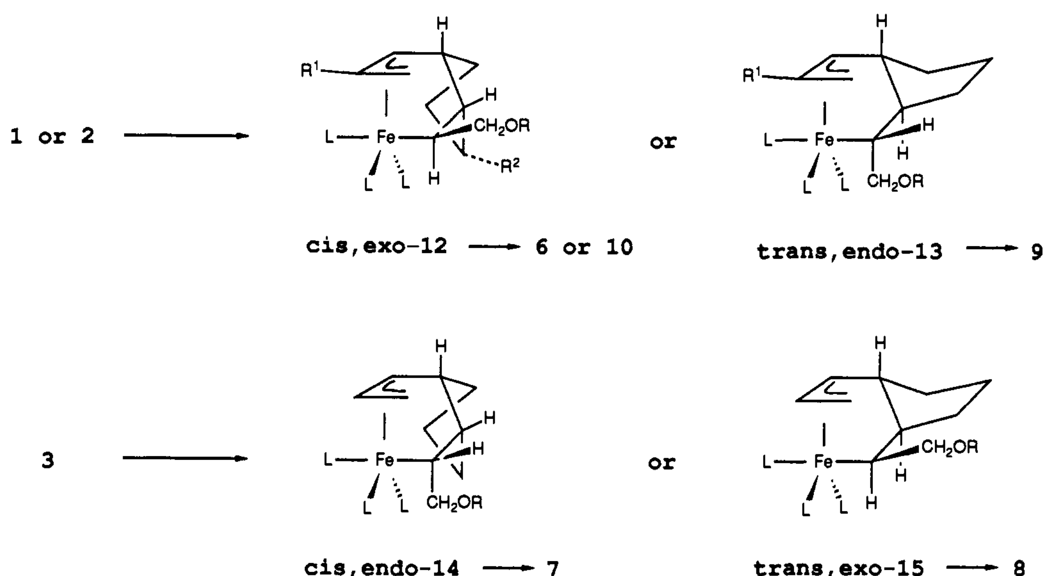
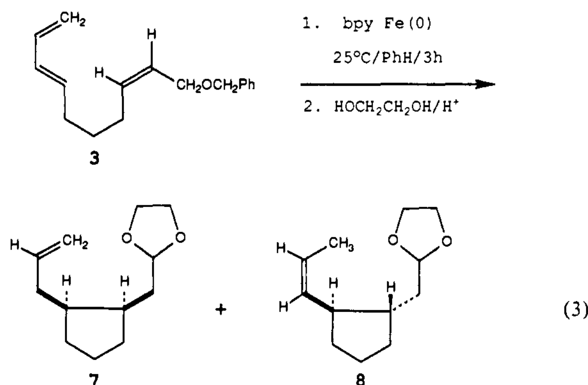
Scheme I. Possible Diastereomeric Intermediates Formed in the Cyclizations of Trienes 1-3



On the basis of our previous mechanistic studies, we anticipated that changing the geometry of the olefin at the 2-position of triene **1** might enable us to exercise a degree of stereocontrol in the cyclization. Accordingly, the 2*Z*,7*E*-triene ether **3** was prepared starting from propargyl alcohol.[8] The bpy·Fe(0)-catalyzed carbocyclization yields after acetalization a 96:4 mixture of two isomeric cyclopentanes (eq 3). The major product **8** is again a



formal [4 + 4] ene product but now is the isomer possessing the trans relative stereochemistry. The minor component is the cis [4 + 2] ene product **7**. Less than 0.2% of the cis [4 + 4] isomer **6** is produced. Thus either *cis*- or *trans*-cyclopentane derivatives can be prepared with high selectivity depending upon the choice of starting olefin geometry.

