NMR and Molecular Modeling Study of the Conformations of Taxol 2′-Acetate in Chloroform and Aqueous Dimethyl Sulfoxide Solutions

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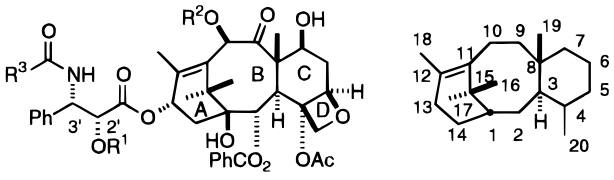
Taxol 2′-acetate, an analog of the antitumor drug taxol, displays no significant in vitro microtubule polymerization activity, thus underscoring the importance of a free 2′-OH group to the biological activity of taxol. Previous work had suggested that the inactivity of taxol 2′-acetate is not due to steric interference by the acetyl group. The present study examined the conformations of taxol 2′-acetate in deuteriochloroform and ^2H_2O−deuteriodimethyl sulfoxide solutions and found them to be essentially the same as the respective conformations adopted by taxol itself. Thus, neither destabilization of an active taxol conformation by the acetyl group nor the formation of an important taxol conformation determining role for the 2′-OH group appears likely. The implication of these findings is that the taxol 2′-OH group interacts directly with a protein residue in the taxol−microtubule complex, perhaps as a hydrogen bond donor.

Introduction

Taxol[1] is a structurally novel antitumor drug that operates at the cellular level through a unique interaction with microtubules[2] in which the dynamic assembly of these polymeric cytoskeletal components from tubulin monomers is shifted toward the polymeric state. The microtubules that result are stabilized and prevented from reorganizing into a functional mitotic spindle apparatus. Inhibition of cell replication ensues and forms the putative basis for taxol's clinical efficacy. Taxol is approved for use in the treatment of drug refractory ovarian and metastatic breast carcinomas and possesses significant activities against nonsmall cell lung and head-and-neck cancers, as well.[3]



TAXOL $R^1 = H; R^2 = Ac; R^3 = Ph$

TAXOL 2'-ACETATE $R^1 = R^2 = Ac; R^3 = Ph$

TAXOTERE $R^1 = R^2 = H; R^3 = tert\text{-}BuO$

The structure−activity profile of taxol has been explored extensively.[4] Among the conclusions reached are that the A-ring side chain is obligatory and more or less optimized for biological activity. An important early structure−activity study by Kingston and Horwitz[5] established that masking the 2′-OH in the form of the acetate ester abolished in vitro microtubule assembly activity, but not cytotoxicity toward J774.2 cells. It was speculated that the latter activity might derive from the intracellular cleavage of taxol 2′-acetate to taxol. Indeed the same study documented the pronounced lability toward hydrolysis of taxol 2′-esters. These seminal observations have formed the basis of much effort toward the development of taxol 2′-ester-based prodrugs.[4]

Whereas a recent low resolution structural study[6] has revealed important information on the taxol−microtubule complex, a detailed three-dimensional structure of the drug−protein interaction at atomic resolution remains unavailable. Such information is essential if so-called rational methods of drug design are to contribute to the development of new taxol analogs with therapeutic and other advantages. To partly fill that void in the near term, two hypotheses for the conformation of taxol that is recognized by its binding site on microtubules have been advanced, one[7] based on the dominant conformation observed for unbound taxol in deuteriochloroform and related solvents[7c,d,8]—the hydrophobic conformation—and a second model[9] based on the dominant conformation observed for taxol in ^2H_2O−deuteriodimethyl sulfoxide and in ^2H_2O[8e,9,10]—the aqueous conformation. The experimentally determined unbound conformations differ significantly only in the conformational features of the A-ring side chain: the hydrophobic conformation is characterized by one of the two possible H−C2′−C3′−H gauche arrangements, projects the 3′-Ph group into the solvent, places the 3′-PhCONH and 2-$PhCO_2$ substituents in close proximity, and is substantially similar to the solid state conformation of the taxol analog Taxotere;[11] the aqueous conformation is characterized by the anti arrangement of H−C2′−C3′−H, projects the 3′-PhCONH into solvent, places the 3′-Ph and 2-$PhCO_2$ substituents in close proximity, and also has been detected in taxol crystals formed from an aqueous solvent mixture.[12]

