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CONFORMATION OF VINBLASTINE IN AQUEOUS SOLUTION DETERMINED BY 2D ^{1}H- AND ^{13}C-NMR SPECTROSCOPY

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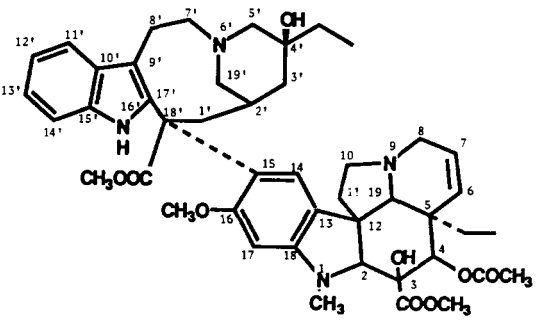
ABSTRACT.—The conformation of vinblastine [**1**] in H_2O solution at pD in the range 4.8–6.6 has been delineated by interpreting ^{1}H-nmr 2D COSY and NOESY maps, ^{1}H-^{13}C 2D correlated spectra, and the pattern of proton-proton scalar couplings. A molecular model of the preferred spatial arrangement has been obtained and compared with the structure previously determined in organic solvents. The dihedral angle between the two indole moieties has been evaluated at ca. 180° as compared to the value of 160° in organic solution.

Vinblastine [**1**] (1–3), an important anti-tumor alkaloid from *Catharanthus roseus*, is known to associate with tubulin, thereby arresting microtubule elongation (4–9). In order to understand this phenomenon at the molecular level we have undertaken nmr studies of the conformation of the dimeric structure. Detailed ^{1}H- (10–12) and ^{13}C- (13–16) nmr investigations of **1** in organic solvents have been reported, and ^{1}H nOe difference spectroscopy has been thoroughly exploited to deduce a preferential solution conformation. However, due to the low solubility no attempt has been made so far to determine the solution conformation in H_2O and to compare this resultant geometry with the structure in organic solvents (16). Moreover, while the thermal stability of **1** at pH 4.5–5.0 has been investigated (17), no report has appeared, to our knowledge, of the effects of pH changes on chemical shift. In the present communication we have utilized 2D ^{1}H- and ^{13}C-nmr techniques for the identification of the H_2O solution structures of vinblastine [**1**] at pD 4.8–6.6. We have used computer graphics to compare the conformations in organic and aqueous solution as a necessary prelude to binding studies with tubulin.

RESULTS AND DISCUSSION

The ^{1}H-nmr assignment for vinblastine [**1**] was acquired by obtaining COSY (18), double-quantum filtered COSY (19), and double quantum phase-sensitive COSY