It has been previously noted that transannular-cyclization processes often proceed with higher stereo- and regioselectivity than simple cyclization processes.[14] The cyclizations of triene ethers **1-3** proceed via the transannular carbon-carbon bond-forming oxidative-coupling reaction of a chelated L·Fe(0)-(diene olefin) complex.[4] The unusual regio- and diastereoselectivity that we observe in the reactions of **1-3** can be accounted for by the selective formation of intermediates **12** (R^1, R^2 = H, CH_3) and **15**. These results suggest an apparent preference for pseudo-exo orientation of the benzyloxymethyl group during the five-membered ring formation (Scheme I). The stereoinduction observed in the cyclization of **2** can be rationalized by placing the methyl substituent (R^2) in the least sterically encumbered position in the intermediate *cis, exo*-**12**. Further mechanistic studies and synthetic applications of this new catalytic carbocyclization procedure are in progress.

Acknowledgment. We thank the National Institutes of Health (GM 34927), the donors of the Petroleum Research Fund, administered by the American Chemical Society, and the University of Utah Research Committee for generous support of this work. We thank Mark W. Creswell and Beckie E. Takacs for assistance in preparing the triene ethers. NMR spectra were obtained by using 300-mHz spectrometers purchased with funds provided by National Science Foundation and Department of Defense Instrumentation Grants.

(14) Delongschamp, P. *Aldrichimica Acta* **1984**, *17*, 59-65.

Resonance Raman Spectra of Colchicinoids: Free and Bound to Tubulin

Richard P. Rava,* Susan Bane Hastie,* and James C. Myslik

Department of Chemistry
State University of New York at Binghamton
Binghamton, New York 13901

Received November 6, 1986

Colchicine binds specifically and with high affinity to tubulin, the major protein subunit of microtubules. The colchicine-tubulin complex substoichiometrically inhibits normal assembly of tubulin into microtubules, resulting in interference with or cessation of normal microtubule-mediated processes, including cell motility, secretion, and growth.[1] The colchicine-tubulin interaction has been extensively investigated since its discovery by Taylor and co-workers in the mid 1960s;[2] however, an understanding of the interaction on a molecular level remains elusive. Two different mechanisms have been proposed to describe the colchicine-tubulin interaction. The first, focusing primarily on the kinetic and spectroscopic features of the association, proposes that a boat-boat interconversion in the colchicine C ring occurs upon binding to tubulin.[3] The second mechanism, based on thermodynamic data, describes colchicine binding in terms of the binding of a bifunctional ligand to two subsites of the protein, requiring protein but

(1) For reviews of the colchicine-tubulin interaction, see: (a) Capraro, H. G.; Brossi, A. In *The Alkaloids*; Brossi, A., Ed.; Academic: New York, 1984; Vol. 23, pp 1-70. (b) Luduena, R. F. In *Microtubules*; Roberts, K., Hyams, J. S., Eds.; Academic: New York, 1979; pp 66-166.
(2) Weisenberg, R. C.; Borisy, G. G.; Taylor, E. W. *Biochemistry*; **1968**, *7*, 4466 and references therein.
(3) Detrich, H. W., III; Williams, R. C., Jr.; Macdonald, T. L.; Wilson, L.; Puett, D. *Biochemistry* **1981**, *21*, 5999.

