Crystal structure of vinblastine

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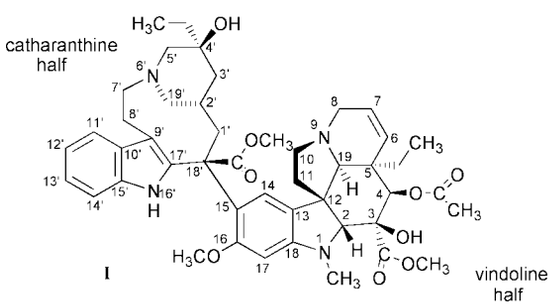
Vinblastine sulfate has been successfully crystallized by the hanging-drop vapor diffusion method against polyethylene glycol and lithium sulfate, and its structure determined by X-ray crystallography. The molecular geometry is rather similar to that of vincristine, with the most notable difference being that the key $COOCH_3$ group in the C18′ position is oriented in an opposite direction from that in vincristine. Half of the 9-membered azacyclononane ring in the catharanthine portion of the molecule is remarkably flat and coplanar with the indole ring, while the other half is in a boat conformation and fused with the piperidine ring in a chair conformation. The title compound crystallizes with an unusually large number of water molecules (nineteen), which form intriguing patterns of interconnected 5-membered rings (and some 6-membered rings) over substantial portions of the unit cell.

Introduction

Vinblastine and vincristine are two potent anti-tumor drugs isolated several decades ago from the tropical plant *Catharanthus roseus* (formerly *Vinca rosea*), commonly known as the Madagascar periwinkle.[1] This plant grows throughout the world, and has been long known in folk medicine as a cure or preventive of many assorted ailments. In 1958 crystalline compounds now known as the vinca alkaloids were isolated[2] from *Vinca rosea* and were found to be active against a wide range of human tumors, and today vinblastine and vincristine are among the most widely-used drugs in chemotherapy. The mode of action of the vinca drugs has been well-studied, and it is now widely believed that they act *via* tubulin inhibition,[1,3] thereby arresting mitosis resulting in cell death.[4] The crystal structure of vincristine was reported by Moncrief and Lipscomb in 1965,[5] but the structure of vinblastine (**I**) has not been reported until now.[6]



I

Results and discussion

Recently we have been successful in applying systematic crystallization procedures, originally developed for macromolecular samples,[7] to grow diffraction-quality crystals of various water-soluble small molecules.[8] Using the technique of hanging-drop vapor diffusion,[7a] a standard procedure in protein crystallography but rarely employed in small-molecule crystallization, we found that crystals of vinblastine sulfate, $[C_{46}H_{60}N_4O_9]^{2+}$-$[SO_4]^{2-}\cdot 19H_2O$, could be grown when diffused against a reservoir solution composed of 2.0 M Li_2SO_4 and 8% poly(ethylene glycol) (molecular weight 8000). The compound crystallizes in the monoclinic space group $P2_1$, with $a = 13.724(3)$ Å, $b = 14.030(4)$ Å, $c = 17.657(5)$ Å, $\beta = 110.19(2)°$, $V = 3190$ Å^3, and $Z = 2$. Data were collected on a Siemens P4 automated diffractometer with Mo-Kα X-rays at −100 °C. The structure was solved by direct methods and refined to a final R factor of 0.053 for 2633 reflections with $I > 2\sigma(I)$.[9] The sulfate group was slightly disordered, but other than that there were no significant problems with the structure determination. A summary of the crystallographic details is given in Table 1, while distances and angles in the molecule are listed in Table 2. Full details of the structural analysis are available.†

