H- and C-nmr Assignments for Taxol, 7-epi-Taxol, and Cephalomannine

Gwendolyn N. Chmurny, Bruce D. Hilton, Susan Brobst, Sally A. Look, Keith M. Witherup, and John A. Beutler

J. Nat. Prod., **1992**, 55 (4), 414-423• DOI: 10.1021/np50082a002 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 55, No. 4, pp. 414-423, April 1992

^{1}H- and ^{13}C-NMR ASSIGNMENTS FOR TAXOL, 7-*epi*-TAXOL, AND CEPHALOMANNINE

GWENDOLYN N. CHMURNY,* BRUCE D. HILTON, SUSAN BROBST, SALLY A. LOOK,[1] KEITH M. WITHERUP,[2] and JOHN A. BEUTLER[3,*]

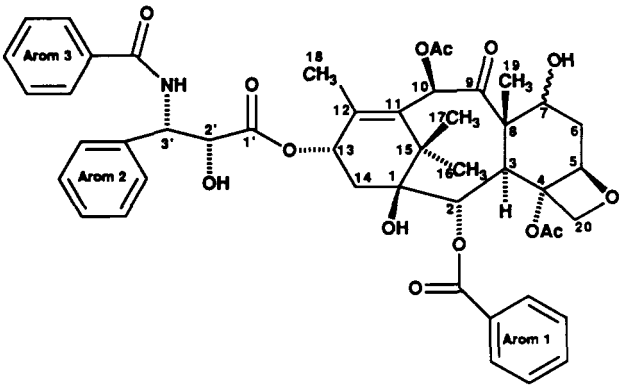
Chemical Synthesis and Analysis Laboratory, PRI/DYNCorp, Inc., National Cancer Institute-Frederick Cancer Research Facility, P.O. Box B, Frederick, Maryland 21702-1201

ABSTRACT.—The ^{1}H- and ^{13}C-nmr spectra of taxol [**1**], 7-*epi*-taxol [**2**], and cephalomannine [**3**] were assigned using modern 1D and 2D nmr methods. Preliminary conformational information was obtained by nOe spectroscopy.

The antineoplastic agent taxol is in phase II clinical trials for ovarian and breast cancer. Its mechanism of action has been shown to involve interference with tubulin depolymerization (1). As a first step toward understanding structural requirements for the biological action of taxol, the ^{1}H- and ^{13}C-nmr spectra of taxol [**1**] and the related strategically important taxanes 7-*epi*-taxol [**2**] and cephalomannine [**3**] have been analyzed using 1D and 2D nmr techniques. Confirmation of the taxol assignments, in addition to preliminary conformational information for taxol, was obtained via nOe spectroscopy.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H- (499.843 MHz) and ^{13}C- (125.697 MHz) nmr spectroscopy were performed on a Varian VXR-500S spectrometer equipped with a Sun 4/110 workstation. ^{1}H chemical shifts are reported relative to TMS; ^{13}C chemical shifts were assigned relative to $CDCl_3$ = 77.00 ppm. Referencing in the indirect detection experiments was accomplished by using the peak positions from the 1D spectra to assign chemical shifts to strong, sharp resonances in the 2D spectra.



1 7-β-OH
2 7-α-OH

[1]Present address: Division of Oncology & Pulmonary Drug Products, Office of Drug Evaluation I, Food and Drug Administration, Rockville, MD 20857.

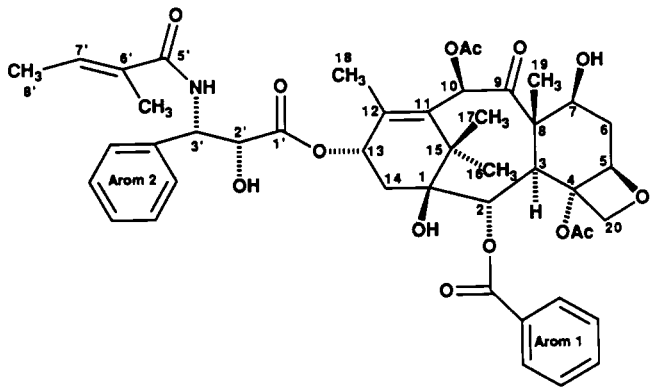
[2]Present address: Department of Medicinal Chemistry, Merck Sharpe & Dohme Research Laboratories, WP-27F-140, West Point, PA 19486.