0002-7863/87/1509-2202$01.50/0 © 1987 American Chemical Society

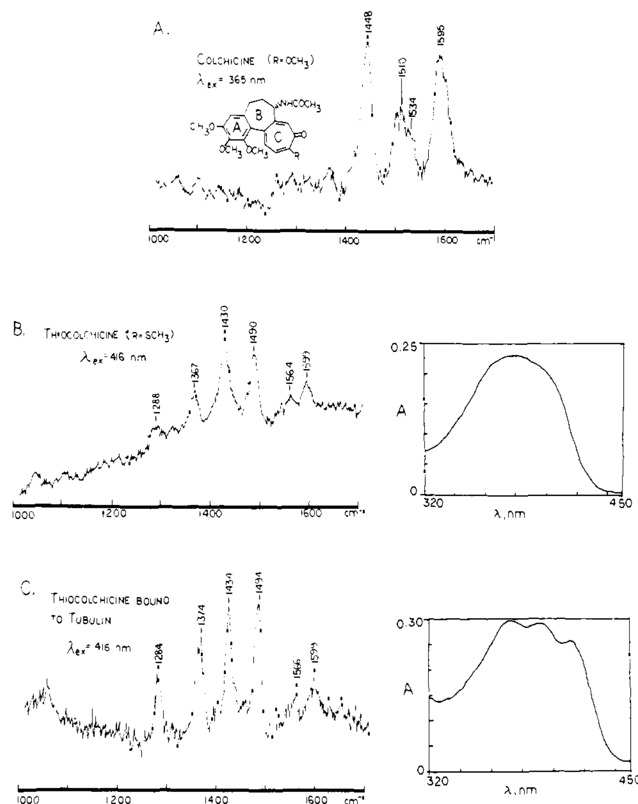


Figure 1. Resonance Raman spectra obtained by using a nitrogen-pumped dye laser (0.010–0.020 mJ/5-ns laser pulse) for excitation and a 1.0-m single spectrometer for scattered light dispersion. (A) Colchicine: 500 μM aqueous solution obtained by using 365-nm excitation. (B) Thiocolchicine: 100 μM aqueous solution obtained by using 416-nm excitation. Absorption spectrum of a 50 μM aqueous solution is shown adjacent to the Raman spectrum. (C) Thiocolchicine bound to tubulin: 60 μM in PMEG buffer (0.1 M PIPES, 1 mM $MgSO_4$, 2 mM EGTA, 0.1 mM GTP, pH 6.90) obtained by using 416-nm excitation. The complex was prepared by incubating 85 μM thiocolchicine and 65 μM tubulin in PMEG at 37 °C for 1.5 h; unbound thiocolchicine was removed from the sample by gel filtration prior to resonance Raman analysis. Absorption spectrum of the complex is shown adjacent to the Raman spectrum.

not ligand conformational changes.[4] Neither mechanism can account for all the known features of the colchicine–tubulin interaction, and the proposed colchicine conformational change has not been experimentally observed.

In order to probe the molecular details of the colchicine–tubulin interaction, we have obtained the first resonance Raman (RR) spectra of colchicine and thiocolchicine free in aqueous solution and of thiocolchicine bound to tubulin. Attempts to obtain the RR spectrum of colchicine bound to tubulin were unsuccessful because of the increased fluorescence of the drug when bound to the protein. The near ultraviolet (UV) spectrum of colchicine shows an absorption maximum at 353 nm in aqueous solution. Though the electronic properties of this transition have not been determined, we would assign this band as due to a $\pi\pi^*$ transition in the C ring, which is in partial conjugation with the rest of the drug. This assignment is supported by the near UV spectrum of thiocolchicine, whose absorption maximum shifts to the red to 380 nm. This shift would be expected from the hyperconjugative effect of the sulfur on the C ring.

The RR spectrum of 500 μM colchicine (Aldrich Chemical) in aqueous solution obtained by using an excitation wavelength of 365 nm, on the red side of the absorption maximum, is shown in Figure 1A. Previous vibrational data on these ring systems are limited to infrared studies containing very little analysis of the vibrational composition.[5] Strong bands are observed at 1448 and 1595 cm^{-1}, and a slightly weaker set of bands appears to overlap at 1510 and 1534 cm^{-1}. Additional weaker bands are observed below 1400 cm^{-1}. Preliminary analysis of these spectra by comparison to the infrared data and Raman data of structurally similar compounds suggests that the strong bands result from vibrations localized on the C ring. This would be expected if the 353-nm transition is indeed localized primarily on the C ring since RR spectra reflect the properties of the resonant excited transition.[6] The 1448- and 1510/1534-cm^{-1} vibrations probably involve C—C and C═C stretching motions in the seven-member ring, while the broad band centered at 1595 cm^{-1} contains a large component of C ring carbonyl stretching character.

