The Crystal Structure of Isocolchicine, an Inactive Isomer of the Mitotic Spindle Inhibitor Colchicine

By L. Lessinger* and T. N. Margulis

Department of Chemistry, University of Massachusetts – Boston, Boston, Massachusetts 02125, USA

Isocolchicine, $C_{22}H_{25}NO_6$, which differs from the powerful mitotic spindle inhibitor colchicine by the interchange of a keto and a methoxy group, shows greatly reduced biological activity of all kinds compared with its isomer. Isocolchicine crystallizes in space group $P2_12_12_1$, $a = 7.612$, $b = 15.832$, $c = 34.40$ Å, $Z = 8$. The crystal structure was solved by the 'magic integer' technique and refined by least-squares to $R = 0.0355$ for 2858 observed X-ray reflections. The conformations of the two independent isocolchicine molecules in the crystal differ both in the orientations of the peripheral N-acetyl and methoxy substituents and in the detailed geometry of the skeletal troponoid rings C. Both troponoid rings have alternating long and short bonds, and one is rather flatter than the other. However, the conformations of the central seven-membered rings B are almost identical with each other and with the conformations of rings B in the two independent molecules in the crystal of colchicine.$2H_2O$.
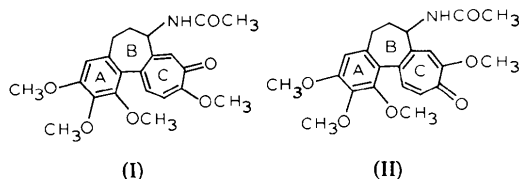
Introduction

Colchicine (I) is a powerful inhibitor of mitosis and is used clinically in the treatment of gouty arthritis. Recent research suggests that it acts by binding specifically and strongly to the protein tubulin, thus preventing the assembly of tubulin into the microtubules which form the mitotic spindle (Soifer, 1975). One of the major tasks in explaining the relation between the structure of colchicine and its activity is to account for the effect of interchanging the positions of the keto and methoxy groups in ring C. Isocolchicine (II) does not bind to tubulin, inhibit mitosis, or relieve gout (Zweig & Chignell, 1973).

Clearly, isocolchicine differs in shape from colchicine because of the keto/methoxy interchange, and this in itself might be sufficient to prevent isocolchicine from fitting neatly into the tubulin binding site, whatever kinds of interactions are responsible for the binding. Lacking at present any detailed knowledge of the binding site, we set out to determine the exact stereochemistry of isocolchicine, colchicine (Lessinger & Margulis, 1978) and various derivatives (Margulis, 1974, 1975, 1977; Koerntgen & Margulis, 1977), using X-ray crystallography, to focus on three questions

which could be answered by crystal structure determinations. (1) What are the effects of the interchange of the keto and methoxy groups on the geometry of ring C? (2) When the double bonds shift in ring C, what are the effects on the conformation of ring B, and therefore on the angle between rings A and C? (3) What are the conformations of the N-acetyl and methoxy substituents in these molecules; that is, is there a single preferred orientation, or are there several possibilities? It has only recently been possible for us to solve the crystal structure of isocolchicine, which is presented here.

Experimental

Isocolchicine, obtained from Smith, Kline & French, was crystallized by slow evaporation of an ethanol/water solution. Crystals grew as pale yellow short needles. Unit-cell parameters and uncertainties were found from least-squares analysis of diffractometer angle measurements. The space group was uniquely determined by systematic absences. The density, measured by flotation, indicated two independent isocolchicine molecules in the asymmetric unit.

Crystal data

Isocolchicine, $C_{22}H_{25}NO_6$, $M_r = 399.45$; orthorhombic, space group $P2_12_12_1$; $a = 7.612$ (3), $b = 15.832$ (5), $c = 34.40$ (2) Å; $D_m = 1.24$ (5), $D_x = 1.28$ g cm^{-3} for $Z = 8$; $V = 4146$ Å^3, $F(000) = 1696$, μ(Cu $K\alpha) = 7.8$ cm^{-1}.

