until bubbling ceased, dried over Na_2SO_4, filtered, and evaporated to give 0.13 g of solid. Recrystallization from hexane gave 0.050 g (64%) of **26** as pale orange crystals in two crops, both of which were homogeneous by TLC. An analytical sample of **26** was prepared by repeated recrystallization from hexane: mp 131–132.5 °C; IR (KBr) 2920, 1835, 1760, 1265, 1210, 1173, 890 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.25 (3 H, t, J = 8 Hz), 2.31–2.50 (2 H, m), 2.91 (2 H, t, J = 6.5 Hz), 3.00 (2 H, q, J = 8 Hz), 6.43 (1 H, dt, J = 10.2, 5 Hz), 7.25–7.31 (1 H, m), 7.28 (1 H, s); ^{13}C NMR ($CDCl_3$) δ 14.6, 22.5, 24.4, 27.5, 121.7, 123.8, 125.6, 132.2, 135.0, 135.2, 144.7, 145.0, 163.0, 163.3; MS m/e 228 (M^+), 200 185, 155 (100), 141, 128, 115; HRMS m/e calcd for $C_{14}H_{12}O_3$ 228.0786, found 228.0809.

3-Ethyl-1,2-bis(hydroxymethyl)-5,6-dihydronaphthalene (27). To a slurry of 0.127 g (3.18 mmol) of $LiAlH_4$ in 2.0 mL of dry THF under N_2 was added a solution of 0.102 g (0.446 mmol) of **26** in 8.0 mL of dry THF. The resulting mixture was heated at reflux for 3 days. The reaction mixture was cooled to room temperature and quenched with 1 mL of water while still under N_2. The mixture was extracted with ether (5 × 20 mL) and ethyl acetate (4 × 25 mL). The combined organic layers were dried over Na_2SO_4, filtered, and evaporated to give 0.080 g of orange semisolid. Flash chromatography (gradient, hexane to ether, ethyl acetate) afforded 0.038 g (39%) of **27** as a white solid. Recrystallization from ether afforded an analytical sample of **27**: mp 81–82 °C; IR (film) 3340, 3060, 2980, 2945, 2895, 1445, 1435, 1275, 1050, 1000, 745 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.19 (3 H, t, J = 7.5 Hz), 2.18–2.27 (2 H, m), 2.64–2.77 (4 H, m), 3.24 (2 H, br s), 4.71 (2 H, s), 4.74 (2 H, s), 6.07–6.16 (1 H, dt, J = 10.1, 4.4 Hz), 6.82 (1 H, d, J = 10.1 Hz), 6.95 (1 H, s); ^{13}C NMR ($CDCl_3$) δ 16.4, 22.6, 26.6, 28.4, 58.2, 58.6, 123.9, 128.6, 129.7, 131.2, 134.7, 135.6, 136.5, 141.9. Anal. Calcd for $C_{14}H_{18}O_2$: C, 77.03; H, 8.31. Found: C, 77.01; H, 8.36.

4-Ethyl-1,2,3,6,7,8-hexahydro-2-phenylcyclohept[*e*]isoindole (28). The following procedure was adapted from that of Oppolzer et al.[16] A slurry of 0.031 g (0.814 mmol) of $LiAlH_4$ in 0.8 mL of dry ether and a mixture of 0.046 g (0.341 mmol) of aluminum trichloride in 0.3 mL of dry ether were combined at 0 °C under N_2 and stirred for several minutes. To this mixture was added dropwise a solution of 0.050 g (0.16 mmol) of **13** in 5 mL of dry ether. The reaction mixture was stirred for 30 min while warming to room temperature and was then heated at reflux for 3 h. The mixture was cooled to room temperature and quenched with saturated Na_2SO_4 while under N_2. The aqueous layer was separated and filtered. The filtrate was extracted with ether (3 × 20 mL), and the combined organic layers were dried over Na_2SO_4, filtered, and evaporated to afford 0.048 g of a white solid which discolored when dissolved in CH_2Cl_2. The crude material was subjected to flash chromatography (hexane, 5:1 hexane–ether) which afforded 0.017 g (39%) of **28** as a pale yellow semisolid that was still nonhomogeneous by TLC. Preparative TLC (4:1 hexane–ether) followed by recrystallization from ether afforded **28**: mp 105.5–107 °C; IR (film) 3020, 2920, 1605, 1505, 1465, 1375, 1260, 1005 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.27 (3 H, t, J = 8 Hz), 1.92–2.07 (2 H, m), 2.36–2.49 (2 H, m), 2.62 (2 H, q, J = 8 Hz), 2.77–2.90 (2 H, m), 4.60 (2 H, s), 4.65 (2 H, s), 5.94–6.06 (1 H, dt, J = 12, 3.6 Hz), 6.47 (1 H, d, J = 12 Hz), 6.60–6.84 (3 H, m), 6.91 (1 H, s), 7.24–7.39 (2 H, m); HRMS m/e calcd for $C_{21}H_{23}N$ 289.1830, found 289.1788.