[3]Present address: Laboratory of Drug Discovery Research & Development, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bldg. 560-15 NCI-FCRDC, Frederick, MD 21702-1201.

TABLE 1. Acquisition and Processing Parameters for 2D Experiments.[a]

Experiment	Data points	Experiments (t_1 increments)	Data size	Transients per experiment	Apodization	Recycle time, sec	Acquisition time, sec	90° ^{1}H Pulse width, μsec	90° ^{13}C Pulse width, μsec
COSY	2K	512	2K × 2K	4	sine bell	1.2	0.214	8.5	—
TOCSY	2K	256 × 2	2K × 1K	16	cosine bell	1.28	0.214	8.3	—
HETCOR	2K	256	4K × .5K	128	Gaussian	1.05	0.054	100.8	11.5
HMQC	2K	512	2K × 1K	140	cosine bell	1.34	0.223	8.3	25.0
HMBC	2K	512	2K × 1K	128	cosine bell	1.34	0.223	8.3	24.0

[a]Points are total points, i.e., 2K = 1K complex points. TOCSY and ROESY were obtained using States Haberkorn phase cycling; HMQC was obtained using TPPI phase cycling; and the COSY was presented in absolute value mode. Broadband ^{13}C decoupling (Waltz modulated) was used during the acquisition time (HMQC). The ROESY spin lock used a Kessler sequence of 30° flip angle pulses.



3

TABLE 2. ^{1}H-nmr Assignments for Taxol [1] in $CDCl_3$ (500 MHz).

Assignment	Shift[a]	Multiplicity (*J*, Hz)[b]	COSY[c]	TOCSY[d]
1-OH	1.98	bs	—	—
H-2	5.67	d (7.1)	H-3	H-3
H-3	3.79	d (1.0), d (7.0)	H-2	H-2
			H_a-20	H_a-20
4-OAc	2.38	s	—	—
H-5	4.94	d (0.8),[f] d (0.9),[f] d (2.3), d (9.6)	$H_{a,b}$-6	H-7
			$H_{a,b}$-20	$H_{a,b}$-6
H_a-6	2.54	d (6.7), d (9.7), d (14.8)	H-5	H-5
			H_b-6	H-7
			H-7	H_b-6
H_b-6	1.88	d (2.3), d (11.0), d (14.7)	H-5 (sm)	H_a-6
			H_a-6	H-5
			H-7	H-7
H-7	4.40	d (6.7), d (10.9), [d (4.3)][e]	$H_{a,b}$-6	H-5
				$H_{a,b}$-6
7-OH	2.48	bs [d (4.4)][e]	—	—
H-10	6.27	s	—	—
10-OAc	2.23	s	—	—
H-13	6.23	q (1.5), t (9.0)	Me-18	Me-18
			$H_{a,b}$-14	$H_{a,b}$-14
H_a-14	2.35	d (9.0), d (15.4)	H_b-14	H-13
			H-13	H_b-14
H_b-14	2.28	d (0.6), d (9.0), d (15.3)	H_a-14	H-13
			H-13	H_a-14
				Me-18
Me-16	1.14	s	—	—
Me-17	1.24	s	—	—
Me-18	1.79	d (1.5)	H-13	H-13
Me-19	1.68	s	—	—
H_a-20	4.30	d (1.1), d (0.8), d (8.4)	H-5 (sm)	H_b-20
			H_b-20	H-3
			H-3	
H_b-20	4.19	d (1.0), d (8.5)	H_a-20	H_a-20
			H-5	

TABLE 2. Continued.

