The Conformation of Vinblastine in Solution as Determined by N.O.E. Difference Spectroscopy

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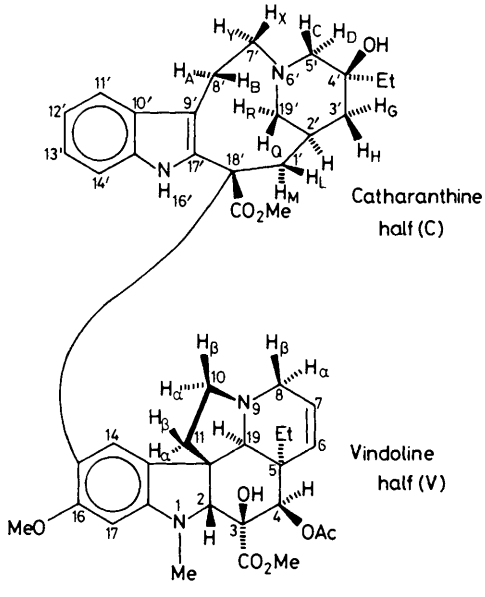
The conformation of the binary alkaloid vinblastine has been determined in solution using n.O.e and decoupling difference spectroscopy. Both positive and negative n.O.e.'s have been observed and exploited. On the catharanthine portion, it has been shown that the piperidine ring takes up a severely flattened conformation with the nitrogen lone pair and the ethyl group being pseudo-equatorial. The preferred conformation of the nine-membered ring has also been determined. The spatial relationship between the catharanthine and vindoline halves has been established from several n.O.e. connectivities between them, notably from the indole NH of the catharanthine side and the aromatic 14-H of the vindoline side. The C(17')—C(18')—C(15)—C(16) dihedral angle is 140—170°. These are the first complete published assignments for the ^{1}H n.m.r. spectra of vinblastine and of vindoline.

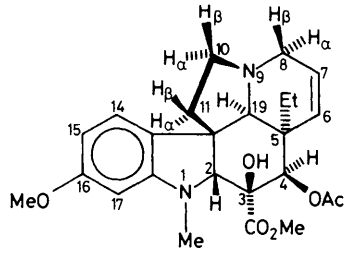
The binary alkaloids such as vinblastine (1) are an important class of chemotherapeutic agent. They act as powerful inhibitors of cell division by interfering with microtubule protein polymerization, but little is known of the mechanism of this action.[1,2] In an attempt to elucidate this, numerous derivatives have been isolated or synthesized and tested for biological activity.[3] It is often assumed that there is a correlation between solution structure and biological activity which could guide further work but there has, until recently, been no reliable method of determining solution geometry. The classical strategy of assigning the ^{1}H n.m.r. spectrum by chemical shift and multiplicity inspection, followed by conformational analysis using coupling constants, ultimately fails for several reasons, not the least being that high fields reveal many signals but chemical shift arguments are of little value for distinguishing them. More serious than this is the fact that these alkaloids contain numerous small spin systems (*e.g.* $\equiv CCH_2CH_2N$) isolated from each other by spectroscopically silent centres. Thus, even if Karplus-type relationships can give reliable conformations *within* spin systems there is no information about spatial relationships *between* spin systems.

These through-space relationships can be best probed [4] using the nuclear Overhauser effect (n.O.e.). We have developed a well defined strategy based on the use of two dimensional (2D) J spectroscopy to resolve and measure individual proton resonances and on n.O.e. and decoupling difference spectroscopy to assign those resonances. This strategy has been applied to steroids,[5,6,7] an enkephalin of known structure [8] and to structure determinations of unknowns.[9,10] We have now applied these procedures (without 2D J spectroscopy) to the complete spectral assignment and conformational analysis of vinblastine as an important representative of the binary alkaloids.

At the outset, we chose to eschew all but the most obvious shift distinctions and we made no use of earlier work on vinblastine or its component halves. We have completely analysed the spectrum of vindoline (2) at 270 MHz for comparison purposes. The rigidity of vindoline may allow useful comparisons with vinblastine but we ultimately assigned the vindoline resonances within vinblastine itself. After we had completed much of this work, De Bruyn, De Taeye, and Anteunis published a conformational study of vinblastine based solely on coupling constants.[11] As their conclusions differed somewhat from ours, we repeated our n.O.e. work in the



(1)



(2)

solvent mixture (C_6D_6–$CDCl_3$) which gave optimum dispersion in their work, allowing more direct comparison of results. Their incomplete assignments are in agreement with ours but their conformational conclusions cannot be sustained by our n.O.e. results.

In spin-decoupling difference spectroscopy a control spectrum is subtracted from a decoupled spectrum. Ideally, the only responses which occur are from protons coupled to the irradiated one, although Bloch-Siegert shifts can be a prob-

