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Chemistry

Crystal and molecular structure of paclitaxel (taxol)

DONALD MASTROPAOLO*†‡, ARTHUR CAMERMAN*†‡, YUOGANG LUO§, GARY D. BRAYER§, AND NORMAN CAMERMAN†‡¶

*Division of Neurology, RG-27, University of Washington, Seattle, WA 98195; †ARDONO Research, 737 Belmont Place East, Seattle, WA 98102; §Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC Canada V6T 1Z3; and ¶Department of Biochemistry, University of Toronto, Toronto, ON Canada M5S 1A8

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ABSTRACT **Paclitaxel (formerly called taxol), an important anticancer drug, inhibits cell replication by binding to and stabilizing microtubule polymers. As drug–receptor interactions are governed by the three-dimensional stereochemistries of both participants, we have determined the crystal structure of paclitaxel to identify its conformational preferences that may be related to biological activity. The monoclinic crystals contain two independent paclitaxel molecules in the asymmetric unit plus several water and dioxane solvent molecules. Taxane ring conformation is very similar in both paclitaxel molecules and is similar to the taxane ring conformation found in the crystal structure of the paclitaxel analogue docetaxel (formerly called taxotere). The two paclitaxel molecules have carbon-13 side-chain conformations that differ from each other and from that of the corresponding side chain in the docetaxel crystal structure. The carbon-13 side-chain conformation of one paclitaxel molecule is similar to what was proposed from NMR studies done in polar solvents, while that of the other paclitaxel molecule is different and hitherto unobserved. The paclitaxel molecules interact with each other and with solvent atoms through an extensive network of hydrogen bonds. Analysis of the hydrogen-bonding network together with structure–activity studies may suggest which atoms of paclitaxel are important for binding to microtubule receptors.**

Since its isolation from the extract of the inner bark of the Pacific Yew tree (1) and the demonstration of its antineoplastic activity against a variety of tumors (2), paclitaxel (formerly called taxol) has become one of the most promising anticancer drugs to appear in decades. Paclitaxel has a complex and novel chemical structure (see Fig. 1) and a unique antitumor mechanism of action. Like the vinca alkaloids vincristine and vinblastine, paclitaxel's site of action is the microtubules. However, unlike the vinca alkaloids, which cause depolymerization of microtubules, paclitaxel promotes microtubule assembly and stabilizes microtubule polymers, thereby blocking cell replication (3).

Clinical development of paclitaxel progressed slowly because of the small amounts of drug obtainable from the crude bark extract and its poor water solubility. Adequate supplies can now be synthesized from a precursor found in the needles or leaves of a variety of yew trees (4–6). Paclitaxel has been approved by the U.S. Food and Drug Administration for the treatment of ovarian and breast cancer, and phase II trials are in progress on a wide variety of carcinomas including lung, colon, prostate, head and neck, cervical, and brain. However, along with the tremendous potential that paclitaxel has shown as an antitumor drug, clinical problems with solubility, toxicity, and development of drug resistance are sufficiently severe that the need for paclitaxel analogues with better therapeutic efficacy and less toxicity is clear.

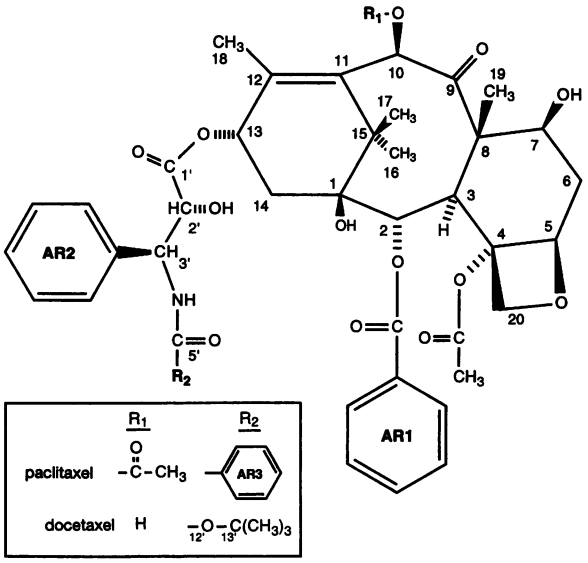

FIG. 1. Chemical structures of paclitaxel and docetaxel with numbering schemes.

