a colorless solid (50 mg) whose HPLC (15% MeCN in 0.1 M NH_4OAc, pH 7, flow rate 1.0 mL/min) revealed two major peaks (4:1 ratio) with retention times of 15.3 and 20 min, respectively. HPLC-homogeneous product was obtained by repeated passage of small samples (1–2 mg) through the analytical column and collecting the faster moving peak. Freeze-drying of pooled eluates afforded **3** as a water-soluble white solid (10 mg): IR (KBr) 3440, 2930, 2860, 1635, 1505, 1445, 1405, 1385, 1310, 1265, 1100, 800, 760 cm^{-1}; UV λ_{max} (95% EtOH) 230 nm (ϵ 30900), 279 inf (9160), λ_{max} (0.1 N HCl) 233 nm (ϵ 29000); high-resolution MS, M + 1 = 576.2479 (calcd 576.2458). The elemental analysis of the product was consistent with a hydrated ammonium salt, but suggested probable contamination with a small amount (5%) of silica from the HPLC column. Since the mass spectrum firmly supported the structure and the C/N ratio was correct, this material was judged to be suitable for bioassays. Sufficient material was not available for repurification. Anal. ($C_{30}H_{33}N_5O_7 \cdot NH_3 \cdot 3H_2O \cdot 0.05SiO_2$) C, H, N. C/N: calcd, 5.00; found, 5.05.

Bioassays. The ability of **1** to serve as a substrate and of **2** to serve as an inhibitor of mouse liver FPGS was evaluated as previously described.[31] Growth inhibition assays against CEM human leukemic lymphoblasts, L1210 murine leukemia cells, and SCC15 human squamous carcinoma cells were also carried out as previously reported.[23]

Acknowledgment. This work was supported in part by Grants CA39867 (R.G.M., A.R.), CA42367 (R.G.M.), and CA19589 (A.R.) from the National Cancer Institute (DHHS). R.G.M. is a Scholar of the Leukemia Society of America. The excellent technical assistance of Paul Colman in carrying out the enzyme assays is gratefully acknowledged. Cell growth inhibition assays at DFCI were performed by Carol Cucchi and Sylvia Holden, and HPLC analyses were carried out with the able assistance of Dorothy Trites. High-resolution mass spectra data were kindly provided by Catherine Costello, Department of Chemistry, Massachusetts Institute of Technology.

Registry No. (6*R*)-L-**1**, 118537-33-0; (6*S*)-L-**1**, 118537-50-1; (6*R*)-L-**2**, 118537-34-1; (6*R*)-L-**2**·Na, 118537-57-8; (6*S*)-L-**2**, 118537-51-2; (6*S*)-L-**2**·Na, 118537-58-9; (6*R*)-L-**3**, 118537-35-2; (6*R*)-L-**3**·NH_3, 118537-59-0; (6*S*)-L-**3**, 118537-52-3; (6*S*)-L-**3**·NH_3, 118537-60-3; (6*RS*)-**4**, 118537-36-3; **5**, 51656-90-7; **6**, 4746-97-8; **7**, 93245-98-8; **8a**, 1253-46-9; **8b**, 118537-46-5; *cis*-**9a**, 118537-37-4; *trans*-**9a**, 118537-53-4; *cis*-**9b**, 118537-47-6; *trans*-**9b**, 118537-54-5; **10a**, 118537-38-5; **10b**, 118537-48-7; **11a**, 118537-39-6; **11b**, 118537-49-8; **13**, 118537-40-9; **14**, 118537-41-0; (6*RS*)-**15**, 118537-42-1; (6*R*)-L-**16**, 118537-43-2; (6*S*)-L-**16**, 118537-55-6; (6*R*)-L-**17**, 118537-44-3; (6*S*)-L-**17**, 118537-56-7; **18**, 118537-45-4; FPGS, 63363-84-8; 4-MeC_6H_4COCl, 874-60-2; 4-MeC_6H_4COOBu-*t*, 13756-42-8; 4-$BrCH_2C_6H_4CH_2COOMe$, 7398-42-7; Ph_3P, 603-35-0; H-Glu(OEt)-OEt·HCl, 1118-89-4; H-Orn(Cbz)-OH, 3304-51-6; N^{δ}-phthaloyl-L-ornithine, 7780-77-0; folic acid, 59-30-3.

Modification of the Hydroxy Lactone Ring of Camptothecin: Inhibition of Mammalian Topoisomerase I and Biological Activity

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Several camptothecin derivatives containing a modified hydroxy lactone ring have been synthesized and evaluated for inhibition of topoisomerase I and cytotoxicity to mammalian cells. Each of the groups of the hydroxy lactone moiety, the carbonyl oxygen, the ring lactone oxygen, and the 20-hydroxy group, were shown to be critical for enyzme inhibition. For example the lactol, lactam, thiolactone, and 20-deoxy derivatives did not stabilize the covalent DNA–topoisomerase I complex. With a few exceptions, those compounds that did not inhibit topoisomerase I were not cytotoxic to mammalian cells. Two cytotoxic derivatives that did not inhibit topoisomerase I were shown to produce non-protein-associated DNA single-strand breaks and are likely to have a different mechanism of action. One of these compounds was tested for antitumor activity and was found to be inactive. The present findings, as well as other reports that the hydroxy lactone ring of camptothecin is critical for antitumor activity in vivo, correlate with the structure–activity relationships at the level of topoisomerase I and support the hypothesis that antitumor activity is related to inhibition of this target enzyme.

