Plant Antitumor Agents. 18.[1] Synthesis and Biological Activity of Camptothecin Analogues

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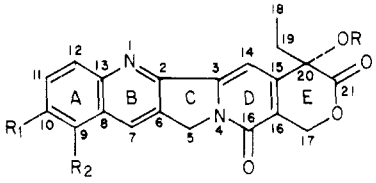
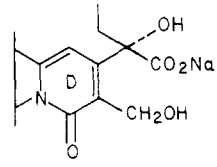
Four analogues, 10-methoxy (**20**), 12-aza (**29**), benz[*j*] (**36**), and 18-methoxy (**38**), of camptothecin were obtained by total synthesis. The two water-soluble analogues, 10-[(carboxymethyl)oxy]- (**24**) and 10-[2′-(diethylamino)-ethoxy]-20(*S*)-camptothecin (**26**), with intact ring E were prepared from natural 10-hydroxycamptothecin (**3**). In general, there was a good correlation between in vitro 9KB cytotoxicity and activity in the P-388 leukemia system. While the aza analogue **29** was active in P-388 only at a much higher dose level than natural camptothecin (**1**), the 18-methoxy analogue **38** exhibited activity comparable to that of **1**. The water-soluble derivative **24** was inactive. The amine hydrochloride **26** showed excellent activity at a high dose level. This could be due to its hydrolysis to **3**. *dl*-Camptothecin (**17**) was roughly half as active as **1**, indicating that the *l* isomer is inactive.

Camptothecin (**1**), a pentacyclic alkaloid isolated from



1, R = R_1 = R_2 = H
3, R = R_2 = H; R_1 = OH
4, R = R_2 = H; R_1 = OMe
5, R = R_1 = H; R_2 = OMe



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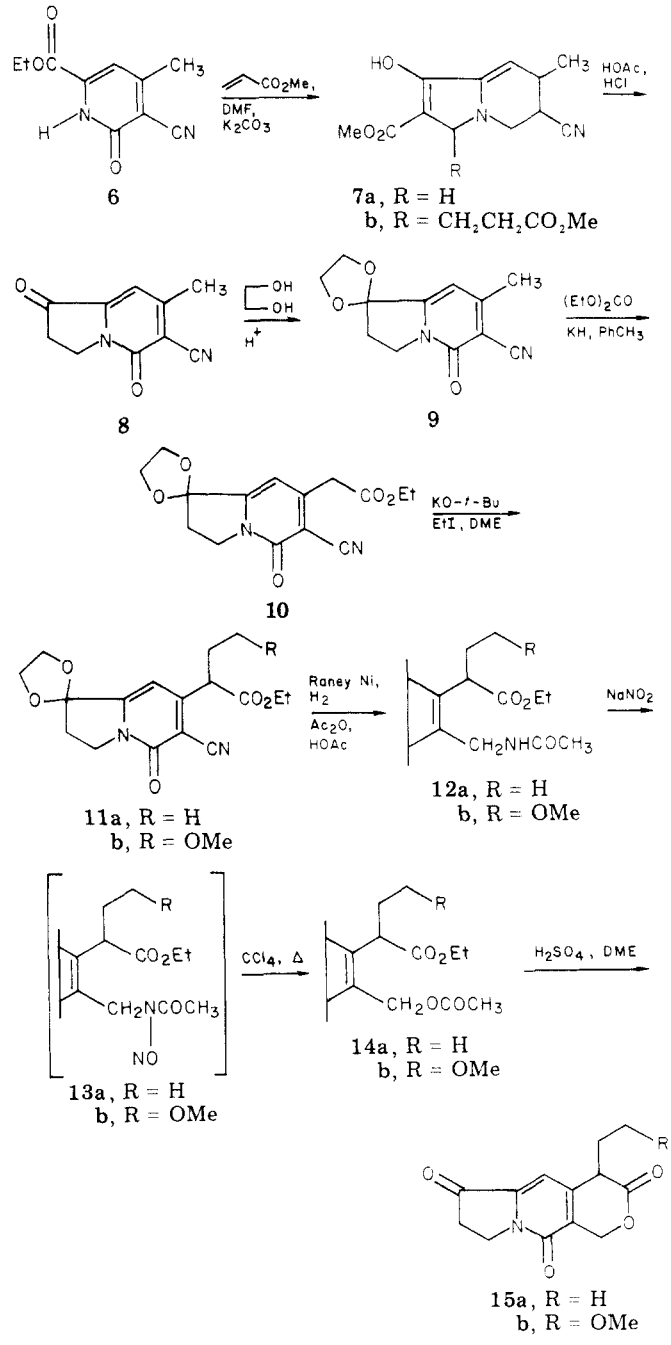
the wood and bark of *Camptotheca acuminata* by Wall and co-workers, displayed excellent antitumor activity in animal testing with therapeutic indices greater than 10.[2] Camptothecin, however, was highly insoluble in water. It was found that the sodium salt (**2**) of camptothecin was water soluble and active in animal trials, although a good comparison of the antitumor activity of the sodium salt and **1** was not conducted until recently.[3] Unfortunately, initially encouraging reports[4] on favorable activity of the sodium salt of **1** in gastrointestinal carcinoma could not be confirmed by later studies.[5,6] As a result, there has been considerable research interest in the synthesis of analogues of **1**.