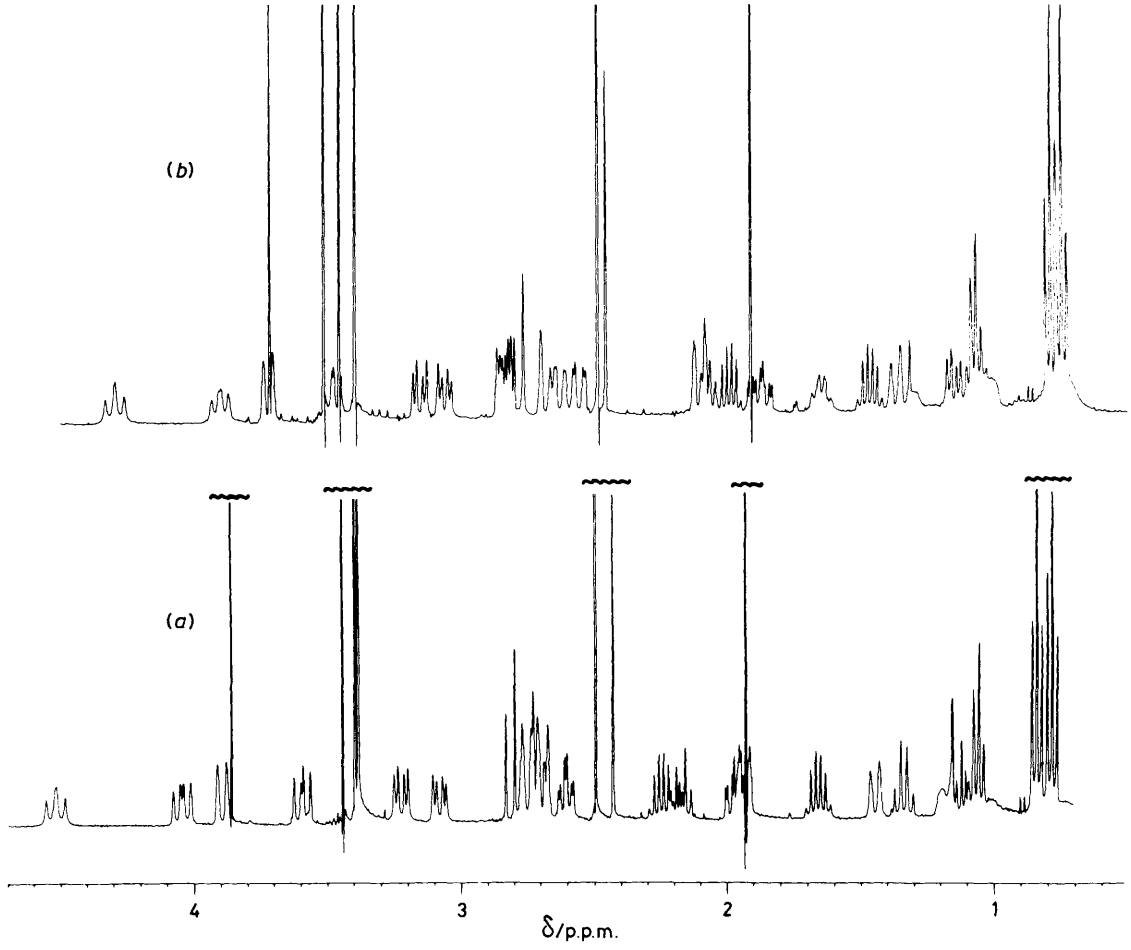


Figure 1. Resolution-enhanced partial 400 MHz ^{1}H n.m.r. spectra of vinblastine (1) in the region δ 0.5—5.0; (*a*) *ca.* 17 mM in C_6D_6 solution, (*b*) *ca.* 13 mM in C_6D_6–$CDCl_3$ (3 : 1) solution

lem.[12] In n.O.e. difference spectroscopy, the control spectrum is subtracted from the enhanced spectrum so that only *changes* appear. In our hands enhancements of 0.5% for multiplets and 0.2% for methyl groups are readily observed and the enhanced signal need not even be resolved in the normal spectrum. Unlike chemical shifts and coupling constants which depend in part, and often unpredictably, on through bond effects, n.O.e.'s are pure through-space effects. In a proton-rich environment providing multiple relaxation pathways, the n.O.e. between two protons is essentially inversely proportional to the sixth power of the distance between them. Thus the ability to see small n.O.e.'s using difference techniques corresponds to the ability to make long range through-space connections between protons. Note that the longest range connections will be made to isolated protons without near neighbours as these protons will rely on distant protons for their relaxation. We have exploited this property with aromatic and methine protons to powerful effect in this work. Certain geometrical arrangements of spins can lead to negative n.O.e.'s by an indirect mechanism [13] and we have used these to provide information on the relative orientation of the separate spin systems in vinblastine.

The 2D J experiment gives complete separation of chemical shifts and homonuclear coupling constants along different frequency axes, provided that all spins are weakly coupled. It is possible to obtain a ' proton-decoupled ' proton spectrum displaying only singlets at each chemical shift and ' partial J spectra ' of each multiplet even when these multiplets are severely overlapped and congested in the one dimensional spectrum. 2D J Spectroscopy has proved valuable in unravelling the otherwise intractable spectra of peptides,[8] steroids,[5,6,7] and oligosaccharides [14] and appeared ideal for complex alkaloids. In this case, however, the n.O.e. and decoupling difference spectra reveal the multiplets in sufficient detail to render the 2D J spectrum unnecessary.

Results

Initially we assigned the spectrum of vindoline in C_6D_6 solution at 270 MHz to establish the applicability of the approach. However, these experiments are not described in detail as they were essentially repeated on vinblastine itself. The assignments for vindoline (2) are collected for comparison in Table 1. Previous assignments [15] for the 8-, 10-, and 11-protons are in error.