Assignment	Shift[a]	Multiplicity (*J*, Hz)[b]	COSY[c]	TOCSY[d]
H-2′	4.78	d(2.7), [d(5.4)][e]	H-3′	3′-NH H-3′ [2′-OH]
2′-OH	3.61	bs [d(5.4)][e]	—	—
H-3′	5.78	d(2.8), d(8.9)	3′-NH H-2′	3′-NH H-2′
3′-NH	7.01	d(8.9)	H-3′	H-3′ H-2′
o-Ph1	8.13	d(1.3), d(8.4)	*p*-Ph1 (sm) *m*-Ph1	*p*-Ph1 *m*-Ph1
m-Ph1	7.51	cm	*o*-Ph1 *p*-Ph1	*o*-Ph1 *p*-Ph1
p-Ph1	7.61	t(1.4), t(7.4)	*o*-Ph1 (sm) *m*-Ph1	*o*-Ph1 *m*-Ph1
o-Ph2	7.48	cm	*m*-Ph2 *p*-Ph2 (sm)	*m*-Ph2 *p*-Ph2
m-Ph2	7.42	cm	*o*-Ph2 *p*-Ph2	*o*-Ph2 *p*-Ph2
p-Ph2	7.35	t(1.6), t(7.3)	*o*-Ph2 (sm) *m*-Ph2	*o*-Ph2 *m*-Ph2
o-Ph3	7.74	d(1.2), d(8.3)	*p*-Ph3 (sm) *m*-Ph3	*p*-Ph3 *m*-Ph3
m-Ph3	7.40	cm	*o*-Ph3 *p*-Ph3	*o*-Ph3 *p*-Ph3
p-Ph3	7.49	cm	*o*-Ph3 (sm) *m*-Ph3	*o*-Ph3 *m*-Ph3

[a]Chemical shifts in ppm high frequency ("low" field) from TMS.
[b]Multiplicity: s = singlet, d = doublet, t = triplet, cm = complex multiplet, b = broad, sm = small.
[c]^{1}H-^{1}H correlation spectroscopy.
[d]^{1}H-^{1}H total correlation spectroscopy.
[e]Information in brackets is additional data from a different sample with slower exchange of hydroxyls.
[f]Spin simulation; see Experimental section.

All spectra were obtained at 25° using a Varian variable temperature controller. During 2D experiments samples were not spun; during 1D experiments samples were spun at 20 Hz. Table 1 contains the important acquisition and processing parameters for the 2D nmr experiments. The HMQC spectra were obtained using a 'Bird' pulse followed by a 300 msec delay; the HMBC spectra were run twice, once with the second delay in the *J* filter segment of the pulse sequence set for $J = 8$ Hz and again set for $J = 4$ Hz. Delays in both HMQC and HMBC experiments used to control the contribution of one-bond couplings were set for $J = 140$ Hz. TOCSY spectra used a MLEV-17 spin locking field of 50 msec during which the 90° proton pulse was 32 μsec.

NOE NMR SPECTROSCOPY.—NOe's in ^{1}H-nmr spectra were measured using 1D difference nOe methods (2) and the 2D pulse sequences NOESY (3) and ROESY (4). Percentage enhancements in the 1D experiments were calculated using peak heights relative to control spectra, while NOESY and ROESY data were not quantitated. One-dimensional experiments were run degassed and non-degassed with interleaved blocks of six irradiations.

RESULTS AND DISCUSSION

NMR ASSIGNMENTS.—^{1}H-nmr assignments for taxol and 7-*epi*-taxol at 500 MHz in $CDCl_3$ are listed in Tables 2 and 3, respectively. Assignments were based on analysis of the 1D, COSY, and TOCSY spectra and are largely consistent with assignments made previously at lower field strengths (5–7). The only change noted is the reversal in assignment of Me-16 and Me-17, which we based on nOe measurements.

The 1D ^{1}H spectra for **1** and **2** (Tables 2 and 3) were assigned using the following strategy. An approximate proton count was obtained by separating general regions of each spectrum and measuring the relative areas. These areas were reassembled by TOCSY connectivities into *J*-coupled groups; for example, the H-5 trace shows protons H-5, $H_{a,b}$-6, and H-7 separated into rows from protons 3′-NH, H-3′, H-2′, and 2′-OH in the H-2′ trace. This technique was especially useful for the aromatic region. The COSY spectrum for taxol illustrates how the TOCSY groups were further assembled into adjacent groups. 3′-NH is coupled only to H-3′, which is coupled only to 3′-NH and H-2′, etc. The absence of the 2′-OH, H-2′ and the 7-OH, H-7 cross peaks in the COSY spectrum can be explained because in this sample the hydroxyl protons are exchanging rapidly on the nmr time scale. Note, however, that the amide proton 3′-NH exchanges more slowly and a cross peak and *J*-connectivities were observed.