The objective of the present study is the investigation through NMR spectroscopy and molecular modeling of the taxol 2′-acetate hydrophobic and aqueous solution conformations to determine their relevance to the biological activity of this taxol analog and thereby to

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Table 1. ^{1}H Chemical Shifts (ppm) and Coupling Constants (Hz) of Taxol 2′-Acetate in Deuteriochloroform and ^2H_2O−Deuteriodimethyl Sulfoxide

^{1}H on	$CDCl_3$	DMSO−^2H_2O
C-2	5.67 (d, 7.0)	5.36 (d, 7.2)
C-3	3.80 (d, 7.0)	3.52 (d, 7.2)
C-5	4.96 (dd, 2.2, 9.6)	4.88 (dd, 2.2, 9.5)
C-6	2.54 (ddd, 6.6, 9.6, 14.7) α	2.32 (ddd, 6.7, 9.5, 14.5) α
	1.87 (ddd, 2.2, 10.9, 14.7) *β*	1.63 (ddd, 2.2, 11.2, 14.5) *β*
C-7	4.43 (dd, 6.6, 10.9)	
C-10	6.27 (s)	6.21 (s)
C-13	6.24 (qdd, 1.2, 9.2, 9.2)	5.76 (qdd, 1.2, 9.4, 9.4)
C-14	2.35 (dd, 9.2, 15.4) α	1.74 (dd, 9.4, 15.5) α
	2.17 (dd, 9.2, 15.4) *β*	1.40 (dd, 9.4, 15.5) *β*
C-16	1.12 (s)	0.94 (s)
C-17	1.22 (s)	0.95 (s)
C-18	1.91 (d, 1.2)	1.71 (d, 1.2)
C-19	1.66 (s)	1.44 (s)
C-20	4.30 (d, 8.7) α	
	4.18 (d, 8.7) *β*	
C-2′	5.49 (d, 3.2)	5.24 (d, 8.8)
C-3′	5.93 (dd, 3.2, 9.2)	5.43 (dd, 8.4, 8.8)
NH	6.86 (d, 9.2)	9.21 (d, 8.4)
OAc-10	2.21 (s)	2.05 (s)
OAc-14	2.43 (s)	2.17 (s)
OAc-2′	2.14 (s)	2.05 (s)
OBz-*o*	8.12 (dd, 8.0, 1.4)	7.91 (dd, 7.3, 1.9)
OBz-*m*	7.49 (dd, 8.0, 7.6)	7.60 (dd, 7.8, 7.3)
OBz-*p*	7.60 (tt, 7.6, 1.4)	7.68 (tt, 7.8, 1.9)
NBz-*o*	7.72 (dd, 8.5, 1.4)	7.72 (dd, 7.3, 1.9)
NBz-*m*	7.39 (dd, 8.5, 7.2)	7.43 (dd, 7.3, 7.2)
NBz-*p*	7.48 (tt, 7.2, 1.4)	7.51 (tt, 7.2, 1.9)
Ph-*o,m*	7.40 (m)	7.37 (m)
Ph-*p*	7.33 (m)	7.12 (m)

probe indirectly the important role of the taxol 2′-OH group in the binding of taxol to microtubules.

Experimental Section

NMR Studies. Taxol 2′-acetate was prepared as previously described.[5] ^{1}H-NMR spectroscopy was performed at 500 MHz on a Bruker AMX-500 with an ASPECTStation data system. Spectra were recorded at room temperature unless otherwise specified. Chemical shifts were referenced to the residual solvent proton peak, i.e. chloroform ($CHCl_3$ = 7.24 *δ*) for the deuteriochloroform samples and dimethyl sulfoxide ($(CH_3)_2SO$ = 2.49 *δ*) for the deuteriodimethyl sulfoxide-containing (1:1 [v/v] deuteriodimethyl sulfoxide−^2H_2O) samples. 1D spectra were recorded with 32K data points (0.2 Hz/point), while 2D spectra were taken with matrix sizes of 2K × 0.5K and a mixing time of 600 ms for both NOESY and ROESY experiments. SYBYL 6.1−TRIAD (Tripos) software was employed for spectral and structural analysis.