In the 400 MHz spectrum of vinblastine in C_6D_6 solution (Figure 1*a*) there are a large number of well resolved signals, some of them recognisably from the vindoline half, but there are also many overlapping multiplets. The regions around δ 7.2, 2.7, and 1.1 appeared particularly intractable.

Titration of a C_6D_6 solution with small amounts of $(CD_3)_2$-CO or $CDCl_3$ and repeated cycles of n.O.e.–decoupling experiments allowed us to assign unambiguously every proton resonance in each solvent. Figure 1*b* shows the spectrum in C_6D_6–$CDCl_3$ (3 : 1) which is close to the solvent mixture used by De Bruyn *et al.*[11] to give optimum dispersion. For con-

Table 1. Chemical shifts in vindoline [a] and the V portion of vinblastine (1)

Proton	Vindoline C_6D_6	V Half of (1) C_6D_6	V Half of (1) C_6D_6–$(CD_3)_2CO$ (2 : 1)	V Half of (1) C_6D_6–$CDCl_3$ (3 : 1)
NMe	2.43	2.49	2.47	2.49
2-H	3.84	3.86	3.61	3.71
3-OH	8.5	8.8		9.5
3-CO_2Me	3.40 [c]	3.43	3.43	3.51
4-H	5.96	6.00	5.54	5.73
4-OAc	1.91	1.92	1.81	1.90
5-CH_2CH$_3$	1.55	1.65	1.5 [b]	1.48
	2.12	2.24	1.95 [b]	1.98
5CH$_2$CH_3	0.47	0.84	0.71	0.80
6-H	5.27	5.30	5.20	5.20
7-H	5.48	5.47	5.47	5.51
8α-H	2.26	1.93	2.02	2.10
8β-H	2.91	2.71 [b]	2.75	2.84
10α-H	1.91	1.33	1.63	1.65
10β-H	2.87	2.60	2.75 [b]	2.83
11α-H	2.03	1.97	2.05 [b]	1.87
11β-H	2.23	2.16	1.95 [b]	2.07
14-H	6.64	7.02	6.79	6.84
15-H	6.37			
16-OMe	3.39 [c]	3.39	3.49	3.45
17-H	6.05	5.95	6.02	5.94
19-H	2.35	2.45	2.42	2.45

[a] δ ±0.01 p.p.m.; *ca.* 0.05M. [b] ±0.05 p.p.m. [c] May be reversed.

Table 2. Chemical shifts for the catharanthine half of vinblastine (1) [a]

Proton	C_6D_6	C_6D_6–$(CD_3)_2CO$	C_6D_6–$CDCl_3$
1′-H_L	4.51	4.30	4.29
1′-H_M	2.71 [b]	2.5 [b]	2.55
2′-H	1.19	0.93	1.02
3′-H_G	1.12	1.14	1.15
3′-H_H	1.44	1.45 [b]	1.36
4′-CH_2CH$_3$	1.15	1.16	1.08
4′-CH$_2$CH_3	0.77	0.79	0.75
4′-OH	*ca.* 1		*ca.* 1
5′-H_C	2.68	2.81	2.68
5′-H_D	2.81	2.81	2.78
7′-H_X	3.07	3.11	3.05
7′-H_Y	3.59	3.42	3.46
8′-H_A	3.22	3.03	3.15
8′-H_B	4.04	3.93	3.95
11′-H	7.56	7.34	7.45
12′-H	7.16	6.91	7.07
13′-H	7.31 [b]	7.26	7.16 [b]
14′-H	7.26 [b]	7.02	7.16 [b]
16′-NH	8.50	8.70	8.35
18′-CO_2Me	3.38	3.33	3.39
19′-H_Q	3.89	3.69	3.72
19′-H_R	2.71 [b]	2.61	2.63

[a] δ ±0.01 p.p.m. [b] ±0.05 p.p.m.

Table 3. Connectivities established by difference spectra on irradiating vindoline portion of vinblastine (1) [a]

Proton irradiated	C_6D_6 DD [b]	C_6D_6 N.O.e.	C_6D_6–$CDCl_3$ DD [b]	C_6D_6–$CDCl_3$ N.O.e.
NMe		3-CO_2Me, 2-H, 17-H		
2-H		NMe, 11β-H, 17-H		NMe, 11β-H
4-H		5-CH_2CH$_3$		5-CH_2CH$_3$
6-H	7-H, 8α-H, 8β-H	5-CH_2CH$_3$, 7-H		
7-H	6-H, 8α-H, 8β-H	6-H, 8α-H, 8β-H		6-H, 8α-H, 8β-H
8α-H	8β-H, 7-H	7-H, 8β-H, 19-H		7-H, 8β-H, 19-H
8β-H				8α-H, 7-H
10α-H			10β-H, 11α-H, 11β-H	8α-H, 10β-H, 19-H
10β-H	10α-H, 11α-H, 11β-H	10α-H		10α-H
11α-H		11β-H, 14-H, 19-H		
11β-H				2-H, 11α-H (NMe) [c]
14-H		16′-NH		11α-H, 19-H, 16′-NH, 19′-H_Q
17-H		2-H, 16-OMe, NMe		
19-H		5-CH$_2$CH_3, 10α-H, 11α-H		

[a] The absence of an entry indicates that the experiment was not carried out. [b] DD is decoupling difference. [c] Parentheses indicate a negative signal.

venience, all chemical shifts are collected in Tables 1 and 2 and the double resonance connectivities in Tables 3 and 4.