1*H*-4-(Dideuterioethyl)-9-deuterio-6,7-dihydro-2-phenylbenz[*e*]isoindole-1,3-dione (29). To a solution of 1.66 g (12.0 mmol) of potassium carbonate in 16 mL of D_2O was added 1.0 g (6.0 mmol) of **6**. The resulting mixture was heated at reflux under N_2 for 5 days and then cooled to room temperature and extracted with ether (4 × 50 mL). The separated aqueous layer was acidified with 2 mL of 37% DCl and was then reextracted with ether (5 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered, and evaporated to give 1.09 g of a mixture of orange oil and white solid. Flash chromatography (gradient, hexane to 4:1 ether–hexane) afforded 0.186 g (18%) of deuterated **6** as a clear oil: IR (film) 2940, 2870, 1780, 1712, 1455, 1295, 1250, 1110, 1055 cm^{-1}, ^1H NMR ($CDCl_3$) δ 1.4–2.5 (m), lacking the resonances at lower field than δ 2.5 characteristic of **6**.[3] To a solution of 0.18 g (1.1 mmol) of the deuterated **6** in 20 mL of dry toluene were added 0.052 g (0.27 mmol) of p-TsOH·H_2O and 1.77 g (10.2 mmol) of **11**. The resulting solution was heated at reflux under N_2 and a Dean–Stark trap for 6 days. After this time an additional 0.026 g (0.13 mmol) of p-TsOH·H_2O was added, and reflux was maintained for 18 h. The reaction mixture was cooled to room temperature, combined with 75 mL of CH_2Cl_2, washed with 20 mL of saturated Na_2CO_3 and 20 mL of brine, dried over Na_2SO_4, filtered, and evaporated to give 0.12 g (39%) of **29** as a white solid which was recrystallized from ether: mp 130–131 °C, IR (film) 2900, 1760, 1700, 1505, 1400, 1120 cm^{-1}; ^1H NMR ($CDCl_3$) 1.13–1.28 (2 H, m), 2.35 (2 H, m), 2.84 (2 H, m), 2.97–3.08 (1 H, m), 6.27 (1 H, m), 7.19 (1 H, s), 7.26–7.47 (5 H, m).

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Synthesis, Photochemical Decomposition, and Tubulin Binding of 10-Azido-10-demethoxycolchicine and 9-Azido-9-demethoxyisocolchicine[1]

Marianne E. Staretz and Susan Bane Hastie*

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

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Introduction

A variety of natural products inhibit cellular division by interfering with the normal assembly of microtubules. Many of these compounds (podophyllotoxin, stegnacin, combretastatin, and colchicine) exert their biological effects by binding to tubulin, a 100 000 Da protein that composes the central core of the microtubule and consists of two similar but nonidentical subunits designated as α and β.[2] The identity of the major drug binding site, known as the colchicine binding site, on the linear sequence of tubulin has been sought using affinity[3] and photoaffinity labeling colchicine derivatives.[4] In both of the photoaffinity labeling colchicine derivatives so far examined, the photolabile group is an aromatic azide attached to the C-7 position of the B ring.[4]

It has been shown that the entire B ring of colchicine participates only minimally in the thermodynamic stability of the colchicinoid–tubulin complex,[5] thus, photolabile

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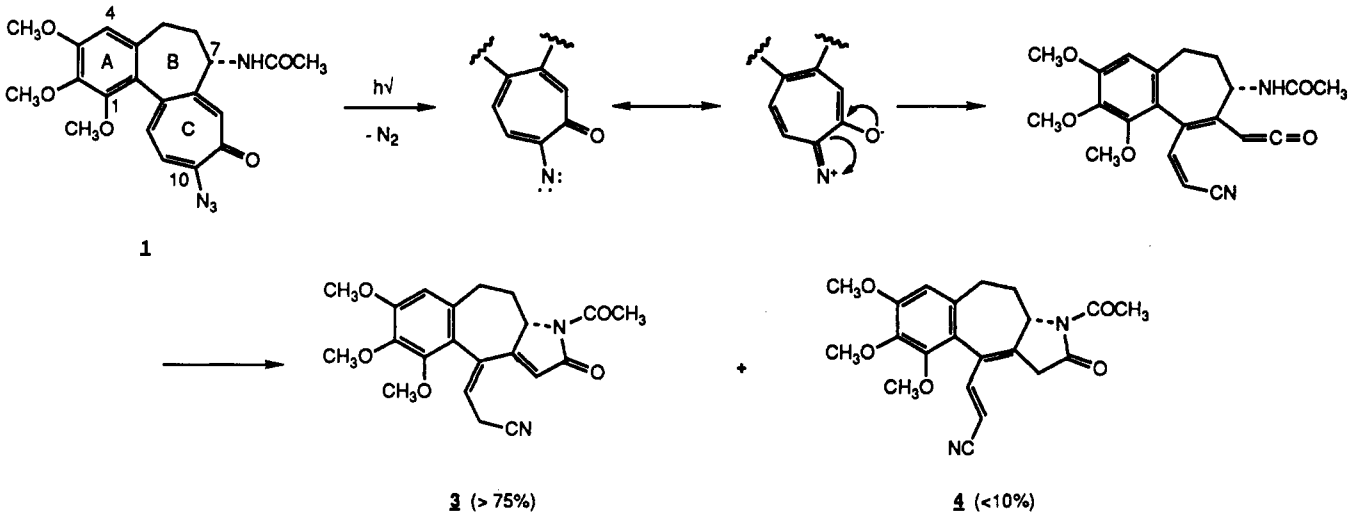
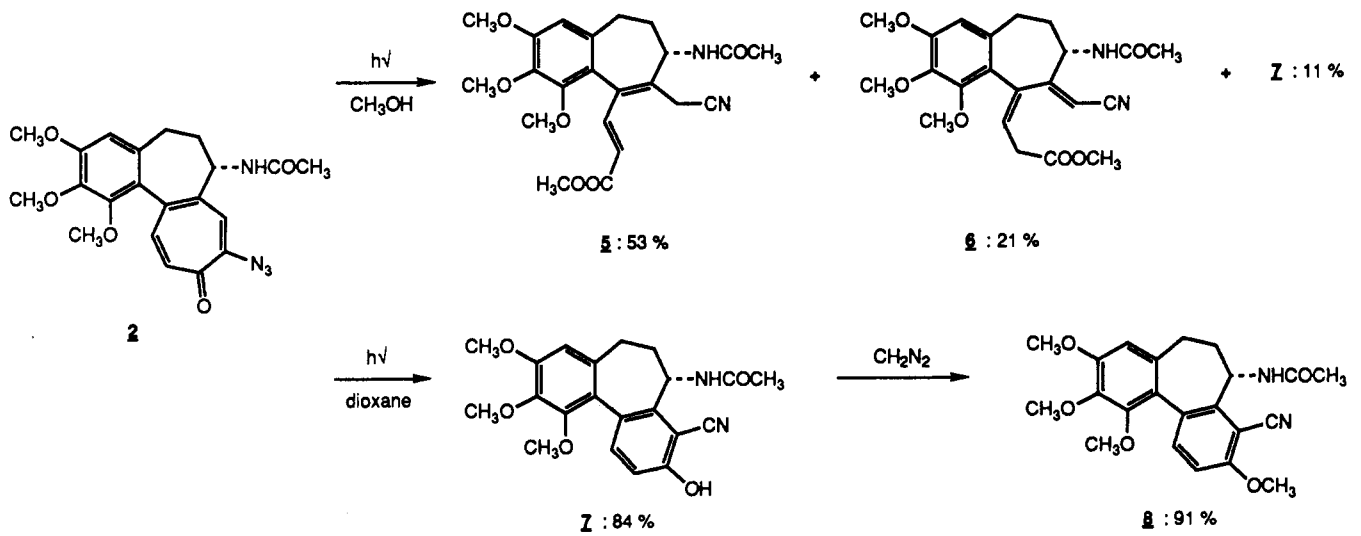
Scheme I



