"Hydrophobic Collapse" of Taxol and Taxotere Solution Conformations in Mixtures of Water and Organic Solvent

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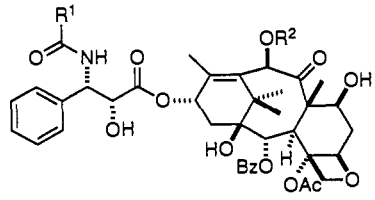
The exceptionally promising antitumor drugs Taxol (**1**) (a natural product) and Taxotere (**2**) (a closely related semisynthetic compound) inhibit the disassembly of microtubules, thereby halting cell division.[1] Although the precise binding site on the



Taxol: R^1 = Ph, R^2 = Ac
Taxotere: R = OBu^t, R^2 = H

microtubules and the bound-state conformation of Taxol or Taxotere are not known, potentially important features of the binding site have been suggested[2] based on the reported crystal structure of Taxotere.[3] The solution conformation of Taxol, as it has been characterized in chloroform[4–7] and methylene chloride,[8] seems to be very similar to that in the crystal structure. There is evidence in all these cases for a network of hydrogen bonding between the 1′ ester carbonyl, the 2′ hydroxyl, and the 3′ NH which structures the phenylisoserine side chain relative to the quite rigid taxane ring system. The apparent robustness of this conformation in the solid state and a variety of organic solvents has contributed to the idea that the Taxol side chain is "preorganized" to bind to microtubules in this conformation.[9,10] Very recently the results of two combined NMR and molecular modeling studies have been published which suggest that in more polar solutions (Taxotere in methanol,[11] Taxol in DMSO/water[12]) the side-chain conformation alters; the conformations favored by the two groups are different, albeit on different molecules in different solvents.

In this communication, we present additional NMR data in support of the alternative hypothesis that water strongly induces the same conformation in both Taxol and Taxotere. The data is consistent with one of four conformations identified as particularly low in energy in solvated molecular modeling studies by Williams *et al.*;[12] the other conformations are inconsistent with the NMR data. The key interaction in this conformation, for which we present direct evidence, is hydrophobic clustering of the 2-benzoyl, 3′-phenyl, and 4-acetyl groups; the nonpolar side chain amide groups (benzoyl for Taxol, *t*-BOC for Taxotere) do not seem to be involved. Additionally, we suggest that these conformational changes are a nonpeptidic example of what Rich and co-workers[13] have termed "hydrophobic collapse" in peptidic enzyme inhibitors, the process whereby some of these bioactive compounds dramatically change their conformation in aqueous compared to organic solvents as a consequence of hydrophobic clustering of nonpolar groups and loss of intramolecular hydrogen bonds. A prominent example is provided by the immunosuppressant peptide cyclosporin A; the structural changes observed for this and other peptides and the importance of controlling or exploiting "hydrophobic collapse" in the design and optimization of enzyme inhibitors, have been reviewed by Rich.[13]

In our studies, we have acquired one- and two-dimensional NMR spectra of Taxol and Taxotere in 75% DMSO/25% water and of Taxotere in chloroform, anhydrous methanol, and 50% methanol/50% water; the spectra from water-containing samples resemble each other but show numerous differences from the spectra taken in nonpolar organic solvents,[4–8] as recently noted by others.[11,12] (The solubility of Taxol and Taxotere in pure water is in the micromolar range, much too low for two-dimensional experiments under such conditions.) Upfield shifts of the 2′ and 3′ protons under these conditions, and a large increase in their *J*-coupling constant, also previously noted, indicate gross conformational changes in the side chain. The shifts of the aromatic signals from the 2-benzoyl and 3′-phenyl groups change substantially as a function of solvent and temperature (Figure 1), reflecting the extent of changes in the conformational ensemble as the solvent polarity increases.

In NOESY and ROESY spectra, cross peaks between the aromatic signals on the different rings are now observed, indicating that the rings are approaching each other and consistent with the explanation that the chemical shift changes of the signals arise from mutual ring-current effects. In our experiments, the strength of these cross peaks proved to be extremely sensitive to the choice of experimental conditions; they get stronger along with the chemical shift perturbations shown in Figure 1. They are prominent in a NOESY spectrum of Taxotere in DMSO/water at −20 °C (Figure 2), where molecular motion in Taxotere is slowed, giving strong positive NOESY cross peaks, and the aromatic protons relax fairly quickly. At room temperature, where ROESY gives substantially stronger cross peaks than NOESY, with ROESY mixing times (300–500 ms) that are quite short compared with the long relaxation times (>2 s) of the aromatic protons, these signals are much weaker. In methanol/water ROESY experiments, and under the conditions employed in ref 12 (1:1 DMSO/D_2O, room temperature, NOESY with t_{mix} = 1 s), they are weaker yet and can be seen as symmetrical cross peaks only at contour levels which also show substantial levels of unsymmetrical artifacts; this likely accounts for these peaks

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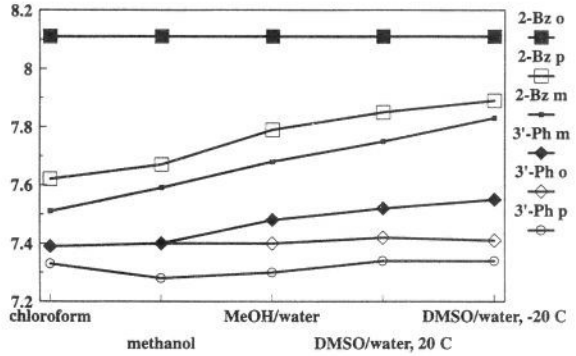

Figure 1. Chemical shifts of the aromatic protons in Taxotere in various solvents. The shifts are normalized to a constant value for the 2-benzyl *ortho* proton, removing small (±0.06 δ) solvent-dependent systematic shifts and thereby clarifying the relative shift trends.