Our starting point was the C_6D_6 solution (Figure 1*a*) assuming only the following assignments; 6-H, 7-H, 4-OAc and NMe on the vindoline half and the indole NH on the catharanthine half. Adding some known assignments from vindoline gave without serious doubt 2-H, the ethyl methylene protons, 14-H and 19-H. These were confirmed unambiguously later. Numerous n.O.e. and decoupling difference spectra were acquired under computer control [6] and the resulting connections with the assigned protons led to the complete assignment of the vindoline half in C_6D_6. These experiments were repeated in C_6D_6–$CDCl_3$ to check that no conformational changes had occurred. Some representative spectra are shown in Figure 2. One notable feature is the separate observation of coincident signals. 10β-H (δ 2.83) and 8β-H (δ 2.84) have been separately enhanced and observed. Similarly 2-H and 19′-H_Q* have been separated on the same figure. Several long range n.O.e. connections are also shown, including H(14)-H(11α), H(11β)-H(2) and two from 14-H to the catharanthine half (16′-NH and 19′-H_Q). The conformation derived from these and other results (Table 3) is shown in Figure 3. The large number of interlocking n.O.e. connections would preclude any substantially different conformations and, in addition, leads to unambiguous assignments at C-10 and C-11 cor-

* It is difficult to assign a surface from which to define α and β. For this reason, and to avoid confusion and simplify diagrams, we have assigned a letter to each methylene proton on the catharanthine skeleton.

Table 4. Difference spectroscopy connectivities established by irradiation of catharanthine portion of vinblastine (1)

Proton irradiated	C_6D_6		C_6D_6–$(CD_3)_2CO$		C_6D_6–$CDCl_3$	
	DD [a]	N.O.e.	DD	N.O.e.	DD	N.O.e.
1′-H_L	δ 2.7, 1.1 [b]	8′-H_B. δ 2.7, 1.1	1′-H_M, 2′-H	1′-H_M		1′-H_M, 8′-H_B, (8′-H_A) [c]
1′-H_M						1′-H_L, 2′-H, 3′-H_H, (8′-H_B)
2′-H				1′-H_M, 3′-H_H, 19′-H_Q, 19′-H_R		1′-H_M, 3′-H_H, 19′-H_Q, 19′-H_R
3′-H_H						1′-H_M, 3′-H_G
5′-H_C					5′-H_D	
5′-H_D		4′-CH_2CH_3				
7′-H_X		7′-H_Y, δ 2.7				5′-H_C, 7′-H_Y
8′-H_A	7′-H_X, 7′-H_Y, 8′-H_B				7′-H_X, 8′-H_B	8′-H_B, 11′-H, (1′-H_L)
8′-H_B	7′-H_X, 7′-H_Y, 8′-H_A	1′-H_L, 8′-H_A				1′-H_L, (1′-H_M), 8′-H_A, (11′-H)
11′-H	12′-H	8′-H_A, 12′-H				8′-H_A, 12′-H
16′-NH		5-CH_2CH_3, 14-H, 14′-H				5-CH_2CH_3, 14-H, 14′-H, 18′-CO_2Me
19′-H_Q	δ 2.7	14-H, δ 2.7	19′-H_R			14-H, 2′-H, 19′-H_R

[a] DD is decoupling difference. [b] Assignment to individual proton is not possible. [c] Parentheses indicate a negative signal.