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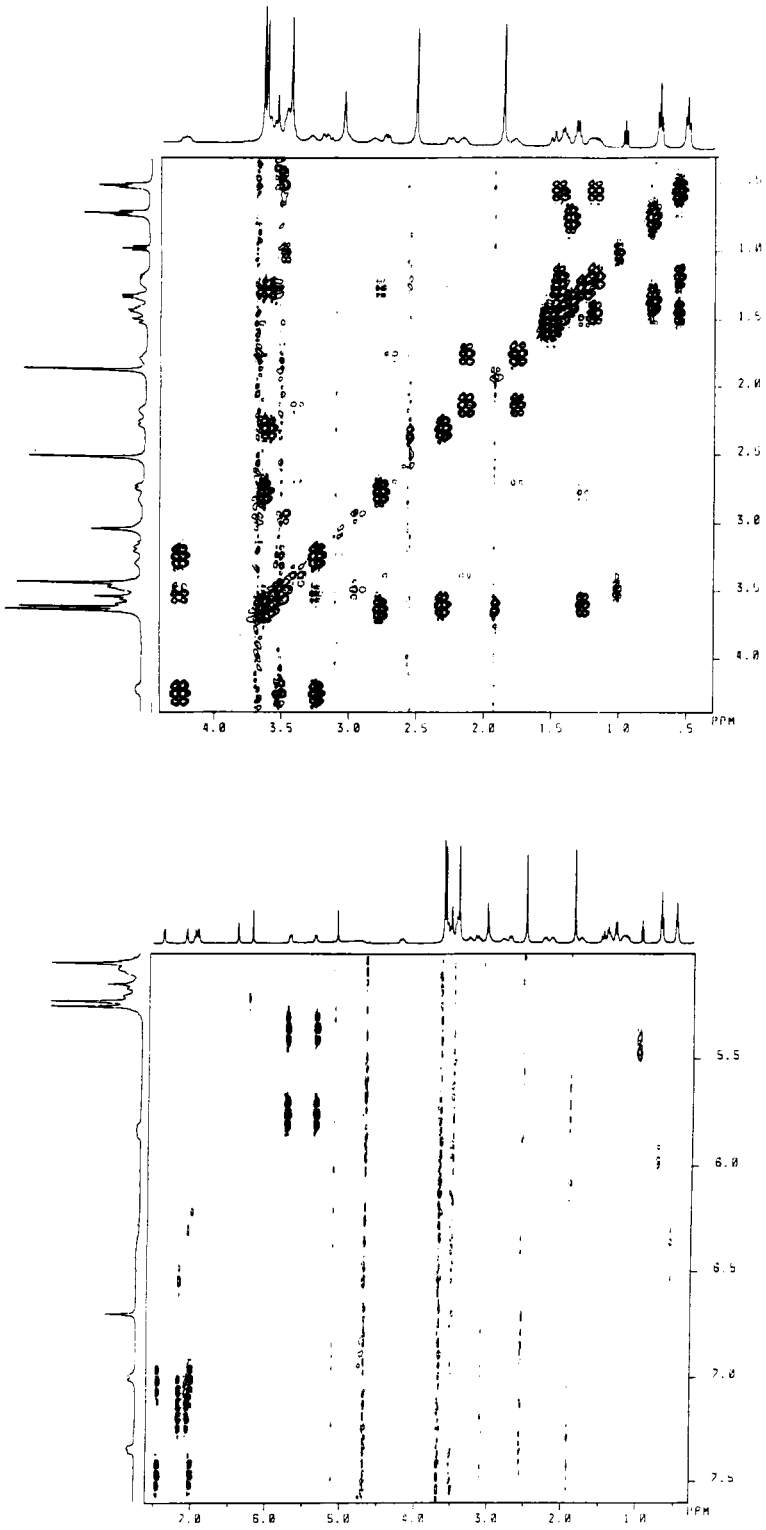

FIGURE 1. Selected regions of the contour plot of the double-quantum filtered phase-sensitive COSY map of vinblastine [**1**] (5 mg in 0.5 ml of D_2O at pD 5.4). A 256 × 1024 data point spectrum was acquired with presaturation of solvent.

spectra (20). ^{13}C-nmr assignments were made from ^{1}H-^{13}C correlation spectroscopy (21) and by comparison with literature data (10–12). The contour plot of the COSY map at pD 5.3 identifies the spin coupling network of individual proton signals (Figure 1), but it contains some ambiguities from overlapping of resonances that are removed at higher pD (Figure 2). The contour plot of the ^{1}H-^{13}C correlation map (Figure 3) allows identification of protonated carbons and verifies the ^{1}H-nmr assignment. ^{1}H and ^{13}C chemical shifts are recorded in Table 1 where a comparison with data reported in the literature (10–12, 16) is also given.

The magnitude of the J couplings (Table 2) potentially provides information about the torsional arrangement of vicinal protons, but due to the double maxima of the angular dependence of $^{3}J_{H\text{-}H}$, the relatively low spectral resolution, and the overlap in the 3.8–3.0 ppm region, an unambiguous assignment of the relative orientation of the two indole moieties was not possible.

However, the identification of spatial proximities as detected by the 2D NOESY (22) map of vinblastine (Figure 4) provides a satisfactory conformational assignment. The comparison between COSY and NOESY maps reveals that the J coupling cross peaks have been effectively suppressed in the NOESY experiment (e.g. the H_1,-H_2, cross peak not observed at $[f_1;f_2] = [3.61;1.20]$). The mixing time of 200 msec allows for the detection of several cross peaks, the most revealing of which connect H_b-19′