Table 1 Crystal data and structure refinement for vinblastine sulfate

Chemical formula	$[C_{46}H_{60}N_4O_9]^{2+}$ $[SO_4]^{2-}\cdot 19H_2O$
Formula weight	1251.46
Crystal system	Monoclinic
Space group	$P2_1$
Unit cell dimensions	$a = 13.724(3)$ Å $\alpha = 90°$
	$b = 14.030(4)$ Å $\beta = 110.19(2)°$
	$c = 17.657(5)$ Å $\gamma = 90°$
Volume	3191(2) Å^3
Z	2
Temperature	173(2) K
Density	1.260 g cm^{-3}
Absorption coefficient	0.139 mm^{-1}
Reflections collected	3569
Independent reflections	3135 [R(int) = 0.0332]
Final R factor [$I > 2\sigma(I)$ data]	$R = 0.0534$ (2633 reflections)
R index (all data)	$R = 0.0615$ (3135 reflections)

The structure of vinblastine is shown in Fig. 1, in which one can clearly see the two halves of the molecule joined by the central C15–C18′ bond: the catharanthine portion (labelled C1′–C26′), which contains an unusual 9-membered ring, and the vindoline portion (labelled N1–C30). The gross molecular geometry is very similar to that of vincristine[5] except for the orientation of the $COOCH_3$ group in the C18′ position, which is rotated by about 170° relative to that in vincristine.

Other features of the catharanthine half of the molecule are worthy of note. Fig. 2 shows the conformation of the 9-membered azacyclononene ring[10] quite clearly: the six-atom C7′–C8′–C9′–C17′–C18′–C1′ portion of the ring is almost flat (within ± 0.04 Å), and is in fact approximately coplanar with the indole ring (C9′–C10′–C11′ ··· N16′–C17′). The other

† CCDC reference number 207/438. See http://www.rsc.org/suppdata/p1/b0/b001855o for crystallographic files in .cif format.

Table 2 Molecular parameters in vinblastine

(a) Bond lengths/Å

N(1)–C(18)	1.407(12)	C(29)–O(31)	1.218(9)	N(1)–C(22)	1.470(11)	O(28)–C(29)	1.363(10)
N(1)–C(2)	1.491(8)	O(32)–C(33)	1.426(10)	C(2)–C(3)	1.556(12)	C(29)–C(30)	1.460(14)
C(2)–C(12)	1.568(12)	C(1′)–C(2′)	1.557(11)	C(3)–O(27)	1.439(8)	C(1′)–C(18′)	1.538(12)
C(3)–C(23)	1.490(12)	C(2′)–C(19′)	1.523(10)	C(3)–C(4)	1.519(12)	C(2′)–C(3′)	1.511(12)
C(4)–O(28)	1.457(10)	C(4′)–O(22′)	1.442(10)	C(4)–C(5)	1.566(13)	C(3′)–C(4′)	1.525(13)
C(5)–C(6)	1.513(12)	C(4′)–C(5′)	1.543(10)	C(5)–C(19)	1.538(12)	C(4′)–C(20′)	1.531(13)
C(5)–C(20)	1.571(10)	N(6′)–C(19′)	1.498(12)	C(6)–C(7)	1.309(12)	C(5′)–N(6′)	1.504(11)
C(7)–C(8)	1.485(12)	N(6′)–H(58)	0.952(16)	C(8)–N(9)	1.514(11)	N(6′)–C(7′)	1.501(12)
N(9)–C(10)	1.494(11)	C(8′)–C(9′)	1.530(12)	N(9)–C(19)	1.499(9)	C(7′)–C(8′)	1.526(12)
N(9)–H(57)	0.955(18)	C(9′)–C(10′)	1.424(11)	C(10)–C(11)	1.486(12)	C(9′)–C(17′)	1.397(11)
C(11)–C(12)	1.572(10)	C(10′)–C(11′)	1.426(12)	C(12)–C(13)	1.509(11)	C(10′)–C(15′)	1.421(11)
C(12)–C(19)	1.536(12)	C(12′)–C(13′)	1.425(14)	C(13)–C(14)	1.378(12)	C(11′)–C(12′)	1.352(12)
C(13)–C(18)	1.402(11)	C(14′)–C(15′)	1.398(11)	C(14)–C(15)	1.377(11)	C(13′)–C(14′)	1.384(13)
C(15)–C(16)	1.403(11)	N(16′)–C(17′)	1.383(10)	C(15)–C(18′)	1.554(13)	C(15′)–N(16′)	1.369(11)
C(16)–C(17)	1.389(13)	C(18′)–C(23′)	1.565(10)	C(16)–O(32)	1.393(10)	C(17′)–C(18′)	1.540(12)
C(17)–C(18)	1.393(11)	C(23′)–O(24′)	1.185(9)	C(20)–C(21)	1.500(15)	C(20′)–C(21′)	1.546(13)
C(23)–O(24)	1.208(10)	O(25′)–C(26′)	1.455(11)	C(23)–O(25)	1.358(9)	C(23′)–O(25′)	1.339(10)
O(25)–C(26)	1.466(12)						