Camptothecin (**1**) is a naturally occurring compound isolated from *Camptotheca acuminata* that exhibits antitumor activity in several experimental tumors including human colon, lung, and mammary tumor lines.[1,2] Several lines of evidence suggest that DNA topoisomerase I is the cellular locus at which camptothecin exerts its antineoplastic effects. Camptothecin and structurally related compounds stabilize a DNA–topoisomerase I covalent complex in which one strand of the DNA helix is broken.[3-6] Studies of camptothecin analogues have suggested a correlation between the ability to induce DNA breakage and antitumor activity.[7] The recent finding that camptothecin-resistant cells contain an altered topoisomerase I that is not inhibited by the drug strongly supports the hypothesis that camptothecin kills cancer cells by inhibiting this enzyme.[8,9]

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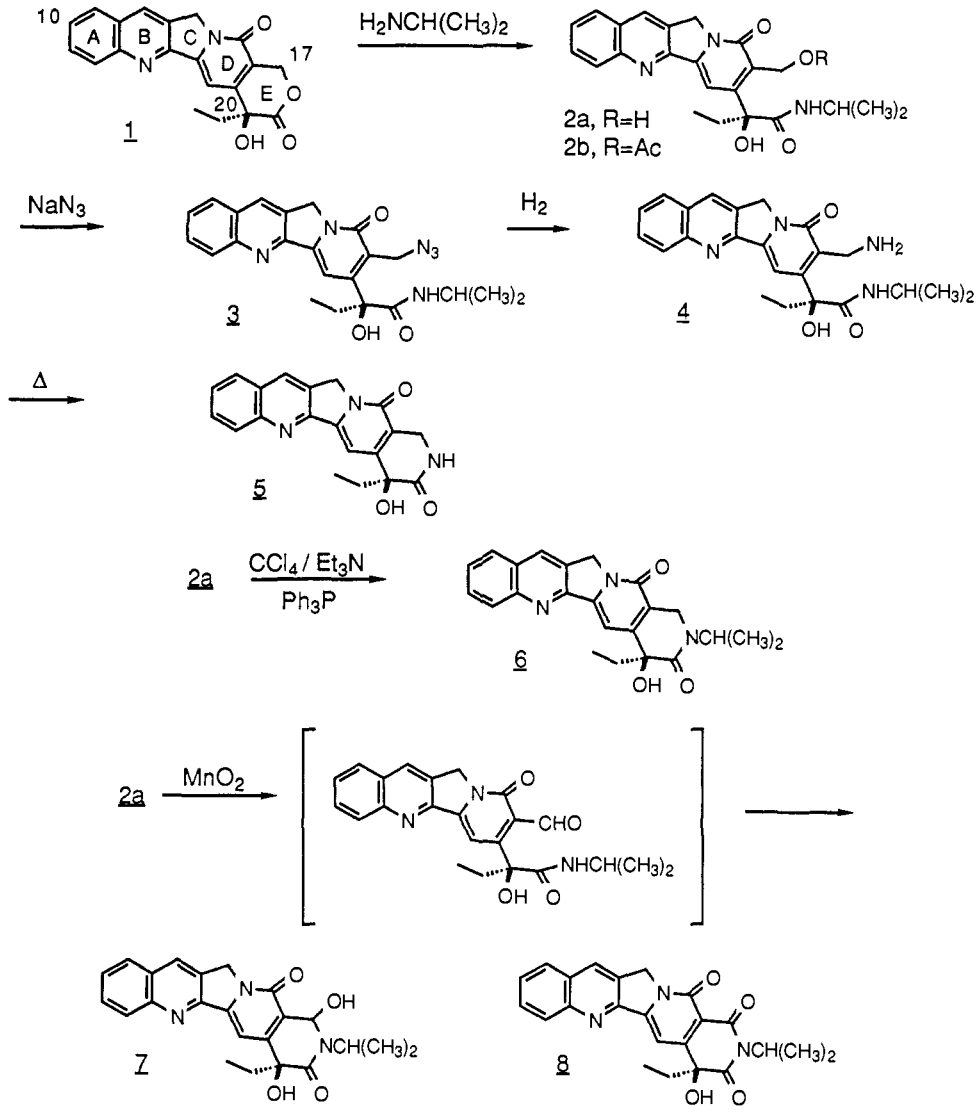
0022-2623/89/1832-0715$01.50/0

Scheme I



The identification of topoisomerase I as the cellular target for camptothecin provides a rational approach to the design and synthesis of useful antitumor agents. We have synthesized several novel E-ring-modified camptothecins and evaluated them for inhibition of purified mammalian topoisomerase I and for cytotoxicity to mammalian cells. These examples provide additional support that the E-ring hydroxy lactone of camptothecin is required for inhibition of topoisomerase I, consistent with its requirement for antitumor activity.[10]

Chemistry

The synthesis of camptothecin lactams **5**, **6**, and **7** as well as the imide **8** are shown in Scheme I. Carbinolamide **2a** and acetate **2b** were prepared as described by Adamovics and Hutchinson.[11a] These authors reported that **2a** was reconverted to camptothecin on attempted purification by silica gel chromatography. While we found that this compound could readily be purified on silica gel by medium-pressure chromatography, we did observe reconversion to camptothecin in aqueous solution. Treatment of the alcohol **2a** with acetic anhydride in pyridine gave acetate **2b** as a yellow solid. Chromatography, using the same technique as was used for **2a**, furnished pure **2b**. Reaction of acetate **2b** with NaN_3 in DMF at room temperature produced azide **3** as a light yellow solid; purification was achieved by recrystallization from acetone–petroleum ether. Reduction of azide **3** over barium sulfate produced **4**, which was converted to lactam **5** on heating. The resulting lactam was purified by preparative silica gel chromatography.

