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The "Hydrophobic Collapse" Conformation of Paclitaxel (Taxol®) Has been Observed in a Non-aqueous Environment: Crystal Structure of 10-Deacetyl-7-epitaxol

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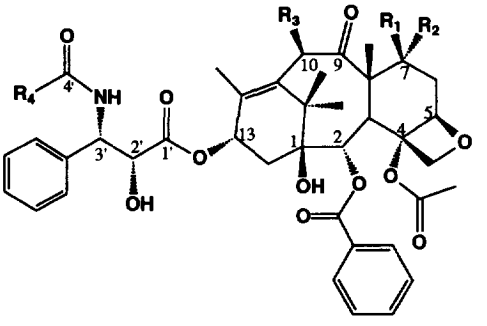
Abstract: Together with the crystal structure of paclitaxel, this single-crystal X-ray diffraction study of 10-deacetyl-7-epitaxol provides examples of the "hydrophobic collapse" conformation of paclitaxel in solid state. More importantly, it gives the first evidence that the "hydrophobic collapse" conformation exists in a non-aqueous environment. This study demonstrates that in solid state, a bioactive molecule could adopt a conformation which is usually held only in an aqueous medium through the process of hydrophobic collapse. The special arrangement of molecules and large solvent channels found in the crystal structure suggest a similarity of the molecular environment between the solid state and the aqueous solution. Comparison to the crystal structure of docetaxel (Taxotere®) reveals that the flexibility around C1'-C2' and C2'-C3' appears to be fully responsible for the orientation of the side chain. Moreover, a careful comparison of the crystal structures has indicated that the non hydrophobic collapse conformation found in the crystal of paclitaxel is probably caused by molecular packing. It is a common feature for molecules of docetaxel, paclitaxel and 10-deacetyl-7-epitaxol that the 2'-hydroxyl and the 4'-carbonyl groups are involved in molecular interactions by hydrogen bonding.

INTRODUCTION

Paclitaxel is a naturally occurring diterpene first isolated from the bark of *Taxus brevifolia*[1] and is regarded as the most promising anticancer agent developed in the last decade. It has shown remarkable activity against ovarian and breast cancer,[2,3] and promising performances in the treatment of non-small cell lung cancer and head and neck cancer.[4,5,6] In addition to clinical results, paclitaxel acts through a unique mechanism of action,[7,8] i.e., instead of inducing microtubule disassembly like the vinca alkaloids, paclitaxel promotes the

polymerization of tubulin to microtubules and stabilizes them against depolymerization and thus prevents cell division. For these reasons, paclitaxel has been the target of intensive biological and chemical studies.[9,10,11,12]



	R1	R2	R3	R4
Paclitaxel	OH	H	Ac	Ph
Docetaxel	OH	H	H	OBu^t
10-deacetyl-7-epitaxol	H	OH	H	Ph

Paclitaxel possesses a side-chain at the C-13 position, which has been demonstrated to be essential for its biological properties.[13] It also has been shown that the receptor binding and activity of paclitaxel are dependent on the conformation of this highly flexible side-chain.[14] To understand the binding mode of paclitaxel, elucidation of the conformation-activity relationship has been an important aspect of paclitaxel-related research.[9,13] The structure and stereochemistry of paclitaxel, reported in 1971,[1] were determined from an X-ray analysis of derivatives of paclitaxel degradants 10-deacetylbaccatin III and *N*-benzoyl-3-phenylisoserine methyl ester. However, X-ray structures of taxoids possessing a side chain at C-13 were not available until 1990. The most important information on conformations of taxoids came from an X-ray analysis of docetaxel,[15] a semisynthetic paclitaxel analog with comparable activity. It remained the only X-ray structure published for active taxoids until the very recent paper regarding the structure of paclitaxel.[16] The conformation of docetaxel in crystals is characterized by the presence of sequential hydrogen bonding between the 1'-ester carbonyl, the 2'-hydroxyl, and the 3'-NH within the side chain as well as interactions between the C-2 benzoate of the taxane core and the t-butyl group of the side chain. This conformation, which was later called the "Apolar" model by Nicolaou *et al.*, was also observed in NMR experiments and modeling studies for both paclitaxel and docetaxel in non-polar solvents. Recently, conformations that differ from the Apolar model have been observed in a series of NMR and computer modeling studies in a more polar environment.[17,18,19,20] In aqueous solutions, a conformation characterized by the presence of interactions between the C-2 benzoyl and the C-4 acetyl groups of the core and 3'-phenyl group of the side chain has been reported by Vander Velde *et al.* and Nicolaou *et al.*. It is also found in the crystal structure of paclitaxel grown from an aqueous solution.[16] All these studies have demonstrated that this conformation occurs only in aqueous solutions and arises due to what is called hydrophobic collapse.[21] Solvent effects are believed to be responsible for differences between the apolar model and this conformation.

