Vol. 83, No. 2, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS

July 28, 1978 Pages 472-478

N-ACETYLCOLCHINOL. STRUCTURE AND HYDROGEN-BONDING IN A BENZENOID COLCHICINE ANALOGUE

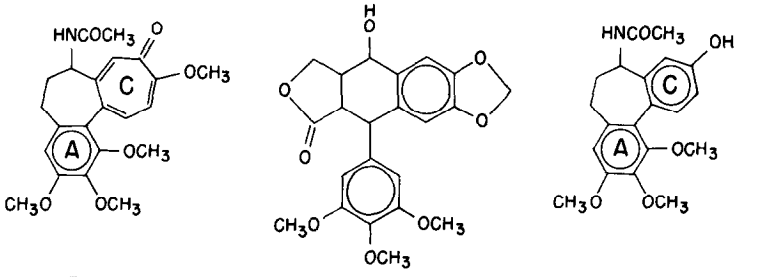
T. N. Margulis and L. Lessinger*

Department of Chemistry
University of Massachusetts-Boston
Boston, Massachusetts 02125

Received June 9,1978

SUMMARY

N-Acetylcolchinol, a benzenoid derivative of colchicine, crystallizes as the monohydrate in space group $P2_1$ with a = 14.334, b = 7.848, c = 17.634 Å; β = 100.6°, Z = 4. The structure was determined from 3978 X-ray reflections and refined to R = 0.039. The molecular shape is like that of colchicine and the crystal shows hydrogen-bonding and hydrophobic regions.



I II III

INTRODUCTION

Colchicine(I) and many of its derivatives are powerful mitotic poisons, anti-inflammatories, and inhibitors of tumor growth(1,2). Many of colchicine's properties may be associated with its binding to the protein tubulin, interfering with assembly into microtubules. The colchicine-tubulin interaction occurs with approximately 1:1 stoichiometry and has been suggested to be "hydrophobic" in nature(3). Cortese et al(4) have shown that the colchicine molecule binds to a site on tubulin with 2 points of attachment, one of which, involving the trimethoxyphenyl ring (ring A), it shares with the equal-

*Present address: Department of Chemistry, Barnard College, New York, NY.

0006-291X/78/0832-0472$01.00/0

ly powerful drug, podophyllotoxin(II). The other site is believed to involve the troponoid ring of colchicine (ring C). We have found (5-6) in crystals of colchicine as well as a number of other derivatives that ring C invariably interacts with other molecules, either water or the colchicine derivative, by hydrogen-bonds involving the carbonyl oxygen atom and often the ring C methoxy group. Ring A, however, usually interacts by van der Waals forces in a water-excluding region of the crystal structure.

N-acetylcolchinol (III) has an -OH containing benzenoid ring instead of a troponoid ring C but still shows much of the same behavior as colchicine. A crystal structure determination was carried out to settle several structural questions including 1) how the change from a 7- to a 6-membered ring C affects the conformation of ring B and therefore the overall shape of the molecule 2) how the -OH group can fulfill the same function as the carbonyl and methoxy groups of colchicine in interaction with other molecules.

METHODS

X-ray diffraction data was collected on a Syntex $P2_1$ diffractometer. A trial structure, requiring location of 54 independent C, N, and O atoms, was obtained with MULTAN modified to use the magic integer representation of unknown phases. Hydrogen atoms were located in difference maps and the final structure was refined by least squares R = 0.039 for 3978 independent non-zero X-ray reflections.

RESULTS

Crystals of N-acetylcolchinol hydrate, $C_{20}H_{23}NO_5 \cdot H_2O$, are monoclinic, space group $P2_1$, with 4 formula weights per unit cell. Cell dimensions are a = 14.334 ± 0.008, b = 7.848 ± 0.005, c = 17.634 ± 0.012, Å; β = 100.6 ± 0.1°. Table 1 gives positional coordinates of the C, N, and O atoms. Bond lengths and angles calculated from these coordinates are all within 0.01 Å and 0.5° of expected values. The overall shape of N-acetylcolchinol, best described by the torsion angles shown in Fig. 1, is very much like that of colchicine and its derivatives. Each of these angles is within 3° of the corresponding value in colchicine, this in spite of the different kind of