Scheme II



groups attached to the B ring may not be contained in the interior of the colchicine binding site. We are attempting to locate the colchicine binding site on tubulin through the use of photoaffinity labeling colchicine derivatives in which the photolabile group is attached to the A or C ring, since these two rings appear to comprise the minimum structural element necessary for high affinity binding to tubulin.[5] In this report, we describe the synthesis and photochemical decomposition of a potential C ring photoaffinity label, 10-azido-10-demethoxycolchicine (1), and the biochemically inactive analogue 9-azido-9-demethoxyisocolchicine (2). The possible use of 1 as a photoaffinity label for tubulin is also explored.

Results and Discussion

The C-ring azido derivatives of colchicine and isocolchicine were prepared in three steps from colchicine. Colchicine was converted to the tropolone derivative colchiceine by mild acidic hydrolysis.[6] Treatment of colchiceine with tosyl chloride resulted in the tosylated forms of the isomers, which are easily separated by chromatography. Each of the isomers was treated with sodium azide to produce the α-azido derivatives in good yield. The tosylated isomers were found to be convenient intermediates for preparing a variety of other α-substituted derivatives. These compounds will be detailed in a separate report.

Compound 1 retains high affinity for the colchicine site on tubulin, as it is a potent competitive inhibitor of colchicine binding to tubulin (K_I = 2.7 μM, K_I = 4.2 μM for colchicine). Photolysis of 1 in either methanol or dioxane resulted in the formation of 3 and a small amount of 4. These products are apparently formed by trapping of an intermediate ketene by the amide nitrogen (Scheme I). The scheme for photolysis of the azidocolchicine derivative involving the formation of an intermediate ketene is based on the results obtained from the photolysis of 1 and 2 and of 2-azidotropone.[7] The products obtained from the photolysis of 1 were found to be independent of the nature of the solvent. The reaction of the ketene with the amide nitrogen presumably occurs quite readily because of their close proximity, thus preventing reaction with other nucleophiles that may be present.

The products obtained from the photolysis of compound 2 were found to be dependent on the nature of the solvent used in the photolysis procedure (Scheme II). In a nucleophilic solvent such as methanol, a conjugated ester 5 and a conjugated nitrile 6 were the major products,

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Table I. Inhibition of [^{3}H]Colchicine Binding to Tubulin by 10-Azido-10-demethoxycolchicine, 9-Azido-9-demethoxyisocolchicine, and Photoproducts

ligand	% inhibition[a]	ligand	% inhibition[a]
colchicine	86	3–6	0
1	94	7	40
1 +. $h\nu$[b]	6	8	94
2	0		

[a] Relative to control without added ligand (see the Experimental Section). [b] Photolyzed for 20 min at room temperature prior to incubation with [^{3}H]colchicine.

probably as a result of attack of the solvent nucleophile on the intermediate ketene. When the solvent was not nucleophilic, an aromatic product **7** which is apparently due to a Cope cyclization of the ketene intermediate was the sole product. This product **7** exists as two atropisomers in approximately a 2:1 ratio. Atropisomers of a similar nature have also been observed with other colchicine derivatives.[1a,8,9] The products from the photolysis of **2** are similar to those expected based on the photochemical and thermal decomposition of 2-azidotropone.[7] In the case of the azidocolchicine and azidoisocolchicine derivatives, however, the products possessing conjugation with the carbonyl predominate, while in the tropone derivative products possessing conjugation with the nitrile predominate.

With the exception of **7** the photolysis products do not bind to the colchicine site on tubulin (Table I). To indirectly assess the extent of covalent incorporation of **1** into tubulin, **1** was irradiated in the presence of tubulin. A small but reproducible inhibition of [^{3}H]colchicine binding was observed after photolysis, indicating that a small amount of the ligand may be covalently incorporated into tubulin. Confirmation of covalent bond formation requires a radioactive **1**, the synthesis of which is currently being pursued in our laboratory.

Alkylation of the azidoisocolchicine photoproduct **7** with diazomethane gave **8**, a derivative of *N*-acetylcolchicinol methyl ether, in an overall yield of 37% from colchicine. Despite the additional C-ring substituent, this compound retains high tubulin-binding activity (Table I). Thus, the isocolchicine derivative **2** is a convenient precursor for entry into the colchicinol skeleton. The colchicinol product can be further derivatized to obtain products of high biological activity and will be useful for future structure–activity studies of colchicinol-based drugs.

