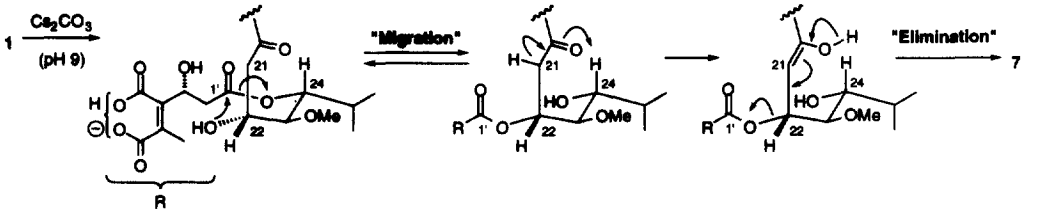


Figure 6. Suggested trans-esterification and elimination mechanism on alkali degradation of tautomycin (**1**).

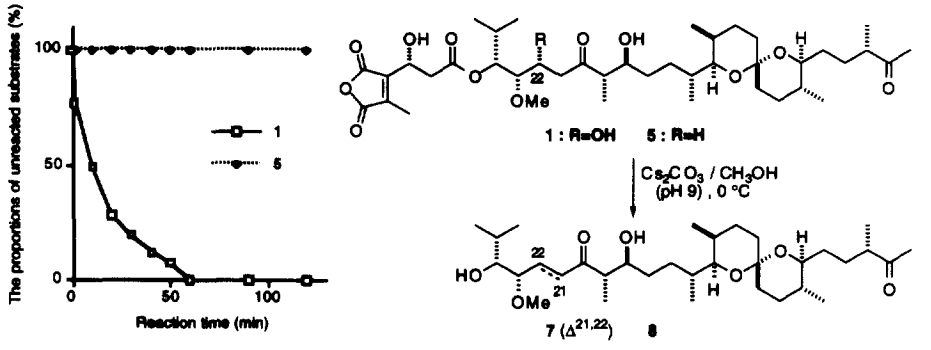


Figure 7. Time-course of alkali degradation of tautomycin (**1**) and 22-deoxytautomycin (**5**).

Before determination of the stable conformation of tautomycin, we examined computer calculation of okadaic acid, whose stable conformation was determined by X-ray analysis of its *o*-bromobenzyl derivative (Figure 8)[10] and by computer modelling.[11] The NMR spectra of okadaic acid were measured in $CDCl_3$ on a Bruker AMX600 at 27 °C. Stable conformation of okadaic acid was calculated by Biograf and NMRgraf programs using 33 NOE data obtained by NOESY experiment in $CDCl_3$ (Figure 9).[12] Obtained conformation was superimposable on that reported.[10,11]

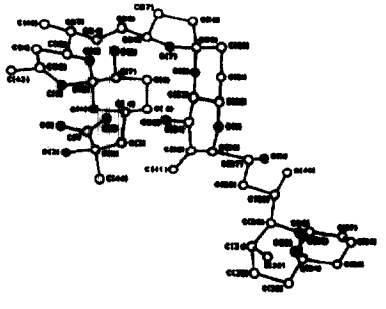


Figure 8. Computer-generated perspective drawing of the X-ray model of okadaic acid. (According to P. J. Scheuer *et. al.*[10])

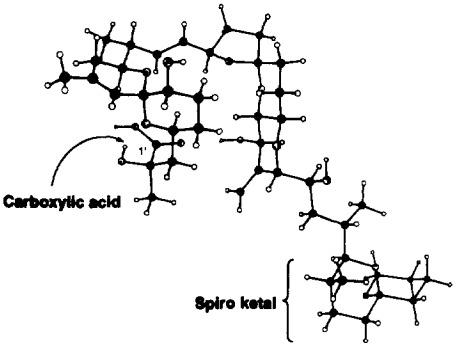


Figure 9. Proposed 3D structure of okadaic acid.

Same strategy was adapted to tautomycin. The NMR spectra of **1b**, active form of tautomycin, were measured in D_2O on a Bruker AMX600 at 27 °C.[13] One of the stable conformations of **1b**, obtained by preliminary computer calculation by Biograf and NMRgraf programs using 35 NOE data (NOESY),[14] is shown in Figure 10. As we expected, **1b** has rigid conformation around the C1 to the C19, whereas it has flexibility around the C20 to the C7'. This conformation supported the trans-esterification mechanism (Figure 6) because oxygen atom at the C22 is placed in near to carbon atom at the C1' (distance 3.65 Å).

