The Conformations of Taxol in Chloroform

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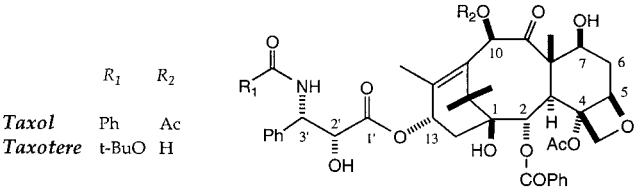
Taxol, isolated from the bark of *Taxus brevifolia* in the late 1960s,[1] and its semisynthetic Taxotere congener[2] have become the drugs of choice for the treatment of ovarian and breast cancer.[3] The availability of active simplified analogues would facilitate shortened synthetic strategies and potentially bypass neurotoxicity[4] and multidrug resistance.[5] The design of such compounds is hampered by an incomplete understanding of Taxol's conformation in the bioactive form. Numerous studies have sought to deduce the drug's three-dimensional state in solution by NMR spectroscopy combined with force-field guided conformational analysis. In both polar and nonpolar solvents, rotamers exhibit hydrophobic collapse[6] between the flexible C-2, C-4, and C-13 side chains, accompanied by variable H2′−C−C−H3′ torsional angles (Table 1).[7,8] Each of the conformational extremes has been proposed as a candidate for the Taxol topology bound to microtubules.[7d,g−j,8b−d]

Table 1. Mole Fractions, Relative $\Delta G(CDCl_3)$ and Selected Geometric Features for the Eight Optimized Conformations of Taxol (**1**−**8**) Derived by NAMFIS Analysis in $CDCl_3$, and for Other Taxol Conformers (**9** and **11**) and Taxotere (**10**)

		C-2 Ph to C-3′ Ph distances, Å					
	MF,[a] %	C-3′ Ph[b]	C-3′ Bza[b]	C-3′ Ph-H[c]	C-3′ Bza-H[c]	$\phi_{H2'CCH3'}$, deg ($J_{H2'-H3'}$, Hz)[d]	$\Delta G(CDCl_3)$, kcal/mol[e]
1	35	9.65	5.41	5.48	2.90	74.3 (0.4)	0.0
2	26	11.06	4.68	7.92	2.87	−41.9 (6.3)	0.2
3	19	10.52	11.32	6.02	7.12	92.7 (0.9)	0.4
4	10	8.63	10.78	4.80	8.16	80.5 (0.4)	0.7
5	4	5.66	12.03	2.86	9.27	171.4 (10.6)	1.3
6	3	9.87	10.92	5.33	7.02	56.9 (1.6)	1.5
7	2	10.87	4.72	7.27	3.09	−57.7 (4.2)	1.7
8	1	11.66	12.87	8.98	8.26	−67.7 (2.6)	2.1
9[g]	−	9.84	4.70	6.83	3.07	62.9 (1.0)	−
10[h]	−	10.72	7.18[f]	7.03	3.68	57.5 (1.5)	−
11[g]	−	5.66	11.26	2.62	7.40	−179.1(10.4)	−

[a] Mole fraction derived from the NAMFIS fitting. [b] Distance between the centroid of the phenyl rings. [c] Distance between the nearest hydrogens. [d] *J*-values calculated from the modified Karplus equation in Macromodel.[23] [e] Energies from a Boltzmann distribution of MF's at 298 K. [f] Distance between the C-2 Ph centroid and $C(CH_3)_3$. [g] The "nonpolar" (**9**) and "polar" (**11**) conformations from ref 7e. [h] From the X-ray crystal structure of Taxotere.[17]



The common thread that links all previously suggested conformers of Taxol and Taxotere in solution is the proposition that only a single or a strongly dominant conformation is consistent with the NMR measurements. Such structures have either been inferred from the data, hand-selected from large conformational searches, or derived by constrained molecular dynamics (MD). Two inescapable facts weaken the proposition. First, Taxol possesses at least seven easily rotated single bonds determining the 3-dimensional disposition of the side chains. Consequently, the existence of only one or two Taxol/Taxotere conformers at 25 °C is highly unlikely. Second, the NMR observables ($J_{H2'-H3'}$ and nOe's) and their corresponding structural correlates (dihedral angles and distances, respectively) are dynamic averages arising from rapid conformer equilibration. Applying the raw observables by limited conformer selection necessarily ignores a significant subset of the data. Constrained MD as a conformational search technique, on the other hand, introduces a severe oversimplification by packing all of the data into a single structure to deliver a "virtual conformation",[9] a high-energy structure present in the computer but not in solution.

As part of a broader effort to understand Taxol conformation in solution and at the β-tubulin binding site,[10] we have reexamined the molecule in chloroform. In the present work, the single and virtual conformation dilemmas are avoided by treating the Taxol system with the NAMFIS methodology (*N*MR *a*nalysis of *m*olecular *f*lexibility *i*n *s*olution).[11] In this way, we identify eight

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Taxol conformations in $CDCl_3$, many of which have gone undetected by previous studies.