Rational design of new analogues would be facilitated if the three-dimensional molecular structures of paclitaxel and related compounds could be determined and correlated with their microtubule-binding affinities, with toxicity and with other pharmacological properties. In attempts to elucidate the three-dimensional structure of paclitaxel, several NMR investigations (7–13) have been undertaken on paclitaxel and docetaxel (formerly called taxotere), a chemically similar analogue of paclitaxel (see Fig. 1) that also shows potent antitumor activity (14). However the NMR studies differ somewhat on their identifications of paclitaxel's preferred conformation in solution. The crystal structure of docetaxel (15) has until now provided the only solid-state three-dimensional structure of a paclitaxel-like compound. In the present study we report the determination of the crystal structure of paclitaxel.‖ The crystal form reported contains two independent paclitaxel molecules in the asymmetric unit plus several water and dioxane solvent molecules. This crystal structure determination provides detailed conformational descriptions of two independent paclitaxel molecules and identifies which regions of the molecular conformations are constant and which are variable. In addition, the presence of solvent molecules may make the crystal structure conformations useful for analysis and interpretation of NMR solution studies. Structure determination also provides prototype three-dimensional conformations against which active and

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‡To whom reprint requests should be addressed.

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inactive paclitaxel analogues may be compared to elucidate structure–activity relationships.

MATERIALS AND METHODS

Crystals of Taxol from Bristol-Myers Squibb were grown by slow evaporation at room temperature of a solution containing a mixture of dioxane, water, and xylene. Thin plate-like crystals usually grew at the water/organic solvent interface. The crystals are monoclinic, space group $P2_1$, with unit-cell dimensions a = 9.661(1), b = 28.275(3), and c = 19.839(2) Å and β = 99.730(4)° with four molecules in the unit cell (two independent paclitaxel molecules in the asymmetric unit). They are sensitive to air and therefore had to be sealed in capillary tubes containing mother liquor. All crystals showed signs of decomposition with time, which was most likely due to a combination of solvent loss and x-ray damage. X-ray diffraction intensity data were collected on a Rigaku *R*-axis image plate detector system. Data collection was attempted on five crystals, with two crystals yielding data of sufficient quality to be used in structure solution and refinement.

Data to 1.2-Å resolution [3040 reflections, 2487 with $I > 2\sigma(I)$, where σ is the standard deviation in intensity (I)] from the second crystal were used to solve the structure. Direct methods were tried initially but without success. A systematic search of rotation and translation space was tried next employing the tricyclic carbon skeleton, the oxetane ring, and the hydroxyl and ester oxygen coordinates of the docetaxel crystal structure (15) as the 28-atom search model. Using data to 2.0-Å resolution, we chose possible solutions on the basis of their crystallographic R (discrepancy) value and then subjected them individually to a rigid-body refinement. The three best possible solutions had approximately equal R values of 53%. Since there are two paclitaxel molecules in the asymmetric unit, translational searches over the unit cell were done on each pair of the three possibilities to determine the placement of the second fragment with respect to the origin defined by the first fragment. The best solution for two fragments gave an R value of 48%. Subsequent difference electron-density maps alternating with restrained least-squares refinements were used to locate the rest of the atoms in the two paclitaxel molecules. During this time we were able to grow better crystals, which diffracted to a resolution of 1.04 Å. Data from one of these crystals [4333 reflections, 4236 with $I > 2\sigma(I)$] were used to complete the structure determination and refinement. All refinements were done with the restrained least-squares program RESLSQ (16) that includes a six-parameter thermal refinement. Hydrogen atoms for each paclitaxel molecule were either calculated or located from difference maps and were included in the final structure. In most cases only one hydrogen atom on each of the methyl groups was visible in the map and the other two were calculated on the basis of ideal tetrahedral geometry. The final structure consists of two paclitaxel molecules, five water oxygens, and three dioxane molecules (one dioxane contains a disordered carbon-atom position) for a total of 250 atom positions. Restraints were imposed on nonhydrogen bond distances and next-bonded distances to lie within 0.02 Å and 0.04 Å, respectively, of their ideal values, and the six phenyl groups were restrained to within 0.02 Å of planarity. Bonds and next-bonded distances involving hydrogen atoms were similarly restrained to 0.05 Å and 0.10 Å, respectively. The final R value based on this model is 0.071 for the 4236 observed data, and the weighted R for all the data is 0.094. There are a small number of reflections, particularly low-angle data, that show large discrepancies between observed and calculated structure factors. These discrepancies are most likely attributable to additional solvent molecules that are grossly disordered and cannot be resolved (17). As might be expected, the solvent atoms included in the structure show high thermal motion: two of the dioxane molecules each have a carbon atom with one anisotropic U_{ij} component as large as 0.51 Å^2, and one water oxygen shows the next highest thermal motion with an anisotropic U_{22} component equal to 0.44 Å^2. The thermal parameters describing the motion of the atoms in the paclitaxel molecules are all reasonable: the phenyl ring atoms exhibit the highest thermal motion relative to the other paclitaxel atoms, with 0.32 Å^2 being the largest anisotropic U_{ij} component observed for one of the phenyl carbons.