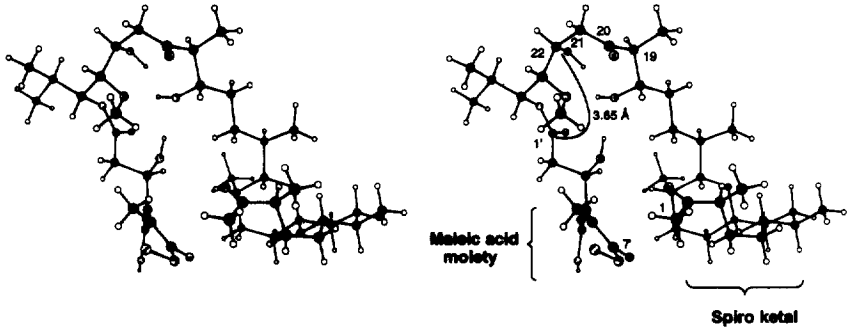


Figure 10. Stereo view of proposed 3D structure of tautomycin diacid (**1b**).

In conclusion, we clarified that tautomycin showed PP2A inhibitory activity as diacid form (**1b**). Tautomycin derivatives, **2-4** (enone) and **5** (22-deoxy-**1**), were prepared for structure-activity relationship studies. None of them showed PP2A inhibitory activities in our assay system, therefore flexibility around the C19 to the C23 and hydroxyl group at the C22 maybe indispensable for PP2A inhibitory activity. Trans-esterification mechanism on alkali degradation of tautomycin (**1**) was proposed. This mechanism proved that hydroxyl group at the C22 is near to the C1' in tautomycin molecule. One of the stable conformation, supported by these evidences, were obtained by preliminary calculation for **1b**.

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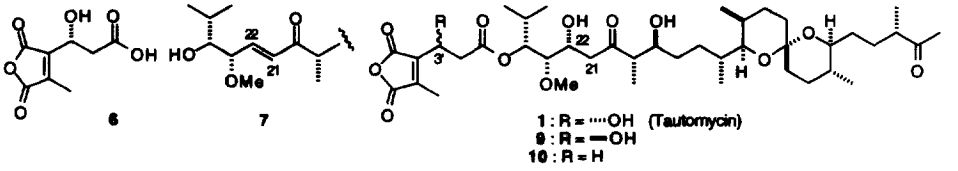
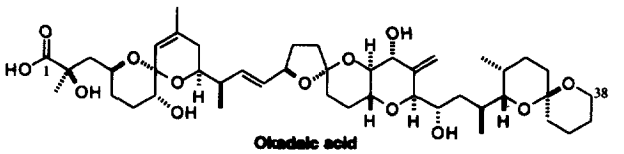
6. The PP inhibitory activity is frequently determined at pH 8.4. [Takai, A.; Mieskes, G. *Biochem. J.* **1991**, *275*, 233-239.]
7. We attempted to prepare **9** and **10** to reveal whether hydroxyl group at C3' is required for activity. Since **7** was only obtained by treatment of **1** with alkali (pH 9), we first synthesized **2-4** by coupling of **7** with synthetic **6** or its analogues. The preparation of **9** and **10** is now under investigation.



8. **5** was prepared from **1** as follows: (i) Cs_2CO_3/CH_3OH, pH 9 (76% yield) (ii) TBDMSOTf, 2,6-lutidine/CH_2Cl_2 (45% yield) (iii) H_2, 10% Pd-C/AcOEt (quant.) (iv) **6**, $Cl_3C_6H_2COCl$, Et_3N, DMAP/$PhCH_3$ (97% yield) (v) HF·Py/THF (76% yield).
9. Hydrolysis of **5** to **8** was not observed under this condition.
10. Tachibana, K.; Scheuer, P. J.; Tsukitani, Y.; Kikuchi, H.; Van Engen, D.; Clardy, J. *J. Am. Chem. Soc.* **1981**, *103*, 2469-2471.
11. Quinn, R. J.; Taylor, C.; Suganuma, M.; Fujiki, H. *BioMed. Chem. Lett.* **1993**, 1029-1034.
12. Molecular mechanics calculations were carried out on a Silicon Graphics Power Iris 220GTXB computer using Biograf and NMRgraf programs version 3.2.1. (Molecular Simulations, Inc.). Dreiding-II was used for force field. [Maya, S. L.; Olafson, B. D.; Goddard III, W. A. *J. Phys. Chem.* **1990**, *94*, 8897-8909.] The *J* values calculated for the most stable conformation by MacroModel version 4 were similar to those observed for corresponding protons.



13. **1a** was insoluble in water and soluble in several organic solvents, such as chloroform and methanol. In contrast, **1b** was soluble in water. The ^1H NMR spectrum of **1b** in D_2O was quite similar to that of **1a** in $CDCl_3$, which suggested that tautomycin has similar conformation in D_2O and $CDCl_3$.
14. Detail calculation is now under investigation.

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