In parallel to the structure-activity relationship study of paclitaxel, we have established a program to study the conformation-activity relationship. This program aims to systematically determine crystal structures of paclitaxel analogs with the original or a modified C-13 paclitaxel side chain, using X-ray

diffraction methods, and to map conformational flexibility of paclitaxel. 10-Deacetyl-7-epitaxol is one of the molecules that we have studied. Its solid state conformation, determined 2 years ago, has served as an important tool in our paclitaxel program. In previous SAR studies, it was demonstrated that modifications at C-10 generally have little effect on the activity, and the C-7 epimer is similar in its ability to inhibit cell proliferation and to promote tubulin polymerization.[14,22] We herein report the X-ray structure of 10-deacetyl-7-epitaxol, a compound with *in vitro* activity and possessing a solid state conformation very similar to what Vander Velde *et al.*, Nicolaou *et al.* and Mastropaolo *et al.* described in their recent papers, but in a non aqueous environment.

EXPERIMENTAL

10-Deacetyltaxol, isolated from extracts of *Taxus brevifolia* bark by chromatography, was epimerized at C-7 by refluxing in 0.2% DBU in toluene. The isolated and purified 10-deacetyl-7-epitaxol was then recrystallized from ethyl acetate by slow evaporation. Large thick colorless plates were formed and were stable at room temperature. A fragment of approximate size 0.18 X 0.36 X 0.54 mm, cut from a large crystal, was mounted on a quartz fiber with epoxy adhesive and used for preliminary examination and diffraction intensity data collection. Diffraction experiments were carried out at room temperature on an Enraf-Nonius CAD4 diffractometer using graphite-monochromated Cu $K\alpha$ radiation (λ = 1.5418 Å). Cell constants were obtained from a least-squares fit to data for 25 well centered reflections in the range $16.32° \le \theta \le 32.57°$. Unit cell constants were a = 16.329(2), b = 17.704(2), c = 17.504(1) Å, β = 100.611(5)° and V = 4973.6(7) Å^3. From the systematic absence, the space group was determined to be $P2_1$. Intensity data were collected with $0 \le h \le 20$, $-21 \le k \le 0$, $-21 \le l \le 21$ to $\theta = 70°$. A total of 9285 unique reflections was measured using a θ–2θ scan mode. The Lorentz, polarization and absorption effects were corrected. After data reduction, the unique data set contained 8336 observed reflections with $I \ge 3\sigma(I)$.

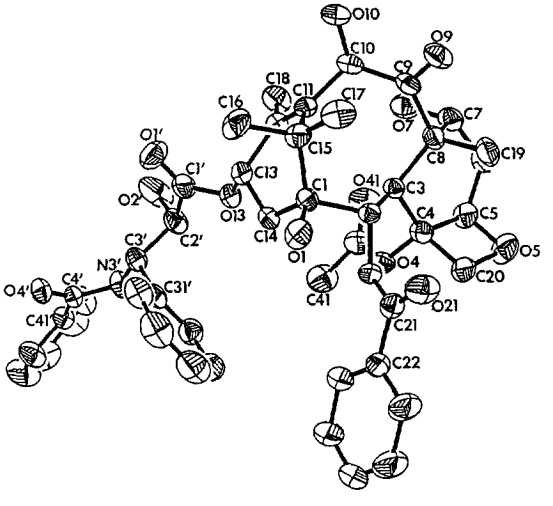


Figure 1. *ORTEP* drawing of conformer A of the crystal structure of 10-deacetyl-7-epitaxol, showing non-H atoms and the atomic labeling scheme. The thermal ellipsoids were drawn at 40% probability level.