Isopropyl lactam **6** was prepared from **2a** with use of carbon tetrachloride, triethylamine, and triphenylphosphine[12] and purified by silica gel chromatography. The carbinol lactam **7** and the imide **8** were prepared as a 1:1 mixture from oxidation of **2a** with MnO_2 and were separated by silica gel chromatography. Thiolactone **10** was prepared by reaction of **2b** with Na_2S to give thiol amide **9**, which was immediately converted to **10** on heating (Scheme II). Compound **11** was prepared by photolysis

(8) (a) Andoh, T.; Ishii, K.; Suzuki, Y.; Ikegami, Y.; Kusunoki, Y.; Takemoto, Y.; Okada, K. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 5565. (b) Kjeldsen, E.; Bonven, B.; Andoh, T.; Ishii, K. Okada, K.; Bolund, L.; Westergaard, O. *J. Biol. Chem.* **1988**, *263*, 3912.

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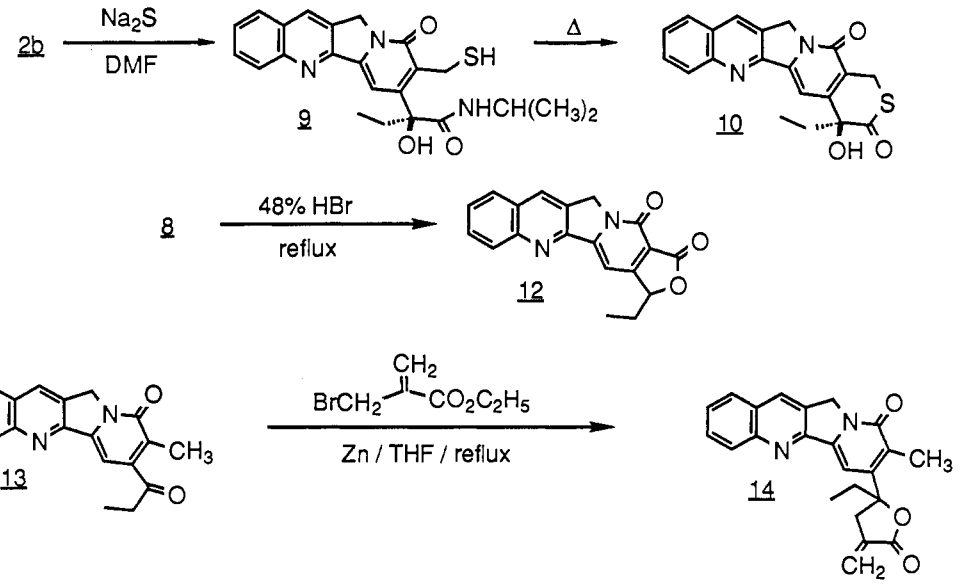
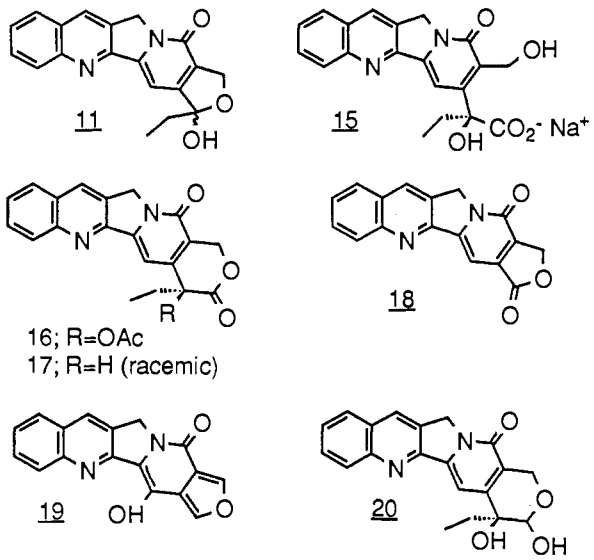
Scheme II



of sodium camptothecin as described.[13] Lactone **12** was obtained by treatment of **8** with 48% HBr at reflux. The methylene lactone **14** was obtained from **13**[14] by a Reformatsky reaction using ethyl α-(bromoethyl)acrylate.[15]

Compounds **15**, **16**, **18**, and **19** were obtained from the National Cancer Institute. Compound **17** was made by total synthesis by using the Wall procedure.[16] Compound **20** was prepared by treating camptothecin with $NaBH_4$ as described.[17]



Biological Results and Discussion

Wall and co-workers[10] have suggested that the α-hydroxy lactone ring of camptothecin is essential for antitumor activity. However sodium camptothecin (**15**), in which the lactone ring is hydrolyzed, has antitumor activity similar to that of **1** but is about 10-fold less potent.[2,16] Since both forms of the drug have similar potency with respect to in vitro cytotoxicity and inhibition of purified topoisomerase I (Table I), it is likely that **15** is slowly converted to **1** in serum and that the difference in in vivo potency of the two administered forms reflects different pharmacokinetic properties. In order to understand the importance of the intact α-hydroxy lactone ring for activity at the proposed enzyme target, we have investigated the inhibition of topoisomerase I by both forms of camptothecin at several pH values.