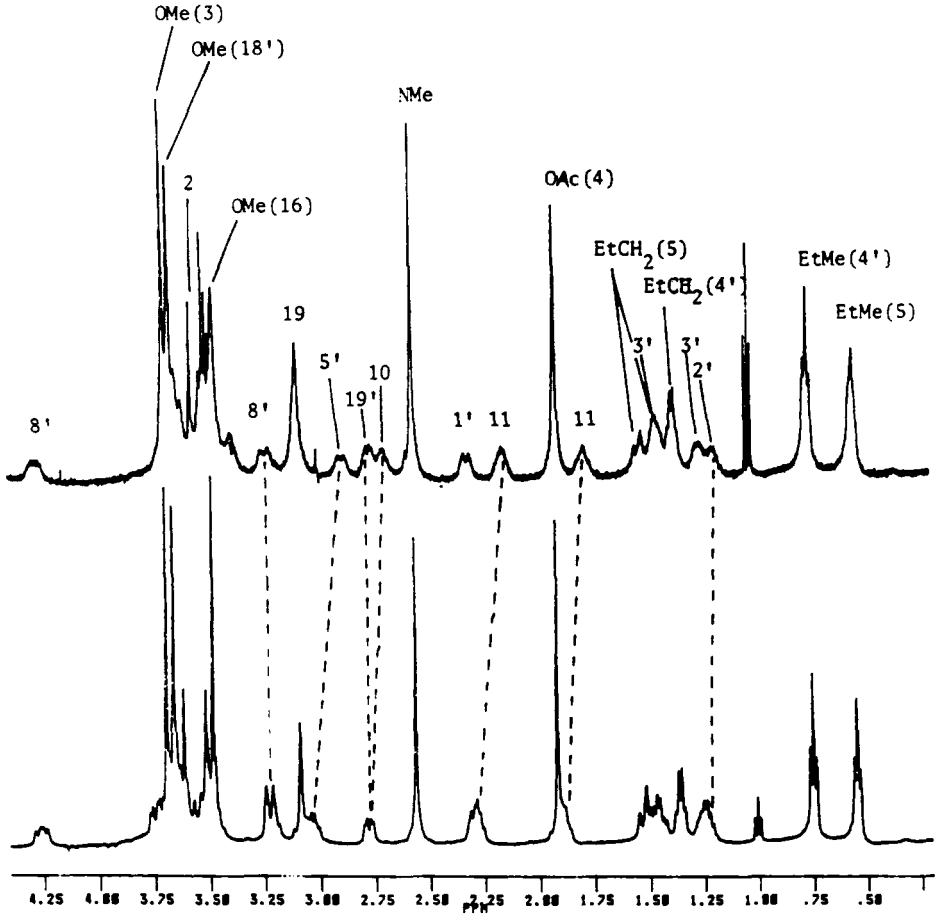

FIGURE 2. pH dependence of ^{1}H-nmr chemical shifts: upper spectrum pD 6.6; lower spectrum pD 5.4.

with H_a-8′, H-11′ with H_a-8, H-17 with 3-OMe, H-14 with 18′-OMe, and H-4 with 5-EtCH_2. The trans-annular interactions position the interacting protons within 2–3 Å of one another, thus severely restricting the conformations that the macrocyclic ring of the catharanthine and vindoline segments can adopt.

The conformation of vinblastine in aqueous solution was approximated using the molecular modelling system Macro Model 3 (23), using the X-ray-derived structure of the closely related compound vincristine (24) as a starting point. Energy minimization was accomplished using the mm2 algorithm employing distance constraints of 3 Å or