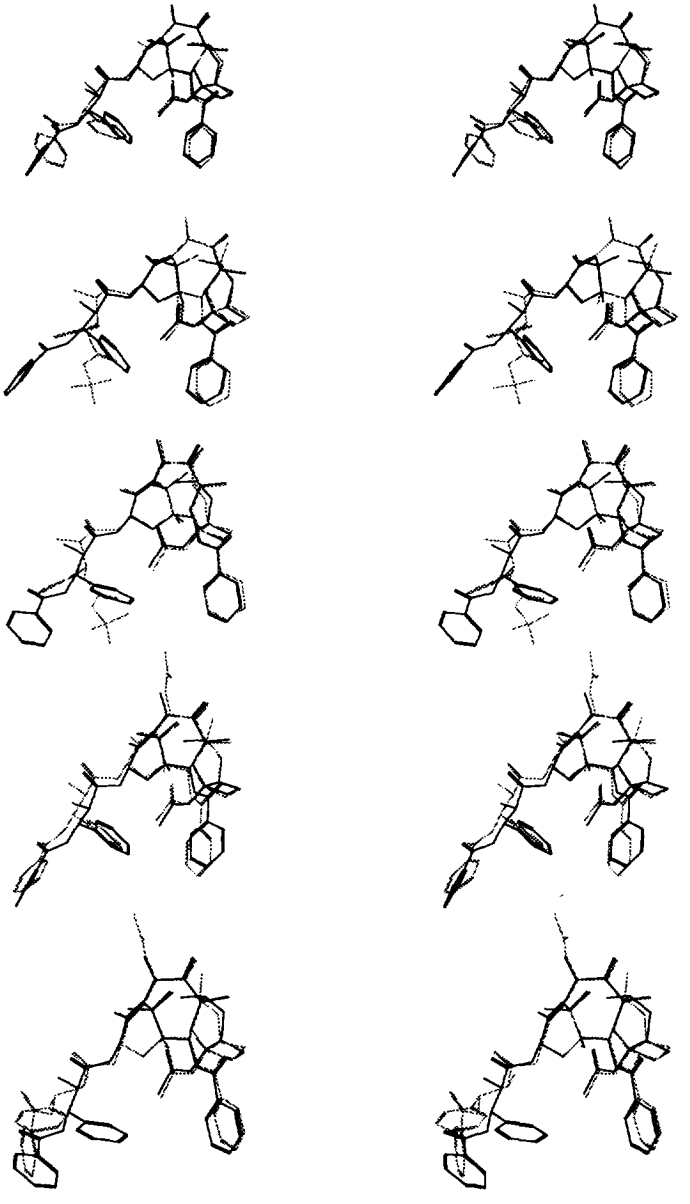


Figure 2. From top to bottom, stereoscopic view of superimposion of conformer A and B, A and docetaxel, B and docetaxel, A and conformer B of paclitaxel, and B and conformer A of paclitaxel, over the tetracyclic ring system.

The structure was solved by direct methods and was refined by full-matrix least-squares techniques using computer software *SHELXTL*.[23] Although all non-hydroxyl hydrogen atoms of the compound molecule were clearly shown in difference Fourier maps, their positions were calculated from an idealized geometry with

standard bond lengths and angles. All hydrogen atoms had isotropic temperature factors and were included in structure factor calculations with fixed parameters. The hydroxyl hydrogen atoms were not located. Due to the rather high temperature factors of the non-H atoms of the solvent molecules, the hydrogen atoms bonded to them were not generated. The final refinements included 1098 parameters, a scale factor and atomic coordinates and anisotropic temperature factors for non-hydrogen atoms of the compound molecule and ordered atoms of solvent molecules. The refinement converged with agreement factors $R(F) = 0.0672$ and $wR(F) = 0.0933$, where $w = 1/[\sigma^2(F) + 0.0007F^2]$, $S = 2.21$ for 8336 reflections. The final difference Fourier map showed a residue of electron density in the range of $-0.45 \leq \Delta\rho \leq 0.90$ eÅ^{-3}, and the recognizable peaks were found only around atoms of the disordered solvent molecule.

