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Crystal and Solution State Conformations of Two Taxoids, Taxinine and Taxinine B

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Abstract: Crystal and solution state conformations of two taxoids, taxinine and taxinine B, inhibiting the drug transport by P-glycoprotein in multidrug-resistant cells, were analyzed by X-ray crystallographic analysis and ROE experiments. This study demonstrated that in solid state, both taxinine and taxinine B took similar cage conformation in which A-ring and the cinnamoyl side chain at C-ring were specially very close to each other. These conformations were also observed in their solution conformations deduced by ROE correlations in $CDCl_3$, Monte Carlo simulation using MM2* force field, and semiempirical molecular orbital calculation using PM3 method.
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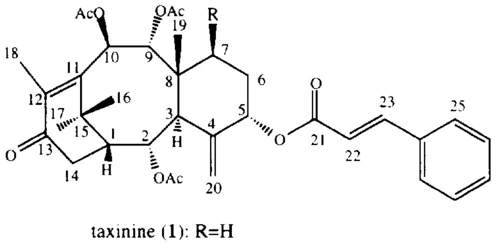
Paclitaxel (Taxol®), a new class of microtubule-stabilizing anticancerous agent, is one of the most promising cancer chemotherapeutic agents today and is currently intensively investigated chemically, biologically, pharmacologically, and clinically.[1] Recently, some of taxoids such as taxinine (**1**) and taxinine B (**2**), naturally occurring taxoids isolated from *Taxus cuspidata*, were shown to inhibit the drug transport activity of P-glycoprotein: these taxoids might be useful agents for overcoming the multidrug-resistance problems.[2]

The conformation of taxoids is recently being watched with interest. The X-ray structure and solution conformation in apolar and polar solvents of taxoids with potent antitumor activities and possessing a side chain at C-13, were published in recent papers,[3] and so were the X-ray structures of some other taxoids, with no side chain at C-13.[4] However, a few reports have referred to the conformation of taxoids possessing a cinnamoyl group at C-5.[5]

We obtained fine colorless needles of taxinine (**1**) and taxinine B (**2**), both possessing a cinnamoyl group at C-5 and assayed their solid state structures by X-ray crystallographic analysis. The solution conformations of **1** and **2** were also analyzed by high field NMR study, including ROE correlations in phase sensitive ROESY spectra to reveal the similarity between the solid state conformations and solution ones of these compounds. The results of the present studies on the solid and solution state conformations of taxinine and taxinine B by X-ray, NMR analysis, and simulation studies may provide basic information about their biological activity.



taxinine (**1**): R=H
taxinine B (**2**): R=OAc

RESULTS AND DISCUSSION

Solid state conformation

Taxinine (**1**) was crystallized from a $CHCl_3$ - MeOH mixture in orthorhombic crystals of space group $P2_12_12_1$, and taxinine B (**2**) from MeOH in orthorhombic crystals of space group $P2_12_12_1$. Crystallographic data are summarized in Table 1. The determination of the lattice constants and collection of intensity data were carried out on a Mac Science MXC18 for **1** and DIP-2020 automated diffractometer for **2**. Because the crystals of **2** deteriorated rapidly upon exposure to air, they were kept in contact with the mother liquor in a sealed thin-walled glass capillary. 3770 and 3107 reflections were collected for **1** and **2**, respectively, and the structures were solved by direct methods.[6] Non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares refinement were based on 2665 observed reflections ($I>3.00\sigma(I)$) with unweighed and weighed agreement factors of R=0.050 and R_w=0.055 for **1**, and on 2015 reflections ($I>2.00\sigma(I)$) with unweighed and weighed agreement factors of R=0.062 and R_w=0.080 for **2**, respectively. Figure 1 shows the stereoscopic view of **1** and **2** by ORTEP drawing.[7] Each skeleton of the molecule consisted of two 6-membered rings (A and C) and a 8-membered ring (B). These views (Fig. 1) show that the 6-membered rings A and C take boat and chair conformations, respectively, whereas, the central 8-membered ring a stable boat-chair conformation. The ring junctions are *cis* and *trans* for A/B and B/C, respectively. The cinnamoyl moiety at C-5 is located very close to ring A, and the molecules of **1** and **2** as a whole adopt cage conformations. All acetyl and cinnamoyl side chains are common planar, and take *s-trans* conformations for each bond between esteric oxygen and carbonyl carbon.