Bond angles/°

C(18)–N(1)–C(22)	114.4(7)	O(24)–C(23)–C(3)	123.1(7)	C(18)–N(1)–C(2)	106.4(6)	O(25)–C(23)–C(3)	114.1(7)
C(22)–N(1)–C(2)	116.5(6)	C(23)–O(25)–C(26)	114.9(6)	N(1)–C(2)–C(3)	113.3(6)	C(29)–O(28)–C(4)	116.4(5)
N(1)–C(2)–C(12)	105.2(6)	O(31)–C(29)–O(28)	121.1(8)	C(3)–C(2)–C(12)	113.0(6)	O(31)–C(29)–C(30)	126.1(8)
O(27)–C(3)–C(23)	108.5(6)	O(28)–C(29)–C(30)	112.8(6)	O(27)–C(3)–C(4)	108.1(6)	C(16)–O(32)–C(33)	119.2(7)
C(23)–C(3)–C(4)	114.7(7)	C(18′)–C(1′)–C(2′)	116.7(7)	O(27)–C(3)–C(2)	106.8(6)	C(3′)–C(2′)–C(19′)	109.5(6)
C(23)–C(3)–C(2)	108.6(7)	C(3′)–C(2′)–C(1′)	112.6(6)	C(4)–C(3)–C(2)	109.8(7)	C(19′)–C(2′)–C(1′)	115.5(7)
O(28)–C(4)–C(3)	105.2(6)	O(22′)–C(4′)–C(3′)	108.6(5)	O(28)–C(4)–C(5)	111.4(6)	O(22′)–C(4′)–C(20′)	110.1(6)
C(3)–C(4)–C(5)	117.0(7)	C(3′)–C(4′)–C(20′)	112.0(8)	C(6)–C(5)–C(19)	107.6(6)	O(22′)–C(4′)–C(5′)	109.7(7)
C(6)–C(5)–C(4)	113.7(7)	C(3′)–C(4′)–C(5′)	109.7(7)	C(19)–C(5)–C(4)	112.9(7)	C(20′)–C(4′)–C(5′)	106.7(6)
C(6)–C(5)–C(20)	106.4(6)	N(6′)–C(5′)–C(4′)	113.8(6)	C(19)–C(5)–C(20)	109.1(7)	C(19′)–N(6′)–C(7′)	116.4(6)
C(4)–C(5)–C(20)	106.8(6)	C(19′)–N(6′)–C(5′)	110.6(7)	C(7)–C(6)–C(5)	125.7(8)	C(7′)–N(6′)–C(5′)	113.0(7)
C(6)–C(7)–C(8)	124.7(9)	N(6′)–C(7′)–C(8′)	114.2(7)	C(7)–C(8)–N(9)	108.2(7)	C(7′)–C(8′)–C(9′)	126.0(7)
C(10)–N(9)–C(19)	103.4(6)	C(17′)–C(9′)–C(10′)	105.8(7)	C(10)–N(9)–C(8)	115.9(7)	C(17′)–C(9′)–C(8′)	139.7(8)
C(19)–N(9)–C(8)	111.0(6)	C(10′)–C(9′)–C(8′)	114.4(7)	C(11)–C(10)–N(9)	104.9(6)	C(15′)–C(10′)–C(9′)	108.4(7)
C(10)–C(11)–C(12)	106.9(7)	C(15′)–C(10′)–C(11′)	117.6(8)	C(13)–C(12)–C(19)	112.1(7)	C(9′)–C(10′)–C(11′)	134.0(8)
C(13)–C(12)–C(2)	101.9(6)	C(12′)–C(11′)–C(10′)	118.6(8)	C(19)–C(12)–C(2)	113.8(7)	C(11′)–C(12′)–C(13′)	123.5(9)