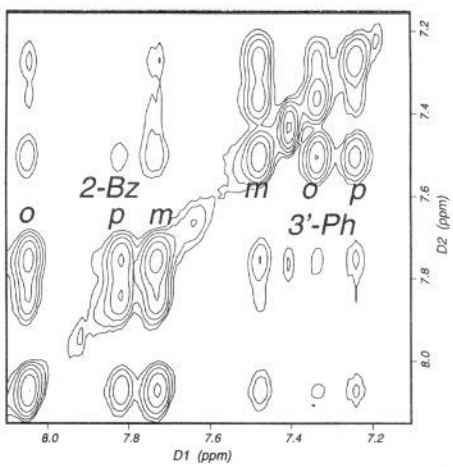

Figure 2. Expansion of aromatic region of Taxotere NOESY, 500-MHz, 75% d_6-DMSO/25% D_2O, –20 °C, t_{mix} = 400 ms, positive contour levels with 2-benzyl *ortho* diagonal peak (8.12 δ) at full scale. Inter-ring, as well as stronger intra-ring, NOEs are observed. Under these conditions, hindered rotation of the *t*-BOC group leads to two sets of signals in the 3′-phenyl ring; one of the smaller *cis* signals is resolved (the isomerism is also seen for the *tert*-butyl and 3′ proton signals; aromatic signals coalesce below room temperature, *tert*-butyl signals by 40 °C).

not being reported previously. These cross peaks are not observed at all in nonpolar solvents[4–8] or in anhydrous methanol[11] at any contour level in our data; adding water seems to be essential if they are to be observed, implying that the conformation producing the new cross peaks is induced by water. The strongest interactions are between the *meta* and *para* protons of the 3′-phenyl and the *ortho* and *meta* protons of the 2-benzoyl. These cross peaks cannot be rationalized with the crystal structure of Taxotere, in which the rings are about 10 Å apart, or with the structure favored by Dubois *et al.*[11] for Taxotere on the basis of NMR and molecular modeling studies. The latter conformation shows hydrophobic clustering of the 2-benzoyl group with the *t*-BOC group rather than the 3′-phenyl, which appear to be mutually exclusive possibilities. The *tert*-butyl to 2-benzoyl cross peaks expected from this conformation were not observed under any experimental conditions employed here.

Very similar chemical shift changes and analogous cross peaks are observed for Taxol in DMSO/water, although the aromatic region is more overlapped because of the third aromatic ring in this compound (data not shown). This findings supports the idea that both of these bioactive compounds have very similar conformational preferences in the presence of water. Williams *et al.*[12] found four low-energy conformations for Taxol in solvated molecular modeling studies. In three of them, the 2′ and 3′ protons are *gauche*, as they are in the crystal structure; in the fourth, they

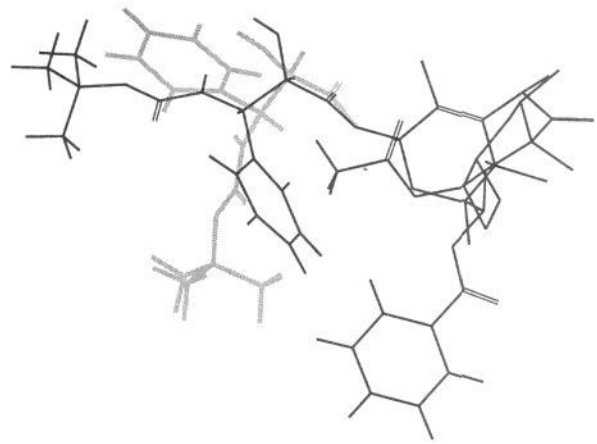

Figure 3. An orientation of the ester groups at positions 2, 4, and 13 in Taxotere consistent with the NMR data, based on a Taxol conformation (identified as **27**) described in ref 12. Starting from the crystal structure of Taxotere[3] (shown in gray), the side-chain torsion angles were modified to the values in ref 12.

are *trans*. On the basis of the large $^3J_{H2'-H3'}$ (8 Hz) observed in DMSO/water solutions of Taxol, they predicted the latter to be the dominant taxol conformation in aqueous solutions. This conformation is also the only one that positions the 2-benzoyl and 3-phenyl rings in close enough proximity (interproton distances ≤5 Å) to produce the type of NOE connectivities and ring-current shifts we have observed (Figure 3, shown for Taxotere). Consistent with the lack of experimentally observable NOEs to the *tert*-butyl group, in this conformation it is relatively isolated from other hydrophobic groups. However, the 4-acetyl methyl group, which does exhibit NOE's to phenylisoserine sidechain protons including the 3′-phenyl group does appear to be part of a new hydrophobic cluster with the two aromatic rings. It is interesting to note that no active taxol analogs are known without 2-benzoyl and 3′-phenyl groups;[1] perhaps these groups actually provide the putative "preorganization" of the side-chain conformation most relevant to binding, via hydrophobic clustering rather than intramolecular hydrogen bonding.

Rich has suggested that in peptides undergoing "hydrophobic collapse", the conformation recognized by the receptor will be induced by water prior to binding.[13] Conformational changes after binding may occur to optimize the fit; there is evidence that this occurs for cyclosporin A. It has been proposed that, for taxol and Taxotere, the taxane ring system is recognized first, followed by additional interactions of the binding site with the side chain.[11] In this case, the highly flexible side chain could conceivably adopt a conformation different from any yet described, depending on the (unknown) hydrophobicity of the binding site and specific interactions with tubulin side chains. Additional experiments, e.g., use of conformationally defined analogs or spectroscopic characterization of the bound state, are required to establish the bioactive conformation(s) of these compounds.

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