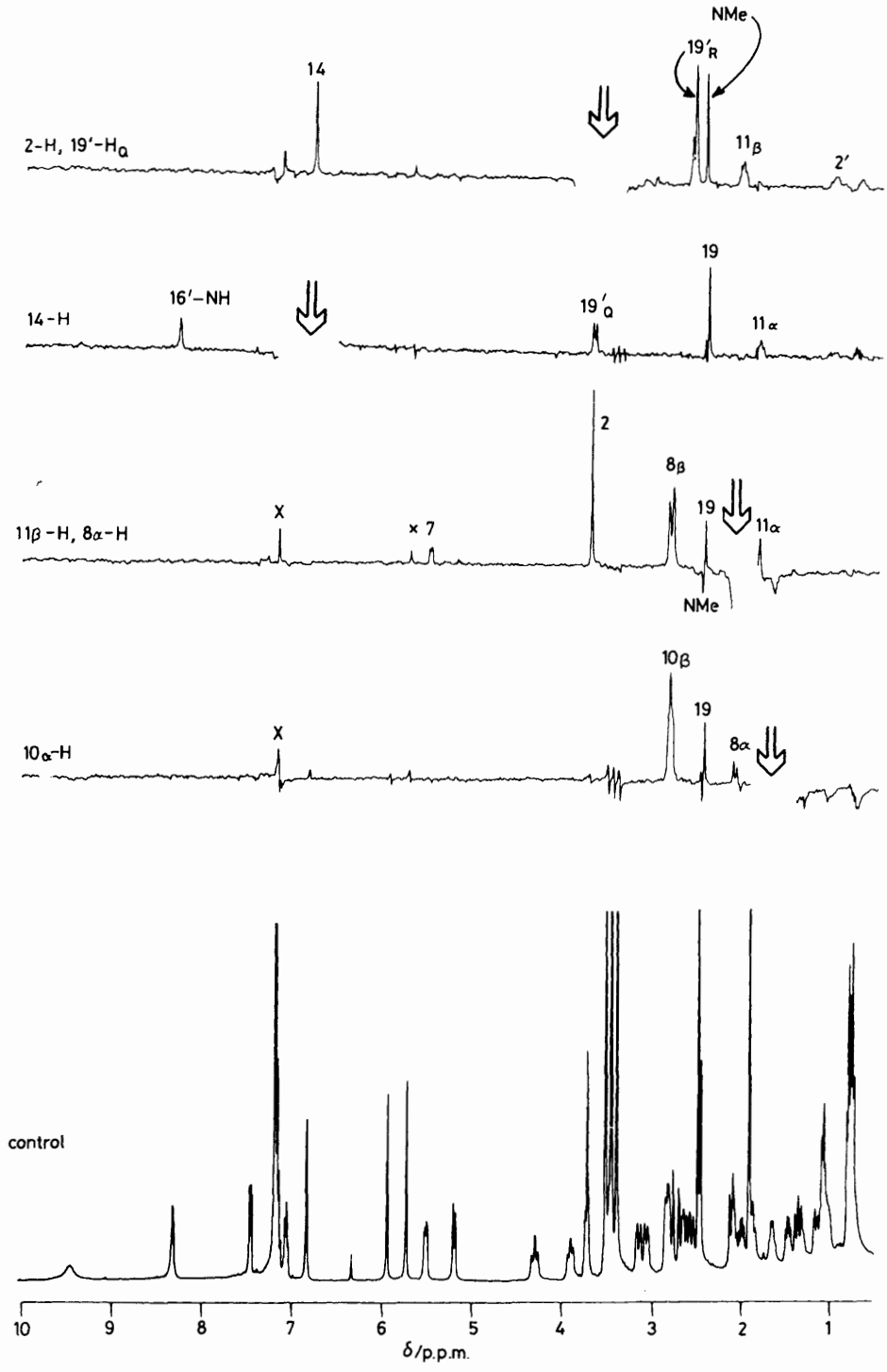


Figure 2. Control spectrum and n.O.e. difference spectra for several irradiations in the V half of vinblastine (1). Irradiated protons are indicated at the left and marked by an arrow. The control spectrum is from the same F.I.D. as Figure 1*b*, but broadened by 1.5 Hz. Difference spectra are × 16 vertical-display scale compared with the control

recting previous incorrect guesses.[15] The crucial experiment in sorting out the H(10α)-H(10β)-H(11α)-H(11β) system is the n.O.e. from 2-H to 11β-H with the n.O.e.'s from 2-H to NCH_3 and 17-H confirming the assignment of 2-H. It is precisely this lack of a remote probe which prevented correct assignments in earlier very careful work.[11] Accurate coupling constants were not obtained in our work but the data agree, within the limits of our digital resolution, with those of De Bruyn *et al.*[11] once a few assignments are corrected. We have collected a selection of relevant coupling constants in Table 5 along with rough estimates of some dihedral angles. Since we have chosen to use a different, more generally accepted, number scheme we have also identified the coupling constants by the scheme used by De Bruyn *et. al.*[11]

The catharanthine half of vinblastine was less amenable. In C_6D_6, the isolated aromatic, C(4')-Et and the C(7')-C(8') system were easily identified by decoupling differences but not assignable in detail by inspection. The remainder of the mole-

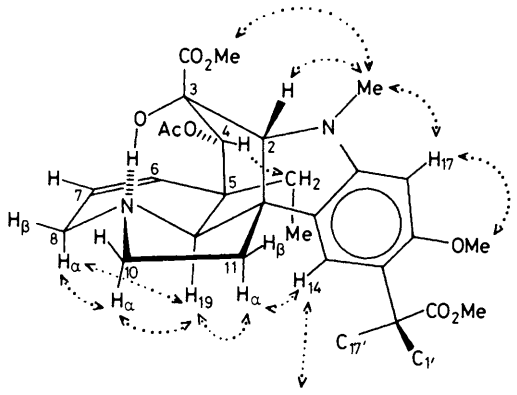


Figure 3. Proposed conformation for the V half of vinblastine (1) showing some of the observed non-geminal n.O.e.'s. H(2)–H(11β), H(14)–H(19), and H(19)–CH_2(5) were also connected by n.O.e., but these have been omitted for clarity

cule is a seven spin system (C-1′, C-2′, C-3′, C-19′) plus a two spin system (C-5′), all spectroscopically entangled around δ 2.7 and 1.1 and containing several vicinal couplings of *ca.* 1 Hz which is approximately the same as 4-bond W couplings and near the natural linewidth. Dropwise titration of a C_6D_6 solution with $(CD_3)_2CO$ or $CDCl_3$ gave sufficiently gradual changes that individual multiplets could be followed from one spectral region to another. The acetone experiments had the advantage of clearly resolving all of the aromatic protons and the $CDCl_3$ titrations gave superior n.O.e. data and better dispersion of the difficult aliphatic protons (see Figure 4).

Irradiation of the 16′-NH proton revealed connections to the vindoline half of the molecule (14-H, 5-Me) and allowed the assignment of 14′-H and the 18′-methoxycarbonyl group (Figure 4). The remaining aromatic doublet is therefore 11′-H and irradiation of this proton located 12′-H and 8′-H_A. The remainder of the C(7′)–C(8′) system was readily assigned by decoupling.