RESULTS AND DISCUSSION

Description of the Molecules. The two independent molecules of paclitaxel are similar in overall shape with the major difference in conformation occurring in the carbon-13 (C13) side chain. Fig. 2 gives a stereoscopic view of each molecule. The taxane ring geometry is fairly constant in the two paclitaxel molecules and agrees with that found in the crystal structure of docetaxel. The C13 side chains are in extended conformations in both molecules, which places the benzamide groups farthest from the taxane ring cluster; this is in contrast to the docetaxel crystal structure wherein the C13 side chain is folded and the *tert*-butyl group, analogous to paclitaxel's benzamide, is positioned closer to the taxane ring (see Fig. 3). The orientations of the C2 benzoate and the acetate groups on taxane ring atoms C4 and C10 are very similar in the two molecules: the C2 benzoate and C10 acetate groups extend away from the ring structure in opposite directions approximately perpendicular to the direction of the C13 side chains, and in each molecule the C4 acetate points away from the C2 benzoate group with the acetate carbonyl oxygen positioned over the U-shaped pocket formed by the rigid ring structure.

A listing of the torsion angles of the C13, 2′-*R*, 3′-*S* ester side chain for each molecule is given in Table 1. The most dramatic difference between the molecules occurs in the geometry about the C1′—C2′ bond; differences in the torsion angles about this bond are on the order of 50°. As a result, the positions and orientations of the C3′ phenyl groups (AR2 in Fig. 1) are markedly different in the two paclitaxel molecules. Comparisons of the two molecules also show differences of 37° and 32° in the torsion angles about bonds C3′—N and C5′—C1 (AR3 in Fig. 1), respectively, which affect the relative positions and orientations of the benzamide group. The carbonyl oxygen of the benzamide is significantly out of the plane of the AR3 phenyl group in molecule A, whereas in molecule B the oxygen atom and phenyl group are almost coplanar.

Although the taxane ring conformation is virtually the same in paclitaxel (both molecules) and docetaxel, conformations of the side chains, particularly that attached to C13, are different in the two compounds. These differences can be seen by comparing the torsion angles listed in Table 1; for example, the C2′—C3′ bond torsion angle differences are on the order of 120°. The lack of similarity in the orientations of the C2′ and C3′ substituents between docetaxel and the two paclitaxel molecules is also depicted in Fig. 3. In this stereo drawing the taxane ring of docetaxel is superimposed upon the taxane rings of paclitaxel molecules A and B, showing the positioning of the C13 side-chain atoms of docetaxel (filled circles) relative to equivalent atoms of the paclitaxel molecules.

As the stereochemistry at C13 is critical for biological activity (18, 19), the conformation in solution of the flexible C13 side chain has been investigated by a number of NMR studies on paclitaxel and docetaxel. These studies suggest that in nonpolar solvents (chloroform, dichloromethane), the solution conformation of paclitaxel is similar to the conformation of docetaxel found in the crystal structure (7–11). In nonpolar solvents the C2 benzoyl group was found to be situated closer to the *tert*-butoxycarbonyl in docetaxel or phenyl amide group in paclitaxel than to the C3′ phenyl group. In more polar solvents there is evidence for a change in the C13