The mean values of the bond distances were calculated to be as follows: for C-C single bonds 1.531Å in **1** and 1.534Å in **2** and for C-O single bonds in the skeleton of the molecule 1.456Å in **1** and 1.468Å in **2**. The bond distances in the O-acetyl group were all normal: for C-C single bonds 1.483Å in **1**, 1.508Å in **2**, for C-O single bonds 1.355Å in **1** and 1.359Å in **2**, and for C=O double bonds 1.193Å in **1** and 1.185Å in **2**, within experimental error, suggesting that the crystal conformation was not under an unusual strain.

Table 1. Crystal data of taxinine (**1**) and taxinine B (**2**).

	taxinine	taxinine B
Empirical Formula	$C_{35}H_{42}O_9$(606)	$C_{37}H_{44}O_{11}$ (664)
Color, Habit	colorless, prismatic	colorless, prismatic
Dimensions	0.30 × 0.35 × 0.20 mm	0.50 × 0.50 × 0.10 mm
System	orthorhombic	orthorhombic
Lattice Type	Primitive	Primitive
Dcalc	1.23 g/cm^3	1.25 g/cm^3
Lattice Parameters (Å)	a=11.42(1) b=29.18(2) c=9.837(5) V=3279(4)Å^3	a=9.280(2) b=18.868(2) c=20.134(3) V=3525(1)Å^3
Space Group	$P2_12_12_1$	$P2_12_12_1$
Z value	4	4
Final R value (R_w)	0.050 (0.055)	0.062 (0.080)

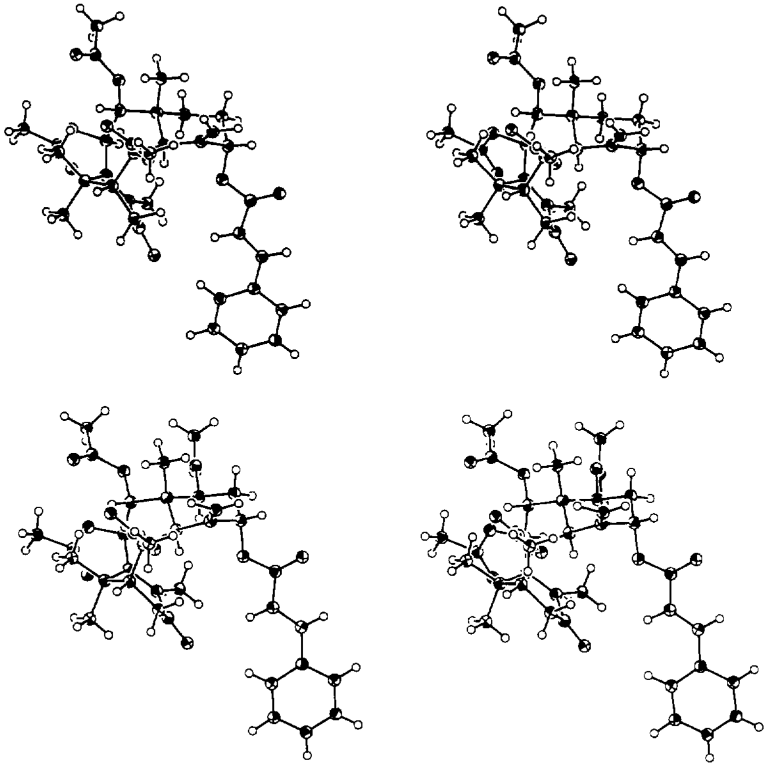


Fig. 1. ORTEP drawing of stereoviews of taxinine (**1**) and taxinine B (**2**), above: taxinine; below: taxinine B.