Molecular Modeling. Internal coordinate Monte Carlo conformational searching[13] was carried out on a Silicon Graphics 4D/35 workstation using MacroModel[14] (version 4.0) with the included MM2 force field[15] and the Still chloroform and water continuum solvation models.[16] All starting structures were generated from the Taxotere crystal structure coordinates.[11a] The clustering of the conformational search results into characteristic conformations was carried out similarly to our earlier work on taxol[8e] and analogs[7e] within the above version of MacroModel. Aqueous conformers **10** and **11** (Table 3) were the only unique types observed within 3 kcal mol^{-1} of the global minimum. However, since the density of distinct low-energy conformers was greater in chloroform (Table 2), the set of low-energy conformers considered below was expanded to include those within approximately 4.5 kcal mol^{-1} of the global minimum. Coordinates for the structures listed herein are available from cswindel@brynmawr.edu.

Results

Deuteriochloroform Solution. Peak positions for the protons in taxol 2′-acetate were within 0.2 ppm of the equivalent proton peaks in taxol itself. The 2′-

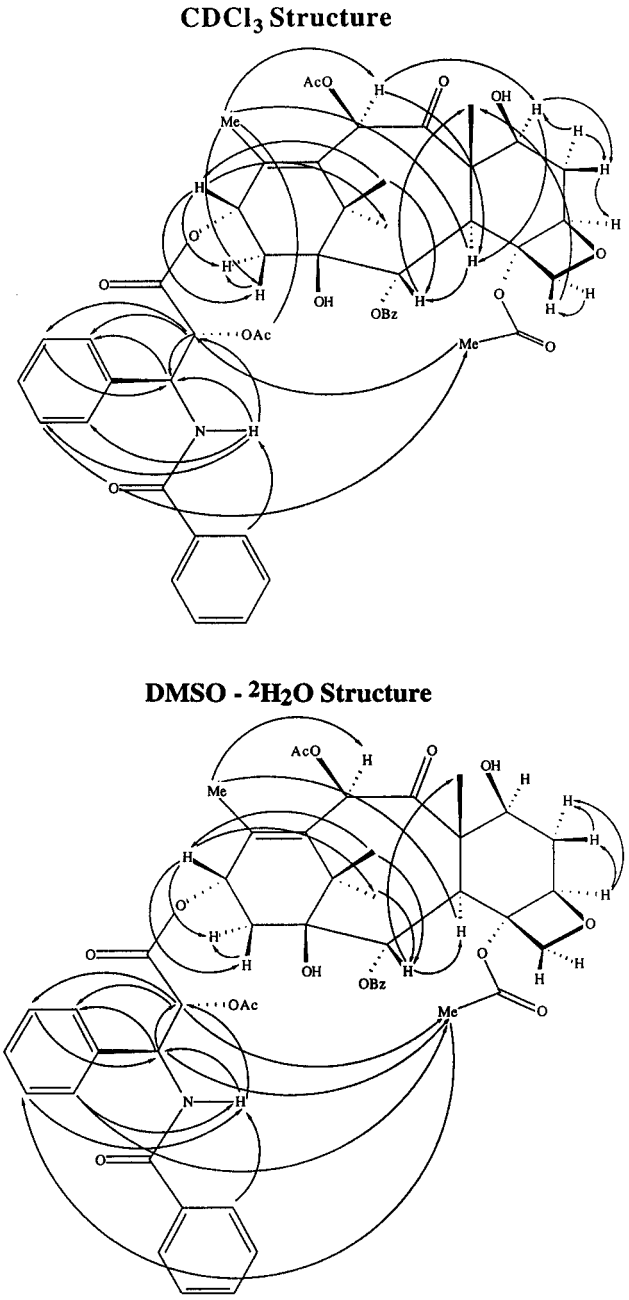

Figure 1. ^{1}H NOE interactions for taxol 2′-acetate in chloroform and aqueous dimethyl sulfoxide solutions.

acetate methyl peak occurs at *δ* 2.14. Table 1 and Figure 1 summarize the NMR data. Some NOE's irrelevant to the conformational questions addressed herein (e.g. some involving the 2-$PhCO_2$, 4-OAc, and oxetane groups) are deleted for clarity.