RESULTS AND DISCUSION

Description of the Molecular Structure of 10-Deacetyl-7-epitaxol. The crystal structure of 10-deacetyl-7-epitaxol revealed two independent molecules (conformer A and B) in each crystallographic asymmetric unit. Figure 1 shows the *ORTEP* drawing of conformer A and the atomic labeling. The two conformers have similar overall structures while there are noticeable differences in the conformation (Figure 2). The principal difference is in the rotational orientation of the benzoyl group at the end of the side chain. The benzoyl group is more *anti* to the adjacent part of the side chain in conformer A (torsion angle C2'-C3'-N3'-C4' = -143.0°) but is in a more *gauche*-like orientation in conformer B (C2'-C3'-N3'-C4' = -109.4°) as listed in Table 1. A more extended conformation is thus observed for the side chain of conformer A. The second most noticeable difference between the two conformers is again seen within the side chain. The torsion angle C31'-C3'-N3'-C4', which corresponds to rotation of the phenyl group around the C3'-N3' single bond, is 92.4° in A but 125.5° in B. Compared to the global structure, these deviations are relatively small and local and are likely due to different orientations of the molecules along different crystallographic axes. There are only slight differences in the conformations of the acyl groups of the taxane core, and the largest deviation is less than 1 Å, occurring at the C-2 benzoate. In both conformers, the *axial* hydroxyl group at C-7 is intramolecularly hydrogen bonded, 2.833 Å and 2.796 Å for A and B respectively, to the carbonyl oxygen of the acetate at C-4. This hydrogen bond with a bond length of 2.787 Å is also found in the crystal structure of Baccatin V,[24] the paclitaxel core structure with an acetyl group at C-10, epimeric at C-7 and lacking the C-13 side chain.

In the crystal, the two conformers are in different environments. The extended side-chains of A conformers are aligned along the opposite directions of crystallographic *c*-axis while the cores are linked by intermolecular hydrogen bonds, O5···H-O1, resulting in infinite long chains along the *b*-axis. For conformer B, although the molecules have a similar arrangement, the long dimension of the side-chain is along the *a*-axis and no hydrogen bonding exists between the cores. It appears that the smaller dimension of the *a*-axis (1.175 Å shorter than the *c*-axis) is responsible for the less extended conformation of conformer B. Table 2 lists the major interactions involving both conformers. There are, in all, four independent hydrogen bonds that do not involve solvent molecules: one core-to-core, two side chain-to-side chain and one core-to-side chain. In both conformers, three atoms of the side chain, the 2'-hydroxyl oxygen, the 3'-amido nitrogen and the 4'-carbonyl oxygen, participate the formation of hydrogen bonding in a similar fashion. Studying the crystal packing also indicates that both conformers are extensively involved in van der Waals contacts in similar manners. These interactions may have contributions to the stabilization of the crystal lattice. However, because they are very

weak as suggested by the distances, they are less likely to have a significant impact on conformations of molecules, and there is no evidence that they have caused different conformations in the two conformers observed at the end of the side-chain. A noticeable short contact is found between two carbonyl oxygens of the two conformers, O9(A)···O21(B) = 2.778 Å. The rather large angle (~120°) between the two dipole moments certainly reduces the strength of the unfavorable interaction.

Comparison of 10-Deacetyl-7-epitaxol and Docetaxel. Our results show that the conformation of the core tetracyclic ring system in 10-deacetyl-7-epitaxol is essentially identical to this portion of the crystal structure of docetaxel. As shown in Figure 2, only slight differences in conformations of the substituent groups at C2 and C4 are observed between the two molecules. It is noteworthy that no significant changes in the conformation of the core are seen as consequence of the change from an *equatorial* to an *axial* O7 group. The intramolecular hydrogen bond between the *axial* hydroxyl group at C7 and the carbonyl oxygen of the acetyl group at C4 in 10-deacetyl-7-epitaxol is absent in docetaxel because the epimeric *equatorial* hydroxyl group is too far from the C4 acetate group. However, the distorted *chair* conformation of the C-ring remains. The largest shift is found at the carbonyl oxygen of the benzoyl group at C2 of conformer B, which is only 0.567 Å away from the same atom of the benzoyl group at C2 in docetaxel.