Intensity data in one octant were collected from a

(I)   (II)

* Present address: Department of Chemistry, Barnard College, Columbia University, New York, NY 10027, USA.

crystal of dimensions $0.60 \times 0.08 \times 0.18$ mm on a Syntex $P2_1$ diffractometer, with monochromated Cu $K\alpha$ ($\lambda = 1.54178$ Å) X-rays, by the 2θ scan technique. Of the 3960 reflections with $\sin \theta/\lambda \leq 0.594$, 3071 were judged measurable by an initial quick scan and, of these, 2858 were considered 'observed' $[I > 3\sigma(I)]$. No corrections were made for either absorption ($\mu r \simeq 0.03$–0.22) or secondary extinction.

Structure determination

The crystal structure of isocolchicine contains 58 independent C, N and O atoms. Repeated attempts to solve this structure with the *MULTAN* direct-methods phasing program (Main, Lessinger, Woolfson, Germain & Declercq, 1976) all failed. The solution was finally achieved by using the 'magic integer' method of unknown phase representation with the multiple-solution approach (Lessinger & Margulis, 1978). In accordance with tactics previously discussed (Lessinger, 1976), only the 290 largest normalized structure factors ($|E| > 1.691$) were chosen, but all the 3183 triple phase relations among them were used. The starting set was as listed below.

	Origin			Unknown	
hkl	0,15,12	0,5,11	130	0,13,14	0,3,31
φ	90	90	90	90, 270	90, 270

		Unknown			
hkl	5,6,10	1,1,30	1,3,30	3,7,23	222	577
φ	$63x$	$62x$	$60x$	$56x$	$48x$	$32x$

The unknown general starting phases were assigned values by sampling x every $360°/(4.63)$ from $x = 360°/(2.4.63)$ to $x = 360° - [360°/(2.4.63)]$. Consideration was restricted to one enantiomorph by looking only at those values of x for which the phase of 5,6,10 was either 45 or 135°. The number of phase sets was therefore $\frac{1}{2}(2.2)(4.63) = 504$. These were developed in the usual way with the tangent formula.

The best set was easily picked out, as it had the highest combined figure of merit (see Table 1). The E

Table 1. *Figures of merit for best 3 of 504 phase sets; ranges for all 504 sets*

Set rank	Combined	Absolute	ψ_0	Residual
1	2.5858	1.0261	157.8	24.01
2	2.4624	1.0268	173.9	24.57
3	1.8896	0.7245	154.9	26.13
Range	Possible	0.5964–	119.5–	24.01–
	0.0–3.0	1.1480	317.7	36.93

map made with those 290 phases showed 50 of the 58 atoms; the first false peak was 43rd in height. The remaining eight atoms were found by Fourier synthesis.

Refinement

The structure was refined with the least-squares program of Gantzel, Sparks & Trueblood. Midway through, all 50 H atoms were located in difference Fourier syntheses. Because of computing limitations, refinement was in three blocks, within which all matrix elements were used: C, N and O, with anisotropic temperature factors, for each of the two independent molecules, and all H, with isotropic temperature factors. Atomic scattering factors were taken from *International Tables for X-ray Crystallography* (1974). The function minimized was $\Sigma w(|F_o| - |F_c|)^2$ with the weight $w = 1/\sigma^2$ where σ is the standard deviation of the structure factor magnitude estimated from counting statistics and machine instability. For the 2858 'observed' reflections on which the refinement was based, the final conventional residual $R = \Sigma||F_o| - |F_c||/\Sigma|F_o| = 0.0355$.*

Results

Final atomic coordinates are listed in Table 2.* Bond distances and angles are shown in Figs. 1 and 2, selected torsion angles in Fig. 3. While the bond lengths and angles show no significant variation between the two independent molecules, the conformations certainly do.