C(13)–C(12)–C(11)	114.0(7)	C(14′)–C(13′)–C(12′)	119.4(9)	C(19)–C(12)–C(11)	102.9(6)	C(13′)–C(14′)–C(15′)	117.7(8)
C(2)–C(12)–C(11)	112.5(6)	N(16′)–C(15′)–C(14′)	129.9(7)	C(14)–C(13)–C(18)	119.7(7)	N(16′)–C(15′)–C(10′)	106.8(7)
C(14)–C(13)–C(12)	130.8(7)	C(14′)–C(15′)–C(10′)	123.3(7)	C(18)–C(13)–C(12)	109.5(7)	C(15′)–N(16′)–C(17′)	109.8(6)
C(15)–C(14)–C(13)	121.7(7)	N(16′)–C(17′)–C(9′)	109.2(7)	C(14)–C(15)–C(16)	117.4(8)	N(16′)–C(17′)–C(18′)	113.5(6)
C(14)–C(15)–C(18′)	121.3(7)	C(9′)–C(17′)–C(18′)	137.3(8)	C(16)–C(15)–C(18′)	120.0(7)	C(1′)–C(18′)–C(17′)	117.7(6)
C(17)–C(16)–O(32)	121.1(7)	C(1′)–C(18′)–C(15)	108.4(7)	C(17)–C(16)–C(15)	123.1(8)	C(17′)–C(18′)–C(15)	109.0(7)
O(32)–C(16)–C(15)	115.6(8)	C(1′)–C(18′)–C(23′)	109.8(6)	C(16)–C(17)–C(18)	117.3(7)	C(17′)–C(18′)–C(23′)	102.1(7)
C(17)–C(18)–C(13)	120.8(8)	C(15)–C(18′)–C(23′)	109.5(6)	C(17)–C(18)–N(1)	127.4(7)	N(6′)–C(19′)–C(2′)	112.9(7)
C(13)–C(18)–N(1)	111.7(7)	C(4′)–C(20′)–C(21′)	113.8(6)	N(9)–C(19)–C(12)	105.8(6)	O(24′)–C(23′)–O(25′)	123.6(7)
N(9)–C(19)–C(5)	112.1(7)	O(24′)–C(23′)–C(18′)	125.1(8)	C(12)–C(19)–C(5)	118.9(7)	O(25′)–C(23′)–C(18′)	111.3(6)
C(21)–C(20)–C(5)	116.6(7)	C(23′)–O(25′)–C(26′)	114.8(6)	O(24)–C(23)–O(25)	122.7(8)		

half of the 9-membered ring (C7′–N6′–C19′–C2′–C1′) is in a boat conformation, and is fused to the piperidine ring (C2′–C3′–C4′–C5′–N6′–C19′) which is itself in a chair conformation (Fig. 2). The C8′–C7′–N6′–C19′ portion of the azacyclononane ring is known from solution NMR studies to exhibit extensive internal motion,[11] and in the present X-ray study we find that the entire pseudo-planar half of the 9-membered ring (C7′–C8′–C9′–C17′–C18′–C1′) has "opened up", with unusually large angles, to accommodate the near-planarity of this six-atom fragment.[12]