Irradiation of the 3-OH proton on the vindoline half produced saturation transfer to two broad signals which were previously buried at δ 1.2 and 0.75.* In addition, there were several n.O.e.'s in both the vindoline and catharanthine fragments. The n.O.e.'s are complicated but they do include both 5′-H_C and 5′-H_D which were assigned later. Thus one of the saturation transfer signals is 4′-OH and the other is presumably water.

There were now essentially three independent n.O.e. routes from securely assigned protons into the difficult area: (i) from and to 8′-H_B there was a set of large positive and associated small negative enhancements to a geminal pair later assigned to C-1′; (ii) there was a large enhancement from a 14 Hz doublet (now known to be 5′-H_D to the ethyl methylene signals; and (iii) there were mutual enhancements of 14-H and a signal now assigned to 19′-H_Q.

Taken together with the other connectivities in Table 4 we have found only one consistent set of assignments and conformation. The conformation is illustrated in several parts in Figure 5.

Discussion

Spectroscopic Features.—We have not reported detailed results on the absolute size of n.O.e.'s for reasons discussed in the Experimental section and previously,[5,6] but it is worth noting that geminal partners were always readily identified by

* This spectrum is published elsewhere.

Table 5. Selected coupling constants in vinblastine (1)

Proton pair	J^a	J^b	ϕ^c	Alternative numbering[b]
10α, 10β	12	11.0		5_A, 5_B [d]
10α, 11α	11	10.6	10	5_B, 6_B [d]
10α, 11β	8	7.2	130	5_B, 6_A [d]
10β, 11α	4	3.6	110	5_A, 6_B [d]
10β, 11β	9	8.8	10	5_A, 6_A [d]
11α, 11β	13	13.6		6_A, 6_B [d]
$1'_L$, $1'_M$	15	14.8		$17'_A$, $17'_B$
$1'_L$, 2′	13	12.4	180	$17'_A$, 14′
$1'_M$, 2′	3	3.6	60	$17'_B$, 14′
2′, $3'_G$	6	6.0	20	14′, $15'_B$
2′, $3'_H$		1	90	14′, $15'_A$
$3'_G$, $3'_H$	14	14.0		$15'_A$, $15'_B$
2′, $19'_Q$		1	90	14′, $3'_A$
2′, $19'_R$	2	2.4	20	14′, $3'_B$
$19'_R$, $19'_Q$	14	13.6		$3'_A$, $3'_B$
$5'_C$, $5'_D$	14	13.6		$21'_A$, $21'_B$
$7'_X$, $7'_Y$	14	13.6		$5'_A$, $5'_B$
$7'_X$, $8'_A$	6	5.6	30	$5'_B$, $6'_B$
$7'_X$, $8'_B$		1	90	$5'_B$, $6'_A$
$7'_Y$, $8'_A$		1	90	$5'_A$, $6'_B$
$7'_Y$, $8'_B$	11	10.8	160	$5'_A$, $6'_A$
$8'_A$, $8'_B$	15	14.6		$6'_A$, $6'_B$

[a] ±1 Hz This work. [b] Ref. 11. [c] Estimated dihedral angle ±10°. [d] Assuming labels 5 and 6 are interchanged on the structure given in ref. 11. See for example the numbering in ref. 16.

large n.O.e.'s of 10—15% in these experiments. More subtly, it is noteworthy that they are smaller in each direction for H(7)-H(8α) than for H(7)-H(8β), providing assignment evidence which is independent of the couplings. In this case, couplings are diagnostic in themselves, $J_{7,8\alpha}$ being *ca.* 1 Hz and $J_{7,8\beta}$ *ca.* 5 Hz. However, this is not the case for the C(10)-C(11) fragment where four of the six coupling constants are very similar. Here n.O.e.'s were crucial but straightforward. Note the important role of 2-H and 19-H, isolated methine protons, in providing long distance information and the ease of assignment of the virtually coincident methoxy-signals. They are not assigned by the difficult task of selective irradiation but by observing very small n.O.e.'s of less than 1% on the methoxy-signals when neighbouring protons are irradiated. This is a complete reversal of the conventional wisdom that methyl groups are good sources of n.O.e. but poor recipients. This wisdom is, of course, quantitatively true when integrals are considered but, using difference spectroscopy, it is easier to see a 0.2% change in a sharp singlet than a 2% change in a broad sixteen-line single-proton multiplet.[9]

The most significant feature of this work is the exploitation of indirect negative n.O.e.'s to orient isolated spin systems.

Conformational Features.—The conformation of the vindoline half is clearly defined by n.O.e.'s as shown in Figure 3. The N-9 lone pair is undoubtedly hydrogen bonded to the 3-OH as evidenced by the δ 9 chemical shift of the latter proton. The methyl protons of the 5-Et group in vinblastine are 0.3 p.p.m. downfield of their position in vindoline. However, the n.O.e. they experience from 19-H clearly indicates that they are still over the aromatic ring of the vindoline half and presumably the downfield shift actually arises from the proximity of the catharanthine indole system. Inspection of other chemical shift differences between vindoline and corresponding protons in vinblastine reveals no obvious pattern other than the fact that they are concentrated around N-9.