Conformations of the side-chains at C13 are different. As suggested by the selected torsion angles of 10-deacetyl-7-epitaxol and docetaxel listed in Table 1, the differences in rotations around the C1'-C2' and C2'-C3' single bonds seem to dominate the changes in the orientation of the side-chain relative to the core. Because of these rotations, the 2' and 3' hydrogen atoms are *trans* in both A and B conformers compared to *gauche* in docetaxel. The 1'-carbonyl oxygen and the 2'-hydroxyl oxygen are no longer *syn* (O1'-C1'-C2'-O2' = -2.2° in docetaxel) but *gauche* to each other, O1'-C1'-C2'-O2' = 47.5°(A) and 56.5°(B). As a result, the intramolecular hydrogen bond between these two atoms observed in docetaxel does not exist in either conformer A or B. For the same reason, it is geometrically impossible to form the second hydrogen bond (3'-N-H···2'-O) within the side-chain. Most importantly, the side-chain and the core interact through the clustering of the hydrophobic 3'-phenyl, the 2-benzoyl and the 4-acetyl groups while the clustering in docetaxel involves different portion of the side-chain, i.e., the t-BOC group. The closest atoms of the 3'-phenyl and 2-benzoyl groups are 3.69 Å in conformer A and 4.22 Å in B. The shortest distance between atoms of 3'-phenyl and 4-acetyl groups is 3.90 Å in A and 4.02 Å in B. In comparison to the corresponding distances between these groups in docetaxel, which are all larger than 5 Å, it is apparent that in solid state the interaction of the side-chain and the core is much stronger in conformer A and B than in docetaxel.

Comparison of 10-Deacetyl-7-epitaxol and Paclitaxel. The crystal structure of paclitaxel determined by Mastropaolo *et al.* also contains two crystallographically independent molecules. Torsion angles listed in Table 1 for the side chain indicate that conformer A of 10-deacetyl-7-epitaxol has a conformation quite similar to conformer B of paclitaxel. Visible deviations occur at both of the core and the side chain although there is no significant change in the side-chain orientation relative to the core. For instance, the largest deviation is 1.1 Å at 2-benzoyl group and is more than 2 Å at the end of the side chain. The shortest distance between atoms of the 3'-phenyl and 2-benzoyl groups is 3.94 Å in conformer B of paclitaxel, but 3.69 Å in conformer A of 10-deacetyl-7-epitaxol. However, the former has a much shorter closest distance between atoms of 3'-phenyl and 4-acetyl groups, 3.63 Å, than the later, 3.90 Å. Unlike conformer B, conformer A of paclitaxel is different from either A or B conformer of 10-deacetyl-7-epitaxol. The torsion angles around C1'-C2' show the most significant differences. Similar to what has been observed when comparing docetaxol to 10-deacetyl-7-epitaxol,

the different stereochemistry at C7 and thus lack of the intromolecular hydrogen bond between the hydroxyl group and the carbonyl oxygen of the acetyl group at C4 apparently have no impact on the C-ring, which again adopts the same distorted *chair* conformation in both conformers of paclitaxel.

Table 1. Selected Torsional Angles (°) for the Side-chain of Conformer A and B of 10-Deacetyl-7-epitaxol, Paclitaxol and Docetaxel

	10-Deacetyl-7-epitaxol		Paclitaxol		Docetaxel
	A	B	A	B	
C13-O13-C1'-O1'	11.8	4.3	2	4	-6.6
C13-O13-C1'-C2'	-166.6	-176.9	180	-177	168.0
O13-C1'-C2'-O2'	-134.0	-122.4	-84	-138	-176.7
O13-C1'-C2'-C3'	108.3	118.0	159	103	60.2
C1'-C2'-C3'-C31'	-66.4	-53.6	-64	-58	-179.4
C1'-C2'-C3'-N3'	168.8	-176.7	176	179	56.4
H2'-C2'-C3'-H3'	173.2	-172.2	-174	-179	57.3
O1'-C1'-C2'-O2'	47.5	56.5	93	41	-2.2
O1'-C1'-C2'-C3'	-70.0	-63.0	-24	-77	-125.3
C2'-C3'- N3'-C4'	-143.0	-109.4	-118	-155	-141.3
C2'-C3'-C31'-C32'	106.8	131.2	-166	102	83.6
O2'-C2'-C3'-N3'	51.7	63.7	60	61	-64.7
O2'-C2'-C3'-C31'	176.4	-173.3	180	-175	59.5
C3'-N3'-C4'-O4'	-3.0	-6.3	1	-1	12.8
C3'-N3'-C4'-C41'[a]	177.0	172.3	-178	-178	-172.4
C31'-C3'-N3'-C4'	92.4	125.5	120	83	97.3
C32'-C31'-C3'-N3'	-130.5	-107.4	-73	-137	-154.6
H3'-C3'-N3'-H'(N3')	153.6	-173.0	158	123	159.4