The more subtle differences occur in the troponoid rings C. The C ring is much flatter in molecule a [r.m.s. deviation 0.013, maximum deviation 0.022 Å from the least-squares plane through $C(1)$–$C(7)$] than in molecule b (r.m.s. deviation 0.058, maximum deviation 0.097 Å). This is evidenced as well in the torsion angles shown in Fig. 3, which provide a more complete description of the conformations of rings C. An alternative view is to describe these rings as very shallow boats, made up of three planes, but with the atoms defining the planes differing between molecules a and b (Table 3). While the boat formed by ring C in isocolchicine molecule b is analogous in conformation to that found in both independent molecules in the crystal structure of colchicine.$2H_2O$ (Lessinger & Margulis, 1978), it is more puckered than the latter. This ring is in fact non-planar to the greatest extent so far observed in structures containing troponoid rings.

Table 2. *Atomic coordinates*

Standard deviations estimated from the least-squares calculations, assuming random errors in the intensity data, are given in parentheses as deviations in the last significant figure.

	Molecule a			Molecule b		
	$x\ (\times 10^4)$	$y\ (\times 10^4)$	$z\ (\times 10^5)$	$x\ (\times 10^4)$	$y\ (\times 10^4)$	$z\ (\times 10^5)$
C(1)	2284 (5)	2006 (2)	19334 (9)	−922 (5)	3485 (2)	26868 (10)
C(2)	1075 (5)	1538 (2)	16797 (10)	−1773 (5)	2659 (2)	26176 (11)
C(3)	1012 (5)	1711 (2)	12689 (10)	−1483 (6)	1959 (2)	28764 (12)
C(4)	1919 (5)	2254 (2)	10396 (9)	−723 (6)	1918 (2)	32318 (11)
C(5)	3274 (4)	2845 (2)	11191 (10)	9 (5)	2555 (2)	34754 (10)
C(6)	3920 (5)	3018 (2)	14844 (9)	272 (5)	3387 (2)	33753 (9)
C(7)	3433 (5)	2627 (2)	18405 (10)	−85 (5)	3771 (2)	30077 (10)
C(8)	4095 (5)	3252 (2)	7707 (10)	291 (5)	2294 (2)	38870 (10)
C(9)	5890 (5)	3205 (2)	7069 (10)	−630 (5)	2680 (2)	41814 (11)
C(10)	6629 (5)	3514 (2)	3651 (10)	−554 (5)	2369 (3)	45605 (11)
C(11)	5573 (5)	3895 (2)	854 (10)	512 (6)	1694 (3)	46494 (11)
C(12)	3765 (5)	3978 (2)	1513 (10)	1629 (5)	1355 (2)	43624 (12)
C(13)	3038 (5)	3653 (2)	4895 (10)	1492 (6)	1635 (2)	39818 (11)
C(14)	7058 (5)	2820 (3)	10134 (10)	−1733 (5)	3461 (3)	40867 (11)
C(15)	7165 (5)	3360 (3)	13833 (11)	−567 (5)	4212 (2)	39665 (11)
C(16)	5365 (5)	3698 (2)	15073 (9)	939 (5)	3960 (2)	36966 (10)
C(17)	4463 (6)	4667 (3)	20283 (12)	3643 (6)	4770 (3)	35941 (13)
C(18)	853 (6)	4519 (3)	7549 (13)	4189 (9)	1590 (5)	36458 (16)
C(19)	2389 (6)	4019 (3)	−4577 (11)	4357 (7)	909 (3)	46201 (17)
C(20)	8029 (6)	4280 (3)	−3047 (12)	−789 (8)	1453 (3)	52674 (13)
C(21)	3097 (6)	2097 (3)	26079 (11)	−400 (6)	4804 (3)	23462 (12)
C(22)	4759 (6)	4922 (3)	24477 (13)	4437 (6)	5589 (3)	34532 (12)
N	5541 (4)	4066 (2)	18935 (8)	1912 (4)	4707 (2)	35648 (8)
O(1)	148 (4)	970 (2)	18166 (7)	−2718 (4)	2570 (2)	23295 (8)
O(2)	2099 (4)	1722 (2)	23018 (7)	−1174 (4)	3975 (2)	23676 (7)
O(3)	1258 (3)	3754 (2)	5505 (7)	2469 (4)	1267 (2)	36928 (9)
O(4)	2729 (3)	4436 (2)	−993 (7)	2733 (4)	694 (2)	44502 (9)
O(5)	6171 (3)	4232 (2)	−2556 (7)	637 (4)	1337 (2)	50136 (8)
O(6)	3276 (4)	4976 (2)	18309 (8)	4570 (4)	4222 (3)	37352 (13)