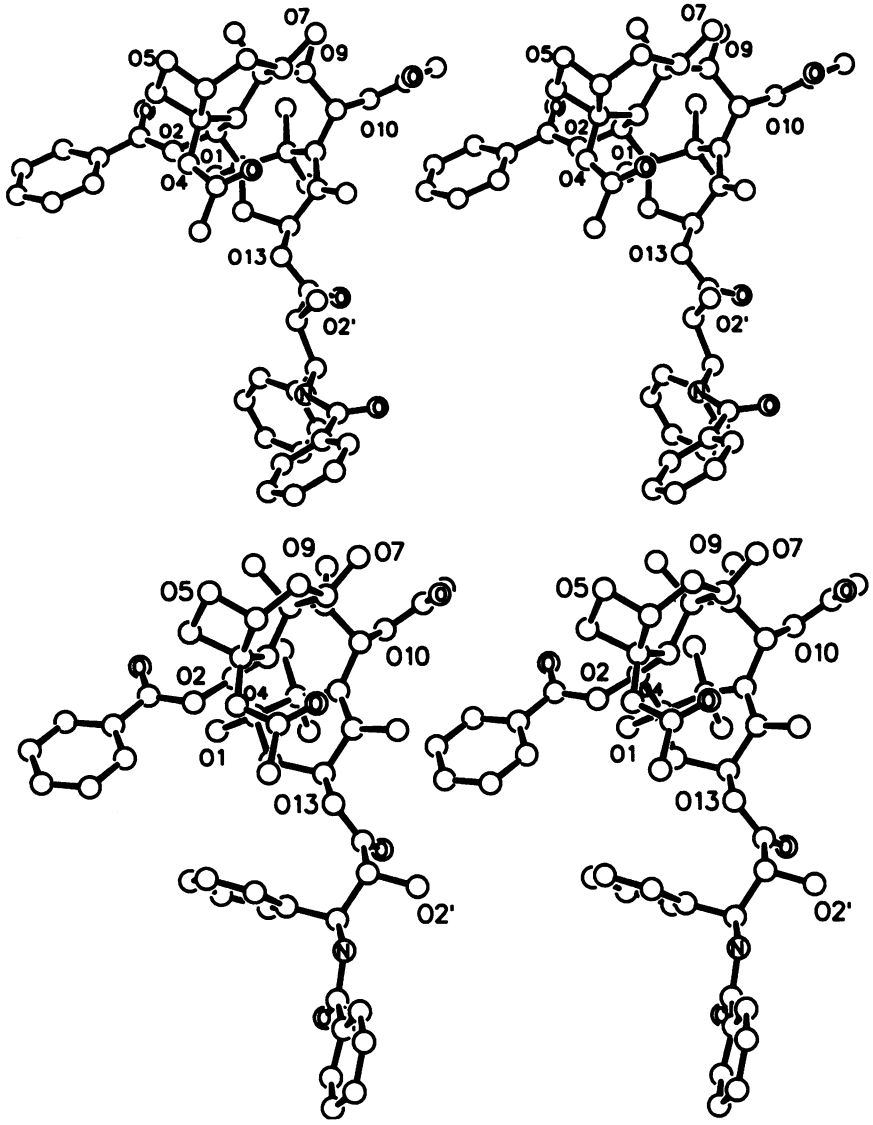

FIG. 2. Stereoscopic view of paclitaxel molecules A (*Upper*) and B (*Lower*). The orientation of molecule B is rotated a few degrees about the vertical axis of the diagram with respect to the view for molecule A to show more clearly all of the atoms in the C13 side chain.

side-chain conformation. In both the NMR and molecular modeling study of paclitaxel in aqueous solution by Williams *et al.* (12) and the NMR studies of paclitaxel and docetaxel in mixtures of water and organic solvents by Vander Velde *et al.* (13), it was found that the C3′ phenyl group is positioned close to the C2 benzoyl and C4 acetyl groups. The crystal structure conformation of paclitaxel molecule B shows the same clustering of the C2 benzoyl, C4 acetyl, and C3′ phenyl groups as suggested by the results of the NMR polar solvent studies. The agreement between paclitaxel conformation in crystals grown from aqueous medium and the NMR polar solution studies may indicate that molecule B exhibits one of the more intrinsically preferred conformations of paclitaxel, especially in an aqueous environment. It is also interesting to note that neither of the paclitaxel molecules in the present study shows any evidence of intramolecular hydrogen bonding between the OH on C2′ (O2′ hydroxyl) and the C1′ carbonyl oxygen or between the O2′ hydroxyl and the amide nitrogen as reported in some of the NMR studies and also in an analysis of the crystal structure results of docetaxel (14).

