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CONFORMATIONAL ANALYSIS OF COLCHICINE AND ISOCOLCHICINE BY MOLECULAR MECHANICS*

William A. Donaldson
Department of Chemistry, Marquette University, Milwaukee, WI 53233 USA

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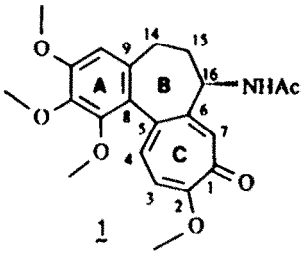
SUMMARY: The structures of isocolchicine (3) and colchicine (1) have been calculated using the MMX routine. The low energy conformations for isocolchicine and colchicine fit well with x-ray crystallographic data. The B ring atropisomer of isocolchicine, which can be spectroscopically observed, is calculated to be <1 kcal/mole higher in energy than 3. The boat-boat inversion conformer of colchicine, which has been predicted to be important in the binding of 1 to tubulin, is also calculated. The B ring geometry of this isomer does not differ to the extent previously predicted.

The tricyclic alkaloid colchicine (1) is the active principle of the toxic meadow saffron (*colchicum automnale*).[1] While colchicine had been isolated as early as the 1800's, the correct tropolonic nature of the C ring was not proposed until 1945 by Dewar.[2] Colchicine has been used as a gout suppressant and more recently as a treatment for glaucoma[3] and in cancer research as an antimitotic agent.[4] These desirable biological properties derive from the slow irreversible 1:1 binding of colchicine to the tubulin protein, which inhibits *in vivo* microtubule formation.[5] Experimental evidence has established that there are two distinct binding sites which individually recognize the A and the C rings of colchicine.[6] For example, either 2-methoxytropone or 1,2,3-trimethoxybenzene will weakly bind to tubulin. Furthermore, 2-methoxy-5-(2',3',4'-trimethoxyphenyl)tropone (2) binds rapidly and reversibly to tubulin.[7] Modification of the B ring *N*-acetamido functionality alters the toxicity as well as the antimitotic activity,[8] however the exact nature of the B ring's contribution to the tubulin binding site is unknown.



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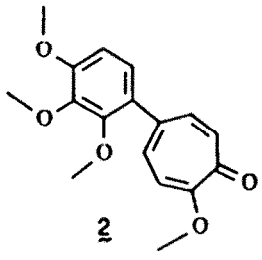
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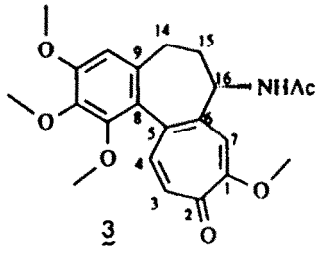

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It has been shown that the dimeric tubulin protein undergoes a conformational (secondary structural) change upon colchicine binding.[9] In addition, the binding to tubulin has been proposed to cause a conformational change for colchicine which involves a boat-boat inversion of the tropolonic C ring.[10] The bound conformational structure has been predicted to exhibit a decrease in the dihedral angle between the A and C rings from 53° to ≤19°, based on examination of Drieding molecular models. The observed increase in fluorescence and decrease in the circular dichroism spectrum of colchicine upon tubulin binding are consistent with this prediction. The slower rate for the binding of 1 compared to the binding for 2 has been attributed to the greater barrier for this conformational change in the tricyclic skeleton of 1. The energy difference between the unbound and bound conformations of colchicine has been estimated to be ≥3 kcal/mole due to unfavorable steric interactions between the C4 hydrogen and the C13 methoxy substituent.[10] In relation to our synthetic efforts toward the preparation of colchicine and suitable analogs, we were interested in modeling this proposed conformational change.

*Dedicated to Professor Michael J.S. Dewar on the occasion of his 70th birthday.

