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THE OXETANE CONFORMATIONAL LOCK OF PACLITAXEL: STRUCTURAL ANALYSIS OF D-SECOPACLITAXEL

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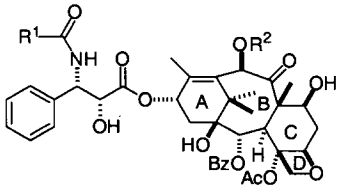
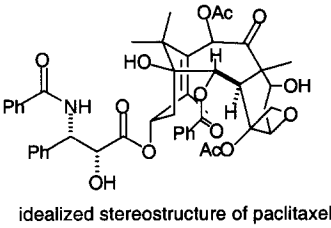
Abstract: Analysis of the ^{1}H NMR data of paclitaxel in comparison with its oxetane ring-opened analogue D-secopaclitaxel suggests that the oxetane moiety (D-ring) of paclitaxel serves as a conformational lock for the diterpene moiety and the C13 side chain.

Paclitaxel (Taxol®, Figure 1), a complex natural product isolated from the bark of *Taxus brevifolia*, is an effective clinical agent for the treatment of a variety of cancers.[3] Due to its importance as an anticancer agent, paclitaxel has been the subject of intense chemical, biological, and clinical investigations.[4-6] Paclitaxel belongs to a growing group of anticancer agents that initiate microtubule formation and stabilize microtubules against depolymerization, thereby blocking mitosis.[7]

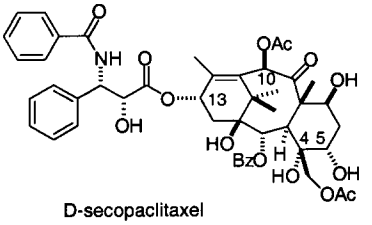
Figure 1. Structures of paclitaxel, docetaxel, and D-secopaclitaxel



R^1 = phenyl, R^2 = Ac paclitaxel
R^1 = *tert*-butylO, R^2 = H docetaxel



idealized stereostructure of paclitaxel



D-secopaclitaxel

The development of a paclitaxel pharmacophore model is of great interest to research aimed at rational drug design efforts.[8-12] Structure-activity relationship information is available from natural and semisynthetic paclitaxel analogues.[13] Further details pertaining to the paclitaxel pharmacophore can be obtained through the analysis of its three dimensional structure.

In this letter, we wish to detail results of an NMR analysis of D-secopaclitaxel (Figure 1) in which the oxetane (D-ring) has been opened by treatment of paclitaxel with Meerwein's reagent.[14] D-Secopaclitaxel is a close structural analogue of paclitaxel that does not stabilize microtubules and is not cytotoxic.[14] The D-secopaclitaxel structure is devoid of the oxetane moiety but also lacks the 4-acetyl group which is crucial for bioactivity.[15,16] In addition, D-secopaclitaxel carries a C5α-hydroxyl group instead of the C5β-oxygen ether moiety. Thus, the lack of activity of D-secopaclitaxel may be the result of one or more structural changes or physical-chemical properties. Recently, the synthesis and evaluation of 5(20)-azadocetaxel analogues in which

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the C5 oxygen of the oxetane ring was replaced by NH, N-benzyl, and N-acetyl groups was reported.[17] Since the analogues did not possess cytotoxicity, a sterically restricted binding site was proposed.[17] Replacement of the oxetane oxygen with sulfur also led to inactive paclitaxel analogues, underscoring the importance of the oxygen atom in the oxetane ring for bioactivity.[18]

We wanted to investigate how the opening of the D-ring would influence the overall conformation of the molecule. We hypothesized that the oxetane ring might serve as a conformational lock for the diterpene scaffold, aiding in the correct display of functional groups for binding site recognition and bioactivity.

The taxane diterpene system is rigid and predominantly occupies a single conformation, while the C13 side chain is highly flexible and can sample many conformations.[13] The conformation of paclitaxel has been primarily investigated using NMR[11,19,20] and X-ray diffractometry.[21,22] Two major models for the paclitaxel conformation emerged from these studies. In DMSO-d_6/D_2O, a predominant solution conformation was determined for paclitaxel and docetaxel (Figure 1) in which the 3'-phenyl, 2-benzoyl and 4-acetyl moieties are in close contact.[19,23] Support for this conformation was subsequently reported for a water-soluble paclitaxel analog in D_2O and in the crystal structure of paclitaxel.[22,24] The second model is based on NMR investigations in $CDCl_3$ and suggests that the 2-benzoyl group is in proximity to the *tert*-butyl group (docetaxel) or the phenyl group of the *N*-benzoyl moiety (paclitaxel).[20,25-27] This conformation was also observed in the crystal structure of docetaxel.[21] Recently, an electron diffraction of Zn-induced tubulin sheets, stabilized with paclitaxel, has been reported.[28] A conformational analysis of the Zn-induced tubulin-paclitaxel complex has led to the conclusion that the bound state is more extended than any of the highly populated solution conformers.[29]