Table 1. Atomic coordinates of non-hydrogen atoms.*

	Molecule A			Molecule B		
Atom	x	y	z	x	y	z
C1	1.1881	0.4857	0.1885	0.6881	0.8058	0.1510
C2	1.2365	0.6185	0.2282	0.7443	0.9512	0.1511
C3	1.2158	0.6703	0.2988	0.7381	1.0837	0.2004
C4	1.1472	0.5847	0.3294	0.6736	1.0739	0.2509
C5	1.0974	0.4478	0.2910	0.6154	0.9320	0.2513
C6	1.1171	0.3974	0.2187	0.6227	0.7975	0.2009
C8	1.0252	0.3546	0.3255	0.5495	0.9151	0.3080
C9	1.0294	0.1766	0.3330	0.5563	0.7732	0.3561
C10	0.9650	0.0887	0.3690	0.4953	0.7558	0.4089
C11	0.8960	0.1779	0.3985	0.4273	0.8792	0.4137
C12	0.8880	0.3540	0.3889	0.4173	1.0174	0.3639
C13	0.9521	0.4402	0.3529	0.4780	1.0353	0.3108
C14	1.1060	0.0789	0.3024	0.6279	0.6356	0.3488
C15	1.0922	0.0782	0.2139	0.6007	0.5311	0.2742
C16	1.0595	0.2512	0.1790	0.5608	0.6417	0.2040
C17	0.9707	0.2468	0.0470	0.4686	0.5526	0.0791
C18	0.8950	0.6648	0.2706	0.3971	1.1458	0.1934
C19	0.8385	0.4917	0.4920	0.3626	1.2964	0.3869
C20	0.8411	-0.0722	0.4536	0.3657	0.7248	0.5103
C22	0.9744	0.2457	-0.0382	0.4651	0.4391	0.0100
N	1.0533	0.2498	0.0953	0.5466	0.5426	0.1331
O2	1.3050	0.7101	0.2018	0.8066	0.9500	0.0999
O3	0.9427	0.6152	0.3446	0.4666	1.1714	0.2610
O4	0.8149	0.4403	0.4131	0.3417	1.1269	0.3629
O5	0.8316	0.1051	0.4373	0.3656	0.8736	0.4649
O6	0.8932	0.2458	0.0698	0.4018	0.6466	0.0846
OW1	0.2262	0.2171	0.0320			
OW2	0.7118	0.3958	0.0201			

*C7, C21, O1 are not used in atomic numbering to be consistent with other derivatives. OW1 and OW2 refer to water oxygen atoms. Estimated standard deviations are 0.0002, 0.0005 and 0.0002 for x, y and z coordinates respectively.

C5-C6 bond. Whereas colchicine may be described as a roughly planar 7-membered ring single-bonded to a planar 6-membered ring with a twist of 52°, N-acetylcolchinol may be described as two planar 6-membered rings twisted by