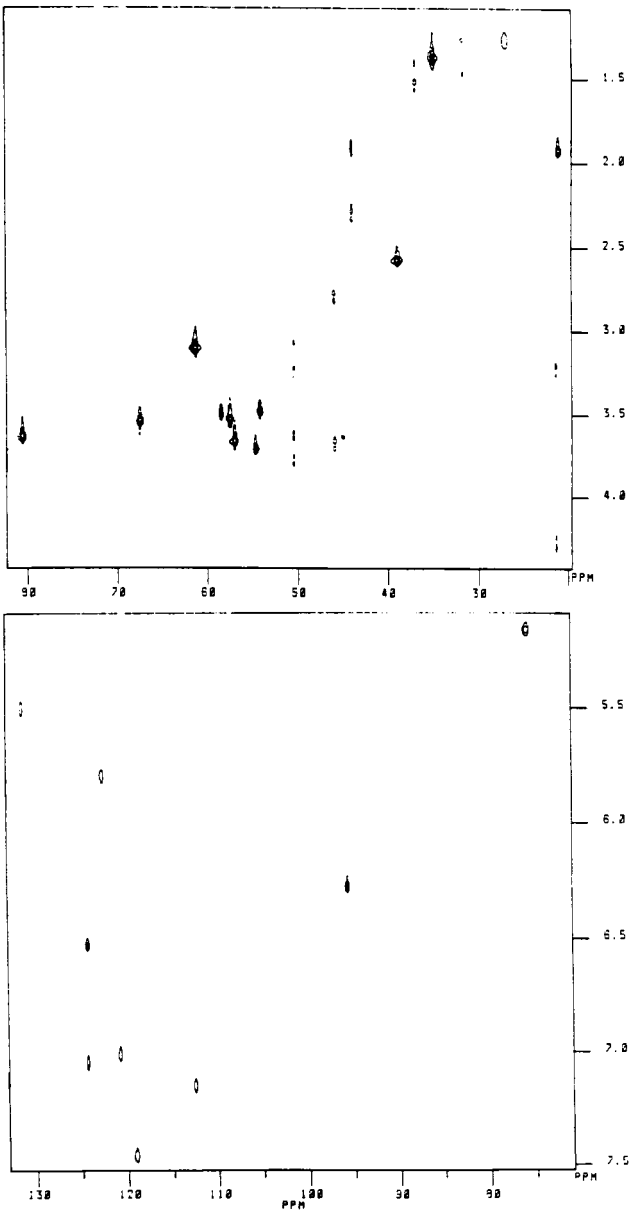

FIGURE 3. Selected regions of the contour plot of the ^1H-^{13}C correlation spectrum of vinblastine [**1**] (5 mg in 0.5 ml of D_2O at pD 5.4). The 256 × 1024 data set was acquired with the pulse sequence reported by Bax and Morris (21).

TABLE 1. ^{1}H-nmr (500.13 MHz) and ^{13}C-nmr (125.71 MHz) Chemical Shifts (ppm) of Vinblastine [**1**] (10 mg/ml in D_2O at 293°).

Proton	D_2O		$C_6D_6/CDCl_3$[a]	Carbon	D_2O	$CDCl_3$[b]
	pD = 5.2	pD = 6.6			pD = 5.6	
H_a-1′	2.28	2.30	2.55	C-1′	35.73	34.4
H_b-1′	3.63	3.61	4.29	C-2′	26.91	30.0
H-2′	1.18	1.21	1.02	C-3′	31.64	41.4
H_a-3′	1.27	1.27	1.15	C-4′	68.58	69.4
H_b-3′	1.44	1.45	1.36	4′-$EtCH_2$	34.82	34.3
4′-$EtCH_2$	1.34	1.40	1.08	4′-$EtCH_3$	6.81	6.8
4′-$EtCH_3$	0.70	0.79	0.75	C-5′	61.15	64.2
H_a-5′	3.02	2.86	2.68	C-7′	58.29	55.6
H_b-5′	3.52	3.45	2.78	C-8′	21.22	28.5
H_a-7′	3.22	3.08	3.05	C-10′	128.77	129.2
H_b-7′	3.52	3.46	3.46	C-11′	119.00	118.3
H_a-8′	3.22	3.21	3.15	C-12′	124.12	122.1
H_b-8′	4.24	4.25	3.95	C-13′	120.87	118.7
H-11′	7.46	7.46	7.45	C-14′	112.48	110.3
H-12′	7.01	6.97	7.07	C-15′	134.30	135.0