In the solid state, the six-membered A-ring of the taxane diterpene assumes a distorted boat conformation flattened by the endocyclic olefin and the flagpole interaction between H13 and the C16 methyl group. Similarly, the six-membered C-ring adopts an envelope-like conformation distorted by the planar oxetane D-ring. The central eight-membered B-ring approximates a boat-chair conformation. The overall conformation of the diterpene ring system can be described as "cup- or cage-like" (Figures 1 and 2).[21,22]

NMR studies suggest that the solution conformation of paclitaxel is similar to the conformations determined from the crystal structure of paclitaxel and docetaxel.[30-32] The coupling constants derived from high field proton NMR spectra of paclitaxel in $CDCl_3$[30,33] and CD_2Cl_2[31] are consistent with the torsion angles obtained from the paclitaxel model structure for the flattened six-membered A- and C-rings (Table 1).

In solution, the flexible C13 side chain also appears to fold under the diterpene ring system as it does in the crystalline state. The small H2', H3' coupling constant (J = 2.6 Hz)[29] indicates hindered rotation in the side chain and is attributed to the hydrogen bonding noted in the crystalline state.[21] The results also suggest that H2' of paclitaxel in solution ($CDCl_3$, CD_2Cl_2) is closer to the 4-acetyl group than to the corresponding atoms in the crystalline structure of docetaxel.[31]

Kingston has reported that the reaction of paclitaxel with Meerwein's reagent (BF_4OEt_3) provides D-secopaclitaxel.[14] We prepared a sample of D-secopaclitaxel, using the conditions described by Samaranayake et al.[14] and carried out a ^1H NMR study.[34] Table 1 summarizes relevant coupling constants in $CDCl_3$ and dihedral angels associated with them as measured from static, energy minimized structures, and Table 2 provides complete peak assignments in $CDCl_3$ and DMSO-d_6/D_2O 3/1.[35] As previously noted,[14] the change in the H5, H6α and H5, H6β coupling constants is consistent with a relaxation of the C-ring from a flattened or strained

Table 1. Selected ^{1}H NMR coupling constants and torsion angles of paclitaxel and D-secopaclitaxel

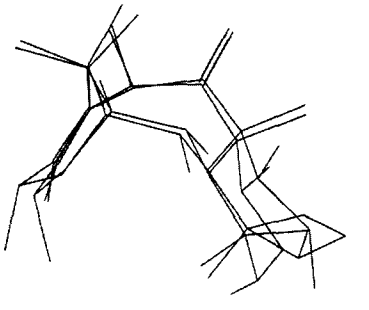
	^{1}H NMR coupling constants[a] and torsion angles[b]						
	$J_{2,3}$	$J_{5,6\alpha}$	$J_{5,6\beta}$	$J_{6\alpha,7}$	$J_{6\beta,7}$	$J_{13,14\alpha}$	$J_{13,14\beta}$
paclitaxel	7.1 Hz	9.6 Hz	2.3 Hz	6.7 Hz	11 Hz	9.0 Hz	9.0 Hz
	-151.7°	7.1°	-108.0°	45.0°	160.3°	150.5°	38.7°
D-secopaclitaxel	5.3 Hz	sm[c]	sm[c]	4.4 Hz	11.0 Hz	4.4 Hz	10.3 Hz
	-138.5°	67.3°	-48.8°	56.5°	171.9°	119.3°	6.2°

[a]$CDCl_3$, 500 MHz; [b]Measured from minimized paclitaxel and D-secopaclitaxel. The structures were constructed from the X-ray data of docetaxel and minimized in the SYBYL molecular modeling program (Tripos force field) to reduce crystal lattice constraints which may be present in the solid state; [c]Coupling constants too small to measure.

chair of paclitaxel to a more normal chair for D-secopaclitaxel (Figure 2), in which the H5 proton now nearly bisects the methylene protons at 6, giving two very small coupling constants.