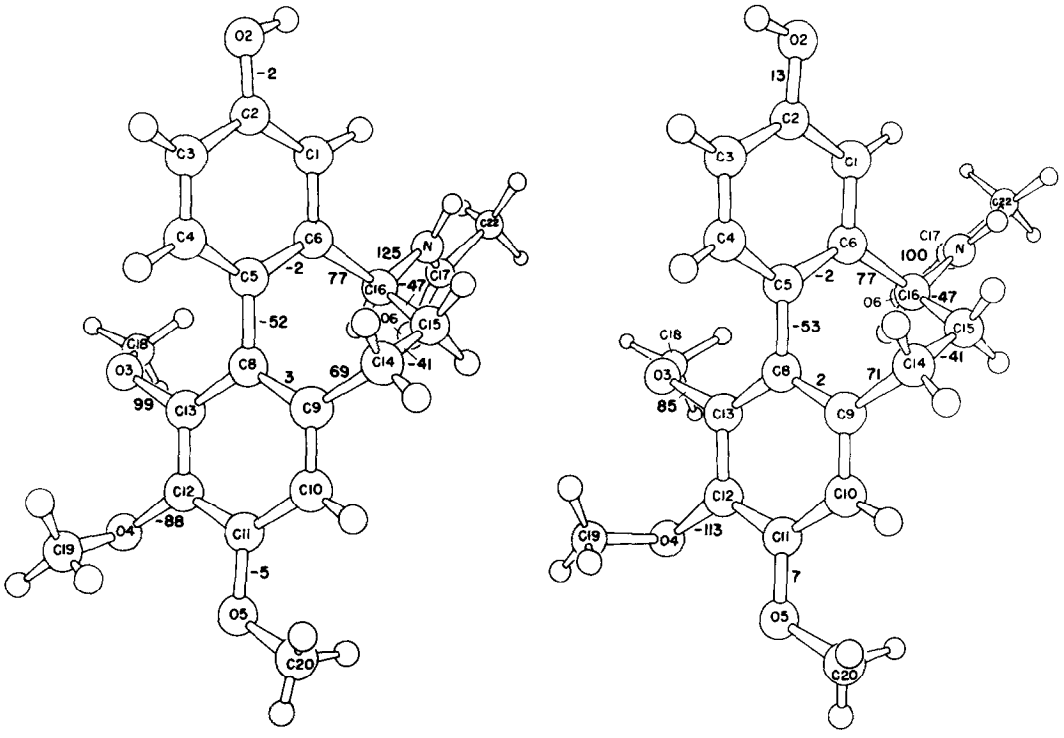


Figure 1. The 2 independent molecules of N-acetylcolchinol with selected torsion angles.

54°. The orientations of the methoxy groups on Ring A are also described by torsion angles (Fig. 1). While the C18 methoxy groups point in the same direction in colchicine and N-acetylcolchinol, the middle group, C19, points in the opposite direction. The arrangement of methoxy groups found in N-acetylcolchinol is, however, exactly like that of the active derivative demecolcine (Colcemid).

The crystal structure (Fig. 2) shows features typical of colchicine and its derivatives. The molecules are held together by a complicated network of hydrogen-bonds in the part of the unit cell near Z = 0. These hydrogen-bonds involve the $-NHCOCH_3$ group, the -OH group, and water of crystallization. At $Z = \frac{1}{2}$ is a region in which the trimethoxy rings come together in a hydrophobic region of the crystal. Similar regions, H-bonding and hydrophobic, are found in crystals of <u>every</u> other colchicine derivative studies in this laboratory.