The vindoline half of the molecule is shown in Fig. 3. The main feature of note is a hydrogen bond between N9 and the C3-hydroxy group (atom O27), the only significant intramolecular H-bond in the vinblastine molecule and a feature which has been noticed before.[5,6b] The resolution of the X-ray data was sufficiently high to clearly locate most of the C–H hydrogen atoms in vinblastine, as well as two distinct peaks near the N6′ and N9 positions. This strongly suggests that the N6′ and N9 atoms are protonated, and that the molecule exists in its dicationic form in the present study.[13]

It is interesting to note that the main conformational features deduced from the present study (on protonated molecules crystallized from a nearly aqueous environment) are virtually the same as those derived from solution state NMR studies[6b] (on neutral molecules in non-polar solvents). This implies a surprising degree of robustness in the overall conformation of vinblastine, which appears to be insensitive to the protonation state or solvent polarity.

The number of water molecules that have co-crystallized with vinblastine in the present structure determination, nineteen, is unusually large for a structure of this size. (In contrast, only two water molecules were found in the structure determination of vincristine.[5]) In fact, examination of packing diagrams shows large portions of the unit cell filled with water, which are remarkably well-defined because of the low temperature of the data collection. Interestingly, many of these water molecules are arranged in the form of interconnected pentagons (Fig. 4), a feature which has been seen before in the structures of crambin[14] and other crystals of small proteins.

Structure–activity studies have pinpointed several key regions of the vinblastine molecule that are crucial for antitumor activity. It is known, for example, that inversion at the C18′ or C2′ positions results in total loss of activity,[3,15] and that epimers at C4′ lack anti-microtubule activity.[16,17] Several studies have focused on the C17′–C18′–C15–C16 dihedral angle, which defines the spatial relationship between the two halves of the molecule. NMR studies have measured this angle to be around 180° in aqueous solution[18] and in the range 140°–170° in

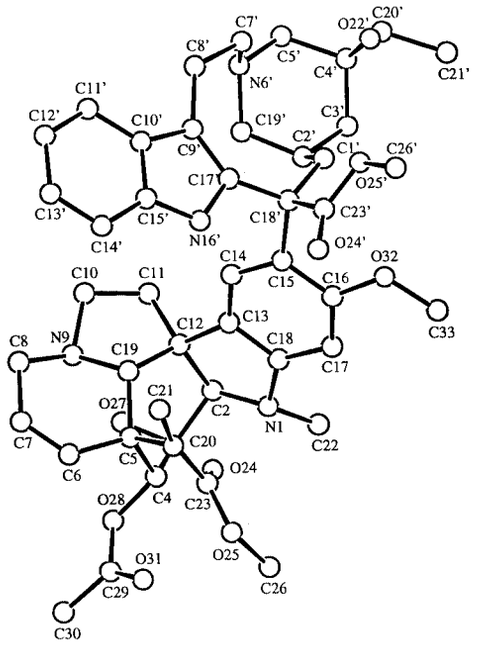


Fig. 1 The molecular structure of vinblastine. Note the unusual 9-membered ring in the catharanthine (upper) portion of the molecule. The orientation around the central C15–C18′ bond places the methoxy group in the C16 position and the methoxy carbonyl group in the C18′ position in close proximity. (Atom C3 is obscured behind C20 in this plot.)

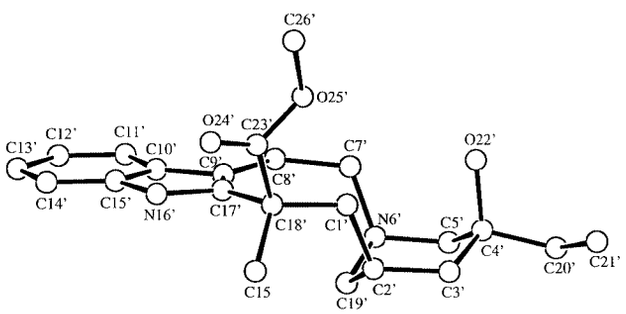


Fig. 2 Alternative view of the catharanthine half of vinblastine, emphasizing the essential flatness of this part of the molecule. Note how the left half of the 9-membered ring is almost coplanar with the indole group, while the right half is distinctly bent in a boat conformation. The piperidine ring at the extreme right is in a chair conformation, with an axial hydroxy group (O22′) and an equatorial ethyl group at the C4′ position.