More interesting, however, is the conformational change that occurs in the A-ring. The change in the H13, H14α and H13, H14β coupling constants from nearly equal values of 9 Hz to widely dissimilar values (10 and 4 Hz respectively) signals a conformational change in the A-ring from a distorted boat conformation in paclitaxel to a pseudo envelope in D-secopaclitaxel (Figure 2).

Figure 2. Overlay of the diterpene moiety of paclitaxel and D-secopaclitaxel. Structures shown without hydrogens and acyl groups for clarity.



This result is in agreement with the highly flattened cyclohexene ring described by Swindell et al. in their molecular modeling study of a tricyclic ring system similar to the paclitaxel ABC-ring system.[36] These maximally different values of the coupling constants, with one near the maximum possible value, are inconsistent with conformational averaging in the A-ring, as has been previously suggested.[14]

In between these sites a subtler change occurs in the B-ring. The flattening of the A-ring upon cleavage of the oxetane ring appears to be transmitted through C2 and to affect the conformation of the B-ring; the *J*-coupling between H2 and H3 decreases from 7.1 to 5.3 Hz (Table 1), which mirrors a decrease in the dihedral angles in the energy minimized models. This modest conformational change at C2 also affects the spatial presentation of the 2-benzoyl moiety which has been identified as an important part of the paclitaxel pharmacophore.[13]

The overall conformational changes bring the C13 side chain closer to the diterpene ring system. The spatial relationships of the functional groups along the "top" of the diterpene ring system (C7, C9, and C10)

Table 2. NMR data and assignments for paclitaxel and D-secopaclitaxel ([a]Couplings were too small to measure even with D_2O shake and sequential decoupling of C-6 protons. [b]Assignments may be reversed.

Protons at	^{1}H Shift-$CDCl_3$ (DMSO-d_6/D_2O 3/1) *paclitaxel shifts in italics*	*J*-couplings-$CDCl_3$ (source)	Long Range (^{1}H HMBC)
1			2, 3, 14α, 14β
2	5.57(5.35) *5.67(5.48)*	d 5.3 (3)	3
3	4.04(3.90) *3.79(3.66)*	d 5.3 (2)	2, 19
4			2, 3
5	3.90(3.80) *4.94(5.00)*	bs (OH, 6α, 6β)[a]	3
6α	2.21(1.99) *2.54(2.41)*	m (5, 7, geminal)	
6β	1.79(1.80) *1.88(1.75)*		
7	4.51(4.26) *4.40(4.14)*	tt 4.4, 11.0 (6α, 6β, OH)	19
8			2, 3, 19
9			10, 19
10	6.59(6.00) *6.27(6.33)*	s	
11			10, 16, 17, 18
12			10, 14β, 18
13	6.03(5.81) *6.23(5.96)*	dd 10.3, 4.4	14α, 14β
14α	3.08(2.98) *2.35(1.92)*	dd 4.4, 15.5 (13, 14β)	2
14β	2.45(2.25) *2.28(1.71)*	dd 10.3, 15.8 (13, 14α)	
15			14α, 16, 17
16	1.14(1.07)[b] *1.14(1.08)*	s	17
17	1.15(0.99)[b] *1.24(1.08)*	s	16
18	2.11(2.10) *1.79(1.82)*	s	
19	1.26(1.16 *1.68(1.82)*	s	3
20α	4.05(3.89) *4.30(4.00)*	qAB	3
20β	3.88(3.82) *4.19(3.93)*		
1'			2', 3'
2'	4.72(4.69) *4.78(4.64)*	dd 1.6 (3', OH)	
3'	5.95(5.68) *5.78(5.41)*	dd 1.6, 9.0 (2', NH)	3'-Ph *o*-
NH	7.20(--) *7.01(--)*	d 9.0 (3')	
10-Ac Me	2.24(2.19) *2.23(2.18)*	s	
20-Ac Me	1.59(1.76)	s	
4-Ac Me	*2.38(2.27)*	s	
2-OBz *o*-	8.05(8.07) *8.13(8.03)*	d	
m-	7.20(7.46) *7.51(7.69)*	t	
p-	7.47(7.66) *7.61(7.80)*	t	
3'-NBz *o*-	7.82(7.96) *7.74(7.91)*	d	
m-	7.48(7.60) *7.40(7.60)*	t	
p-	7.58(7.55) *7.49(7.55)*	t	
3'-Ph *o*-	7.49(7.52) *7.48(7.46)*	d	
m-	7.40(7.45) *7.42(7.46)*	t	
p-	7.34(7.37) *7.35(7.24)*	t	

undergo very little change when paclitaxel is converted to D-secopaclitaxel. It is at these same functional groups that the molecule can tolerate extensive changes without affecting biological activity.[13] The overlapped structures of paclitaxel and D-secopaclitaxel are shown in Figure 2.