The significant parameters for the characteristic low-energy taxol 2′-acetate chloroform conformers are listed in Table 2. The interproton distances are calculated from the methyl group carbon atoms and, where relevant, from the closest 3′-Ph group ortho proton. Whereas the NOESY data indicate H2′ and one of the 3′-Ph group ortho protons, and H2′ and the NH proton to be in close contact, all of the low-energy modeled conformers separate these protons by 4.3 Å or less and 3.9 Å or less, respectively. Therefore, these interproton distances are not listed in Table 2 since the corresponding NOESY data cannot be used to distinguish among the conformers calculated for taxol 2′-acetate in chlo-

Table 2. Calculated $J_{H2'-H3'}$ Values, Selected Dihedral Angles and Interproton Distances, and Relative Energies of Taxol 2′-Acetate Chloroform Conformers **1**−**9**

conformer	calcd $J_{H2'-H3'}$ (Hz)	N−C3′−C2′−O (deg)	O−C2′−C1′=O (deg)	Cl′−O−C13−C12 (deg)	H2′−4-O_2CCH_3 (Å)	H3′−4-O_2CCH_3 (Å)	3′-Ph-H−4-O_2CCH_3 (Å)	relative E (kcal mol^{-1})
1	3.7	−180	−179	−158	3.4	5.5	6.4	0
2	1.9	−70	−154	−91	5.2	5.8	3.6	1.6
3	10.5	62	36	−94	3.0	5.7	3.3	2.1
4	1.6	−60	−174	−161	3.5	5.7	6.4	2.6
5	10.7	54	179	−158	3.3	5.7	5.6	2.6
6	3.9	178	22	−91	3.1	4.4	5.6	2.7
7	2.8	−78	25	−153	4.4	2.8	4.2	3.3
8	3.7	178	−175	−95	5.2	5.3	4.0	3.6
9	1.5	−63	19	−96	3.1	3.3	3.0	4.2

roform solution. Conformer **3** is very similar to the previously detected taxol major aqueous solution conformation,[8e,9,10] and conformer **7** likewise resembles the previously observed taxol major hydrophobic solution conformation.[7c,d,8]

The observed value for $J_{H2'-H3'}$ allows the rejection of modeled conformers **3** and **5** as significant contributors to the low-energy conformational manifold of taxol 2′-acetate in this solvent. The relatively significant NOESY cross peak observed for H3′ and the 4-OAc methyl group suggests **1**, **2**, **4**, and **8** to be less important given their larger separations of these protons. An analogous case can be made for the relegation of **2** and **8** to secondary importance on the basis of the significant NOESY cross peak observed for H2′ and the 4-OAc methyl group. Conformers **1**, **4**, and **6** are less consistent with the somewhat weaker NOE interaction between one of the 3′-Ph ortho protons and the 4-OAc methyl group, and **2**, **4**, and **9** are less consistent with the observed $J_{H2'-H3'}$ value. Of the conformers most compatible with the NMR parameters—**6**, **7**, and **9**—only **7** reproduces reasonably well all of the data. Indeed the most economical interpretation of the NMR data in terms of the modeling results is that the dominant taxol 2′-acetate hydrophobic conformer is **7** (Figure 2). This indicates that the replacement of the taxol 2′-OH proton with the acetyl group causes no profound conformational perturbation of the A-ring side chain in chloroform solution and that intra side chain hydrogen bonding of the 2′-OH as a donor is unnecessary for the stabilization of the hydrophobic conformation.[7,8e,11]