The catharanthine half is conformationally less clear cut from molecular models. However, the large number of ob-

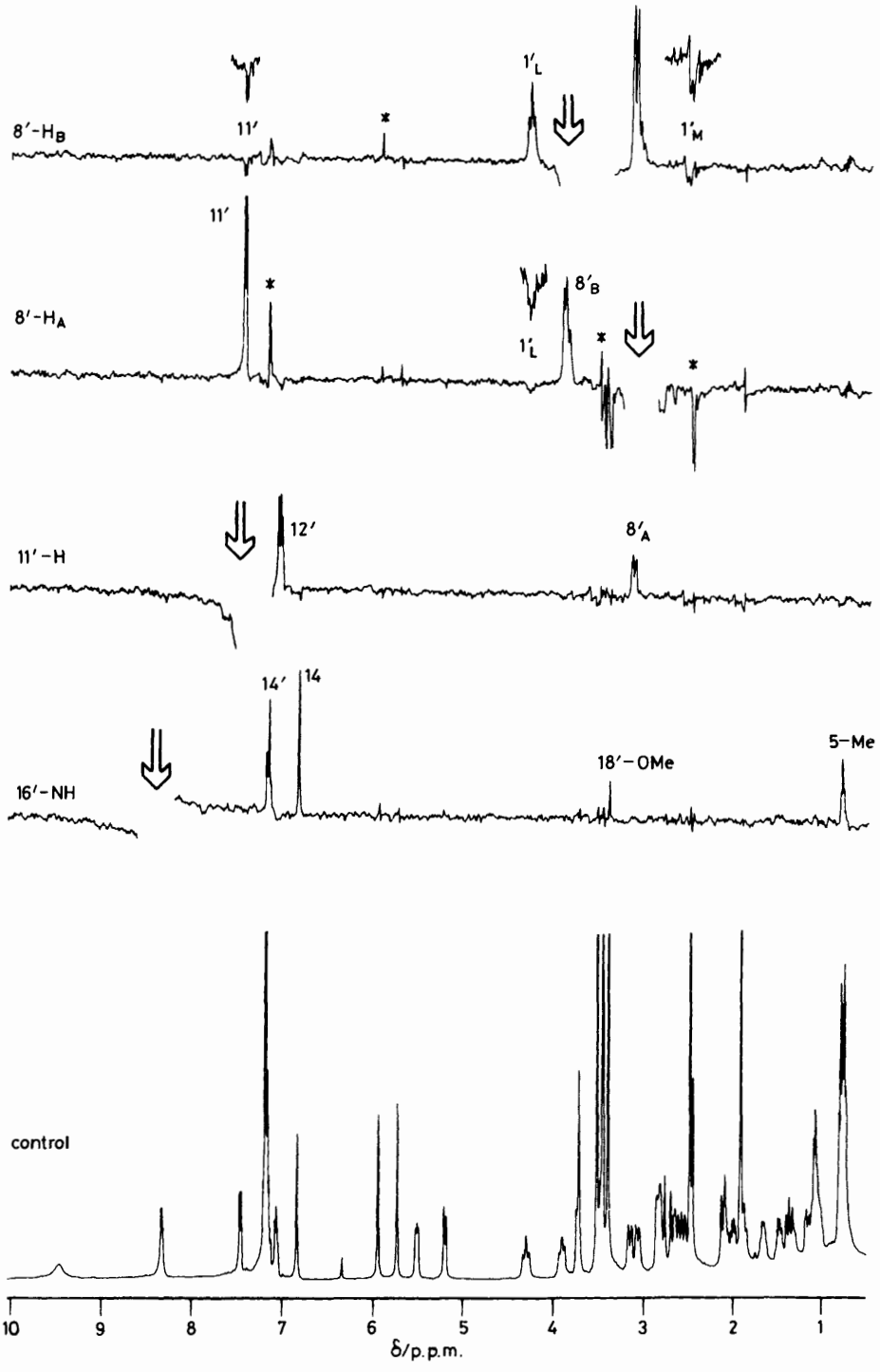


Figure 4. Control spectrum and n.O.e. difference spectra for some irradiations in the C half of vinblastine (1). Difference spectra are × 32 vertical-display scale, and inset negative signals are further expanded vertically. Other conditions are as for Figure 2

served n.O.e.'s give us some confidence in the essential correctness of the proposals in Figure 5. In particular, the interlocking set of positive and negative enhancements in the C(8′)-C(1′) system detailed in Table 3 and summarized in Table 6 provides overwhelming evidence for the linearity and extraordinary compactness of arrangement of two pairs of protons separated by five carbon atoms. The conformations allowed by these observations are very few. Thus, although our assignments are in agreement with De Bruyn,[11] our n.O.e. results rule out any significant population of the conformation they proposed in which C-8′ and C-1′ are far apart.

Our data indicate that the piperidine ring is severely flattened with C-19′, C-2′, C-3′, and C-4′ virtually coplanar with C-5′, and N-6′ only slightly out of the plane. This structure places the OH group and the two ring junctions in axial positions (De Bruyn [11] has C-7′ in an equatorial position) and the ethyl group equatorial. In addition to satisfying all of the n.O.e. evidence, the structure gives reasonable dihedral angles