While the conformation of paclitaxel molecule B is similar to the solution conformations of paclitaxel and docetaxel deduced from NMR experiments performed in polar solvents, the conformation of paclitaxel molecule A found in this structure determination has not been seen or suggested in any other structural study to date to our knowledge. This study has thus identified another conformation of paclitaxel that should prove useful in modeling experiments.

Hydrogen Bonding. As hydrogen bonds are the usual modes by which drugs bind to receptors, analysis of the hydrogen bonding network in the crystal (in conjunction with structure–activity studies) may allow one to speculate as to which paclitaxel atoms are likely to interact with microtubule receptors. Hydrogen bonds are observed in the crystal structure between the two paclitaxel molecules and between paclitaxel molecules and solvent molecules. Fig. 4 displays a schematic representing the hydrogen bonding between the paclitaxel molecules. It should be noted that the paclitaxel molecules form two different kinds of hydrogen-bonded dimers, both of the molecule A–molecule B type. One type may be described as a head-to-tail dimer and the other as a head-to-head interaction. (The head here is used to describe the rigid ring portion of the molecule and the tail is composed mostly of the C13 side chain.) The specific interactions in the head-to-tail dimer are between the O7 A hydroxyl and O2′ B hydroxyl (2.71 Å) and between the O2′ A hydroxyl and C10 B acetyl oxygen (2.7 Å). Each molecule in this pair is also hydrogen bonded to a symmetry-related molecule of the opposite kind, forming the

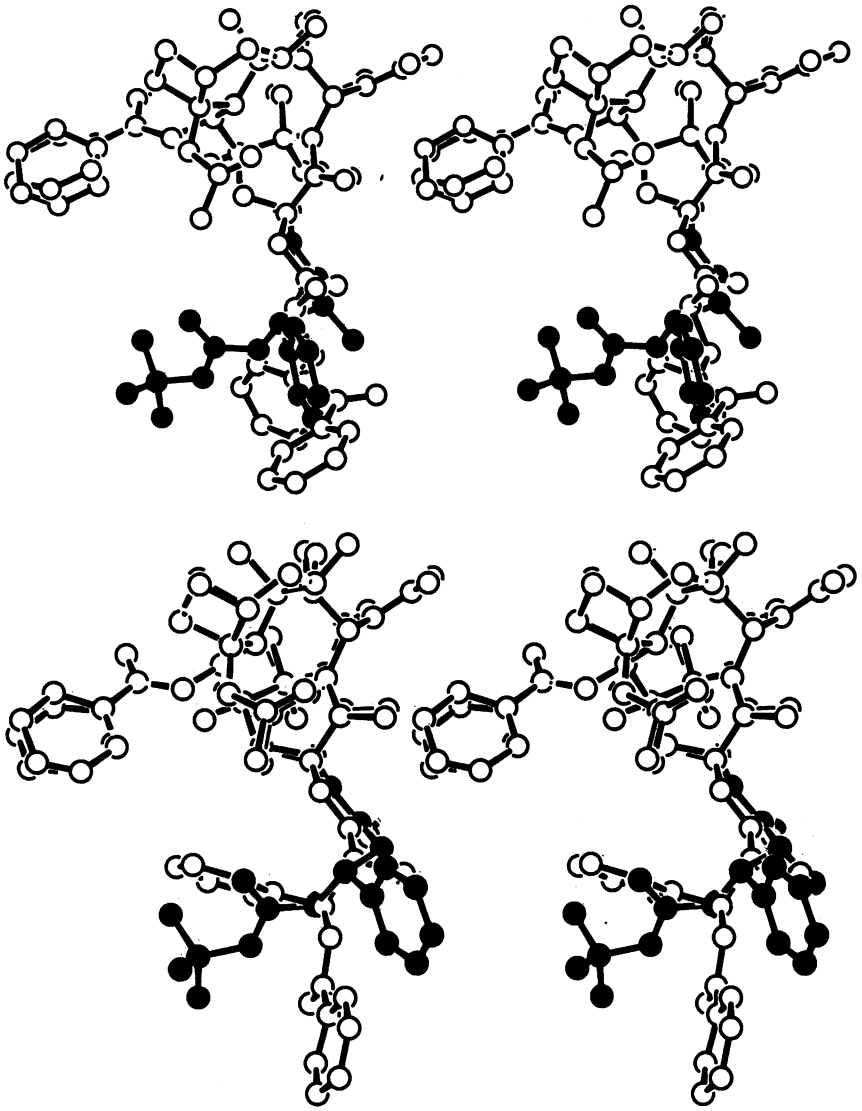

FIG. 3. Stereoscopic drawing of the superimpositions of the docetaxel taxane ring with the taxane ring of paclitaxel molecules A (*Upper*) and B (*Lower*). The docetaxel C13 side chain is shown with filled circles.