	$x\ (\times 10^3)$	$y\ (\times 10^3)$	$z\ (\times 10^4)$	$x\ (\times 10^3)$	$y\ (\times 10^3)$	$z\ (\times 10^4)$
H(C3)	9 (4)	137 (2)	1146 (7)	−207 (5)	143 (2)	2784 (8)
H(C4)	173 (4)	221 (2)	778 (8)	−60 (5)	136 (2)	3354 (9)
H(C7)	420 (4)	283 (2)	2049 (8)	16 (4)	437 (2)	3000 (7)
H(C10)	777 (4)	343 (2)	308 (8)	−138 (5)	266 (2)	4751 (10)
H1(C14)	684 (6)	226 (3)	1087 (11)	−255 (5)	331 (2)	3863 (9)
H2(C14)	808 (5)	276 (2)	887 (10)	−248 (5)	362 (2)	4308 (9)
H1(C15)	756 (4)	302 (2)	1595 (8)	−140 (5)	462 (2)	3834 (11)
H2(C15)	781 (5)	389 (2)	1333 (11)	−5 (4)	447 (2)	4210 (9)
H(C16)	508 (4)	418 (2)	1330 (9)	183 (4)	369 (2)	3854 (8)
H1(C18)	131 (7)	505 (3)	620 (14)	478 (8)	185 (4)	3892 (17)
H2(C18)	−42 (6)	456 (3)	794 (12)	503 (7)	135 (3)	3419 (13)
H3(C18)	177 (7)	456 (3)	1007 (15)	412 (10)	214 (4)	3536 (19)
H1(C19)	179 (6)	442 (3)	−611 (12)	444 (10)	101 (5)	4894 (21)
H2(C19)	354 (6)	380 (3)	−581 (12)	490 (7)	142 (3)	4499 (14)
H3(C19)	199 (7)	343 (3)	−377 (12)	502 (10)	42 (5)	4598 (21)
H1(C20)	843 (6)	373 (3)	−311 (11)	−77 (7)	213 (3)	5376 (14)
H2(C20)	878 (7)	457 (3)	−107 (14)	−52 (6)	109 (3)	5513 (13)
H3(C20)	807 (6)	457 (3)	−558 (12)	−198 (7)	132 (3)	5140 (13)
H1(C21)	263 (5)	184 (2)	2844 (9)	−71 (6)	513 (3)	2566 (13)
H2(C21)	447 (6)	201 (2)	2576 (11)	93 (7)	470 (3)	2338 (13)
H3(C21)	282 (5)	271 (2)	2613 (10)	−56 (6)	505 (3)	2079 (12)
H1(C22)	610 (9)	499 (4)	2493 (17)	497 (7)	587 (3)	3663 (14)
H2(C22)	409 (11)	538 (5)	2531 (20)	514 (6)	548 (3)	3245 (12)
H3(C22)	474 (8)	451 (4)	2628 (16)	352 (8)	600 (4)	3310 (16)
H(N)	649 (5)	387 (2)	2018 (9)	136 (6)	517 (3)	3469 (12)

On the other hand, ring C in molecule a, adopting a different conformation, is flatter than either ring C in the colchicine crystal. The pattern of alternating long and short bonds, and internal angles, is that commonly found in troponoid rings and is consistent only with incomplete delocalization of the π electrons of the ring and keto O atom [see list of references given in Lessinger & Margulis (1978)].

The N-acetyl side chain [C(16), N, C(17), O(6), C(22), group D] is roughly planar in molecule a (r.m.s. deviation 0·024, maximum deviation 0·033 Å), more nearly so in molecule b (r.m.s. deviation 0·008, maximum deviation 0·013 Å, from the least-squares plane through the five atoms).