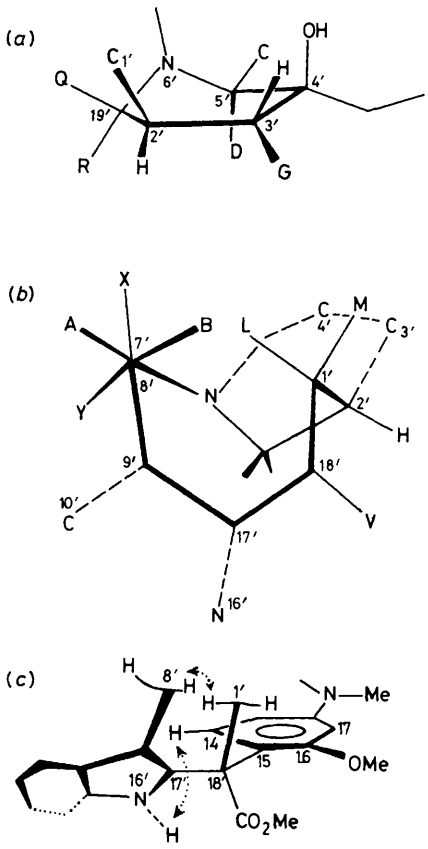


Figure 5. Proposed conformation for the C half of vinblastine (1). For clarity several different views are shown. (*c*) Shows the relationship between the V and C halves

for the observed vicinal couplings and 5′-H_C is broadened relative to 5′-H_D presumably by W-rule coupling to 19′-H_Q and 3′-H_H. Furthermore, 19′-H_Q is very close to the edge of the indole system which would produce the observed downfield shift relative to 19′-H_R.

The proposed conformation is further supported by two other pieces of evidence. First, we observe an n.O.e. from the indole NH to the 18′-methoxycarbonyl group. Secondly, we observe saturation transfer from the 3-OH on the vindoline half to two broad high-field signals at δ 1.2 and 0.75. The signals must be water and the 4′-OH but not necessarily in that order. Whichever is 4′-OH, the chemical shift is typical for dilute non-hydrogen bonded hydroxy-protons in non-polar solvents and is well upfield of the position of hydrogen-bonded OH. Together, these observations rule out any significant interaction between 4′-OH and 18′-CO_2Me.

In order to accommodate the conformation of the nine-membered ring, it is necessary that the structure be substantially flattened at N-6′. This flattening has the effect of rotating the C(7′)–C(8′) bond to produce a set of dihedral angles which is consistent with the observed coupling constants between the 7′- and 8′-protons (Tables 5, Figure 5, and ref. 11). In the quaternized systems cleavamine methiodide,[17] vincristine methiodide,[18] and vinblastine dihydrochloride,[19] there is considerable distortion about the piperidine nitrogen, N-6′, with bond angles reported from 102 to 126°.

At first sight the most striking conformational result is the observation of a strongly preferred orientation of the two halves of the molecule. Inspection of models reveals no obviously favourable interaction although the proximity of the 16-methoxy and 18′-methoxycarbonyl groups is notable. Our

Table 6. Positive and negative n.O.e.'s in the C(11′)–C(8′)–C(1′) system

Proton irradiated	Proton observed				
	11′-H	8′-H_A	8′-H_B	1′-H_L	1′-H_M
11′-H	Irr[a]	+[b]			
8′-H_A	+	Irr	+	−	
8′-H_B	−	+	Irr	+	−
1′-H_L		−	+	Irr	+
1′-H_M			−	+	Irr

[a] Irr = proton irradiated. [b] + = positive; − = negative.

estimate of 140—170° for the dihedral angle C(17)-C(18)-C(15)-C(16) agrees remarkably well with the corresponding angles of 140° in vincristine methiodide [18] and 165° in vinblastine dihydrochloride [19] as determined by *X*-ray crystallography. The observed orientation does give a compact, roughly spherical molecule which corresponds remarkably closely to the solid state structure. Even if this orientation is favoured as much as 90% of the time, and we have not quantified this in any way, this corresponds to less than 5 kJ mol^{-1} of stabilization and no functional group interaction is required.

The work described here demanded access to high quality equipment. However, the derived spectroscopic and conformational conclusions have a rare degree of rigour. As a result future investigations of this or related compounds will require very much less time to establish the precise conformational features of interest.

Experimental

In Vancouver, vinblastine (as free base) and vindoline were gifts from Dr. B. R. Worth. In Cambridge, vinblastine sulphate (Sigma Chemical Company) in dichloromethane solution was neutralized with dilute aqueous sodium hydroxide and the resulting free-base solution was washed with water, dried and evaporated to dryness. Solutions for n.m.r. study were 5—50 mM in alkaloid and were not degassed. All spectra were run at ambient temperature (*ca.* 17 °C for the 270 MHz and *ca.* 20 °C for 400 MHz spectra). The 270 MHz spectra were obtained on a home-built instrument at U.B.C. operating under Nicolet 1180–293A control and 400 MHz spectra on Bruker WH400 instruments at U.B.C. and in Cambridge. Double-resonance difference spectra were acquired using automated programs described elsewhere.[5,6] Generally, 8 K data points were collected over 4 000 Hz spectral widths with 50—1 000 transients being collected for each irradiation. Subsaturating power levels were used to achieve maximum frequency selectivity but some 'spill-over' into adjacent resonances still occurred in crowded spectral regions. N.O.e.'s from spillover (marked with * in Figures 2 and 4) were identified by comparing spectra from irradiation of adjacent signals. Pre-irradiation times in n.O.e. experiments were 1—2 s. Where necessary, interfering solvent signals were suppressed by inserting a 180° inverting pulse near the beginning of the irradiation sequence. The alkaloid T_1 values were all less than 1 s.

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