The mean values of the C-C-C bond angles of **1** and **2** were calculated to be 112.4° and 112.6°, respectively, for ring A and 112.0° and 112.3°, respectively, for ring C. The C8-C9-C10 bond angles (120.5° and 121.1°) in the eight-membered ring B and the C2-C3-C4 bond angles (118.5° and 118.8°) of **1** and **2** tended to be greater than the expected values.

Two bond lengths (C2-C3: 1.557 and 1.583Å; C3-C8 1.588 and 1.579Å for **1** and **2**, respectively) were significantly longer and the C8-C9-C10 bond angle (120.5° and 121.1° for **1** and **2**, respectively) deviates significantly from the idealized sp3 value. In addition, the ring A was also distorted which took a boat form much flattened at one end. The double bond in the ring A was distorted from planarity (torsion angle C15-C11-C12-C13: -17.2° and -14.8° for **1** and **2**, respectively), which was reflected by the length of the double bond (C11-C12 1.341 and 1.368Å for **1** and **2**, respectively).

Solution state conformation

NMR spectra were measured in $CDCl_3$ and the solution conformations of **1** and **2** were deduced on the basis of the data from ROE correlations in phase sensitive ROESY spectra. The ROE correlations of **1** are shown in Fig. 2. The ROE enhancements were observed between H-2, H-9, Me-16, and Me-19, suggesting that ring B took a boat-chair conformation. In addition, the ROEs between H-3 and H-7α, and between Me-19 and H-20 implied that ring C took a chair conformation, as in the crystal form. Furthermore, the ROE correlations between Me-18 and H-22, regulated the whole conformations of **1** and **2**, suggested that **1** and **2** took similar conformations to those of the crystals. Since no difference was observed between the ROE correlations of **1** and **2**, they are both considered to take similar conformations in $CDCl_3$.

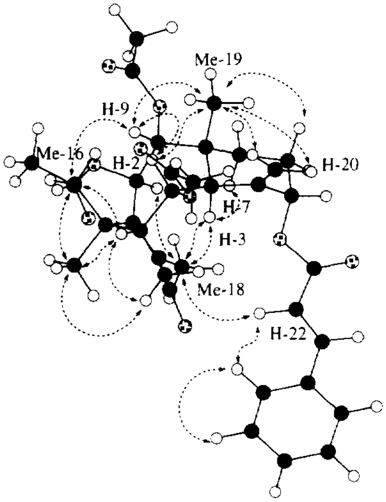


Fig. 2. Selected ROE correlations observed in **1**

Monte Calro Simulation and Molecular Orbital Calculation

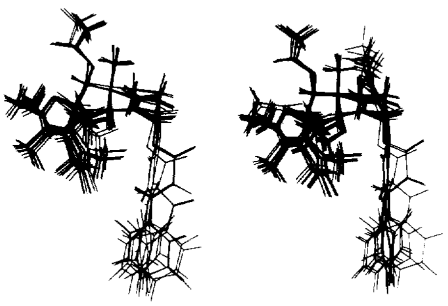
Intensive conformational studies of **1** and **2** were conducted by using the systematic pseudo Monte Carlo (MC) search,[8] followed by molecular orbital calculation. Each conformation generated by each MC calculation was minimized by the use of molecular mechanics calculation of MM2* force field implemented in MacroModel/Batchmin (Ver. 4.5).[9]