Experimental Section

Melting points were determined with an Electrothermal IA6304 open-capillary melting point apparatus and are uncorrected. Infrared (IR) spectra were measured in chloroform on a Perkin-Elmer 1420 ratio recording infrared spectrophotometer. ^{1}H NMR spectra and ^{13}C spectra were recorded on a Brucker AM 360 spectrometer. ^{1}H NMR spectra were recorded in $CDCl_3$ with Me_4Si as internal standard. ^{13}C NMR were recorded in $CDCl_3$ ($CDCl_3$ δ 77.0 as internal standard). Low-resolution mass spectra (LRMS) were obtained on a Hewlett-Packard 5995 gas chromatograph/mass spectrometer. High-resolution mass spectra (HRMS) were recorded on a VG 70-70 spectrometer at an ionization voltage of 16 eV. Optical rotations were measured with a Perkin-Elmer Model 243B polarimeter in $CHCl_3$ at the concentrations specified.

Column chromatography was done on Baker silica gel (60–200 mesh). Radial chromatography was performed on a Chromatotron using Merck silica gel PF-254 with $CaSO_4 \cdot {}^1/_2H_2O$. Silica gel plates (Merck F_{245}, 0.25 mm thickness) were used for thin-layer chromatography. Colchicine was purchased from the U.S. Biochemical Corporation and was found to be >95% pure by thin-layer chromatography (TLC) and ^{1}H NMR and ^{13}C NMR spectroscopy. Spectrograde methanol (Fisher Scientific Co.) and spectrograde dioxane (Aldrich Chemical Co.) were used for the photolysis experiments. Pyridine was dried by refluxing over calcium hydride. Dimethyl sulfoxide (DMSO) was purified by distillation under reduced pressure and storage over molecular sieves. All other reagents were purchased from Aldrich Chemical Co. and were used without further purification.

Colchiceine. Colchiceine was prepared from colchicine by mild acidic hydrolysis.[6] The structure of the product was confirmed by ^{1}H NMR, IR, and mass spectral analyses.

10-Demethoxy-10-tosylcolchicine and 9-Demethoxy-9-tosylisocolchicine. The tosylated derivatives were prepared according to a modified literature procedure previously described for a colchicine analogue.[10] Colchiceine (1 g, 2.59 mmol) was stirred with 1.2 equiv (0.49 g) of *p*-toluenesulfonyl chloride in dry pyridine (1 mL) at room temperature for 24 h. Following completion of the reaction, the solution was diluted with 15 mL of cold water and extracted three times with 50-mL portions of methylene chloride. The organic extracts were combined, washed three times with a 10% cupric sulfate solution, and twice with water. The organic extracts were dried over sodium sulfate and evaporated to yield a greenish-yellow solid. The colchicine and isocolchicine derivatives were easily separated by radial chromatography (95:5 ethyl acetate:methanol) and were obtained in yields of 22% and 67%, respectively. **10-Demethoxy-10-tosylcolchicine**: mp 138–139 °C; ^{1}H NMR ($CDCl_3$) δ 7.91 (d, 2 H, tosyl protons, J = 8 Hz), 7.80 (d, 1 H, NH, J = 7 Hz), 7.65 (s, 1 H, C8), 7.35 (d, 2 H, tosyl protons, J = 8 Hz), 7.31 (d, 1 H, C12, J = 10.5 Hz), 7.14 (d, 1 H, C11, J = 10.5 Hz), 6.53 (s, 1 H, C4), 4.65 (p, 1 H, C7), 3.92 (s, 3 H, 3-OCH_3), 3.90 (s, 3 H, 2-OCH_3), 3.66 (s, 3 H, 1-OCH_3), 2.55 (m, 1 H, C5), 2.45 (s, 3 H, tosyl CH_3), 2.38 (m, 1 H, C5), 2.23 (m, 1 H, C6), 1.93 (s, 3 H, $COCH_3$), 1.84 (m, 1 H, C6); ^{13}C NMR ($CDCl_3$) δ 178.8, 170.0, 154.2, 153.4, 152.8, 151.2, 145.5, 144.5, 141.7, 135.4, 134.2, 133.8, 132.9, 129.7, 129.3, 128.3, 124.9, 107.4, 61.7, 61.3, 56.1, 52.3, 36.0, 29.8, 22.7, 21.7; IR ($CHCl_3$) 3650, 3340, 3028–2850, 1686, 1632, 1355, 1181 cm^{-1}; LRMS (m/z) M^+ not observed, 385, 357, 326. **10-Demethoxy-10-tosylisocolchicine**: mp 134–135 °C; ^{1}H NMR δ 7.81 (s, 1 H, C8), 7.75 (d, 2 H, tosyl protons, J = 8 Hz), 7.26 (d, 1 H, C12, J = 13 Hz), 7.20 (d, 2 H, tosyl protons, J = 8 Hz), 6.87 (d, 1 H, C11, J = 13 Hz), 6.87 (d, 1 H, NH), 6.51 (s, 1 H, C4), 4.42 (p, 1 H, NH), 3.84 (s, 4 H, 3-OCH_3), 3.83 (s, 3 H, 2-OCH_3), 3.59 (s, 3 H, 1-OCH_3), 2.46 (m, 1 H, C5), 2.33 (s, 3 H, tosyl CH_3), 2.27 (m, 2 H, C5 and C6), 2.02 (m, 1 H, C5), 1.99 (s, 3 H, $COCH_3$); ^{13}C NMR 177.7, 170.5, 154.1, 152.7, 150.8, 145.3, 142.7, 141.3, 141.1, 137.1, 135.1, 132.4, 129.2, 128.7, 127.8, 124.9, 107.5, 61.4, 61.1, 55.9, 52.2, 37.6, 29.6, 22.5, 21.5; IR 3460, 3330, 3028–2850, 1686, 1631, 1355, 1179 cm^{-1}; LRMS (m/z) M^+ not observed, 385, 357, 326.