RESULTS AND DISCUSSION

Since MM2 calculations will not correctly predict geometries or energies for structures containing non-planar conjugated double bonds, the MMX program, involving a non-planar pi subroutine, was used.[11] In order to gain confidence in this programs's ability to model realistic geometries and energies, the structure of the biologically inactive isocolchicine (*3*) was first attempted. The x-ray crystal structure of *3* is known,[12] and an additional minor conformation of isocolchicine (ie. *3'*), differing by rotation about the biaryl bond, has been spectroscopically observed.[13] From the ratio of atropisomers *3* and *3'* the energy difference has been approximated to be 1.0-1.3 kcal/mole.

The x-ray analysis of *3* resulted in two structures, *3A* and *3B*, which differ primarily in the orientation of the A ring methoxy substituents and the extent of the puckering of the tropolonic C ring.[12] Molecular mechanics calculations generate two similar structures, *3C* and *3D* (Figure 1). The literature and MMX calculated B and C ring dihedral angles for structures *3A-D* appear in Table I.[14] A comparison of the two matching structures (*3A* with *3C* and *3B* with *3D*) indicates that the A and B ring calculated geometries fit well with the x-ray crystallographic data; notably the torsional angles defining the central B ring are nearly identical. The tropolonic C ring geometries are also comparable, however the MMX calculations predict a ring with more alternant single and double bond character, and consequently *greater C ring puckering* than is observed in the crystalline state.

The geometry of the isocolchicine atropisomer (*3'*) was calculated and the B and C ring torsional angles appear in Table I (*3E*).[15] Since this isomer has not been experimentally isolated it is only possible to compare this structure with NMR spectral data.[13] The literature B ring vicinal coupling constants and those calculated by PCMODEL (from dihedral angles[16]) appear in Table II. While the calculated and observed coupling constants for the isocolchicine atropisomer are not an identical match, they are of the correct magnitude. Thus structure *3E* is consistent with the spectroscopic data.

TABLE I.
Geometric Parameters for Isocolchicine[a]

TORSIONAL ANGLES	*3A*	*3B*	*3C*	*3D*	*3E*
C9-C8-C5-C6	+53	+57	+50	+52	-52
C8-C5-C6-C16	+6	+4	+7	+5	-2
C5-C6-C16-C15	-78	-79	-80	-80	+74
C6-C16-C15-C14	+44	+46	+46	+46	-43
C16-C15-C14-C9	+44	+42	+42	+42	-44
C15-C14-C9-C8	-68	-67	-71	-70	+71
C14-C9-C8-C5	-7	-9	-1	-4	+2
C1-C2-C3-C4	-1	-12	-37	-37	+41
C2-C3-C4-C5	-1	-2	-4	-4	+4
C3-C4-C5-C6	+4	+8	+22	+22	-26
C4-C5-C6-C7	-3	+2	+7	+6	-1
C5-C6-C7-C1	0	-9	-31	-30	+28
C6-C7-C1-C2	+3	-1	+4	+4	-3
C7-C1-C2-C3	-1	+13	+39	+38	-41
C6-C16-N-C17	+80	106	+68	+70	+73
ENERGY (calc., kcal/mole)			53.31	54.50	53.74
EQUIL. PERCENT (25°C)			62.1%	8.1%	29.8%

[a]Values for *3A* and *3B* taken from Ref. 12.

TABLE III.
Geometric Parameters for Colchicine[a]

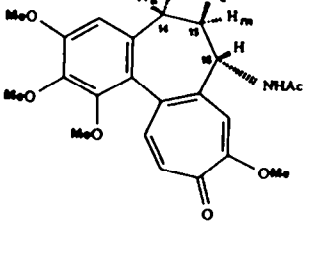
TORSIONAL ANGLES	*1A*	*1B*	*1C*	*1D*	*1E*
C9-C8-C5-C6	+53	+53	+57	+60	+49
C8-C5-C6-C16	+5	+5	0	+5	+13
C5-C6-C16-C15	-79	-81	-77	-73	-84
C6-C16-C15-C14	+48	+49	+48	+49	+44
C16-C15-C14-C9	+43	+42	+42	+41	+46
C15-C14-C9-C8	-73	-70	-71	-71	-70
C14-C9-C8-C5	-4	-5	-5	-5	-6
C1-C2-C3-C4	+1	-4	+1	+1	-1
C2-C3-C4-C5	+4	+10	+3	+12	-23
C3-C4-C5-C6	-1	-1	0	0	+5
C4-C5-C6-C7	-2	-7	-3	-11	+19
C5-C6-C7-C1	-3	+2	-3	+2	-4
C6-C7-C1-C2	+9	+8	+11	+24	-32
C7-C1-C2-C3	-8	-6	-9	-23	+35
C6-C16-N-C17	-88	-86	-78	-82	-78
ENERGY (calc., kcal/mole)			55.39	55.38	54.55