TABLE 3. ^{1}H-nmr Assignments for 7-*epi*-Taxol [**2**] in $CDCl_3$ (500 MHz).

Assignment	Shift	Multiplicity	*J*
H-2	5.76	d	7.5
H-3	3.92	d	7.5
4-OAc	2.50	s	
H-5	4.91	dd	9.0, 3.5
H-6	2.33	dd AB	2.1, 9.2, 16.1
	2.27	dd AB	3.7, 5.0, 16.0
H-7	3.70	brd [ddd][a]	[2.1, 5.0, 11.6][a]
7-OH	4.68	bs [dd][a]	[4.49, 11.5][a]
H-10	6.80	s	
10-OAc	2.19	s	
H-13	6.23	qt	1.5, 9.0
H-14	2.42	d AB	9.3, 15.5
	2.25	d AB	9.1, 15.4
H-16	1.15	s	
H-17	1.19	s	
H-18	1.79	d	1.5
H-19	1.67	s	
H-20	4.39	AB	8.7, $J_{CH} \cong 155$ Hz
2′-OH	2–2.5		
H-2′	4.81	d	2.6
H-3′	5.81	dd	9.0, 2.5
3′-NH	7.00	d	9.1
o-PH1	8.18	dd	1.3, 8.5
o-Ph3	7.72	dd	1.3, 8.4
p-Ph1	7.62	tt	1.6, 7.4 not 1st order
aromatic	7.34–7.56	m	

[a]Couplings seen in slow exchanging sample only.

With the proton chemical shifts in hand, *J*-coupling constants and chemical shifts were assembled into Tables 2 and 3 with the use of our program ZFD, which is used to simplify the handling of large amounts of data.[4] Varian's version of the LAOCOON spin simulation program was also applied. The exchangeable protons 7-OH and 2′-OH were identified by comparison of two samples of either **1** or **2**; one sample did not exchange rapidly on the nmr time scale, and the other sample did, presumably as a consequence of differing H_2O content or pH. For example, the 7-OH in **2** was identified by

[4]G.N. McGregor and G.N. Chmurny, manuscript in preparation.

the 11.5 Hz coupling constant observed for H-7/7-OH which occurred in the slow exchanging sample of 7-*epi*-taxol. Note that this coupling can be seen in both the 7-OH and H-7 peaks. In taxol [**1**], the H-7/7-OH coupling is 4.4 Hz, and for H-2′/2′-OH it is 5.4 Hz.

On the occasions where the J coupling was strong, (e.g., $\Delta\delta/J<1$), spin simulation was performed and the calculated spectrum compared to the experimental spectrum. The simulated spectrum justified the assignments. The chemical shift values and scalar coupling constants were then assigned to the structures of taxol and 7-*epi*-taxol.

Of the six methyl groups each in **1** and **2**, only Me-18 showed J-coupling: 1.5 Hz to H-13. Methyl groups 19, 4-OAc, and 10-OAc were readily assigned by HMBC connectivities (see discussion below). Me-16 and Me-17 could only be assigned using nOe spectra (Figure 1), because HMBC connectivities were the same for the two groups. ^{1}H-nmr assignments for **3** were made in similar fashion.

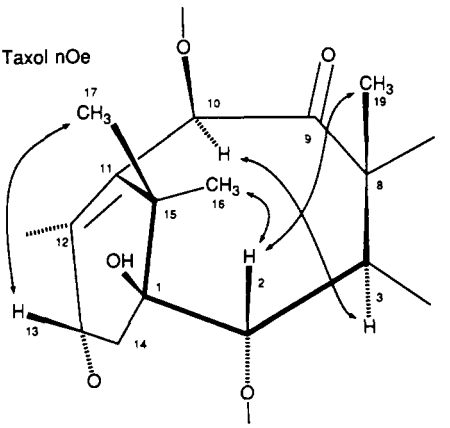


FIGURE 1. Diagram illustrating the key nOe connectivities for Me-16, -17, and -19. (Me-18 is assigned by scalar coupling to H-13.)