As shown in Figure 1a, when either **1** or **15** was added to a reaction mixture containing DNA and topoisomerase I, DNA cleavage was observed at pH values below 9. This represents stabilization of a DNA–topoisomerase I covalent complex.[3-6] Addition of the lactone form (**1**) produced slightly more DNA strand scission than addition to the open-ring form (**15**). DNA cleavage induced by the addition of either camptothecin form increased as the pH was decreased. DNA cleavage was not observed in reaction mixtures of pH 9 or above (Figure 1a), even though topoisomerase I is catalytically active in this pH range (Figure 1b). Since low pH induces lactone ring closure,[1] these results suggest that the closed-ring form is more inhibitory to topoisomerase I than the open-ring form. In

Table I. Topoisomerase I Inhibition and Cytotoxicity of E-Ring-Modified Camptothecin Derivatives

compd	CC_{50},[a] μM	IC_{50},[b] μM
1	0.8	0.014
15	0.9	0.014
16	neg[c]	0.39
17	neg	0.59
2a	5.0	nd[d]
5	neg	>2
10	neg	2.9
20	neg	2.7
11	neg	>5.9
12	neg	>5.9
14	neg	0.094
18	neg	>5.9
19	neg	0.092

[a] Concentration that produced 50% DNA cleavage mediated by topoisomerase I under standard reaction conditions; none of the compounds cleaved DNA in the absence of enzyme. [b] Concentration that inhibited the proliferation of L1210 cells upon continuous exposure. [c] No enzyme-mediated DNA cleavage was observed at the highest concentration tested (50 μM). [d] Not determined.

(13) Lown, J. W.; Chen, H.-H. *Biochem. Pharmacol.* **1980**, *29*, 905.
(14) Mappicine ketone **13** was prepared by treating camptothecin with NaN_3 (Kingsbury, W. *Tetrahedron Lett.*, in press).
(15) Drewes, S. E.; Loiyou, G.; Roos, G. H. P. *Synth. Commun.* **1987**, *17*, 291.
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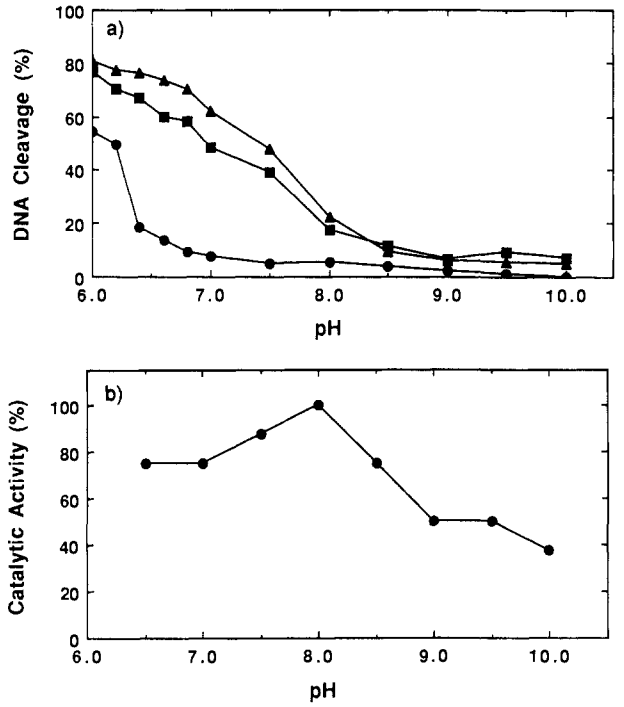

Figure 1. (a) Effect of pH on the cleavage of supercoiled pDPT2789 DNA by 17 ng of topoisomerase I in the presence of 5 μM camptothecin (1, ▲), 5 μM sodium camptothecin (**15**, ■), or in the absence of drug (●). (b) Effect of pH on the catalytic relaxation of supercoiled pDPT2789 DNA by topoisomerase I; values are reported relative to the activity at pH 8.0 (100%).

addition, it is likely that the two forms of camptothecin interconvert in aqueous solution to an equilibrium mixture that is pH-dependent. DNA cleavage observed in drug-free reaction mixtures at pH values between 6 and 7 (Figure 1) was most likely the result of conformational changes in DNA or topoisomerase I induced by protonation.

In an effort to produce more efficacious camptothecin derivatives and to permit a better understanding of the role of the α-hydroxy lactone ring, the E-ring-modified camptothecins were evaluated for topoisomerase I inhibition and cytotoxicity to mammalian cells (Table I). With a few exceptions (see below), those compounds that were unable to stabilize DNA cleavage by topoisomerase I were not cytotoxic. Compounds that retained the intact lactone ring but lacked the 20-hydroxy group (**16** and **17**) were found to be inactive as inhibitors of topoisomerase I. Wall and Wani[10] established that the 20-hydroxy group of camptothecin is an absolute requirement for antitumor activity. The small degree of cytotoxicity observed with **16** and **17** may be due to small amounts of **1** in the samples. We have observed that **17** is slowly converted to **1** upon standing in the presence of oxygen.