Studies from this laboratory have established some important parameters for antitumor activity in the camptothecin series.[7] It has been shown that the α-hydroxy lactone moiety present in ring E is an absolute requirement for antitumor activity. The 20-ethyl substituent in ring E can be replaced by groups such as allyl and propargyl, with the allyl analogue being more active than **1**.[8] Oxygenated ring A analogues **3**–**5** are highly active in both L-1210 and P-388 leukemia systems, thus indicating that substitution in ring A is compatible with biological activity. A large number of ring DE and CDE analogues are inactive[9-12] as antitumor agents, indicating that factors other than the α-hydroxy lactone moiety are required for biological activity. In order to further delineate the structural

Scheme I



(1) For paper 17, see M. E. Wall and M. C. Wani, *J. Med. Chem.*, **21**, 1186 (1978).
(2) M. E. Wall, M. C. Wani, C. E. Cook, K. H. Palmer, A. T. McPhail, and G. A. Sim, *J. Am. Chem. Soc.*, **94**, 3888 (1966).
(3) Unpublished results from this laboratory show that the sodium salt **2** is only 10–20% as active as **1**.
(4) J. A. Gottlieb, A. M. Guarino, J. C. Call, V. T. Oliverio, and J. B. Block, *Cancer Chemother. Rep.*, **54**, 461 (1970).
(5) C. G. Moertel, A. J. Schutt, R. J. Reitmeier, and R. C. Hahn, *Cancer Chemother. Rep.*, **56**, 95 (1972).
(6) J. A. Gottlieb and J. K. Luce, *Cancer Chemother. Rep.*, **56**, 103 (1972).
(7) M. E. Wall and M. C. Wani, *Annu. Rev. Pharmacol. Toxicol.*, **17**, 117 (1977).
(8) T. Sugasawa, T. Toyoda, N. Uchida, and K. Yamaguchi, *J. Med. Chem.*, **19**, 675 (1976).

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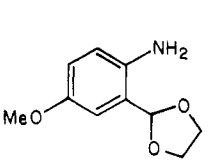
parameters for biological activity, we have continued our work on the synthesis of camptothecin analogues. This report describes the synthesis of several analogues from the key tricyclic intermediates **15a** and **15b**. It also describes the biological activity of these analogues and of some new water-soluble analogues.

Chemistry. The synthesis of the key tricyclic intermediate **15a** is shown in Scheme I. This synthetic sequence is a modification of the procedure used by the Chinese workers.[13,14] The known pyridinone **6**[15] was converted to the bicyclic pyridinone **7** by treatment with methyl acrylate and K_2CO_3 in dimethylformamide. When this was done using the Chinese conditions of a higher temperature (70 °C instead of 45 °C), it was found that 2 mol of methyl acrylate was added, forming **7b** as the exclusive product instead of **7a**. By reducing the temperature to 45 °C, a 75% yield of **7a** could be realized. Hydrolysis and decarboxylation of **7a** to the ketone **8** were effected by refluxing in a mixture of HOAc and concentrated HCl under nitrogen. Without a nitrogen atmosphere, the product **8** quickly decomposed. Ketone **8** was, however, stable in a crystalline form. Ketalization did not proceed unless a two-phase system of toluene and ethylene glycol was used. The ketone was converted to the ketal **9** plus a side product, the enol ether of the ketone **8**. The enol ether remained in the ethylene glycol phase and the ketal, being somewhat more hydrophobic, partitioned into the toluene layer. The toluene layer containing nearly pure ketal could be decanted and fresh toluene added so that the enol ether in the ethylene glycol layer could be converted to the ketal. Using this procedure, the original yield of 40% was increased to more than 90%. Construction of the E ring began with functionalization of the methyl group. Unless the ketal **9** was absolutely pure, its reaction with diethyl carbonate in the presence of potassium hydride would not proceed. Trace amounts of impurity **7a** completely suppressed the reaction, resulting in recovered ketal **9** instead of the product **10**. An important improvement over the literature procedure[13b] was the use of potassium *tert*-butoxide, dimethoxyethane, and EtI for the ethylation of **10**. The yield was increased from 80 to 98% but, more importantly, the difficult separation of **10** and **11a** was avoided.