H-13′	7.06	7.06	7.16	C-17′	131.68	131.4
H-14′	7.18	7.18	7.16	C-18′	55.58	55.6
18′-OMe	3.67	3.68	3.39	18′-CO	175.55	174.8
H_a-19′	2.81	2.74	2.63	18′-OMe	54.54	52.2
H_b-19′	3.59	3.57	3.72	C-19′	45.83	47.8
NMe	2.55	2.55	2.49	NMe	38.72	38.2
H-2	3.61	3.60	3.71	C-2	80.38	83.2
3-OMe	3.68	3.72	3.51	C-3	80.08	79.5
H-4	5.11	5.09	5.73	3-CO	171.49	170.7
4-OAc	1.92	1.90	1.90	3-OMe	56.84	52.0
5a-$EtCH_2$	1.49	1.49	1.48	C-4	75.71	76.3
5_b-$EtCH_2$	1.54	1.55	1.98	4-CO	170.86	171.5
5-$EtCH_3$	0.54	0.59	0.80	4-OAc	20.95	20.9
H-6	5.36	5.28	5.20	C-5	42.91	42.5
H-7	5.75	5.70	5.51	5-$EtCH_2$	36.72	30.6
H_a-8	3.22	3.14	2.10	5-$EtCH_3$	7.89	8.2
H_b-8	3.79	3.41	2.84	C-6	130.87	129.8
H_a-10	2.97	2.68	1.65	C-7	122.48	124.4
H_b-10	3.68	3.36	2.83	C-8	50.38	50.1
H_a-11	1.86	1.76	1.87	C-10	57.39	50.1
H_b-11	2.24	2.11	2.07	C-11	43.81	44.5
H-14	6.42	6.41	6.84	C-12	52.58	53.1
16-OMe	3.50	3.47	3.45	C-13	121.36	122.6
H-17	6.27	6.23	5.94	C-14	124.05	123.3
H-19	3.09	3.12	2.45	C-15	121.19	121.1
				C-16	157.73	157.9
				16-OMe	54.03	55.6
				C-17	95.71	94.1
				C-18	152.11	152.5
				C-19	67.33	65.4