head-to-head symmetrically bonded A–B dimer. The head-to-head type hydrogen bonds, which involve the O1 A hydroxyl and C2 B benzoyl oxygen (2.97 Å) and the O1 B hydroxyl and C2 A benzoyl oxygen (2.96 Å), are slightly longer than normal. These increased hydrogen-bonded distances are likely caused by the proximity of the C1 hydroxyl and C2 benzoate substituents on the taxane ring. This proximity brings the A and B carbonyl oxygens to just within normal van der Waals contact at the observed hydrogen-bond distances. A stronger head-to-head dimer interaction is therefore prevented by the close contact of these carbonyl oxygens. The action of the 2-fold screw axis thus propagates a ribbon of A and B hydrogen-bonded dimers along the crystallographic *b* axis. Finally, dimers related by a unit cell translation along the crystallographic *a* direction are linked by hydrogen bonds between the C5′ A carbonyl oxygen and the O7 B hydroxyl group (2.89 Å).

Table 1. Selected torsion angles in the C13 side chain for paclitaxel molecules A and B and docetaxel

Atoms defining torsion angle*	Angle, degrees (SD)		
	Molecule A	Molecule B	Docetaxel
⟨C13—O13—C1′—O1′⟩	2 (2)	4 (2)	−6.6 (9)
⟨C13—O13—C1′—C2′⟩	180 (1)	−177 (1)	168.0 (5)
⟨O13—C1′—C2′—C3′⟩	159 (1)	103 (2)	60.1 (7)
⟨O1′—C1′—C2′—O2′⟩	93 (2)	41 (2)	−2.2 (9)
⟨C1′—C2′—C3′—N⟩	176 (1)	179 (1)	56.6 (6)
⟨O2′—C2′—C3′—C1(AR2)⟩	180 (1)	−175 (1)	59.5 (6)
⟨O2′—C2′—C3′—N⟩	60 (1)	61 (2)	−64.6 (6)
⟨C2′—C3′—N—C5′⟩	−118 (2)	−155 (2)	−141.4 (6)
⟨C1(AR2)—C3′—N—C5′⟩	120 (2)	83 (2)	97.4 (6)
⟨C3′—N—C5′—C1(AR3)⟩	−178 (2)	−178 (2)	−172.4 (5)
⟨C3′—N—C5′—O5′⟩	1 (2)	−1 (3)	12.8 (12)
⟨N—C5′—C1(AR3)—C2(AR3)⟩	−153 (2)	174 (2)	−176.5 (6)
⟨O5′—C5′—C1(AR3)—C2(AR3)⟩	28 (3)	−3 (4)	−1.6 (12)

*For docetaxel C1(AR3) = O12′, C2(AR3) = C13′.

Other hydrogen bonds connect the paclitaxel molecules with solvent molecules. Water molecule 1 (O1w) forms hydrogen bonds with the O9 B carbonyl oxygen (2.96 Å) and with the C5′ A carbonyl oxygen (2.92 Å). O2w hydrogen bonds with the O7 A hydroxyl group (2.73 Å), O3w bonds with the O2′ B hydroxyl group (3.03 Å), O4w bonds with the C10 A acetyl oxygen (2.96 Å), and O5w hydrogen bonds to the C5′ B carbonyl oxygen (2.80 Å). An oxygen of one of the dioxane molecules is an acceptor in a hydrogen bond with the amide nitrogen on molecule A (2.89 Å). All of the hydroxyl oxygens on both paclitaxel molecules participate in hydrogen bonds, with O7 A