At this stage, our synthetic scheme deviated from that of the Chinese workers. Catalytic hydrogenation of **11a** in the presence of Raney Ni in a mixture of Ac_2O and HOAc gave the amide **12a**. Removal of the catalyst by filtration followed by the addition of $NaNO_2$ to the filtrate gave the *N*-nitroso amide **13a**. If decomposition of the *N*-nitroso amide **13a** was attempted in HOAc and Ac_2O, no product resulted. However, by isolating the *N*-nitroso derivative which was relatively stable and decomposing it by heating in an inert solvent (CCl_4), a good yield (>90%) of the acetate **14a** resulted. The Chinese workers attempted to prepare this intermediate but were unsuccessful.[13b] The diester **14a** was lactonized by 2 N H_2SO_4 in dimethoxyethane with simultaneous deketalization to give the intermediate **15a** (40% overall yield from **6**).

Friedlander condensation of the ketone **15a** with *N*-(*o*-aminobenzylidene)-*p*-toluidine[16] in the presence of an acid catalyst gave the known 20-deoxycamptothecin (**16**),[16]



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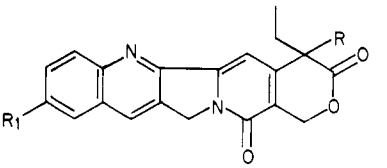
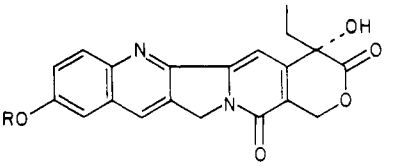


16, R = R_1 = H
17, R = OH; R_1 = H
19, R = H; R_1 = OMe
20, R = OH; R_1 = OMe
21, R = R_1 = OH

which was oxidized to *dl*-camptothecin (**17**) by passing oxygen through a solution of **16** in dimethylformamide in the presence of $CuCl_2$.[16] Similarly, Friedlander condensation of the *o*-amino acetal **18** with **15a** gave *dl*-10-methoxy-20-deoxycamptothecin (**19**). The latter on oxidation[16] gave *dl*-10-methoxycamptothecin (**20**), which was identical with natural 10-methoxycamptothecin (**4**)[17] by TLC and spectral properties. Demethylation of **20** in refluxing hydrobromic acid gave *dl*-10-hydroxycamptothecin (**21**), which was identical with natural 10-hydroxycamptothecin (**3**)[17] by TLC and spectral properties.

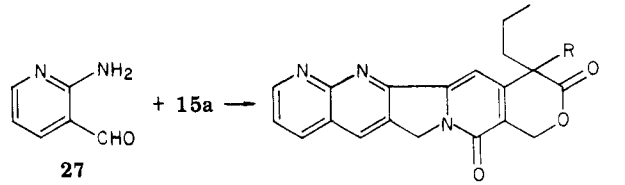
The water-soluble analogues **24** and **26** were prepared from the natural 10-hydroxycamptothecin (**3**) rather than from the racemic isomer **21**. Thus, alkylation of **3** with ethyl bromoacetate in refluxing acetone in the presence of anhydrous K_2CO_3 gave **22**. In dilute base the carb-



22, R = CH_2CO_2Et
23, R = CH_2CO_2H
24, R = CH_2CO_2Na
25, R = $CH_2CH_2NEt_2$
26, R = $CH_2CH_2NEt_2$·HCl

ethoxymethyloxy derivative **22** was hydrolyzed to the acid **23**, which was then converted to the sodium salt **24** by treatment with $NaHCO_3$. Alkylation of **3** with 2-(diethylamino)ethyl chloride in dimethylformamide in the presence of anhydrous K_2CO_3 gave the diethylaminoethyl derivative **25**, which was then converted to the hydrochloride **26** by treatment with anhydrous HCl in Et_2O.