We also carried out a NOESY experiment in 75% *d*6-DMSO/25%D_2O to examine whether the 3'-phenyl and the 2-benzoyl moieties of D-secopaclitaxel are forming a hydrophobic cluster, as seen for many bioactive paclitaxel analogues.[19,37] However, no aromatic inter-ring NOE cross peaks were observed.

In order to understand the conformational changes more completely, molecular dynamics simulations of paclitaxel and D-secopaclitaxel were undertaken. For D-secopaclitaxel, the A-ring is found to be in an envelope conformation most of the time, with brief and relatively infrequent adoption of a boat conformation. For paclitaxel, the A-ring much more frequently samples both boat and envelope conformations, with the average values of the dihedrals obtained from the dynamics simulations closely paralleling both the static, energy minimized structures and the observed coupling constants in the NMR spectrum.

Whether these conformational changes are responsible or contribute to the lack of activity of D-secopaclitaxel needs further study. This is especially true in light of the results by Appendino et al. who recently demonstrated that a conformationally flexible C-ring opened C-secodocetaxel analogue, possessing an intact oxetane ring, retained excellent activity in a tubulin assembly assay and was highly cytotoxic against the MDA-MB231 breast cancer cell line.[38]

In summary, our studies have demonstrated that the oxetane moiety significantly influences the overall conformation of the diterpene moiety of paclitaxel, including the spatial orientation of the 2-benzoyl group and the C13 side chain. Additional studies are needed to ascertain in more detail the influence of conformational, structural, and physical-chemical properties of the oxetane moiety for the bioactivity of paclitaxel.

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References and Notes

(1) Present address: University of Missouri-Kansas City, Division of Pharmaceutical Sciences, Kansas City, MO 64110-2499.
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(3) For review: Holmes, F. A.; Kudelka, A. P.; Kavanagh, J. J.; Huber, M. H.; Ajani, J. A.; Valero, V. In *Taxane Anticancer Agents: Basic Science and Current Status*; Georg, G. I., Chen, T. T., Ojima, I., Vyas, D. M., Eds.; American Chemical Society: Washington D. C., 1995; Vol. ACS Symposium Series 583, p 31.
(4) For review: *Taxane Anticancer Agents: Basic Science and Current Status*; Georg, G. I.; Chen, T. T.; Ojima, I.; Vyas, D. M., Eds.; American Chemical Society: Washington, DC, 1995.
(5) For review: *Taxol® Science and Applications*; Suffness, M., Ed.; CRC: Boca Raton, FL, 1995.
(6) *The Chemistry and Pharmacology of Taxol® and Its Derivatives*; Farina, V., Ed.; Elsevier: Amsterdam, 1995.
(7) Jordan, A.; Hadfield, J. A.; Lawrence, N. J.; McGown, A. T. *Med. Res. Rev.* **1998**, *18*, 259.
(8) Czaplinski, K.-H. A.; Grunewald, G. L. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2211.
(9) Morita, H.; Gonda, A.; Wei, L.; Takeya, K.; Itokawa, H. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2387.
(10) Zhu, Q.; Guo, Z.; Huang, N.; Chu, F. *J. Med. Chem.* **1997**, *40*, 4319.
(11) Ojima, I.; Chakravarta, S.; Inoue, T.; Lin, S.; He, L.; Horwitz, S. B.; Danishefsky, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 4256.
(12) Wang, M.; Xia, X.; Kim, Y.; Hwang, D.; Jansen, J. M.; Botta, M.; Liotta, D. C.; Snyder, J. P. *Org. Lett.* **1999**, *1*, 43.