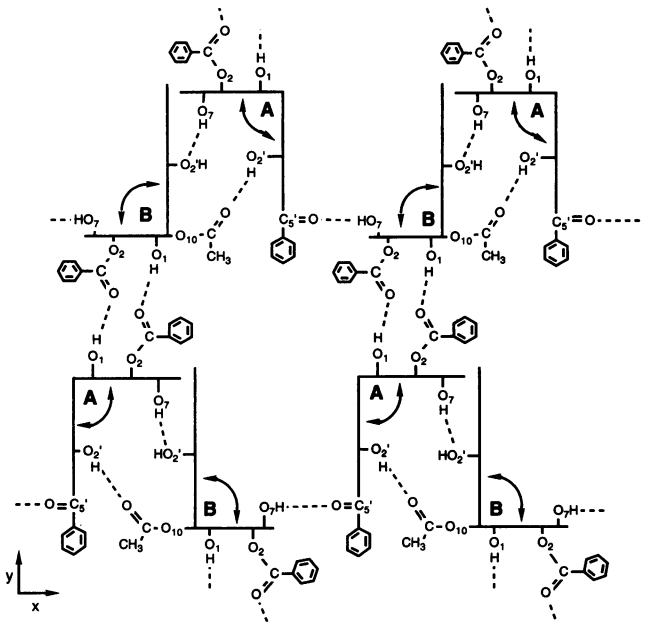

FIG. 4. Schematic diagram of the hydrogen-bonding interaction between paclitaxel molecules A and B. Each molecule is depicted with a horizontal and vertical line indicating the rigid ring structure (head) and the C13 side chain (tail), respectively. The arrows point to the head and tail belonging to a particular molecule. Hydrogen bonds are shown as dashed lines.

and O2′ B participating in two each. It should be noted that the C5′ A carbonyl oxygen is also involved in two hydrogen-bond interactions. On the other hand the O9 A carbonyl oxygen and the amide nitrogen on molecule B do not participate in any hydrogen bonds in this crystal structure.

Structure–activity studies (18, 19) on the C13 side chain of paclitaxel have shown that the C2′ hydroxyl group is critical for biological activity. The C2′ hydroxyl groups are observed in the present structure determination to participate in hydrogen bonds as both acceptors and donors. Two of these hydrogen bonds are responsible for the formation of the head-to-tail dimer association of paclitaxel molecules discussed above and another is with a water molecule. The results of the structure–activity studies suggest that this observed hydrogen-bonding capability is utilized in paclitaxel–microtubule binding.

A number of studies have indicated the importance of the C2 benzoate group for biological activity. All active derivatives from the crude plant extract contain the benzoate group at C2 (14), and deletion of the group causes a significant reduction in antitumor activity (20). It has been suggested (13, 14) that the hydrophobic nature of the benzoate is important for stabilizing the orientations of the C3′ substituents. This type of hydrophobic interaction is evident in the conformation of paclitaxel molecule B, where the C2 benzoate and C3′ phenyl are near each other and possibly influence each others' orientation. It has been suggested that the C2 benzoate may also contribute to biological activity by binding to a hydrophobic pocket of the tubulin protein (14). We also point out that the carbonyl oxygen of the C2 benzoate is capable of hydrogen-bond formation as demonstrated in this structure and may interact with tubulin polar residues as well. In addition, although structure–activity studies on the 1 position of the taxane ring have not yet been reported, the head-to-head dimer interaction of the paclitaxel molecules observed in this structure suggests that the O1 hydroxyl group might also contribute to antitumor activity by hydrogen bonding together with the C2-benzoyl carbonyl oxygen to complementary donor–acceptor residues on the microtubules. However, less speculative and more definitive information on the exact nature of paclitaxel binding to microtubules must await further crystallographic or spectroscopic data on the paclitaxel–microtubule complex itself.

Addendum. It has come to our attention that the crystal structures of two chemically modified and biologically inactive paclitaxel analogues have recently been published (21, 22). One of these, the 2′-carbamate form of paclitaxel (21), crystallized from methylene chloride, exhibits a C13 side-chain conformation different from those found in the crystal structures of paclitaxel and docetaxel. The other, a 2-desbenzoyl-2-acetoxy derivative of paclitaxel (22), crystallized from methanol/2-propanol, has a C13 side-chain conformation similar to that of docetaxel. These crystal structures support our observation (and those of the NMR studies) that C13 side-chain conformation is strongly influenced by environment. They also demonstrate that C13 side-chain conformation is unimportant if a paclitaxel analogue is biologically inactive because of chemical modification.

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