10-Azido-10-demethoxycolchicine (1). 10-Demethoxy-10-tosylcolchicine (0.2 g, 0.371 mmol) dissolved in 1 mL of dry DMSO was added slowly to a solution of 48 mg (0.74 mmol) of sodium azide in 1 mL of DMSO. After stirring in darkness under nitrogen for 3 h at room temperature, the mixture was diluted with cold water and extracted three times with methylene chloride. The organic extracts were combined, dried over sodium sulfate, and evaporated to yield a brownish oil. The product was purified by column chromatography using 95:5 ethyl acetate–methanol. After drying under vacuum and trituration with diethyl ether, a yellow solid was obtained in a yield of 76%. Compound **1**: mp 108–109 °C; $[\alpha]^{25}_D$ −171° (c 1.0); ^{1}H NMR δ 7.35 (s, 1 H, C8), 7.27 (d, 1 H, C12, J = 10.7 Hz), 7.08 (d, 1 H, C11, J = 10.7 Hz), 6.81 (d, 1 H, NH), 6.54 (s, 1 H, C4), 4.65 (p, 1 H, C7), 3.94 (s, 3 H, 3-OCH_3), 3.93 (s, 3 H, 3-OCH_3), 3.66 (s, 3 H, 1-OCH_3), 2.54 (m, 1 H, C5), 2.39 (m, 1 H, C5), 2.26 (m, 1 H, C6), 2.02 (s, 3 H, $COCH_3$), 1.85 (m, 1 H, C6); ^{13}C NMR δ 180.1, 169.7, 154.0, 153.0, 150.1, 149.2, 141.8, 141.4, 138.6, 134.7, 132.8, 125.6, 123.2, 107.6, 61.6, 61.4, 56.2, 52.4, 38.5, 29.8, 23.0; IR 3470, 3320 (br), 3030–2960, 2120, 1680, 1613, 1310 cm^{-1}; LRMS (m/z) M^+ not observed, 382 ($M^+ - N_2$), 339, 308; HRMS calcd for $C_{21}H_{22}O_5N_2$ ($M - N_2$) 382.1527, found 382.1499.

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9-Azido-9-demethoxyisocolchicine (2). Compound **2** was prepared from 10-demethoxy-10-tosylisocolchicine by the same procedure as above in an 87% yield. Compound **2**: mp 149–150 °C dec; $[\alpha]^{25}_D$ −249° (*c* 0.86); ^{1}H NMR δ 7.44 (d, 1 H, C12, *J* = 12.8 Hz), 7.14 (s, 1 H, C8), 7.01 (d, 1 H, C11, *J* = 12.8 Hz), 6.58 (s, 1 H, C4), 5.81 (d, 1 H, NH), 4.55 (p, 1 H, C7), 3.93 (s, 3 H, 3-OCH_3), 3.90 (s, 3 H, 2-OCH_3), 3.67 (s, 3 H, 1-OCH_3), 2.51 (m, 1 H, C5), 2.33 (m, 2 H, C5,C6), 2.04 (s, 3 H, $COCH_3$), 1.91 (m, 1 H, C6); ^{13}C NMR δ 180.1. 169.7, 154.0, 151.1, 150.0, 144.7, 141.8, 141.4, 138.6, 134.7, 132.8, 125.6, 120.0, 107.6, 61.6, 61.4, 56.2, 52.4, 38.5, 29.8, 23.0; IR 3450, 3280 (br), 3030–2960, 2130, 1680, 1610, 1320 cm^{-1}; LRMS (*m*/*z*) M^+ not observed, 382, 339.

General Procedure for Photolysis. The sample to be photolyzed was dissolved in the selected solvent at a concentration of 2.5 mg mL^{-1}. This solution in Pyrex (under nitrogen) was irradiated for 2 h at 5 cm from a Spectroline (Model ENF-24) ultraviolet lamp using the short wave lamp (254 nm).

Photolysis of Compound 1 in Methanol. Compound 1 was submitted to the above photolysis procedure. The solution was then evaporated in vacuo, and the resulting solid was purified by column chromatography using 3:2 hexanes–ethyl acetate. The product was a whitish solid and was obtained in a yield of 82%. Only one spot was visible by thin-layer chromatography under various solvent conditions. Spectral data indicate that two products are formed. Compound **3** was the major product with a small amount of compound **4** (<10%) being formed. These two products were inseparable by radial and column chromatography. **(10a*S*)-1-Acetyl-4-(2-cyanoethylidene)-2,9,10,10a-tetrahydro-5,6,7-trimethoxybenzo[4,5]cyclohepta[1,2-*b*]pyrrol-2-one (3)**: ^{1}H NMR δ 6.63 (dd, 1 H, C10), 6.62 (s, 1 H, C4), 6.34 (s, 1 H, C9), 4.40 (t, 1 H, C7), 3.92 (s, 3 H, 3-OCH_3), 3.88 (s, 3 H, 2-OCH_3), 3.69 (s, 3 H, 1-OCH_3), 3.34 (dd, 1 H, C11), 3.07 (dd, 1 H, C11), 2.96 (m, 1 H, C5), 2.75 (m, 1 H, C5), 2.65 (m, 1 H, C6), 2.52 (m, 1 H, C6), 2.52 (s, 3 H, $COCH_3$); ^{13}C NMR δ 169.8, 169.3 160.7, 154.2, 150.4, 140.8, 133.6, 132.0, 124.7, 118.7, 116.9, 116.7, 108.6, 61.3, 61.1, 59.8, 59.7, 55.9, 55.8, 30.9, 29.7, 24.8, 18.6; IR 3200–2950, 2260, 1730, 1700 cm^{-1}; LRMS (*m*/*z*) M^+ 382 (383 also observed), 325, 324, 309, 308; HRMS calcd for $C_{21}H_{22}O_5N_2$ 382.1527, found 382.1518. **(10a*S*)-1-Acetyl-4-(2-cyanoethylene)-2,3,3a,9,10,10a-hexahydro-5,6,7-trimethoxybenzo-[4,5]cyclohepta[1,2-*b*]pyrrol-2-one (4).** ^{1}H NMR and ^{13}C NMR signals are barely detectable. Many of the signals due to compound **4** are obscured by the signals of compound **3**. ^{1}H NMR: δ 7.25 (d, 1 H, C10, *J* = 16.2 Hz), 5.14 (d, 1 H, C11), 4.56 (t, 1 H, C7); ^{13}C NMR δ 171.8, 170.2, 145.0, 137.8, 135.9, 128.3, 122.0, 121.0, 118.1, 118.4, 107.8, 98.9, 61.0, 38.9, 36.2, 30.2, 25.5. The remaining ^{1}H NMR and ^{13}C NMR signals are obscured by the signals of compound **3**. IR: Only the —C≡N stretch (2200 cm^{-1}) is distinguishable from the stretches of compound **3**. Complete characterization of compound **4** was not possible due to the small percentage formed during photolysis and because it could not be separated from the major product. The proposed structure of compound **4** is based on similarities between the above spectral data and spectra of **6**.