Firstly, the X-ray coordinates of **1** and **2** were selected as the starting stereostructures for the MC search. The pseudosystematic MC procedure of Still and Goodman[8] was performed, in a pseudosystematic way, by changing the torsion angles of the side chains in the 8-membered B and 6-membered C rings in the range of 0 - 180°. After the 5000 MC conformational search steps, each of the resulting conformations was subjected to the energy-minimization calculation by the MM2* force field to reduce the gradient rms to less than 0.001 kcal/Å mol. Of them, those conformers whose energy level was less than 50 kJ/mol above the global minimum-energy conformation were chosen. To eliminate possible duplicate conformations, comparison was performed on the heavy atoms only. Total 174 conformers for **1** and 96 for **2** were obtained. Among them, 9 conformers for **1** and 16 conformers for **2** had the energy levels within a range of 3 kcal/mol from the respective global minimum. All these conformers possessed similar 3-dimensional structures including the backbone ring and the side chains (Fig. 3: mean heavy atom RMSD: 0.85 Å for **1**; 0.83 Å for **2**). Major differences between these conformers are in the conformation of cinnamoyl side chains: for **1** and **2**, two types of conformations were obtained, one having *s-trans* conformation and the other *s-cis* for a C21-C22 bond. Total energies for the two types were comparable.

To compare the energetical stability of these two types of C21-C22 bond conformers, semiempirical molecular orbital calculation by the PM3 method was carried out.[10] 4 conformers of **1** and 5 conformers of **2**, having the energy levels within 1 kcal/mol of the global minima were chosen and subjected to geometry optimization by the PM3 method. The heat of formation (kcal/mol) of the *s-trans* isomers was shown to be lower than that of *s-cis* isomers both in **1** and **2** (average energy of 3 conformers for *s-trans* of **1**: -298.862

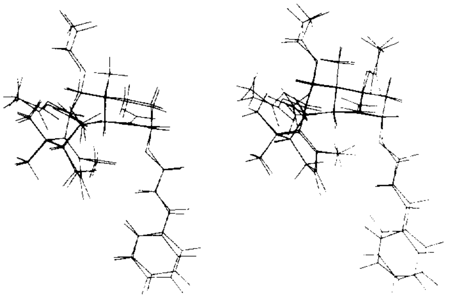
kcal/mol, 1 conformer for *s-cis* of **1**: -297.474 kcal/mol; average energy of 2 conformers for *s-trans* of **2**: -373.559 kcal/mol, 3 conformers for *s-cis* of **2**: -371.2523 kcal/mol). Fig. 4 shows superposition of the X-ray structures, and the energetically most stable *s-trans* conformers, indicating that the two structures matched fairly well in both **1** and **2** (heavy atom RMSD of **1**: 0.30 Å; that of **2**: 0.78 Å).

It is interesting to note that the cinnamoyl moiety at C-5 both in **1** and **2** are all directed into the interior of the ring A, resulting in forming similar cage conformations. Such conformational features might closely be related to their biological function.



taxinine (**1**) taxinine B (**2**)

Fig. 3. 9 and 16 superimposed conformers of **1** and **2**, respectively, within 3 kcal/mol of the global minima.



taxinine (**1**) taxinine B (**2**)

Fig. 4. Superimposed structures of X-ray structure and the energetically most stable conformer of **1** and **2**.

EXPERIMENTAL

Materials and Methods

Taxinine (**1**) and taxinine B (**2**) were isolated from the stem of *Taxus cuspidata* Sieb. et. Zucc. *var. nana* Rehder. and were identified by comparison with authentic samples. Proton NMR spectra were recorded on Varian Unity 400 spectrometer. 5 mg of **1** and **2** in a 5mm tube (0.5ml $CDCl_3$, degassed) was used for the homonuclear 2D NMR measurements. The spectra were recorded at 303K. A phase sensitive ROESY experiment was performed with mixing times of 200 msec.

Crystallographic Analysis of Structures of 1 and 2

The crystal data are shown in Table 1. **1** was mounted on a Mac Science MXC18 automated diffractometer with graphite-monochromated CuKα radiation (λ=1.54178Å) at 23°C and **2** on a DIP-2020 automated diffractometer with MoKα radiation (λ=0.71073Å) at 23°C. Each structure was determined by the direct method using the SIR program[6] and the refinement was carried out by the full-matrix least-squared method [Function minimized: $\Sigma w(|Fo|-|Fc|)^2$, $w=1/\sigma^2(Fo)$]. The molecular structures determined by these methods are illustrated in Fig. 1. The refined fractional atomic coordinates, bond lengths, bond angles, hydrogen-atom coordinates and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre (CCDC).