Once the proton assignments were made, we proceeded to assign all of the carbon resonances based on the heteronuclear experiments HMQC and HMBC (J filter set for 8 Hz and 4 Hz). ^{13}C-nmr assignments for **1**, **2**, and **3** are given in Table 4. The heteronuclear correlation results for taxol are tabulated in Table 5.

The HMBC connectivities obtained led to the assignment of the 4-OAc and the 10-OAc methyl groups (Table 5); H-10 correlates to C-9, and the 10-OAc methyl to its carbonyl, while the 4-OAc methyl correlates to its carbonyl. Since C-4 is quaternary, the 4-OAc carbonyl is assigned by difference. The HMBC correlations between Me-18 and C-8, C-7, C-9, and C-3 establish the assignment of Me-18. The Me-16 and Me-17 resonances both show connectivities to C-15, C-1, and C-11 and to each other; they were assigned by nOe as mentioned above.

The remaining assignments were made using the same strategy and techniques. Previous ^{13}C-nmr assignments for several taxanes (8) were based on chemical shift arguments; modern nmr techniques and strategies lead to more reliable assignments for molecules of this complexity.

TAXOL VS. 7-*epi*-TAXOL.—In Figures 2 and 3 and Tables 2 and 3, the 1D ^{1}H-nmr spectra for taxol and 7-*epi*-taxol clearly show chemical shift and coupling differences between **1** and **2** that are consistent with epimerization at C-7. Most dramatic are the

TABLE 4. ^{13}C-nmr Assignments for Taxol [**1**], 7-*epi*-Taxol [**2**], and Cephalomannine [**3**] in $CDCl_3$ (500 MHz).[a]

Carbon	Compound		
	1[b]	**2**	**3**
C-1	79.0	79.1	79.0
C-2	74.9	75.3	74.9
C-3	45.6	40.3	45.5
C-4	81.1	82.1	81.1
C-5	84.4	82.8	84.4
C-6	35.6	36.1	35.5
C-7	72.2	75.7	72.17
C-8	58.6	57.6	58.5
C-9	203.6	207.4	203.8
C-10	75.5	78.1	75.6
C-11	133.2	133.5	133.1
C-12	142.0	139.7	142.2
C-13	72.3	72.3	72.24
C-14	35.7	35.3	35.5
C-15	43.2	42.6	43.1
C-16	21.8	21.2	21.8
C-17	26.9	25.9	26.8
C-18	14.8	14.7	14.7
C-19	9.5	16.1	9.5
C-20	76.5	77.6	76.5
C-1′	172.7	172.9	172.9
C-2′	73.2	73.1	73.3
C-3′	55.0	54.8	54.8
4-OAc C=O	170.4	172.4	170.4
Me	22.6	22.5	22.5
10-OAc C=O	171.2	169.5	171.4
Me	20.8	20.8	20.8
C=O Ph1	167.00	167.3	167.1
q-Ph1	129.1	133.7	129.15
o-Ph1	130.2	130.3	130.3
m-Ph1	128.71	128.4[c]	128.3
p-Ph1	133.7	133.8	133.8
q-Ph2	133.6	138.1	131.3
o-Ph2	127.03	127.0	127.0
m-Ph2	128.68	128.8[c]	128.8
p-Ph2	131.9	128.9	129.03
C=O Ph3	167.02	167.2	—
q-Ph3	138.0	129.4	—
o-Ph3	127.04	127.1	—
m-Ph3	129.0	129.1[c]	—
p-Ph3	128.3	132.0	—
5′			169.1
6′			138.2
7′			132.0
8′			13.9
6′-Me			12.3

[a]^{13}C chemical shifts are in ppm from $CDCl_3$ assigned as 77.00 relative to TMS.

[b]Assignments based on HMQC, HMBC (8 and 4 Hz), approximate integrals, line widths, and coupled ^{13}C spectra.

[c]May be interchanged.