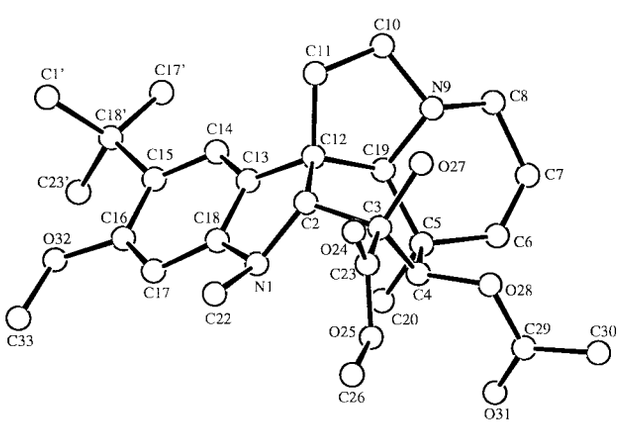


Fig. 3 A plot of the vindoline half of the molecule, with one atom (C21) removed for clarity. Atom N9 is protonated, and forms the only intramolecular hydrogen bond (N9–H···O27) in the molecule.

organic solvents,[6b] while prior X-ray studies have yielded the values of 160° in vincristine methiodide[5] and 165° in vinblastine chloride.[6a] Our value of the C17′–C18′–C15–C16 dihedral angle, for vinblastine sulfate, is 162°, a value which places the

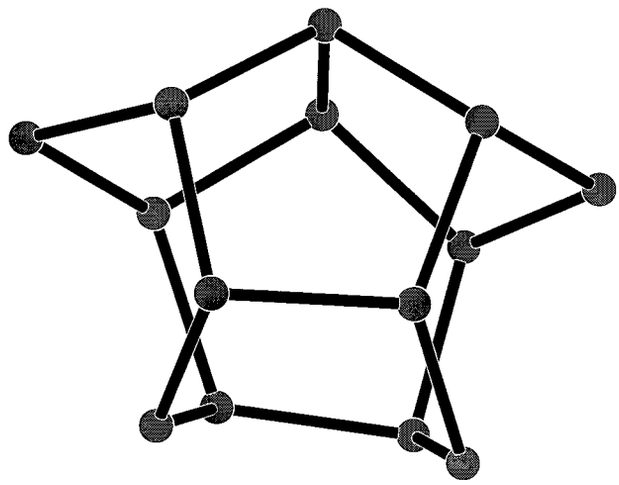


Fig. 4 A portion of the extensive hydrogen-bonding network between water molecules in the unit cell of vinblastine sulfate. The tendency of the water solvate molecules to form 5-membered and 6-membered rings in the solid state is well established (ref. 14).

C16-methoxy and C18′-methoxycarbonyl groups in close proximity. Thus, it appears that the orientation around the critical C18′–C15 bond is essentially maintained both in the solid state and in various solvents.

Finally, we comment on the main difference between the structures of vincristine and vinblastine, which is the orientation of the $COOCH_3$ group in the C18′ position. This is a crucial substituent, essential for anti-tumor activity.[3,15] In vincristine the carbonyl group of this ester substituent is oriented away from the indole ring, towards the C18′–C1′ bond (O=C–C18′–C1′ torsion angle + 13°),[5] while in vinblastine it is in a reversed direction (O24′=C23′–C18′–C1′ torsion angle –158°). It is unclear if this orientational difference is a significant feature, or simply an artifact of crystal-packing forces.