Photolysis of Compound 1 in Dioxane. A solution of compound 1 in dioxane was submitted to photolysis. After 2 h, the reaction mixture was diluted with water and extracted three times with methylene chloride. The extracts were dried over sodium sulfate and evaporated to dryness. Thin-layer chromatography (3:2 hexanes–ethyl acetate) revealed one spot having the same R_f (0.24) value as compound **3**. Spectral data indicated that the product was compound **3** containing a small amount (<10%) of compound **4**.

Photolysis of Compound 2 in Dioxane. Compound **2** in dioxane was subjected to the same photolysis procedure and workup as above. Column chromatography using 95:5 ethyl acetate–methanol resulted in an 84% yield of a white solid (compound **7**). The product appeared as one spot by thin-layer chromatography (95:5 ethyl acetate–methanol and 9:1 methylene chloride–methanol). The NMR spectra indicated that the product exists as two atropisomers in a 2:1 ratio (estimated from the relative integration intensities of the ^{1}H NMR signals). **(5*S*)-5-(Acetylamino)-4-cyano-6,7-dihydro-9,10,11-trimethoxy-5*H*-dibenzo[*a*,*c*]cyclohepten-3-ol (7)**: mp 157–158 °C; $[\alpha]^{25}_D$ −28.0° (*c* 1.0); IR 3500–2600 (br), 3428, 3010–2850, 2218, 1654 cm^{-1}; LRMS (*m*/*z*) M^+ 382 (383 also observed), 341, 340, 339, 326, 325, 324, 310, 309, 308; HRMS calcd for $C_{21}H_{22}O_5N_2$ 382.1527, found 382.1518. Major conformer: ^{1}H NMR δ 7.53 (d, 1 H, C11, *J* = 8.5 Hz), 6.89 (d, 1 H, C10, *J* = 8.5 Hz), 6.58 (s, 1 H, C4), 6.56 (d, 1 H, NH), 4.81 (p, 1 H, C7), 3.93 (s, 3 H, 3-OCH_3), 3.89 (s, 3 H, 2-OCH_3), 3.62 (s, 3 H, 1-OCH_3), 1.9–2.7 (m, C5 and C6 protons from both conformers), 2.17 (s, 3 H, $COCH_3$); ^{13}C NMR δ 175.0, 154.5, 152.4, 146.1, 142.7, 139.3, 138.3, 129.2, 122.6, 120.9, 112.3, 111.4, 97.9, 64.4, 64.3, 58.9, 58.9, 53.8, 39.5, 32.4, 24.3. Minor conformer: ^{1}H NMR δ 7.55 (d, 1 H, C11, *J* = 8.6 Hz), 6.78 (d, 1 H, C10, *J* = 8.6 Hz), 6.65 (s, 1 H, C4), 5.44 (t, 1 H, C7), 5.33 (d, 1 H, NH), 3.92 (s, 3 H, 3-OCH_3), 3.89 (s, 3 H, 2-OCH_3), 3.56 (s, 3 H, 1-OCH_3), 1.9–2.7 (m, C5 and C6 from both conformers), 1.66 (s, 3 H, $COCH_3$); ^{13}C NMR 175.2, 154.1, 149.5, 145.7, 142.9, 123.0, 122.8, 121.7, 64.4, 63.9, 54.6, 40.2, 32.5, 24.4. The remaining peaks are obscured by the peaks of the major conformer.