Our analysis makes use of the Taxol ROESY dataset carefully assembled by Hilton and co-workers.[12] It also depends on a thorough search of Taxol conformation space with the AMBER*/GBSA/$CHCl_3$ protocol.[13] Thus 30 000 steps of Monte Carlo searching delivered 2995 unique and fully optimized conformers. Those ≤12 kcal/mol were clustered into 509 families with XCluster.[14] The 509 leading conformers were then subjected to a NAMFIS fit of 42 nOe-derived H- - -H distances and a $J_{H2'-H3'} = 2.7$ Hz. Eight Taxol conformations with estimated solution populations ranging from 1 to 35% were obtained as the "best fit" of the data (Table 1). For example, for six key side-chain/core or side-chain/side-chain NMR distances from 2.75 to 3.42 Å, all are matched with an accuracy ≤0.5 Å and an average deviation of 0.24 Å. $J_{H2'-H3'}$ is fitted precisely at 2.7 Hz.[15,16]

The eight Taxol conformers **1**−**8** fall into three subsets. The most populated form (**1**, 35%), the "nonpolar" form, resembles all previous suggestions in halocarbon solvents in that the C-2 and benzamide (Bza) C-3′ hydrophobes are clustered, while H-2′ and H-3′ adopt a gauche relationship. Table 1 compares **1** to structure **9** previously derived by Scott, Swindell, and co-workers[7e] and the X-ray structure of Taxotere (**10**)[17] routinely cited as the model for the nonpolar conformation. Conformations **2** and **7** (26 and 2%, respectively), with very different torsional angles along the C-13 side chain, likewise adopt the nonpolar motif raising the total nonpolar contribution in the equilibrium to 63%. Remarkably, the fifth most populated conformer (**5**, 4%) is the so-called "polar form" routinely observed in DMSO-d_6/D_2O and CD_3OD (compare **11** in Table 1).

The third and second largest subset of Taxol conformers includes **3**, **4**, **6**, and **8**. In these "open" conformers comprising 33% of the equilibrium mixture, phenyl−phenyl hydrophobic collapse is absent. As reported by Table 1, the benzoyl phenyl proton to C-13 terminal phenyl proton distances range from 4.8 to 9.0 Å. While these structures are all unique, as a class they resemble the single conformer proposal of Balasubramanian et al.[7f] and the second of two conformations obtained from constrained MD by Cachau et al.[7h]

For the low population conformers (≤4%, Table 1), what nOe distances would be violated by their exclusion? The Hilton study[12] reports a weak nOe between the *ortho* protons of C-3′ Ph and the methyl protons of C-4 OAc ($r = 3.35$ Å). Since C-4 OAc and C-2 Ph also interact ($r = 3.42$ Å), a subset of conformations (e.g., **5**, **11**) experience C-3′ and C-2 phenyl hydrophobic collapse. Removal of all conformers with C-3′ Ph and C-4 OAc H- - -H distances ≤4.5 Å from the set of 509, eliminates **5** from the list in Table 1, while raising the sum of square differences (SSD)[15] from 93 to 150. The presence of **6**−**8** can be understood similarly.

The final column in Table 1 lists $\Delta G(CDCl_3)$ for each conformation. It should be noted that these energies are not from the AMBER* force field, but are derived from a Boltzmann distribution of the calculated populations in $CDCl_3$. To the extent that the NAMFIS mole fractions accurately describe the populations in solution, the energies are empirical.[18] With respect to binding at β-tubulin or microtubules, the open conformations offer two important properties. First, the energies ($\Delta G(CDCl_3)$) are only 0.4−2.1 kcal/mol above the low-energy hydrophobically collapsed conformer. Second, the outstretched C-13 termini are available for interaction with the tubulin protein rather than self.

The results of the present NAMFIS analysis neither assert nor require that non-hydrophobically collapsed conformers of Taxol or Taxotere bind to β-tubulin. They do, however, illustrate that single or "virtual" conformation hypotheses are incomplete. Several studies,[7,8] in particular the recent and elegant fluorine probe work of Ojima and colleagues,[7i] have recognized the dynamic behavior and time averaging of Taxol analogues in solution. However, in any given solvent system it has not been possible to identify more than two specific conformations. For flexible molecules, deconvolution of averaged NMR spectra is essential for providing both a realistic assessment of conformational equilibria and the detection of novel conformations. The multi-conformer outcome, if it carries over to water, thus raises the possibility that low-energy forms (≤2 kcal/mol) unperceived by interpretation of averaged NMR spectra are viable candidates for ligand protein binding. Finally, the presence in solution of energetically similar Taxol conformers with diverse 3-D topologies eliminates the need to suppose that the molecule's side chains exist in a specific "preorganized" conformational state[8c,20] in preparation for binding to microtubules. NMR studies on highly active cyclohexyl analogues of Taxol and Taxotere have led to the same conclusion.[21] The poly-protein need only select from the conformational palette a state congruent with the bound form. Nevertheless, whatever the latter may be,[10] Taxol's relatively weak association with tubulin ($IC_{50} \approx 15$ μM)[22] may, in part, be due to the presence of an ensemble of nonproductive conformers.[6]

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