TABLE 5. ^{1}H, HMQC, and HMBC Data for Taxol [**1**] in $CDCl_3$ (500 MHz).

Assignment	Delta (ppm)[a]	HMQC	HMBC (8 Hz)	HMBC (4 Hz)
1-OH	1.98	—	—	—
H-2	5.67	75.1	Ph 1 C=O, C-1, C-8, C-3, C-14	—
H-3	3.79	45.8	C-4, C-10, C-8, C-19	C-4, C-1, C-2, C-7, C-8
4-OAc	2.38	22.7	4-C=O	4-C=O
H-5	4.94	84.6	C-4, C-7	—
H_a-6	2.54	35.6	C-5, C-4, C-7, C-8	—
H_b-6	1.88	35.6	C-5, C-7	—
H-7	4.40	72.2	—	—
7-OH	2.48	—	—	—
H-10	6.27	75.70	C-9, 10-C=O, C-12, C-11, C-15	C-9, 10-C=O, C-12, C-11, C-15
10-OAc	2.23	21.0	10-C=O	10-C=O
H-13	6.23	72.4	C-1′, C-14	—
H_a-14	2.35	35.65	C-15	C-1, C-2, C-15
H_b-14	2.28	35.65	C-12	C-1, C-2, C-13
Me-16	1.14	21.8	C-1, C-15, C-17, C-11	C-1, C-15, C-17, C-11
Me-17	1.24	26.9	C-1, C-15, C-16, C-11	C-1, C-15, C-16, C-11
Me-18	1.79	15.0	C-13, C-12, C-11	C-11, C-12, C-13
Me-19	1.68	9.6	C-9, C-7, C-8, C-3	C-9, C-7, C-8, C-3
H_a-20	4.30	76.7	C-4	C-4, C-3
H_b-20	4.19	76.7	C-5, C-4	C-5, C-4, C-3
H-2′	4.78	73.26	—	—
2′-OH	3.61	—	—	—
H-3′	5.78	55.2	Ph3-C=O, C-2′, *q*-Ph3, *o*-Ph2	C-1′, C-2′, Ph3-C=O
3′-NH	7.01	—	Ph3-C=O	Ph3-C=O
o-Ph1	8.13	130.5	Ph1 C=O, *p*-Ph1	Ph1 C=O
m-Ph1	7.51	128.9	*o*-Ph1	*o*-Ph1
p-Ph1	7.61	133.8	*o*-Ph1	—
o-Ph2	7.48	126.9		
m-Ph2	7.42	128.8		
p-Ph2	7.35	132.0		
o-Ph3	7.74	127.00		
m-Ph3	7.40			
p-Ph3	7.49	128.4		

[a]Chemical shifts in ppm high frequency from TMS.

changes in chemical shifts of 7-OH, H-7, and $H_{a,b}$-6. The major coupling differences are between H-7 and $H_{a,b}$-6 (from 6.7 and 10.9 Hz in taxol to 5.0 and 2.1 Hz in 7-*epi*-taxol). The C-6 geminal coupling changes from 14.8 Hz in taxol to 16.1 Hz in 7-*epi*-taxol. The observation that the H-5 to $H_{a,b}$-6 couplings change by less than 1.2 Hz, in conjunction with the preceding data, clearly reflects the epimerization at C-7. Epimerization at C-7 is further supported by the lack of significant changes in chemical shifts and scalar couplings at C-14. In addition, no change in geminal coupling for the protons at C-20 is observed; however, epimerization causes a slight chemical shift change for these protons (Figure 3).

In 7-*epi*-taxol, the 4-OAc carbonyl is hydrogen-bonded to the 7′-OH [Huang *et al.* (6) and modeling results in this laboratory]. The ^{13}C-nmr spectrum of 7-*epi*-taxol shows distinct differences from that of taxol; this reflects the conformational consequences of hydrogen bonding.