[a] In docetaxel, the corresponding torsional angle is C3'-N3'-C4'-O5'.

Although paclitaxel and 10-deacetyl-7-epitaxol crystallized in the same space group, the crystals are of different unit cells and molecular packing. Interestingly, the involvement of functional groups in forming hydrogen bonding and thus in molecular interactions is of great similarity. For the four conformers of both structures, the C4 acetyl oxygen is the only atom that may but does not participate in any H-bonds. An important feature, when comparing the two crystal structures, found in the crystal of paclitaxel is the intermolecular clustering between 3'-phenyl group and 2-benzoyl group as consequence of a side-by-side alignment of molecules of conformer A along the crystallographic *a*-axis. The closest distance is 3.83Å. This observation may have suggested that this conformation of paclitaxel, which differs from the "hydrophobic collapse" model, is likely induced by crystal packing.

Conformer A and B of 10-deacetyl-7-epitaxol are basically the same as the conformation that Vander Velde *et. al* and Nicolaou *et al.* observed in aqueous solutions. The only difference we have noticed is the relative orientations of the two phenyl groups involved in the clustering. Nicolaou *et al.* found that the two aromatic rings of the phenyl groups are nearly parallelly aligned, and concluded that this parallel arrangement is particularly favorable for π-π interactions. Such special alignment has not been seen in solid state. A comparison of crystal structures of taxoids with or without a C-13 side-chain demonstrates that the 2-benzoyl and 4-acetyl groups are relatively rigid and have shown very small conformational changes from structure to structure. Thus, in crystals, differences in conformation between paclitaxel analogs may occur only within the side chain. In other words, the hydrophobic clustering is realized mainly through conformational changes of the side chain.

Table 2. Intermolecular Hydrogen Bonds and short Contacts (Å) for 10-Deacetyl-7-epitaxol

Atoms	Distance	Symmetry	Atoms	Distance	Symmetry
O5(A)···O1(A)	2.650	VII	O1(B)···O10(A)	2.882	III
O1(B)···O(EtOAc3)	2.832	I	O10(B)···O2'(A)	3.071	V
O21(B)···O9(A)	2.778	III	O4'(B)···N3'(A)	2.796	VI
O4'(A)···N3'(B)	2.851	I	O(EtOAc1)···O2'(A)	2.721	IV
O(EtOAc2)···O2'(B)	2.785	II			

Symmetry codes:

(I)	x, y, z	(V)	1-x, 0.5+y, 2-z
(II)	x, y, -1+z	(VI)	2-x, 0.5+y, 2-z
(III)	1-x, 0.5+y, 1-z	(VII)	2-x, -0.5+y, 1-z
(IV)	1-x, -0.5+y, 1-z		

Solvents and Molecular Packing in the Crystal. In the crystal of 10-deacetyl-7-epitaxol, each asymmetric unit contains three ethyl acetate molecules in addition to the two conformers. These solvent molecules are all found in channels that are formed by the molecular packing of conformer A and B and are infinite along crystallographic *a*-axis. In the channel, approximately one third of the surface is hydrophobic and the rest is hydrophilic. The 4-acetyl group is found near the center of the solvent channel as shown clearly in Figure 3. All the three solvent molecules are hydrogen bonded to either conformer A or B. The carbonyl oxygen of solvent 1 is hydrogen bonded to the 2'-hydroxyl group of conformer A with a distance of 2.721Å. The same oxygen of solvent 2 makes a hydrogen bond of 2.785 Å with the 2'-hydroxyl group of conformer B. The third hydrogen bond is formed between solvent 3 and the hydroxyl group at C-1 of the core in conformer B with a bond length of 2.832 Å. The N3'-benzoyl and C2- benzoyl groups do not interact with the solvent molecules though they are also on the floor of the channels and exposed to the solvents. It is of particular interest to note that all of the functional groups that are important for the activity of paclitaxel, i.e., the side chain and the southern portion of the molecule, are found on the surface of the channels. The much looser molecular packing in the channals assures the side-chain to adopt the most intrinsically prefered conformation.