There are two possible explanations for the importance of the 20-hydroxy group for topoisomerase I inhibition: the formation of a hydrogen bond between this hydroxy group and the enzyme–DNA complex, or the presence of an intramolecular hydrogen bond with the lactone carbonyl of camptothecin that could facilitate lactone ring opening. Other investigators have suggested that the 20-hydroxy group increases the electrophilicity of the lactone carbonyl, since its removal or acetylation reduced the reactivity of the lactone ring.[1,11] In addition, we observed that camptothecin incorporated ^{18}O in dilute solutions of $^{18}OH_2$, suggesting that the lactone ring opening was facile, while 20-deoxycamptothecin did not incorporate ^{18}O under the same conditions (unpublished results). This result, coupled with the pH study and the observation that the 20-hydroxy group was necessary for topoisomerase I inhibition, suggests that camptothecin binds to the enzyme–DNA complex as the closed lactone form but that subsequent ring opening plays a role in the covalent complex stabilization. Additional derivatives that contain open E rings (**2b**, **3**, and **4**) were found to be inactive. One exception, however, is compound **2a** in which the 17-position is hydroxylated. The activity of **2a** may be due to conversion to **1** in situ; analysis of the reaction mixture used for the enzyme-inhibition assay confirmed that significant amounts of **1** were formed under assay conditions.

Table II. DNA Strand Breakage Produced in L1210 Cells upon Treatment with Camptothecin Derivatives, As Measured by Alkaline Elution Techniques[6]

compd	concn, μM (for 1 h)	PASSB,[a] rad equiv	non-PASSB,[b] rad equiv
1	2	342	240
14	2	<100	<100
14	20	<100	1360
19	3.5	nd[c]	175
19	35	<100	1000
5	10	<100	<100
20	10	<100	<100

[a] Protein-associated single-strand breaks, measured after cell lysis and proteinase K treatment. [b] Non-protein associated single-strand breaks, measured after cell lysis in the absence of proteinase K treatment. [c] Not determined.

To further investigate the role of the E ring, we prepared compounds that retained the 20-hydroxy group but lacked the lactone oxygen. The lactam derivative **5** did not inhibit topoisomerase I and was not cytotoxic. **5** differs from **1** in two respects: the lactam ring is more stable to hydrolysis than the lactone ring and the lactam NH is a hydrogen bond donor while the lactone oxygen is a hydrogen bond acceptor. Thiolactone **10**, whose E ring more closely resembles the lactone ring in **1**, is expected to be more reactive than a lactone but sulfur is a poorer hydrogen bond acceptor than oxygen and also represents increased steric bulk and ring size. The inactivity of **10** reinforces the strict requirement for an α-hydroxy lactone ring; clearly this region of the molecule is in very close contact with the enyzme–DNA complex. Compound **20** was also found to be inactive, consistent with the requirement for a carbonyl group at position 20; compounds **6–8** were also found to be inactive.

Consistent with our findings, Pommier and co-workers have recently reported that the lactam **5** does not inhibit topoisomerase I and lacks antitumor activity in vivo (L1210).[18] In addition, Wall and co-workers have prepared (20*S*)- and (20*R*)-camptothecin[19] and have shown that the 20*R* analogue was significantly less active than the natural 20*S* analogue with respect to cytotoxicity, antitumor activity in vivo, and topoisomerase I inhibition.[18] These results provide additional support for the importance of the 20-hydroxy group and demonstrate a requirement for the correct steric configuration at C-20.

Several other camptothecin derivatives containing a modified lactone ring (**11**, **12**, **14**, **18**, **19**) were evaluated. All of these derivatives were found to be inactive with respect to topoisomerase I inhibition and, with the exception of **14** and **19**, were not cytotoxic. The observation that **14** and **19** were cytotoxic to mammalian cells without inhibiting topoisomerase I prompted a further investiga-

(18) Pommier, Y.; Jaxel, C.; Covey, J. M.; Kerrigan, D.; Wani, M. C.; Wall, M. E.; Kohn, K. W. *Proc. Am. Assoc. Cancer Res.* **1988**, *29*, 1080.
(19) Wani, M. C.; Nicholas, A. W.; Wall, M. E. *J. Med. Chem.* **1987**, *30*, 2317.

tion of their mechanism of action. Using alkaline elution techniques to measure DNA strand breakage,[6] it was found that **14** and **19** produced DNA single-strand breaks that were not associated with topoisomerase I covalent complexes (Table II). In contrast, greater than half of the DNA single-strand breaks produced by campothecin were associated with protein.[6] Noncytotoxic compounds such as **5** and **20** did not produce detectable DNA strand breaks of either type. Therefore, it is likely that the cytotoxicity of **14** and **19** is unrelated to inhibition of topoisomerase I. The presence of a Michael acceptor in **14** suggests the possibility of nonselective alkylation of cellular macromolecules.

Compound **14** was evaluated for antitumor activity in mice bearing ip L1210 leukemia; this compound was toxic at 125 mg/kg (ip on days 1 and 5) and was inactive at lower dose levels. Camptothecin, in the same experiment, produced 131% increase in life span at a maximally tolerated dose of 7.5 mg/kg. Since **14** and other E-ring-modified camptothecin derivatives have no antitumor activity in vivo,[10] it would appear that the antitumor activity of **1** is directly related to topoisomerase I inhibition.