One-dimensional nOe difference spectroscopy as well as the 2D experiments NOESY and ROESY were used to measure nOe's for taxol (Table 6). The most notable

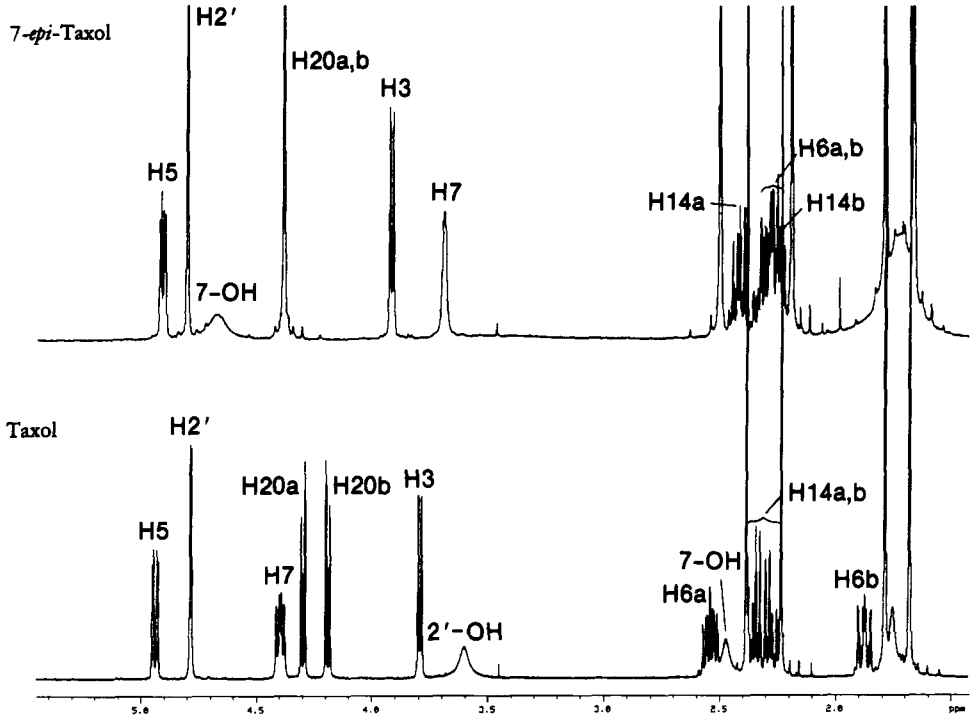


FIGURE 2. 1-D proton spectrum of taxol and 7-*epi*-taxol in $CDCl_3$ at 500 MHz showing the differences in chemical shift and coupling constants between the two isomers.

TABLE 6. NOe Data for Taxol [**1**] ($CDCl_3$, 500 MHz).[a]

Proton	Proton	Enhancement	N/ROESY
H-2	H-16	1.4%, 5.9%	NR
H-2	H-19	1.1%, 2.4%	NR
H-3	H-7	2.7%, 4.0%	R
H-3	H-10	1.0%, 1.0%	R
4-OAc	H-2′	0.9%, 0.5%	R
4-OAc	H-3′	1.4%, 0.9%	R
H-5	H-7	none	N
H-7	H-10	4.1%, 3.2%	none
H-10	H-18	1.9%, 4.4%	NR
10-OAc	H-13	none	NR
H-13	H-17	2.4%, 5.0%	NR
H-16	H-19	none	none
H-18	H-2′	1.1%, 0.3%	R
H-19	H_b-20	2.3%, 1.0%	NR
H_a-20	*o*-Ph1	1.0%, 0.8%	R
H-2′	H-3′	3.5%, 3.6%	NR
H-3′	3′-NH	3.2%, none	NR
H-3′	*o*-Ph2	1.2%, 2.5%	R
3′-NH	*o*-Ph2	1.1%, 1.8%	R
3′-NH	*o*-Ph3	4.6%, 4.6%	R

[a]Enhancements refer to difference nOe enhancements; percentrages are for the enhancement in the forward and reverse direction respectively. N indicates a NOESY cross peak was observed; R indicates a ROESY cross peak was observed.