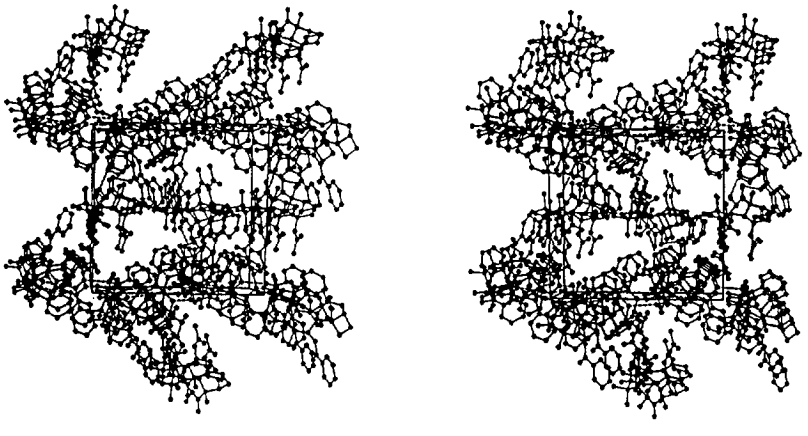


Figure 3. Stereoscopic view of crystal packing of 7-epi, 10-deacetyl paclitaxel down the *a*-axis. The solvent molecules are not present to show solvent channels clearly.

According to reported data[15,16,27] and our unpublished results, solvents are present in all crystals of paclitaxel analogs containing a side chain at C-13 with only one exception.[26] Obviously, solvents play an important role in crystallization of these compounds by stabilizing the crystal lattice. However, such large solvent channels as found in the crystal of 10-deacetyl-7-epitaxol are not evidenced in crystals of either docetaxel or 2-debenzoyl, 2-acetoxy paclitaxel, both molecules adopt a conformation different from what has been observed in aqueous solutions. Therefore, it seems to be conclusive that the rather large space in the solvent channels provides the molecule a solution-like environment and allows the functional groups to adopt the conformation found in aqueous solutions. A careful comparison of the solvent structures in these crystals has also shown that the 2' hydroxyl group is actively involved in interactions with solvent molecules. The presence of a hydrogen bond between the 2' hydroxyl group and a solvent molecule with the hydroxyl oxygen as the donor has been a common feature in all these crystals.

CONCLUSION

The crystal structure of 10-deacetyl-7-epitaxol is the second X-ray diffraction study reported for active paclitaxel analogs which adopt a "hydrophobic collapse" conformation. However, it provides, for the first time, evidence for the "hydrophobic collapse" conformation of paclitaxel in a non aqueous environment. Interestingly, this conformation was not observed in crystal structures of inactive analogs, such as 2-debenzoyl, 2-acetoxy paclitaxel and 2'-carbamate paclitaxel, determined earlier in our laboratory.[26,27] The conformation held by clustered 2-benzoyl, 4-acetyl and 3'-phenyl groups and by hydrogen bonding between the side chain and solvents appears to be structurally very stable and might remain in the bound state with only relatively small deviations. This study also indicates that this conformation, induced in aqueous solutions by the hydrophobic collapse process,[17] can be adopted in crystals of non-aqueous medium and, perhaps, in an organic solvent such as ethyl acetate prior to the formation of crystals. The hydrophobic clustering involves the 2-benzoyl, 4-acetyl and 3'-phenyl groups. Comparison to the crystal structure of docetaxel has demonstrated that large solvent channels could significantly minimize possible influences of the molecular packing in solid state on the conformation of molecules and may provide an environment similar to that in aqueous solutions. It also indicates that the conformation adopted by docetaxel in crystals are mainly due to the specific molecular packing, which somehow mimics the effect of a non-polar solvent on conformation.

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