[a]Values for *1A* and *1B* taken from Ref. 17.

TABLE II. Coupling constants (Hz) of isocolchicines.

compd	$J_{14a,15c}$	$J_{14a,15m}$	$J_{14b,15c}$	$J_{14b,15m}$	$J_{15c,16}$	$J_{15m,16}$
3[a]	6.5	ca. 0	13.0	6.5	6.5	12.5
3C[b]	6.4	1.0	13.4	6.6	5.4	11.0
3D[b]	6.4	1.0	13.4	6.6	5.3	11.0
3'[a]	7.5	c	ca. 0	c	7.5	ca. 0
3E[b]	6.2	13.0	1.2	6.3	5.2	1.2

[a]Ref. 13; [b]calculated from dihedral angle, Ref. 14; [c]obscured by overlapping signals.



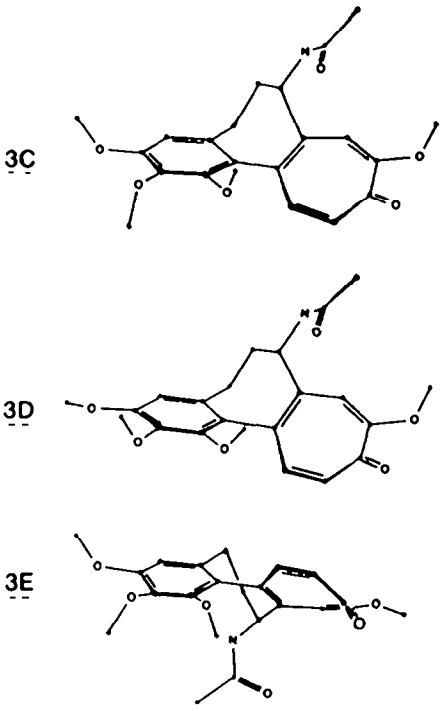


Figure 1. Graphical representations of the MMX calculated geometries for isocolchicine and isocolchicine atropisomer.

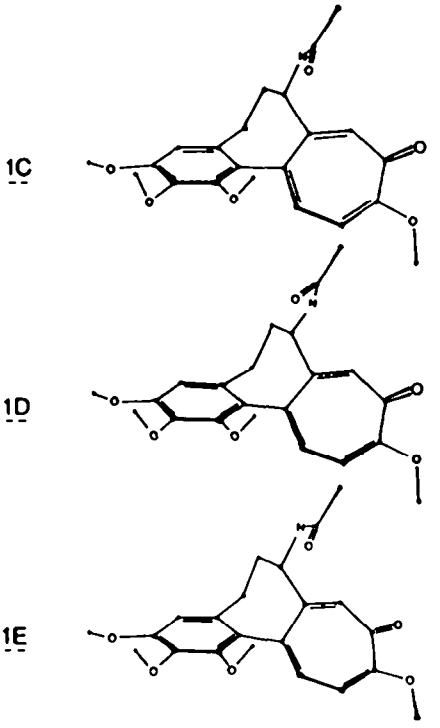


Figure 2. Graphical representations of the MMX calculated geometries for colchicine.