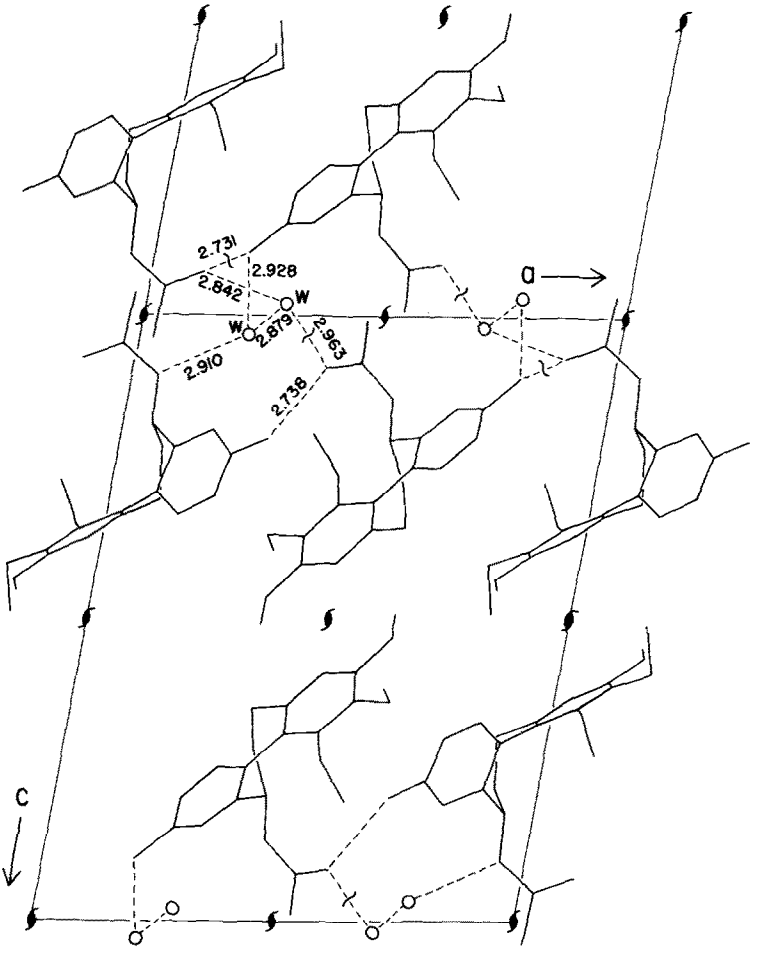


Figure 2. The crystal structure of N-acetylcolchinol. Hydrogen-bond distances given in Å.

N-Acetylcolchinol as well as other colchicine derivatives thus show two ways in which they interact with other molecules--by hydrogen-bonds involving Ring C and the acetyl group, and by van der Waals or hydrophobic interactions involving the trimethoxyphenyl ring. The structure of N-acetylcolchinol suggests that hydrogen-bonding must not be dismissed as a method of binding to other molecules e.g. tubulin. In Fig. 3 a molecule of N-acetylcolchinol is shown superimposed on a molecule of colchicine projected in the same direction. Aside from demonstrating the similarities of structure described above this drawing shows that the -OH group of N-acetylcolchinol falls precisely

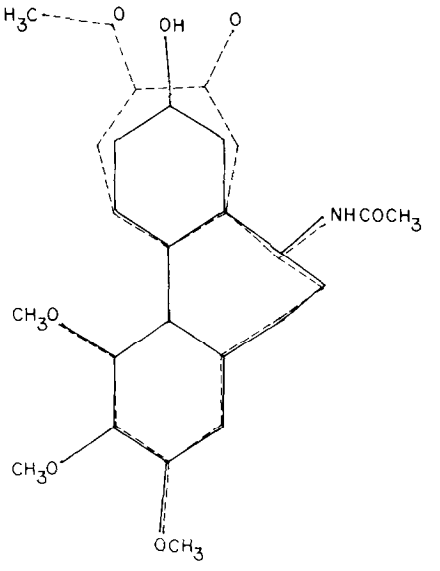


Figure 3. A molecule of N-acetylcolchinol (solid lines) superimposed on a molecule of colchicine (dashed lines).

between the carbonyl and methoxy groups of colchicine. In several structures, including colchicine (5), we have found bifurcated hydrogen-bonds with the donor atom almost equidistant from O1 and O2. The -OH group of N-acetylcolchinol is in a perfect position to receive a hydrogen-bond from such a donor. If colchicine should bind to tubulin by hydrophobic interactions with Ring A and a hydrogen-bond to Ring C, then N-acetylcolchinol has perfect geometry to bind in exactly the same way.

ACKNOWLEDGEMENT

This investigation was supported by Public Health Service Research Grant No. CA-17436 from the National Cancer Institute. We are indebted to Dr. G. E. Ullyot of Smith, Kline and French for his generous gift of a sample of N-acetylcolchinol and to the staff of the University of Massachusetts Computer Center for their assistance.

REFERENCES

1. Eigsti, O. J. and Dustin, P. Jr. (1955) "Colchicine-in Agriculture, Medicine, Biology, and Chemistry," Ames, Iowa, Iowa State College Press.

2. Zweig, M. H. and Chignell, C. F. (1973) Biochemical Pharmacology, 22, 2141-2150.

3. Wilson, L., (1975) Ann. N.Y. Acad. Sci., 253, 213-231.

4. Cortese, F., Bhattacharyya, B. and Wolff, J. (1977) J. Biol. Chem., 252, 1134-1140.

5. Lessinger, L. and Margulis, T. N. (1978) Acta Cryst. B34, 578-584.

6. Koerntgen, C. and Margulis, T. N. (1977) J. Pharm. Sci., 66, 1127-1131.