^2H_2O−Deuteriodimethyl Sulfoxide Solution. Peak positions for the protons in taxol 2′-acetate were within 0.2 ppm of the equivalent proton peaks in taxol itself in this solvent system also. The 2′-acetate methyl peak occurs at 2.05 δ. Table 1 and Figure 1 summarize the NMR data. Again, some NOE's irrelevant to the conformational questions addressed herein (e.g. some involving the 2-$PhCO_2$, 4-OAc, and oxetane groups) are deleted for clarity. The weak 2-$PhCO_2$−3′-Ph cross peaks that have been observed for taxol in this solvent mixture in NOESY and ROESY experiments conducted under special conditions[9] were not observed in the experiments reported herein.

The significant parameters for the characteristic low-energy taxol 2′-acetate aqueous conformers are listed in Table 3. The interproton distances are calculated from the methyl group carbon atoms. The NOESY data indicate H2′ and one of the 3′-Ph group ortho protons, H2′ and the NH proton, and one of the 3′-Ph group ortho protons and the 4-OAc methyl group protons to be in close contact. However, both **10** and **11** show these respective proton sets to be separated by 3.1 Å or less,

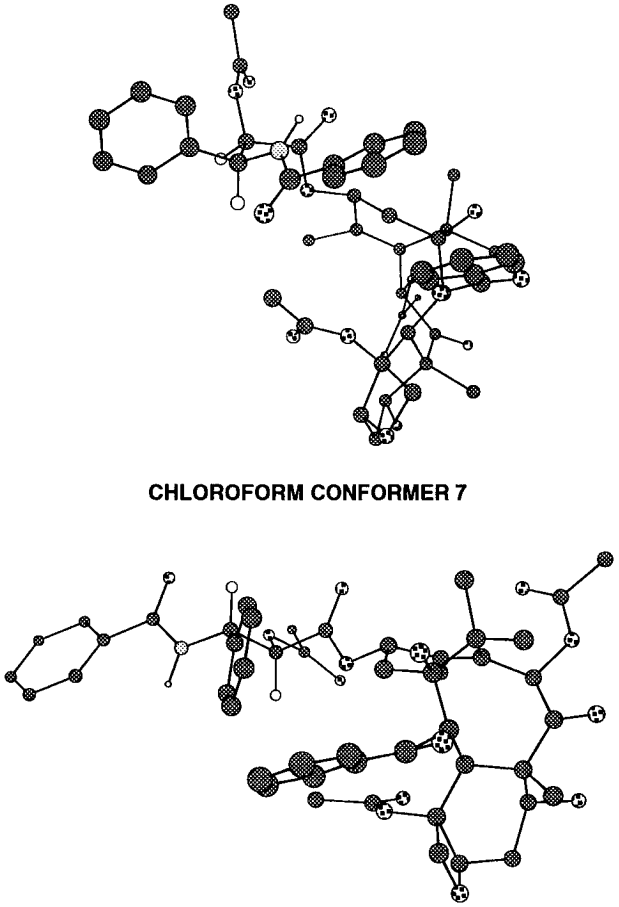

Figure 2. The dominant conformations of taxol 2′-acetate in chloroform and aqueous dimethyl sulfoxide solutions.

4.0 Å or less, and 3.4 Å or less, respectively. Therefore, these interproton distances are not listed in Table 3 since the corresponding NOESY data cannot be used to distinguish among the conformers calculated for taxol 2′-acetate in aqueous solution. Conformer **10** is very similar to the previously detected taxol major chloroform solution conformation,[7c,d,8] and conformer **11** likewise resembles the previously observed taxol major aqueous solution conformation.[8e,9,10]

The measured $J_{H2'-H3'}$ value eliminates **10** from contention as the major conformer for taxol 2′-acetate in this medium. Not only does **11** (Figure 2) largely account for the high observed value of $J_{H2'-H3'}$, but **11** also is more consistent with the relatively strong NOE interaction between H2′ and the 4-OAc methyl group. Both the modest discrepancy between the observed $J_{H2'-H3'}$ value and that calculated for **11** and the contact between H3′ and the 4-OAc methyl group indicated by