dl-12-Aza-20-deoxycamptothecin (**28**) was obtained in



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28, R = H
29, R = OH

(9) M. E. Wall, H. F. Campbell, M. C. Wani, and S. G. Levine, *J. Am. Chem. Soc.*, **94**, 3632 (1972).
(10) S. Danishefsky, J. Quick, and S. B. Horwitz, *Tetrahedron Lett.*, 2525 (1973).
(11) J. J. Plattner, R. D. Gless, G. K. Cooper, and H. Rapoport, *J. Org. Chem.*, **39**, 303 (1974).
(12) S. Danishefsky and S. J. Etheredge, *J. Org. Chem.*, **39**, 3432 (1974).
(13) (a) *Chem. Abstr.*, **84**, 171 221*m* (1976); (b) Shanghai No. 5 Pharmaceutical Plant, *Sci. Sin.*, **21**, 87 (1978).
(14) At the time we began our work (1976) experimental details of the Chinese scheme (ref 13a) were not available. After we had completed our work, the Chinese published full details of their experimental. In many instances, our procedures give better yields than those of the Chinese, so we have described our experiments in full detail.
(15) H. Henk, *Chem. Ber.*, **82**, 36 (1949).
(16) C. Tang, J. C. Morrow, and H. Rapoport, *J. Am. Chem. Soc.*, **97**, 159 (1975).
(17) *Chem. Abstr.*, **87**, 184 742*U*; Shanghai Fifth Pharmaceutical Plant, *K'φ Hsueh T'ung Poa*, **22**, 269 (1977).

Table I. Antileukemic Activity against the P-388 System and Cytotoxicity against 9KB Cells of Camptothecin and Its Analogues

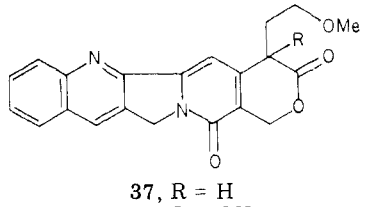
no.	dose range, mg/kg	optimal % T/C	optimal dose, mg/kg	lowest toxic dose, mg/kg	therapeutic index	9KB ED_{50}, μg/mL
1	8.0-0.5	197	4.0	8.0	8	2×10^{-2}
2	80.0-2.5	212	40.0	80.0	4	2×10^{-1}
3	8.0-0.5	314	4.0	8.0	8	2×10^{-2}
4	4.0-0.5	145	0.5	1.0	1	2×10^{-2}
17	16.0-1.0	222	8.0	16.0	8	1×10^{-1}
24	32.0-4.0	*a*				$>1 \times 10^{0}$
26	32.0-2.0	234	32.0		16	$>1 \times 10^{0}$
29	32.0-2.0	175	32.0		2	$>1 \times 10^{0}$
36	32.0-1.0	198	16.0		4	2×10^{-1}
38	8.0-0.5	160	4.0	8.0	2	2×10^{-1}

[a] Inactive.

50% yield by condensing 2-amino-3-formylpyridine (**27**)[18] with ketone **15a**. Oxidation[16] of **28** gave *dl*-12-azacamptothecin (**29**) in poor yield (24%) partly because of the difficulty in separating **29** from the cupric salts used in the oxidation step.

The hexacyclic analogue, *dl*-benz[*j*]camptothecin (**36**), was made starting with 3-nitro-2-naphthoic acid (**30**)[19] (Scheme II). Reduction of **30** with diborane in tetrahydrofuran gave the alcohol **31**, which was oxidized with activated MnO_2 in chloroform to the aldehyde **32**. The latter upon treatment with ethylene glycol in refluxing toluene in the presence of *p*-toluenesulfonic acid gave the acetal **33**. Reduction of the nitro acetal **33** with H_2 in the presence of Pt gave the amino acetal **34**, which condensed smoothly with ketone **15a** to give *dl*-benz[*j*]-20-deoxycamptothecin (**35**). Oxidation[16] of **35** gave the hexacyclic analogue **36**.