Rings A, while of course quite flat, are not as precisely planar as anticipated. The deviations from planarity, listed in Table 3, follow the same pattern, with different magnitudes, in both molecules, and may be ascribed to the substitution on five of the six ring atoms of groups which crowd one another.

The overall shape of the molecule, illustrated in Fig. 3, is governed by the dihedral angles between the planar regions, which are listed in Table 3. Corresponding dihedral angles vary much more here than in colchicine, where they all agreed to within 3°. Dihedral angles involving the planes through the puckered rings C, however, overestimate somewhat the difference between isocolchicine molecules a and b, because the

Fig. 1. Bond distances (Å), those for molecule a listed above those for molecule b. Standard deviations for bonds not involving H are approximately 0·004–0·006 Å; for bonds to H approximately 0·03–0·07 Å. Numbered atoms (large circles) without element symbol are carbon. Small circles are hydrogen atoms.

Fig. 2. Bond angles (°), those for molecule a listed above those for molecule b. Standard deviations for angles not involving H are approximately 0·5°. Numbered atoms (large circles) without element symbol are carbon. Small circles are hydrogen atoms.

Table 3. Atoms defining the planes in molecules a and b and deviations from the planes (Å)

Molecule a	r.m.s. deviation	Molecule b	r.m.s. deviation
Plane 1 C(1),C(2),C(3),C(7)	0·0024	Plane 1 C(1),C(2),C(3)	–
Plane 2 C(3),C(4),C(6),C(7)	0·0018	Plane 2 C(1),C(3),C(4),C(7)	0·0084
Plane 3 C(4),C(5),C(6)	–	Plane 3 C(4),C(5),C(6),C(7)	0·0056

Dihedral angles

Plane 1–plane 2	1·2°	Plane 1–plane 2	9·6°
Plane 2–plane 3	2·5	Plane 2–plane 3	6·2

	Deviations from the least-squares plane through						r.m.s.
	C(8)	C(9)	C(10)	C(11)	C(12)	C(13)	deviation
Molecule a	0·014	−0·014	0·001	0·012	−0·012	−0·001	0·011
Molecule b	0·043	−0·038	−0·006	0·044	−0·039	−0·004	0·034

Dihedral angles between planar regions

Plane	Plane	Molecule a	Molecule b
Ring A	Ring C	54°	66°
Ring A	Group D	56	69
Ring C	Group D	89	124

conformations of rings C themselves differ. Alternative measures of the first and third quantities in the above list are the torsion angles C(6)–C(5)–C(8)–C(9) (−53, −57°) and C(6)–C(16)–N–C(17) (80, 106°), respectively. While the imino H atoms point toward the same side in both molecules a and b of isocolchicine as they did in colchicine, the orientations of groups D do differ considerably, whether measured by the dihedral angle with ring A, the dihedral angle with ring C, or the torsion angle C(6)–C(16)–N–C(17).

As in colchicine, rings A and C are not parallel but are considerably twisted about the C(5)–C(8) bond, which is necessary in order to accommodate the fused seven-membered ring B. We have shown that in isocolchicine C(5)–C(6) is essentially a double bond, whereas in colchicine it is essentially a single bond. This might have been expected to lead to significant differences between the conformations in rings B, and thus perhaps to marked differences in the twists of rings A and C about the C(5)–C(8) bond, as measured by the torsion angle C(6)–C(5)–C(8)–C(9). In fact, all corresponding torsion angles remain remarkably close to constant, whether ring B is found in colchicine or isocolchicine derivatives, for all but one of the compounds we have examined. The single exception is N-deacetylthiocolchicine.HCl.2H₂O, in which the N atom attached to ring B carries a charge, apparently a large perturbation. Table 4 compares the torsion angles in ring B for two isocolchicine derivatives and five colchicine derivatives. It seems likely that the differences in activity between colchicine derivatives and isocolchicine derivatives do not stem from any pronounced differences in the conformation of ring B or the twist of ring A with respect to ring C, even though ring C is not aromatic and the pattern of single and double bonds does shift in going from one group to the other.