Experimental Section

General Procedures. ^{1}H NMR and ^{13}C NMR spectra were obtained on a JEOL FX-90Q spectrometer; all values are reported in ppm (δ) downfield from $(CH_3)_4Si$. IR spectra were obtained on a Perkin-Elmer 783 spectrophotometer. Elemental analysis and mass spectra were performed in the Analytical, Physical and Structural Chemistry Department of Smith Kline & French Laboratories. Elemental results were within 0.4% of the theoretical values. J. T. Baker silica gel (average particle size 40 μm) was used for flash and HPLC chromatography and Whatman Partisil 40 ODS-3 was used for reversed-phase HPLC chromatography.

17-Azidocamptothecin 21-Isopropylamide (3). Compound **2b**[11a] (30 mg, 0.066 mmol) was dissolved in DMF (3 mL) and treated with sodium azide (80 mg, 1.2 mmol). After stirring of the mixture at room temperature for 23 h, solvent was removed in vacuo and the residue was triturated with water. The light yellow precipitate was collected and dried in a vacuum desiccator, giving 27 mg of crude product containing a trace of the starting material (94%). Reprecipitation from acetone–petroleum ether gave **3** (17 mg, 60%): mass spectrum, m/z 433 (M + H); ^{1}H NMR ($CDCl_3$) δ 1.2 (m, 9), 2.4 (m, 2), 4.1 (m, 1), 4.7 (q, 2), 4.8 (d, 2), 6.8 (d, 1), 7.1–7.8 (m, 6).

17-Aminocamptothecin 21-Isopropylamide (4). Compound **3** (234 mg, 0.54 mmol) in CH_3OH (30 mL) was treated with 5% Pd on $BaSO_4$ (100 mg) and hydrogenated in a Parr shaker at 5 psi for 5 h. Catalyst was removed by filtration through supercel and the filtrate was concentrated in vacuo to give 180 mg of an oily residue (82% crude yield). A small sample was purified by reversed-phase MPLC; the product was eluted with 40% CH_3OH in water: mass spectrum, m/z 407 (M + H); ^{1}H NMR (CD_3OD) δ 1.2 (m, 9), 2.4 (m, 2), 4.1 (m, 1), 5.1 (s, 2), 5.4 (s, 2), 6.9 (d, 1), 7.4–8.2 (m, 5), 8.7 (s, 1).

Camptothecin Lactam (5). Compound **4** (90 mg, 0.22 mmol) was dissolved in DMF (1 mL) and heated at 80–85 °C for 3 h. The reaction mixture was cooled and the precipitate was collected and washed with CH_3OH, giving **5** as a slightly impure yellow solid. Purification was achieved by flash chromatography (silica gel, 5% CH_3OH in CH_2Cl_2; 20 mg, 26%): mass spectrum, m/z 348 (M + H); ^{1}H NMR δ 0.94 (t, 3), 1.9 (m, 2), 4.5 (q, 2), 5.3 (s, 2), 7.3–8.2 (m, 5), 8.4 (s, 1). Anal. ($C_{20}H_{17}N_3O_3$) C, H, N.

***N*-Isopropylcamptothecin Lactam (6).** Compound **2a**[11a] (26 mg, 0.06 mmol) was dissolved in distilled, dry THF (8 mL) and dry CH_3CN (4 mL) and treated with CCl_4 (0.011 mL, 0.12 mmol), Et_3N (0.015 mg, 0.15 mmol), and triphenylphosphine (31 mg, 0.12 mmol). The contents were stirred under argon at room temperature for 72 h. All volatiles were removed in vacuo; after trituration with Et_2O/petroleum ether (50/50), the crude precipitate was purified (MPLC, silica gel, 2% CH_3OH in CH_2Cl_2), giving **6** (9 mg, 39%); starting material **2a** (15 mg, 57%) was also recovered. **6**: mass spectrum, m/z 390 (M + H); ^{1}H NMR ($CDCl_3$) δ 1.2 (m, 9), 2.3 (m, 2), 4.0 (m, 1), 5.2 (s, 2), 5.3 (s, 2), 7.5–8.3 (m, 6).

***N*-Isopropyl-17-hydroxycamptothecin Lactam (7) and *N*-Isopropyl-17-oxocamptothecin Lactam (8).** Compound **2a** (0.7 g, 1.7 mmol) in CH_2Cl_2 (100 mL) was treated with activated manganese dioxide (7.0 g) and the mixture was stirred at room temperature for 3 days. The brown oxidant was removed by filtration and washed with CH_3OH. The dark filtrate was concentrated in vacuo to give 0.5 g of a dark residue. After purification (MPLC, silica gel, 1–3% CH_3OH in CH_2Cl_2) the following were eluted: **8** (80 mg, 12%), **1** (20 mg, 3.4%), **7** (56 mg, 8.1%), and starting material **2a** (15 mg, 2.1%).

8: mass spectrum, m/z 404 (M + H); ^{1}H NMR ($CDCl_3$) δ 1.2 (m, 9), 2.3 (m, 2), 4.1 (m, 1), 5.4 (s, 2), 7.5–8.3 (m, 5), 8.4 (s, 1); ^{13}C NMR ($CDCl_3$) δ 7.8, 22.4, 22.6, 31.0, 42.0, 50.4, 87.6, 95.8, 111.0, 128.1, 128.5, 128.7, 129.7, 130.2, 130.9, 131.2, 149.3, 153.4, 155.9, 166.4, 167.6. Anal. ($C_{23}H_{21}N_3O_4 \cdot 1.5H_2O$) C, H, N.