Computational Methods

Computer modeling experiments were carried out by using the MacroModel program (version 4.5) on an IRIS 4D computer (Indigo^2 R4400). Molecular mechanics calculations without any constraints were performed with the MM2* force field with a distance-dependent dielectric, $\varepsilon=R_{ij}$. The extended cutoff distances employed were 8 Å for van der Waals, 20 Å for charge/electrostatics and 10 Å for charge/multipole electrostatics. The obtained structures were energy minimized by the use of the derivative convergence criteria at a value of 0.001 kJ/Å-mol *in vacuo*.

Pseudo Monte Carlo Calculation. Each MC search was carried out by using the Pseudo Monte Carlo routine in MacroModel. Three closure bonds were chosen at each taxane ring with a closure limit of 1 - 4 Å. A total of 174 unique conformers for **1** and 96 conformers for **2** were obtained whose energy levels were within 50 kJ/mol of the lowest energy conformer. Furthermore, 4 and 5 conformers for **1** and **2**, respectively, were obtained whose energy levels were within 1 kcal/mol of the lowest energy conformer.

Molecular Orbital Calculation Geometries of the above conformers within 1 kcal/mol of the global minima were optimized by semiempirical molecular orbital calculation with the PM3 method and the heat of formation was calculated for each conformer.

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REFERENCES AND NOTES

1. Kingston, D.G.I.; Molinero, A.A.; Rimoldi, J.M. *Progress in the Chemistry of Organic Natural Products*, **1993**, *61*, 1-206.
2. Kobayashi, J.; Ogiwara, A.; Hosoyama, H.; Shigemori, H.; Yoshida, N.; Sasaki, T.; Li, Y.; Iwasaki, S.; Naito, M.; Tsuruo, T. *Tetrahedron*, **1994**, *50*, 7401-7416.
3. Gao, Q.; Parker, W.L. *Tetrahedron*, **1996**, *52*, 2291-2300; Mastropaolo, D.; Camermam, A.; Luo, Y.; Brayer, G.D.; Camerman, N. *Proc. Natl. Acad. Sci. USA*, **1995**, *92*, 6920-6924; Dubois, J.; Guenard, D.; G.-Voegelein, F.; Guedira, N.; Potier, P.; Gillet, B.; Beloeil, J.-C. *Tetrahedron*, **1993**, *49*, 6533-6544; Williams, H.J.; Scott, A.I.; Dieden, R.A.; Swindell, C.S.; Chirlian, L.E.; Francl, M.M.; Heerding, J.M.; Krauss, N.E. *Tetrahedron*, **1993**, *49*, 6545-6560.
4. Shiro, M.; Koyama, H. *J. Chem. Soc.* (B), **1971**, 1342-1346; Ho, T.-I.; Lin Y.-C.; Lee, G.-H.; Peng, S.M.; Yeh, M.-K.; Chen, F.-C. *Acta Cryst.*, **1987**, *C43*, 1380-1382.
5. Begley, M.J.; Frecknall, E.A.; Pattenden, G. *Acta Cryst.*, **1984**, *C40*, 1745-1747.
6. Attomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A.; Burla, M.C.; Polidori, G.; Camalli, M. *J. Appl. Cryst.*, **1994**, *27*, 435-436.
7. Johnson, C. K. ORTEP II Report ORNL-5138 Oak Ridge National Laboratory, Tennesse, USA, 1976.
8. Goodmann, J.M.; Still, W.C. *J. Comput. Chem.*, **1991**, *12*, 1110-1117.
9. Mohamadi, F.; Richards, N.G.J.; Guida, W.C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W.C. *J. Comput. Chem.*, **1990**, *11*, 440-467.
10. Stewart, J.J.P. *J. Computational Chem.*, **1989**, *10*, 221-264.

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