[a]Data in this column are from De Bruyn *et al.* (10).
[b]Data in this column are from Wenkert *et al.* (13).

less for groups giving NOESY cross peaks. From this process, a value of 180° for the dihedral angle C-17′–C-18′–C-15–C-16 was obtained. Using the same method and the nOe difference data previously reported (16), a value of 160° was arrived at for the same angle in organic solvents in excellent agreement with the 140–170° value obtained by

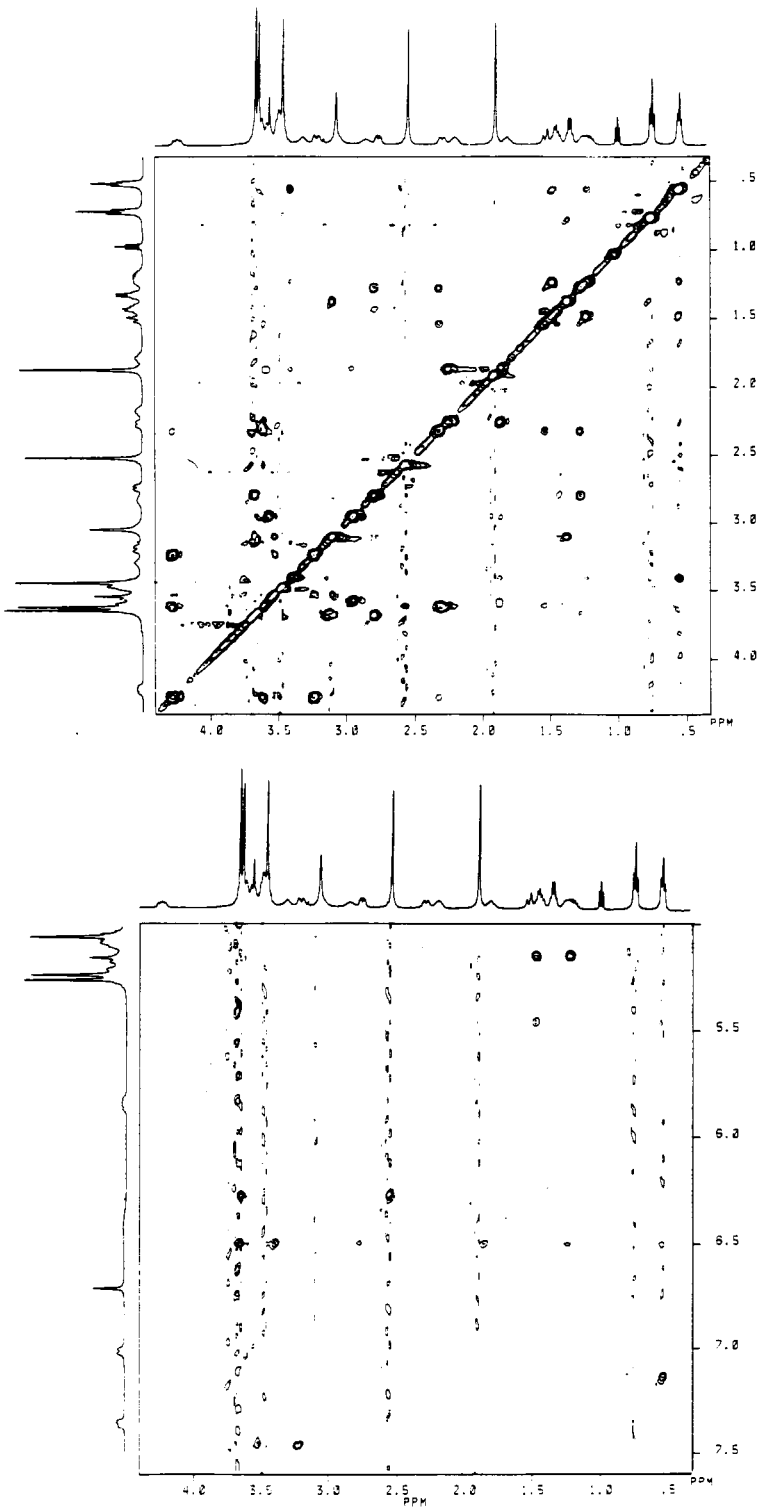

FIGURE 4. Selected regions of the contour plot of the phase-sensitive NOESY map of vinblastine [**1**] (5 mg in 0.5 ml of D_2O at pD 5.4). The 256 × 1024 data set was acquired with the pulse sequence reported by Bodenhausen *et al.* (22) with a mixing time of 200 msec and presaturation of solvents. Only negative peaks are displayed.

the authors (16) (Figure 5). The conformation in H_2O is more compact and globular, with polar groups on the outside surface. The conformation in organic solvents is more open. The dihedral angle found in the crystalline state for vincristine was 160° (3) and for vinblastine was 165° (16).

The conformation assigned in aqueous solution is relevant for understanding the chemical reactivity and biological mode of action of vinblastine and its congeners. Be-