Photolysis of Compound 2 in Methanol. A solution of compound **2** in methanol was submitted to photolysis. After 2 h the solution was evaporated in vacuo. The resulting product was first purified by column chromatography using 9:1 methylene chloride–methanol. TLC under these solvent conditions showed two spots with R_f values of 0.47 and 0.20. The product with the lower R_f value was isolated in 11% yield. Spectral data indicated that this product was compound **7**. The spot with higher R_f value consisted of compounds **5** and **6**. These two products were separated by radial chromatography using 94:6 ethyl acetate–methanol. Compound **5** (R_f 0.44) was the major component and was obtained in a 53% yield. Compound **6** (R_f 0.36) was obtained in a yield of 21%. Both compounds were off-while solids after purification. **(7*S*)-7-(Acetylamino)-8-(cyanomethyl)-6,7-dihydro-1,2,3-trimethoxy-9-[2-(methoxycarbonyl)ethylene]-5*H*-benzocycloheptene (5)**: mp 142–143 °C; ^{1}H NMR δ 7.69 (d, 1 H, C9, *J* = 15 Hz), 6.54 (s, 1 H, C4), 6.25 (d, 1 H, NH), 5.62 (d, 1 H, C10, *J* = 15 Hz), 4.36 (p, 1 H, C7), 3.87 (s, 3 H, 3-OCH_3), 3.85 (s, 3 H, 2-OCH_3), 3.77 (d, 1 H, C8, *J* = 17.4 Hz), 3.71 (s, 3 H, $COOCH_3$), 3.69 (s, 3 H, 1-OCH_3), 3.19 (d, 1 H, C8), 2.51 (m, 2 H, C5), 2.19 (m, 2 H, C6), 2.01 (s, 3 H, $COCH_3$); ^{13}C NMR δ 170.5, 167.3, 153.7, 151.3, 141.3, 138.5, 135.1, 134.8, 132.7, 122.6, 121.2, 117.7, 107.4, 61.3, 61.1, 56.2, 51.7, 51.5, 38.9, 30.2, 23.0, 16.2; IR 3430, 3341, 3023–2838, 2248, 1710, 1660 cm^{-1}; LRMS (*m*/*z*) M^+ 414 (415 also observed), 382, 355, 341, 340, 339, 329, 323; HRMS calcd for $C_{22}H_{26}O_6N_2$ 414.1789, found 414.1766. **(7*S*)-7-(Acetylamino)-8-(cyanomethylene)-6,7-dihydro-1,2,3-trimethoxy-9-[2-(methoxycarbonyl)ethylidene]-5*H*-benzocycloheptene (6)**: mp 91.92 °C; ^{1}H NMR (at 312 K) δ 6.45 (br, 1 H, NH), 6.35 (s, 1 H, C4), 5.94 (dd, 1 H, C9), 5.49 (s, 1 H, C8), 4.79 (m, 1 H, C7), 3.86 (s, 3 H, 3-OCH_3), 3.78 (s, 3 H, 2-OCH_3), 3.76 (s, 3 H, $COOCH_3$), 3.68 (s, 3 H, 1-OCH_3), 3.34 (dd, 1 H, C10), 3.18 (dd, 1 H, C10), 2.82 (m, 1 H, C5), 2.59 (m, 1 H, C5), 1.96 (s, 3 H, $COCH_3$), 1.90 (m, 2 H, C6). At 298 K the signals at 5.49, 4.79, 2.82, 2.59, and 1.90 are broad. This is most likely due to the greater conformational flexibility of the cycloheptane ring as opposed to the cycloheptene ring: ^{13}C NMR δ 172.7, 169.2, 152.8, 151.7, 141.6, 135.3, 133.0, 128.5, 128.4, 123.9, 115.5, 107.9, 108.0, 61.7, 60.5, 56.0, 54.6, 52.1, 34.9, 32.5, 52.1, 34.9, 32.5, 31.1, 23.0, 22.9; IR 3458, 3352, 3050–2860, 2238, 1739, 1674 cm^{-1}; LRMS (*m*/*z*) M^+ 414 (415 also observed), 382, 355, 341, 340, 339, 329, 323; HRMS calcd for $C_{22}H_{26}O_5N_2$ 414.1789, found 414.1752.

Methylation of Compound 7. Fifty milligrams of compound **7** dissolved in 10 mL of methylene chloride was added to an excess of ethereal diazomethane. After stirring the reaction mixture on ice for 18 h, the excess diazomethane was quenched with glacial acetic acid, and the ether was evaporated to yield a white solid. The product was purified by column chromatography (9:1 methylene chloride–methanol) and was obtained in a yield of 91%. The NMR spectra indicated that this compound also exists as two atropisomers in a ca. 3:1 ratio. **(5*S*)-5-(Acetylamino)-4-cyano-6,7-dihydro-3,9,10,11-tetramethoxy-5*H*-dibenzo[*a*,*c*]-cycloheptene (8)**: mp 116–117 °C; $[\alpha]^{25}_C$ −28.2° (*c* 0.78); IR 3468, 3100–2860, 2220, 1675 cm^{-1}; LRMS (*m*/*z*) M^+ 396 (397 also observed), 354, 353, 338, 337. Major conformer: ^{1}H NMR δ 7.63 (d, 1 H, C11, *J* = 8.8 Hz), 6.91 (d, 1 H, C10, *J* = 8.8 Hz), 6.57 (s, 1 H, C4), 6.37 (D, 1 H, NH), 4.88 (m, 1 H, C7), 3.96 (s, 3 H, 9-OCH_3), 3.93 (s, 3 H, 3-OCH_3), 3.89 (s, 3 H, 2-OCH_3), 3.56 (s, 3 H, 1-OCH_3), 2.70–2.05 (m, C5 and C6 protons from both conformers), 2.07 (s, 3 H, $COCH_3$); ^{13}C NMR δ 169.8, 161.9, 153.2, 145.1, 141.5, 136.2, 135.1, 134.5, 128.5, 123.3, 116.3, 110.1, 108.1,

97.2, 61.1 (2 signals), 56.2 (2 signals), 50.5, 37.8, 30.5, 22.8. Minor conformer: ^{1}H NMR δ 7.63 (d, 1 H, C11, J = 8.8 Hz), 6.93 (d, 1 H, C10, J = 8.8 Hz), 6.65 (s, 1 H, C4), 5.56 (t, 1 H, C7), 5.13 (d, 1 H, NH), 3.96 (s, 3 H, 9-OCH_3), 3.93 (s, 3 H, 3-OCH_3), 3.90 (s, 3 H, 2-OCH_3), 3.53 (s, 3 H, 1-OCH_3), 2.70–2.05 (m, C5 and C6 protons from both conformers), 1.57 (s, 3 H, $COCH_3$); ^{13}C NMR δ 167.8, 161.0, 151.0, 144.1, 134.8, 126.9, 124.5, 114.5, 109.2, 61.3, 60.9, 39.7, 22.9. The remaining peaks are obscured by the peaks of the major conformer.