How does vinblastine interfere with microtubule assembly? Some investigators suspect that it modifies certain cysteine residues on tubulin, although it is not known which ones.[19] A three-dimensional structure of tubulin at 3.7 Å resolution, determined by electron crystallography, has recently been reported[20] and the taxol binding site has been identified. Whether or not this is also the binding site for the vinca alkaloids is not clear, but one hopes that with further structural work the situation will be clarified in the not too distant future.

Acknowledgements

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References and notes

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2 R. L. Noble, C. T. Beer and J. H. Cutts, *Ann. N.Y. Acad. Sci.*, 1958, **76**, 882.
3 R. H. Himes, *Pharmacol. Ther.*, 1991, **51**, 257.
4 By binding to the protein tubulin with high affinity, the vinca alkaloids inhibit microtubule assembly (they prevent cells from making the spindles needed to pull the chromosomes apart during cell division). This action is similar to that of colchicine, but is different from that of taxol, which also binds to tubulin but interferes with cell division in an opposite way, by preventing the spindles from being broken down.
5 (*a*) J. W. Moncrief and W. N. Lipscomb, *J. Am. Chem. Soc.*, 1965, **87**, 4963; (*b*) J. W. Moncrief and W. N. Lipscomb, *Acta Crystallogr.*, 1966, **21**, 322.
6 (*a*) An unpublished account of an X-ray structure determination of a different salt of the title compound (vinblastine dihydrochloride)

has been mentioned in reference 6(*b*) as footnote 19 ("O. Kennard and J. Nachman, unpublished observations"), but no details were given other than the fact that the C17′–C18′–C15–C16 dihedral angle in that molecule had a value of 165°; (*b*) B. K. Hunter, L. D. Hall and J. K. M. Sanders, *J. Chem. Soc.*, *Perkin Trans. 1*, 1983, 657.

7 (*a*) *Crystallization of Nucleic Acids and Proteins*, eds., A. Ducruix and R. Giegé, Oxford University Press, Oxford, UK, 1992; (*b*) *Protein Crystallization*: *Techniques*, *Strategies and Tips*, ed. T. M. Bergfors, International University Line Press, Oxford, UK, 1999.

8 (*a*) R. Bau, *J. Am. Chem. Soc.*, 1998, **120**, 9380; (*b*) R. Bau and I. Tsyba, *Tetrahedron*, 1999, **55**, 14839.

9 (*a*) G. M. Sheldrick, SHELXS-86, University of Göttingen, Germany, 1986; (*b*) G. M. Sheldrick, SHELX-76, University of Cambridge, England, 1976.

10 The azacyclononane ring has been described as being in a boat–chair conformation in epivinblastine, a molecule related to vinblastine but one in which the absolute configuration at the $C^{18'}$ position is inverted (V. M. Lynch, A. Stamford, P. Magnus and B. E. David, *Acta Crystallogr.*, *Sect. C*, 1991, **47**, 1563.

11 C. W. Andrews, J. Wisowaty, A. O. Davis, R. Crouch and G. E. Martin, *J. Heterocycl. Chem.*, 1995, **32**, 1011.

12 In other words, in order to place atoms C1′ and C7′ in a roughly coplanar arrangement with atoms C8′, C9′, C17′, and C18′ (Fig. 2), steric factors make it necessary to expand the C–C–C angles of the latter four atoms beyond their normal expected values. Thus, C7′–C8′–C9′ = 126.0(7)°, C8′–C9′–C17′ = 139.7(8)°, C9′–C17′–C18′ = 137.3(8)°, C17′–C18′–C1′ = 117.7(6)°. Similarly distorted angles were also found in the structure of vincristine.[5]

13 Peaks corresponding to H positions were found 0.95 Å from N6′ (peak H58) and 1.16 Å from N9 (peak H57). This not only indicates that both nitrogen atoms are protonated, but it also suggests that the hydrogen bond between N9 and the C3 hydroxy group is of the type N9–H · · · O27 and not N9 · · · H–O27.

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