The orientations of the N-acetyl group and the four methoxy groups determine the exact shape of much of the periphery, and the accessibility of rings A and C, of isocolchicine. While a difference in the location of one methoxy group on ring C results in the greatly diminished activity of isocolchicine when compared with colchicine, specific orientations of some or all of these groups may also be necessary for binding to tubulin to occur. Methoxy groups tend to lie roughly parallel or perpendicular to the rings on which they are substituted, and this is true in isocolchicine as well, as seen in Fig. 3, which also gives the angles between the COCH₃ planes and the ring planes. The methoxy
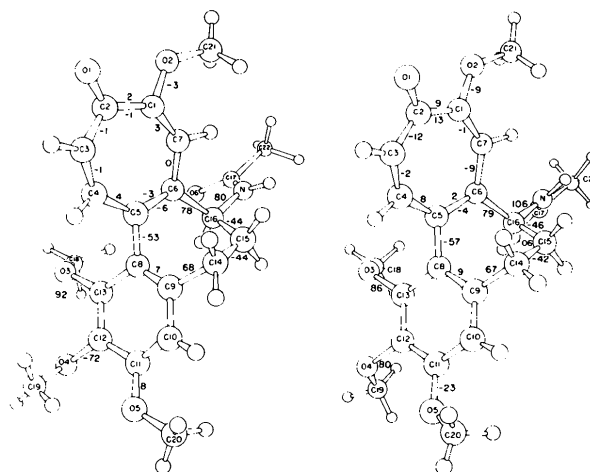

Fig. 3. Experimentally determined conformations of molecule a (left) and molecule b (right), viewed normal to the planes defined by C(5), C(8), and C(15). Torsion angles are given within rings B and C, for O(1)–C(2)–C(1)–O(2), for C(6)–C(16)–N–C(17), and for the methoxy groups. The latter are taken to be the angles between the planes COCH₃ and the least-squares planes for rings A and C.

Table 4. *Torsion angles* (°) *in ring B*

	Isocolchicine derivatives		Colchicine derivatives				
Torsion angle	ISOC	DMIT	COLC	CMID	THIOC	TSIDE	DTC
C(9)–C(8)–C(5)–C(6)	−53, −57	−54	−53, −53	−54	−55	−56	−60
C(8)–C(5)–C(6)–C(16)	−6, −4	−3	−5, −5	−5	−3	−3	+7
C(5)–C(6)–C(16)–C(15)	78, 79	79	79, 81	81	82	81	74
C(6)–C(16)–C(15)–C(14)	−44, −46	−46	−48, −49	−48	−50	−51	−52
C(16)–C(15)–C(14)–C(9)	−44, −42	−44	−43, −42	−41	−39	−40	−39
C(15)–C(14)–C(9)–C(8)	68, 67	72	73, 70	71	68	70	70
C(14)–C(9)–C(8)–C(5)	7, 9	4	4, 5	4	7	6	4

ISOC: Isocolchicine (this paper).
DMIT: Demethylisothiocolchicine (Margulis, 1975, 1977).
COLC: Colchicine.2H₂O (Lessinger & Margulis, 1978).
CMID: Colcemid (Margulis, 1974).
THIOC: Thiocolchicine.6H₂O (Koerntgen & Margulis, 1977).
TSIDE: Thiocolchicoside.2C₂H₅OH.H₂O (Clark & Margulis, 1978).
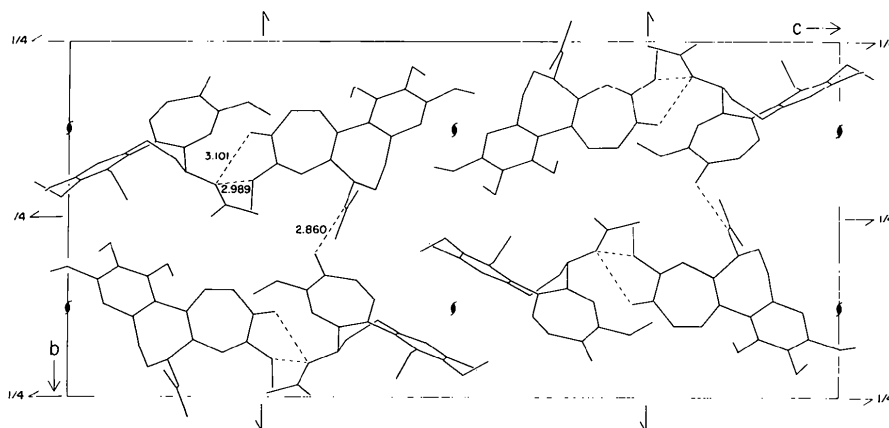DTC: N-Deacetylthiocolchicine.HCl.2H₂O (Koerntgen & Margulis, 1977; Margulis, 1975).