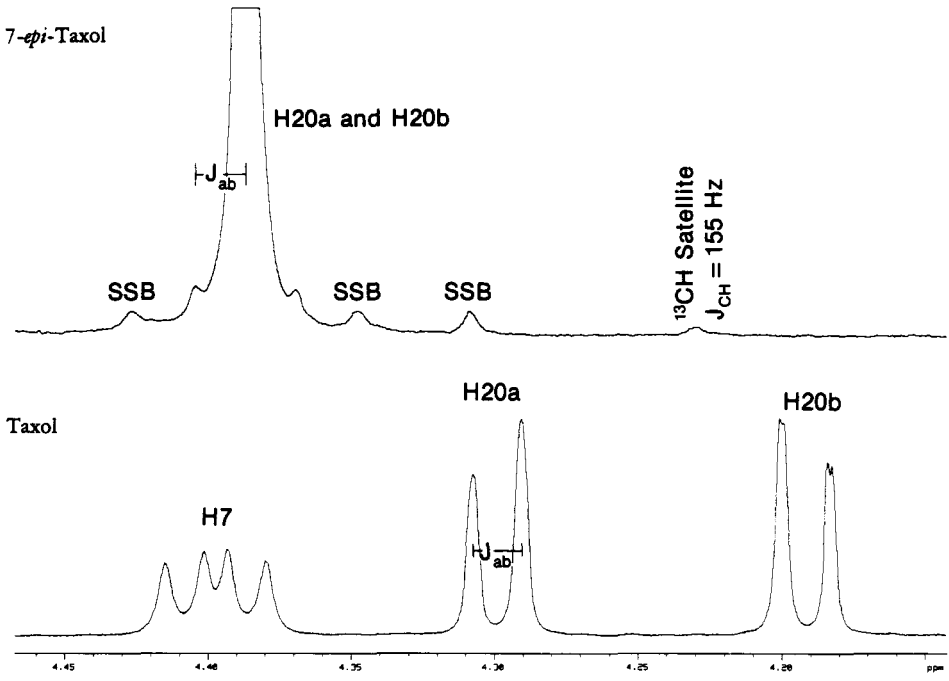


FIGURE 3. Expansions of spectra in Figure 2 showing the lack of change from taxol to 7-*epi*-taxol in the H_a-20 and H_b-20 geminal coupling constant. The differences in chemical shifts are also illustrated; in 7-*epi*-taxol these chemical shifts are the same to within 10^{-3} ppm.

finding of these experiments is that there are few detectable nOe enhancements between side-chain protons and protons on the taxane skeleton, although weak effects were seen between H-3′ and Me-18 as well as between H-2′ and the 4-OAc Me. No nOe was seen between H-13 and H-2′. Difference nOe, NOESY, and ROESY experiments gave consistent results (Table 6) which were further supportive of the ^{1}H-nmr assignments.

CONCLUSIONS.—The preceding ^{1}H- and ^{13}C-nmr assignments and nOe data form the basis for our ongoing investigations of the conformational behavior of taxol. These data provide an experimental foundation for testing molecular modeling results obtained for this important natural product.

ACKNOWLEDGMENTS

Research was supported in part by the National Cancer Institute, DHHS, under contract NO1-CO-74102 with Program Resources, Incorporated. The contents of this publication do not necessarily reflect the views or policies of the DHHS, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

LITERATURE CITED

1. P.B. Schiff, J. Fant, and S.B. Horwitz, *Nature*, **277**, 665 (1979).
2. M. Kinns and J.K.M. Saunders, *J. Magn. Reson.*, **56**, 518 (1984).
3. J. Jeener, B.H. Meier, P. Bachman, and R. Ernst, *J. Chem. Phys.*, **71**, 4546 (1979).
4. A. Bax and D.G. Davis, *J. Magn. Reson.*, **63**, 207 (1985).
5. D.G.I. Kingston, D.R. Hawkins, and L. Ovington, *J. Nat. Prod.*, **45**, 466 (1982).
6. C.H.O. Huang, D.G.I. Kingston, N.F. Magri, G. Samaranayake, and F. Boettner, *J. Nat. Prod.*, **49**, 665 (1986).
7. R.W. Miller, R.G. Powell, C.R. Smith Jr., E. Arnold, and J. Clardy, *J. Org. Chem.*, **46**, 1469 (1981).
8. A.C. Rojas, D. de Marcano, B. Mendez, and J. de Mendez, *Org. Magn. Reson.*, **21**, 257 (1983).

Received 10 April 1991