Biochemical Analyses. Pipes, EGTA, GTP (Type II-S), Sephadex G-50 were obtained from Sigma Chemical Co. Phosphocellulose (Whatman P11, Whatman Inc.) was precycled according to the manufacturer's instructions. [^{3}H]Colchicine was purchased from Dupont-New England Nuclear Research Products. Scintillation counting was performed on Beckman LS 7500. All experiments were done in PMEG buffer (0.1 M PIPES 1 mM $MgSO_4$, 2 mM EGTA, 0.1 mM GTP, pH 6.90 at 23 °C). If the ligand was not soluble in the buffer at the concentrations used, a small amount (<5%) of DMSO was included in the solutions.

Tubulin Purification. Tubulin was purified from bovine brain by two cycles of assembly/disassembly followed by phosphocellulose chromatography as previously described[11] and stored in liquid nitrogen. Prior to use, the frozen pellets were gently thawed, centrifuged at 5000g for 10 min at 4 °C and desalted into PMEG buffer on 1 mL Sephadex G-50 columns according to the method of Penefsky.[12] Tubulin concentrations were determined spectrophotometrically by the use of an extinction coefficient of 1.23 $(mg/mL)^{-1}$ at 278.5 nm in PMEG buffer.[13]

Inhibition of [^{3}H]Colchicine Binding to Tubulin. Solutions containing 50 μM of the ligand to be tested, 5 μM tubulin, and 5 μM [^{3}H]colchicine were incubated at 37 °C for 1.5 h. After incubation the ligand–protein complex was separated from unbound ligand by rapid gel filtration according to the method of Penefsky[12] as described previously.[13,14] The effluent was analyzed for [^{3}H]colchicine by scintilliation spectrometry. The percent inhibition of [^{3}H]colchicine binding was calculated relative to a control without added ligand. To indirectly assess the extent of covalent incorporation of compound 1 into tubulin, a sample containing compound 1 (50 μM) and tubulin (5 μM) was incubated for 45 min at 37 °C and then photolyzed at room temperature for a period of 20 min. (It was determined that 20 min was a sufficient amount of time to photolyze a 50 μM solution of 1.) A control was treated in the same manner. After photolysis [^{3}H]colchicine was added to achieve a final concentration of 5 μM, the sample was incubated for 1.5 h, and treated as described above.

Competitive Binding Assay. The ability of a ligand to competitively inhibit the binding of [^{3}H]colchicine was determined by a previously described procedure.[14,15] Tubulin (5 μM), the ligand to be tested (at concentrations of 0, 5, 10, 15, and 20 μM), and [^{3}H]colchicine (1, 2, and 4 μM) were incubated at 37 °C for 1.5 h. After incubation the ligand–protein complex was separated from unbound ligand by the method of Penefsky.[12] The effluent was then analyzed for [^{3}H]colchicine by scintillation spectrometry. The competitive inhibition constant (K_I) was determined by modified Lineweaver–Burke analysis of the data.[14,15]

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Registry No. 1, 129467-54-5; 2, 129467-55-6; 3, 129467-56-7; 4, 129467-57-8; 5, 129467-58-9; 6, 129467-59-0; 7, 129467-60-3; 8, 129467-61-4; 10-demethyl-10-tosylcolchicine, 129467-62-5; 9-demethyl-9-tosylisocolchicine, 129491-66-3; colchiceine, 477-27-0.

Supplementary Material Available: Original ^{1}H and ^{13}C NMR spectra of 10-demethoxy-10-tosylcolchicine, 9-demethoxy-9-tosylisocolchicine, and compounds 1–8 (18 pages). Ordering information is given on any current masthead page.

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Optically Active Amines. 35.[1] A Sector Rule for the Circular Dichroism of the Benzene Chromophore

Howard E. Smith* and Luca P. Fontana

Department of Chemistry, Vanderbilt University, Nashville, Tennessee 37235

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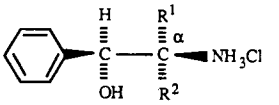
The circular dichroism (CD) of (αR)-norephedrine hydrochloride [($\alpha R,\beta S$)-**1a**] and (αS)-norpseudoephedrine hydrochloride [($\alpha S,\beta S$)-**1b**] both show a number of nega-



($\alpha R,\beta S$)-**1a**, $R^1 = CH_3$; $R^2 = H$

($\alpha S,\beta S$)-**1b**, $R^1 = H$; $R^2 = CH_3$

tive Cotton effects (CEs) from about 255 to 270 nm[2] associated with transitions from the lowest energy vibrational mode in the ground state to totally symmetric vibrational modes in the ^1L_b electronically excited state of the benzene chromophore,[2-4] the lowest energy CE being associated with the ^1L_b band origin. Occasionally, for other benzene compounds, additional, weak CD maxima are observed within the ^1L_b band with signs opposite to that of the ^1L_b band origin. These latter CD maxima are associated with transitions to nontotally symmetric vibrational modes in the electronically excited state.[4]

For benzene compounds without an additional substituent, vibronic borrowing from benzene transitions at shorter wavelength[5-8] gives the sign to the ^1L_b CEs, and the sign depends only on the chirality of the chiral center immediately attached to the benzene ring and is the same as that observed in the optical rotatory dispersion[9] (ORD) and CD spectra[10-13] of other phenylalkylcarbinols of the same generic configuration, also with a second chiral center contiguous to that attached to the benzene ring. The sign of the ^1L_b CEs for transitions to totally symmetric vibrational modes in the excited state can be predicted provided

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