7: mass spectrum, m/z 406 (M + H). ^{1}H NMR ($CDCl_3$) δ 0.9 (t, 3), 1.3 (m, 6), 2.2 (m, 2), 4.1 (m, 1), 5.0 (s, 2), 6.7 (d, 1), 7.4–8.0 (m, 5), 8.3 (s, 1); ^{13}C NMR ($CDCl_3$) δ 7.8, 18.5, 22.3, 22.9, 30.3, 41.4, 41.7, 50.1, 91.7, 97.2, 100.1, 105.8, 127.7, 127.9, 128.7, 129.4, 130.3, 131.0, 148.3, 156.3, 170.4. Anal. ($C_{23}H_{23}N_3O_4 \cdot 1.75H_2O$) C, H, N.

Camptothecin-17-thiol 21-Isopropylamide (9). To a solution of **2b** (0.45 g, 1.0 mmol) in DMF (60 mL) was added sodium sulfide (0.78 g, 10 mmol), and the contents were stirred overnight at room temperature. The solvent was removed in vacuo and the residue was triturated with a small volume of water and filtered from insolubles. The filtrate was acidified to pH 1.2 with 3 N HCl. The precipitate was collected and dried, giving 200 mg of solid product (47%): mass spectrum, m/z 423 (M + H); ^{1}H NMR (DMSO-d_6) δ 1.0 (t, 3), 1.2 (m, 6), 2.3 (m, 2), 4.0 (s, 2), 4.2 (m, 1), 5.1 (s, 2), 7.3 (s, 1), 7.7 (m, 1), 7.4–8.1 (m, 4), 8.3 (s, 1); ^{13}C NMR (DMSO-d_6) δ 8.0, 21.8, 22.2, 30.5, 31.9, 41.3, 50.0, 79.9, 99.7, 127.3, 127.7, 128.3, 128.6, 129.5, 129.7, 130.2, 131.5, 141.6, 147.7, 151.2, 152.8, 160.3, 171.6.

Camptothecin Thiolactone (10). Compound **9** (100 mg, 0.23 mmol) was dissolved in DMF and the solution was heated at 80 °C under argon for 8 h. Solvent was removed in vacuo and the residue was triturated in water and filtered. The crude solid was purified (MPLC, silica gel, 1% CH_3OH in CH_2Cl_2) to give **10** (15 mg, 18%); starting material **9** was also recovered (40 mg, 40%). **10**: mass spectrum, m/z 365 (M + H); ^{1}H NMR ($CDCl_3$) δ 0.98 (t, 3), 1.9 (m, 2), 4.2 (s, 2), 5.3 (s, 2), 7.7–8.3 (m, 5), 8.4 (s, 1).

***d,l*-1*H*,3*H*-Furo[3′4′:6,7]indolizino[1,2-*b*]quinolin-1,13-(11*H*)-dione (12).** Compound **8** (90 mg, 0.22 mmol) was treated with freshly distilled 48% HBr (9 mL) and the solution was refluxed under argon for 24 h. The contents were concentrated in vacuo, giving a residue which was purified by flash chromatography on silica gel (1% CH_3OH in CH_2Cl_2 eluted the starting material, 9 mg, 10%; 2% CH_3OH in CH_2Cl_2 eluted **12**, 25 mg, 36%). Similar results were obtained with 49% H_2SO_4 in place of 48% HBr. **12**: mass spectrum, m/z 319 (M + H); ^{1}H NMR (CD_3OD) δ 1.0 (t, 3), 2.0 (m, 2), 5.3 (s, 2), 5.4 (m, 1), 7.4 (s, 1), 7.8 (m, 4), 8.4 (s, 1); ^{13}C NMR (CD_3OD) δ 8.2, 26.3, 50.5, 80.4, 94.3, 112.8, 128.1, 128.5, 129.2, 129.7, 130.9, 131.7, 148.6, 150.5, 152.7, 168.4.

***d,l*-8-Methyl-7-(2-ethyl-4-methylene-5-oxotetrahydrofuran-2-yl)indolizino[1,2-*b*]quinolin-9(11*H*)-one (14).** To a mixture of **13**[14] (304 mg, 1.0 mmol) activated zinc dust (85 mg), and hydroquinone (2 mg) in anhydrous THF (20 mL) at room temperature was slowly added ethyl α-(bromomethyl)acrylate[15] (0.3 mL) in THF (8 mL) over 1 h with vigorous stirring. The reaction was refluxed for 1.5 h; the light orange solution was cooled, poured into 100 mL of ice-cold 5% HCl, neutralized with solid $NaHCO_3$, and extracted with $CHCl_3$. The combined organic extract was dried over Na_2SO_4, concentrated in vacuo to a volume of 8 mL, diluted with 40 mL of hexane, and filtered to give 308 mg of a light orange powder. This was purified by flash chromatography (silica gel, EtOAc) to give **14** (235 mg, 66%): mp 272–274 °C; mass spectrum, m/z 373 (M + H). IR (KBr) 1765, 1660, 1600 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.0 (t, 3), 2.2 (q, 2), 2.4 (s, 3), 3.4 (m, 2), 5.3 (s, 2), 5.7 (m, 1), 6.3 (m, 1), 7.5–7.9 (m, 4), 8.1–8.3 (m, 2). Anal. ($C_{23}H_{20}N_2O_3$) C, H, N.