Fig. 4. Projection of the crystal structure down **a**. Hydrogen atoms are omitted for clarity. Dashed lines indicate hydrogen bonds. N(a), O(1b), and O(2b) are connected by a single bifurcated hydrogen bond.

conformations in molecule b are much like those in colchicine, but in molecule a the central methoxy group O(4)–C(19) on ring A points 'up' instead of 'down'! In this conformation, access to ring A is somewhat restricted from both sides, and any interaction with O atoms O(3), O(4), O(5) would have to come from directions different from those possible with the conformation of molecule b.

Isocolchicine, crystallized from ethanol/water, does not bind any solvent in the crystal, so the only hydrogen-bond donors are the imino H atoms. The hydrogen-bond scheme is shown in Fig. 4. A simple hydrogen-bond is formed between N on molecule b (xyz) and O(1) on molecule a ($1 - x$, $\frac{1}{2} + y$, $\frac{1}{2} - z$): N$\cdots$O 2·860, H$\cdots$O 1·97 Å, N–H$\cdots$O 166°. The second hydrogen bond is bifurcated, donated from N on molecule a (xyz) to both O(1) and O(2) on molecule b ($1 + x$, y, z). Distances and angles are N$\cdots$O(1) 3·101, H$\cdots$O(1) 2·39 Å, N–H$\cdots$O(1) 136°, N$\cdots$O(2) 2·989, H$\cdots$O(2) 2·15 Å, N–H$\cdots$O(2) 154°. The H atom is essentially in the plane of N, O(1), O(2), as the sum of angles about H is 357·6°.

The crystal is made up of hydrogen-bonded ribbons, two molecules wide, extended along **b** around a 2_1 axis. The ribbons are stacked together along **c** by a 2_1 screw operation, along **a** by simple translation.

References

CLARK, J. & MARGULIS, T. N. (1978). To be published.
International Tables for X-ray Crystallography (1974). Vol. IV, pp. 73, 102. Birmingham: Kynoch Press.
KOERNTGEN, C. & MARGULIS, T. N. (1977). *J. Pharm. Sci.* **66**, 1127–1131.
LESSINGER, L. (1976). *Acta Cryst.* A**32**, 538–550.
LESSINGER, L. & MARGULIS, T. N. (1978). *Acta Cryst.* B**34**, 578–584
MAIN, P., LESSINGER, L., WOOLFSON, M. M., GERMAIN, G. & DECLERCQ, J.-P. (1976). *MULTAN 76. A System of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data.* Univs. York, England, and Louvain, Belgium.
MARGULIS, T. N. (1974). *J. Am. Chem. Soc.* **96**, 899–902.
MARGULIS, T. N. (1975). *X-ray Analysis of Microtubule Inhibitors*, in *Microtubules and Microtubule Inhibitors*, edited by M. BORGERS & M. DE BRABANDER. Amsterdam: North-Holland.
MARGULIS, T. N. (1977). *Biochem. Biophys. Res. Commun.* **76**, 1293–1298.
SOIFER, D. (1975). Editor, *The Biology of Cytoplasmic Microtubules, Ann. NY Acad. Sci.* **253**, 213–231.
ZWEIG, M. H. & CHIGNELL, C. F. (1973). *Biochem. Pharmacol.* **22**, 2141–2150.