Table 3. Calculated $J_{H2'-H3'}$ Values, Selected Dihedral Angles and Interproton Distances, and Relative Energies of Taxol 2′-Acetate Aqueous Chloroform Conformers **10** and **11**

conformer	calcd $J_{H2'-H3'}$ (Hz)	N−C3′−C2′−O (deg)	O−C2′−C1′═O (deg)	Cl′−O−C13−C12 (deg)	H2′−4-O_2CCH_3 (Å)	H3′−4-O_2CCH_3 (Å)	relative E (kcal mol^{-1})
10	1.6	−64	23	−157	4.8	2.9	0
11	10.4	60	33	−94	3.4	6.0	2.4

the NOESY data can be explained by a minor contribution by **10** to the conformational ensemble characteristic of taxol 2′-acetate in aqueous media. The time-averaged reduction through conformational flexibility of the proximity of the 2-$PhCO_2$ and 3′-Ph groups in aqueous conformer **11** likely accounts for the absence of strong and readily detectable NOE's involving these groups. Thus, as appears to be the case in chloroform solution, the replacement of the taxol 2′-OH proton with the acetyl group fails to result in significant perturbation of the A-ring side chain conformation in aqueous solution.

Discussion

One explanation for the inactivity in vitro of taxol 2′-acetate is that the taxol binding site on microtubules is too restricted to accept the ligand when the 2′-acetate group is present.[5] However, Kant et al.[17] have investigated 2′-methoxytaxol, 2′-deoxytaxol, and 2′-fluorotaxol and found their cytotoxicities toward a human colon carcinoma cell line (HCT116) to be 70−200-fold less than that observed for taxol. To the extent that cytotoxicity data reports indirectly on microtubule assembly activity, these analogs, which have altered 2′-groups smaller than acetoxy, possess diminished activities compared to that of taxol. Thus, it seems unlikely that steric interference is responsible for the inactivity in vitro of taxol 2′-acetate.

Masking the taxol 2′-OH as the acetate ester could destabilize either or both of the hydrophobic and aqueous taxol conformations. Indeed it has been proposed that one of the determinants of the hydrophobic solution conformation of taxol (and the solid state structure of Taxotere) is hydrogen bonding of the 2′-OH with the adjacent side chain carboxyl carbonyl oxygen.[7,8e,11] Our results indicate that the respective major A-ring side chain conformations of taxol 2′-acetate in hydrophobic and aqueous solutions are essentially the same as those adopted by taxol in the same media. A recent X-ray crystallographic study of taxol 2′-carbamate[18] likewise showed that the 2′-carbamate group fails to perturb significantly the solid state A-ring side chain local conformation relative to that observed for Taxotere.[11] Thus, an important conformation organizing role for the 2′-OH group[5] can be ruled out. The logical alternative explanation of the prominent position of the 2′-OH group in the structure−activity profile of taxol is that it plays a direct role in stabilizing the interaction of taxol and microtubules[5]—for example, by acting as a hydrogen bond donor to a protein residue. If this were the case, the inactivity in vitro of taxol 2′-acetate could result simply from the elimination of this ligand−protein interaction. The results of Kant et al.[17] further support this explanation.

Our studies provide no new information concerning the relevance of the taxol hydrophobic and aqueous conformations to microtubule assembly activity. However, if the 2′-OH group is functioning as a hydrogen bond donor toward a tubulin residue in the taxol−microtubule complex, it is entirely possible that this interaction occurs with taxol bound in the hydrophobic conformation. Since the 2′-OH group apparently need not play a major role in the stabilization of this conformation, it would thus be free for strong hydrogen bonding with the protein. Further support for the possible importance of the hydrophobic conformation is provided by observations from the Kansas group,[19] which has shown recently that two highly active taxol analogs (with free 2′-OH groups) preserve the "hydrophobic" A-ring side chain conformation in aqueous solution. Thus, not only is preorganization of the taxol A-ring side chain "aqueous" conformation unnecessary for effective recognition by the microtubule binding site but preorganization of the "hydrophobic" conformation might well be beneficial.

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