(13) For review: Georg, G. I.; Boge, T. C.; Cheruvallath, Z. S.; Clowers, J. S.; Harriman, G. C. B.; Hepperle, M.; Park, H. In *Taxol® Science and Applications*; Suffness, M., Ed.; CRC: Boca Raton, FL, 1995, p 317.
(14) Samaranayake, G.; Magri, N. F.; Jitrangsri, C.; Kingston, D. G. I. *J. Org. Chem.* **1991**, *56*, 5114.
(15) Datta, A.; Jayasinghe, L.; Georg, G. I. *J. Med. Chem.* **1994**, *37*, 4258.
(16) Neidigh, K. A.; Gharpure, M. M.; Rimoldi, J. M.; Kingston, D. G. I.; Jiang, Y. Q.; Hamel, E. *Tetrahedron Lett.* **1994**, *35*, 6839.
(17) Marder-Karesenti, R.; Dubois, J.; Bricard, L.; Guenard, D.; Gueritte-Voegelein, F. *J. Org. Chem.* **1997**, *62*, 6631.
(18) Gunatilaka, A. A. L.; Ramdayal, F. D.; Sarragiotto, M. H.; Kingston, D. G. I. *J. Org. Chem.* **1999**, *64*, 2694.
(19) Vander Velde, D. G.; Georg, G. I.; Grunewald, G. L.; Gunn, K.; Mitscher, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 11650.
(20) Williams, H. J.; Scott, A. I.; Dieden, R. A.; Swindell, C. S.; Chirlian, L. E.; Francl, M. M.; Heerding, J. M.; Krauss, N. E. *Can. J. Chem.* **1994**, *72*, 252.
(21) Guéritte-Voegelein, F.; Guénard, D.; Mangatal, L.; Potier, P.; Guilhem, J.; Cesario, M.; Pascard, C. *Acta Crystallogr.* **1990**, *C46*, 781.
(22) Mastropaolo, D.; Camerman, A.; Luo, Y.; Brayer, G. D.; Camerman, N. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 6920.
(23) Ojima, I.; Kuduk, S. D.; Chakravarty, S.; Ourevitch, M.; Begue, J.-P. *J. Am. Chem. Soc.* **1997**, *119*, 5519.
(24) Paloma, L. G.; Guy, R. K.; Wrasidlo, W.; Nicolaou, K. C. *Chem. Biol.* **1994**, *1*, 107.
(25) Williams, H. J.; Scott, A. I.; Dieden, R. A.; Swindell, C. S.; Chirlian, L. E.; Francl, M. M.; Heerding, J. M.; Krauss, N. E. *Tetrahedron* **1993**, *49*, 6545.
(26) Dubois, J.; Guénard, D.; Guéritte-Voegelein, F.; Guedira, N.; Potier, P.; Gillet, B.; Beloeil, J.-C. *Tetrahedron* **1993**, *49*, 6533.
(27) Cachau, R. E.; Gussio, R.; Beutler, J. A.; Chmurney, G. N.; Hilton, B. D.; Muschik, G. M.; Erickson, J. W. *Intl. J. Supercomput. Appl.* **1994**, *8*, 24.
(28) Nogales, E.; Wolf, S. G.; Downing, K. *Nature, (London)* **1998**, *391*, 199.
(29) Snyder, J. P.; Cornett, B.; Downing, K.; Nogales, E. **1999**, (Submitted for publication). We thank Professor Snyder and collaborators for informing us of their results prior to publication.
(30) Chmurny, G. N.; Hilton, B. D.; Brobst, S.; Look, S. A.; Witherup, K. M.; Beutler, J. A. *J. Nat. Prod.* **1992**, *55*, 414.
(31) Falzone, C. J.; Benesi, A. J.; Lecomte, J. T. J. *Tetrahedron Lett.* **1992**, *33*, 1169.
(32) Grunewald, G.; Vander Velde, D. G.; Gunn, C. W. In *203rd National Meeting of the American Chemical Society*; American Chemical Society, Washington, D. C.: San Francisco, CA, 1992, MEDI 31.
(33) Georg, G. I.; Cheruvallath, Z. S.; Himes, R. H.; Mejillano, M. R.; Burke, C. T. *J. Med. Chem.* **1992**, *35*, 4230.
(34) The sample of D-secopaclitaxel prepared in our laboratory has the H5 signal at a different chemical shift than reported in reference 14. The signal closest to the shift reported was, in our sample, a hydroxyl proton as confirmed by a D_2O shake.
(35) Shifts of directly bonded protons identified from HMQC. *J*-couplings identified from COSY and D_2O shake spectrum. HMBC correlations useful for establishing assignments, which extend and in a few cases differ with those given in reference 14, are also given.
(36) Swindell, C. S.; Isaacs, T. F.; Kanes, K. J. *Tetrahedron Lett.* **1985**, *26*, 289.
(37) Georg, G. I.; Harriman, G. C. B.; Hepperle, M.; Clowers, J. S.; Vander Velde, D. G.; Himes, R. H. *J. Org. Chem.* **1996**, *61*, 2664.
(38) Appendino, G.; Danieli, B.; Jakupovic, J.; Belloro, E.; Scambia, G.; Bombardelli, E. *Tetrahedron Lett.* **1997**, *38*, 4273.