Support for this preliminary analysis comes from the RR spectra of thiocolchicine (a sulfur substituted for the methoxy oxygen on the C ring) obtained from a 100 μM aqueous solution by using 416-nm excitation (Figure 1B, absorption spectrum adjacent to the Raman spectrum). The vibrational spectrum is perturbed to a greater degree than might be expected if the colchicine RR bands were due to vibrations of the A or B ring. The π electron delocalizing effect of the sulfur tends to lower the frequencies of the vibrations assigned as carbon skeletal vibrations of the C ring (e.g., 1448–1430 cm^{-1} for the strong vibrations assigned to carbon stretching modes). Also, note the strong band found in colchicine at 1595 cm^{-1} appears to have split in thiocolchicine into two weaker bands at 1599 and 1564 cm^{-1}.

The RR spectrum of thiocolchicine bound to bovine brain tubulin obtained by using 416-nm excitation is shown in Figure 1C and the absorption spectrum shown adjacent.[7] Several features of the bound drug spectrum compared to that of the free drug should be noted. The 1430-cm^{-1} band has decreased in intensity relative to the 1490-, 1367-, and 1288-cm^{-1} bands. In addition, a small frequency upshift (7 cm^{-1}) is observed for the 1367-cm^{-1} vibration while the frequencies of other vibrations remain essentially constant. Studies of thiocolchicine in carbon tetrachloride indicate these changes cannot be completely due to a change in environment hydrophilicity. Since the 1430-cm^{-1} band must involve the carbon skeletal portion of the C ring, the decreased intensity may be interpreted as indicating stacking of the C ring with other π bonds, possibly from aromatic amino acid residues in tubulin. The lack of vibrational frequency shifts is consistent with this type of binding interaction. Hydrogen bonding or covalent interactions would perturb the vibrational mode forms which would lead to frequency changes for several of the vibrations.

Absorption data also indicate an electronic perturbation upon binding of thiocolchicine as shown in Figure 1B and 1C. The near UV spectrum of the free drug is broad and diffuse, peaking at 380 nm, while the bound form contains at least three peaks at 374, 392, and 412 nm. If the excitation laser line is changed, the relative enhancement of the bands in the RR spectrum is observed to change, suggesting greater than one excited state is responsible for the enhancement.

In summary, the RR spectra of the colchicinoid drugs provide the first molecular information on the binding of these drugs to tubulin. The results suggest that stacking of thiocolchicine with tubulin aromatic amino acid residue(s) occurs and that the electronic spectra in the near UV region may be the result of several electronic transitions. It does not appear to be necessary to invoke a colchicine conformational change to explain the RR data, but additional experiments are being carried out to fix the nature of the electronic, vibrational, and conformational properties of these systems.

Acknowledgment. We thank K. M. Hahn and R. J. Sundberg for providing a sample of thiocolchicine. This work has been partially supported by the SUNY Research Foundation.

(4) Andreu, J. M.; Timasheff, S. N. *Biochemistry* **1982**, *21*, 534.
(5) Scott, G. P.; Tarbell, D. S. *J. Am. Chem. Soc.* **1950**, *72*, 240.

(6) Carey, P. R. *Biochemical Applications of Raman and Resonance Raman Spectroscopies*; Academic: New York, 1982.
(7) Both thiocolchicine and colchicine have approximately the same affinities for the colchicine binding site and in vitro antitumor activities. See: (a) Kerekes, P.; Sharma, P. N.; Brossi, A.; Chignell, C. F.; Quinn, F. R. *J. Med. Chem.* **1985**, *28*, 1204. (b) Shiau, G. T.; De, K. K.; Harmon, R. E. *J. Pharm. Sci.* **1975**, *64*, 646.