The calculated energies and populations at 25°C for *3C*, *3D*, and *3E* appear in Table I. Since the structures *3C* and *3D* are derived from the same biaryl atropisomer (ie. *3*), the predicted ratio of *3*:*3'* is 7:3 (lit.[13] 10:1). While the predicted ratio of *3*:*3'* is slightly less than the observed value, this ratio along with the geometry match indicate that the MMX calculations reasonably model reality for the isocolchicine case. With these results in hand, we could confidently approach the colchicine structural solution.

The x-ray analysis of colchicine results in two structures, *1A* and *1B*, which differ from each other primarily in the pucker of the C ring.[17] Molecular mechanics calculations generate two similar structures, *1C* and *1D* (Figure 2). The literature and MMX calculated B and C ring dihedral angles appear in Table III.[14] The calculated geometries for the A and B rings fit well with the crystal structure data; notably the B ring torsional angles of *1C* are nearly identical with the x-ray structure *1A*. The tropolonic C ring geometries are comparable, and again MMX predicts structures with greater tropolonic ring pucker.

The structure of the heretofore unobserved, yet predicted, colchicine boat-boat inversion conformer was likewise calculated. The B and C ring dihedral angles appear in Table III (*1E*).[14] As predicted, this boat-boat inversion causes a decrease in the dihedral angle between the A and C rings. *However, the A-C dihedral angle of 3E (49°) is considerably greater than that predicted[10] from Drieding molecular models (19°). We believe that the calculated geometry more closely approximates the structure of this conformer, since the MMX calculations will consider bond lengths and angles which are not ideal (ie. non-120° sp^2 hybridized carbon).*[18]

It should be noted that the calculated torsional angles defining the B ring of *1E* are reasonably close to those found in *1A-D*. The similarity between B ring dihedral angles of colchicine, its derivatives, and isocolchicine has previously been reported.[12] On these grounds, it would appear that the magnitude of the dihedral angle change in the boat-boat colchicine conformer (*1'*) has previously been overstated. Considering the numerous citations of this proposed conformational change[19], alternative explanations for the increased fluorescence and decrease in circular dichroism of colchicine upon binding to tubulin should be considered. The increase in fluorescence may be due to a decrease in collisions between colchicine and quenching molecules, such as solvent, upon tight binding with the receptor site on the tubulin protein.[20]

In addition, the decrease in the circular dichroism spectrum of colchicine might be due to cancellation resulting from the intrinsic asymmetry of tubulin.[10]

The calculated energies for *1C*, *1D*, and *1E* appear in Table III. While the energies of *1C* and *1D* are nearly identical, the boat-boat conformer *1E* is predicted to be 0.83 kcal/mole *lower* in energy. Therefore, this conformer should be spectroscopically observable. However, the B ring dihedral angles for *1C-E* are not greatly different and it would be difficult to distinguish among these structural possibilities on the basis of NMR coupling data. In addition, the transition state for the *1* ⇌ *1'* equilibrium is the structure with a planar C ring. Since the C ring in the crystal structure is already nearly planar (within 0.06 Å for *1A* and 0.07 Å for *1B*) the activation energy for this conformational isomerization is probably not large. Thus the NMR spectrum of colchicine should be a weighted average of the spectra for the two conformers and it is not surprising that a second distinct solution conformer of colchicine has not been, and probably will not be observed.[21]

In view of the calculated accessibility of the colchicine boat-boat conformer (*1E*), it would seem prudent to consider both the x-ray crystal structure as well as calculated structure *1E* in molecular modeling of the binding site on tubulin.[22] Most importantly, the position of the tropolonic carbonyl oxygen, a functionality which is known to be crucial for tubulin binding,[8] changes by about 1.2-1.4 Å in the isomerisation of conformer *1C* to conformer *1E*.

In summary, we have found MMX molecular mechanics suitable for the modeling of the tropolonic compounds colchicine and isocolchicine. One important result of these calculations is that the boat-boat inversion isomer of colchicine does not have as large a decrease in the A-C biaryl bond dihedral angle as previously predicted[10] and that this isomer should be energetically accessible.

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