Topoisomerase I Assays. Purification of topoisomerase I from calf thymus and enzyme-inhibition assays were performed as

described.[9] Briefly, reaction mixtures containing supercoiled DNA, topoisomerase I, and drug were incubated for 30 min at 37 °C followed by treatment with proteinase K to remove covalently bound enzyme. The resultant nicked DNA plasmids were separated from supercoiled and covalently closed circular relaxed DNA by ethidium bromide/agarose gel electrophoresis. Negatives of the gel photographs were scanned with a densitometer to quantitate DNA cleavage. The pH profile for topoisomerase I catalytic activity was determined by the relaxation of supercoiled pDPT2789 DNA as described,[20] substituting 50 mM HEPES buffer of the appropriate pH for Tris·HCl.

Growth-Inhibition Assay. L1210 murine leukemia cells were incubated at 37 °C for 60 min in the presence of various concentrations of drug. Treated cells were diluted into soft agar (0.6% noble agar, 20% fetal calf serum in Fischer's medium) and incubated for 7 days to permit development of macroscopic colonies. Colonies were stained with tetrazolium salts (0.1% for 1–2 days and enumerated with a Biotran III automatic totalizer (New Brunswick Scientific). Cloning efficiency of L1210 cells in this assay is 8–10%. The IC_{50} is the concentration of drug (in the soft agar during the 7-day incubation) that resulted in a 50% reduction in the number of cells that survive to form colonies.

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Registry No. 1, 7689-03-4; **2a**, 69203-72-1; **2b**, 69181-16-4; **3**, 118514-58-2; **4**, 118514-59-3; **5**, 118514-60-6; **6**, 118514-61-7; **7**, 118514-62-8; **8**, 118514-63-9; **9**, 118514-64-0; **10**, 118514-65-1; (±)-**11**, 118514-66-2; (±)-**12**, 118514-67-3; **13**, 55854-89-2; (±)-**14**, 118514-68-4; **15**, 25387-67-1; **16**, 7688-64-4; (±)-**17**, 34141-35-0; **18**, 26862-74-8; **19**, 118514-69-5; **20**, 35903-44-7; CH_2=C(CH_2Br)C-$O_2C_2H_5$, 17435-72-2; topoisomerase I, 80449-01-0.

2*H*-[1]Benzopyrano[3,4-*b*]pyridines: Synthesis and Activity at Central Monoamine Receptors

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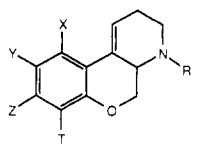
Two general synthetic approaches to a novel series of 2*H*-[1]benzopyrano[3,4-*b*]pyridines are described together with their receptor binding profile at a variety of monoamine receptors in mammalian brain tissue. The biologically active members of this series fall into into one of two broad classes: 3,4,4a,5-tetrahydro-2*H*-[1]benzopyrano[3,4-*b*]pyridines or *trans*-1,3,4,4a,5,10b-hexahydro-2*H*-[1]benzopyrano[3,4-*b*]pyridines. By appropriate pharmacophoric modification potent selective ligands for D_2, α-2, $5HT_{1A}$, and $5HT_2$ receptors may be obtained. The previously published in vivo data on certain key representatives of these series are also summarized.

During the course of investigations directed toward the discovery of novel, direct-acting modulators of central monoamine receptor function, we have synthesized a series of 2*H*-[1]benzopyrano[3,4-*b*]pyridines and studied their binding to a variety of monoamine receptors in mammalian brain tissue. Most of the biologically active members of this series fall into one of two broad classes: 3,4,4a,5-tetrahydro-2*H*-[1]benzopyrano[3,4-*b*]pyridines such as **1** or *trans*-1,3,4,4a,5,10b-hexahydro-2*H*-[1]benzopyrano-[3,4-*b*]pyridines such as **2**. The former class of compounds may be viewed as tricyclic analogues of lysergic acid (**3**) in which the pyrrole ring has been eliminated and an oxygen has been introduced at the former 3-position of the indole moiety. Lysergic acid derivatives have a long history of central nervous system (CNS) activity. They are also known to have affinity for central monoamine receptors.[1] The hexahydro analogues **2** are structurally related to **4** (PHNO, MK 458)[2] and to the 1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinolines, which have been previously described by Cannon[3] and Carlsson.[4] All of these derivatives are rigid structures that lock the phenethylamine pharmacophore in the extended presumed bioactive conformation.[5] It was envisioned that such templates could be



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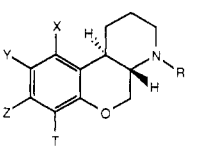

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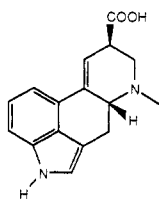

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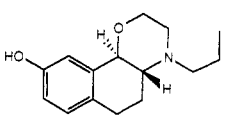

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modified to give selective binders to a variety of monoamine receptors as well as being effective probes to explore the relative similarities and differences in the topology of a number of central monoamine receptors. In practice, the modification of the benzopyranopyridine template **2** has led to the discovery of four compounds that have been selected for extensive biological evaluation and toxicological assessment, namely, **2k** (CGS 15855A),[6] **2m** (CGS 15873A),[6,7] and **2u** (CGS 19845)[8] as selective presynaptic

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