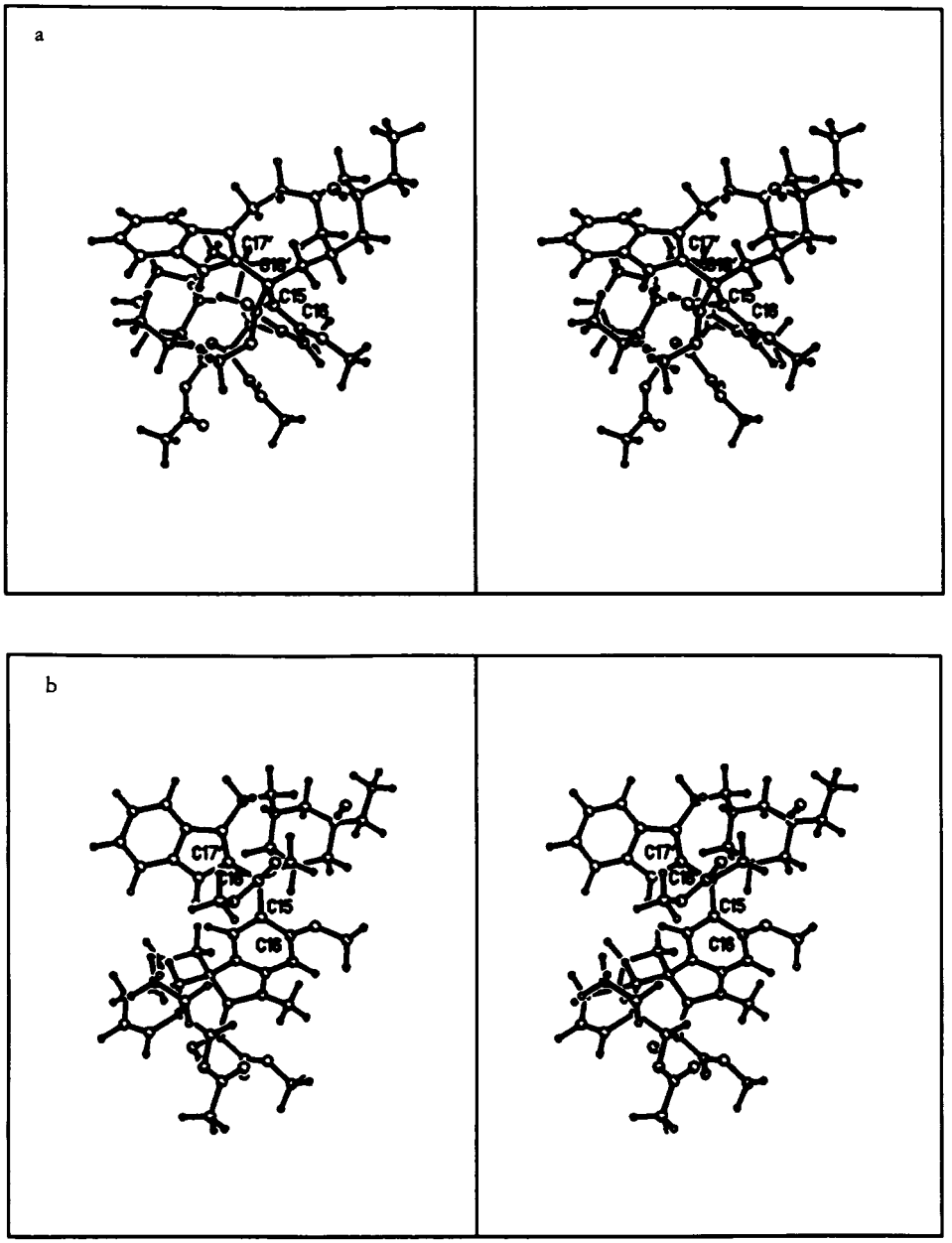

FIGURE 5. Perspective views of the solution and solid state structures of vinblastine [**1**] as obtained using the molecular modeling system Molecular Model 3 (23); (a) relative orientation of indole moieties in water solution; (b) relative orientation of indole moieties in organic solvents [calculated from Hunter *et al.* (16)].

TABLE 2. Vinblastine [1] Proton Coupling Constants (Hz) in H_2O at pD = 5.8 and 293°.

Proton	Observed couplings[a]
H_a-1′	H_b-1′ (>13); H-2′ (13)
H_b-1′	H-2′ (<4)
H-2′	H_a-3′ (<4); H_b-3′ (10); H_a-19′ (12); H_b-19′ (<4)
H_a-3′	H_b-3′ (>13)
H_a-5′	H_b-5′ (>13)
H_a-7′	H_b-7′ (>13); H_a-8′ (7); H_b-8′ (<4)
H_b-7′	H_a-8′ (<4); H_b-8′ (<4)
H_a-8′	H_b-8′ (>13)
H_a-19′	H_b-19′ (>13)
H_a-10	H_b-10 (≅9); H_a-11 (6); H_b-11 (<4)
H_b-10	H_a-11 (<4); H_b-11 (6)
H_a-11	H_b-11 (>13)

[a]In most cases couplings were measured using selective decoupling to simplify patterns. When necessary, couplings were estimated from double quantum filtered spectra optimized for 7 or 12 Hz (8), or from patterns from double quantum 2D spectra.

cause the highly negatively charged carboxyl-terminal domain of tubulin is involved in a regulatory role in modulating the interactions responsible for self-association (24,25), Magnus *et al.* (26) have recently hypothesized that a reversible ipso protonation of vinblastine is the starting event for the biological action at the molecular level, and solution variables such as pH and ionic strength have been shown to largely affect binding of vinblastine to tubulin (27). The fact that the preferred conformation delineated by nmr data is not appreciably different from that of the solid state structure demonstrates that, at least in this case, energy levels are not greatly affected by solvation. ^{1}H-nmr relaxation and conformation studies of vinblastine-tubulin complex will be reported in a subsequent paper.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were recorded at ambient temperature on a Bruker AM 500 Fourier transform instrument equipped with an ASPECT 3000 data system and a process controller, utilizing a 5 mm C/H switchable probe. Samples were prepared in 99.96% D_2O (Isotec) solution, and pD was adjusted to the given value by addition of dilute DCl or NaOD in D_2O. Programs for acquiring and processing data were supplied by the manufacturer. Vinblastine was supplied by OmniChem Company.

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