The synthesis of the ring E analogue, *dl*-18-methoxycamptothecin (**38**), was accomplished by using a sequence similar to that used for *dl*-camptothecin (**17**). Instead of alkylating the bicyclic pyridinone **10** with EtI, a different set of conditions employing 2-methoxyethyl bromide in dimethylformamide in the presence of potassium *tert*-butoxide gave the alkylated product **11b** in 61% yield. The same sequence outlined in Scheme I then gave the tricyclic ketone **15b**, which upon condensation with *N*-(*o*-aminobenzylidene)-*p*-toluidine[16] gave the deoxy analogue **37**. Oxidation[16] of the latter as before led to the desired analogue **38**.



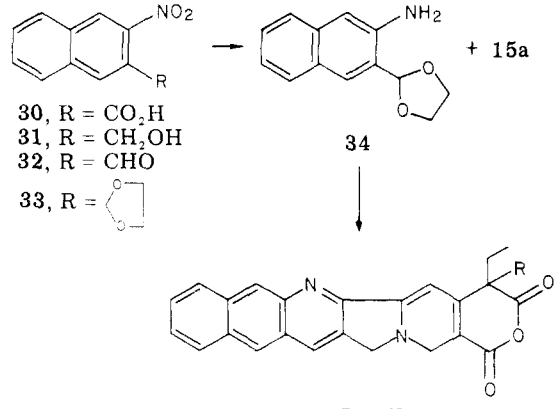
37, R = H
38, R = OH

Biological Activity. The results of in vitro cytotoxicity (9KB) and in vivo P-388 leukemia (PS) bioassays are shown in Table I. The 9KB tests were conducted at the Research Triangle Institute and the PS assays by National Cancer Institute contractors using standard procedures.[20] In general, there was excellent correlation between 9KB and PS data in the compounds under study.

Discussion

In the course of our studies it has become evident that the antitumor activity of camptothecin involves two

Scheme II



35, R = H
36, R = OH

structural factors: (1) the flat, planar aromatic structure inherent in rings A, B, C, and D which may be responsible for intercalation and has been shown by Horwitz[21] to be responsible for the inhibition of RNA synthesis and DNA depolymerization, and (2) the α-hydroxy lactone moiety in ring E which Wall[22] has shown to be a requisite for antitumor activity. Recently, Adamovics and Hutchinson[23] reported the synthesis of several camptothecin analogues in which ring E was converted to open-chain amide derivatives. These compounds were somewhat less active than **1** and their activity could be due to relactonization to **1** in vivo. One of the amide analogues was converted to another derivative which could no longer be relactonized and was completely inactive in "in vivo" leukemia systems. Analogues can be highly active as DNA and RNA inhibitors but inactive as antitumor agents; for example, deoxycamptothecin which lacks the α-hydroxy group is about equipotent to camptothecin in the inhibition of RNA synthesis and production of DNA depolymerization[21] but is totally inactive in in vivo leukemia systems.[22]

Camptothecin and the analogues described in this paper could be placed in three categories: (1) Analogues with the highest in vivo and in vitro activity. In this category were the various naturally occurring optically active alkaloids, including camptothecin **1** and its hydroxy (**3**) and methoxy (**4**) analogues. These compounds all had 9KB activity of the order of ED_{50} = 0.02 μg/mL. PS leukemia activity in this group was generally very high and was associated with high potency. Compound **3** was clearly the

(18) R. Majewicz and P. Caluwe, *J. Org. Chem.*, **39**, 720 (1974).
(19) M. Look, *Aldrichim. Acta*, 7, 23 (1974).
(20) R. I. Geran, N. H. Greenberg, M. M. McDonald, A. M. Schumacher and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, 3, 1 (1972).
(21) S. Horwitz, *Antibiotics*, **3**, 48-57 (1975).
(22) M. E. Wall, *Biochem. Physiol. Alkaloide, Int. Symp., 4th*, 1969, pp 77-87.
(23) J. A. Adamovics and C. R. Hutchinson, *J. Med. Chem.*, **22**, 310 (1979).

most potent and active compound in this group. (2) Analogues with intermediate activity. This group included compounds with 9KB activity of the order of ED_{50} = 0.1 μg/mL, e.g., the synthetic camptothecin analogues **17**, **36**, **38**, and the optically active water-soluble sodium salt **2**. PS activity was frequently high, but potency was lower than that of group 1. (3) Analogues which are weakly active or inactive. This group included inactive or weakly active compounds with ED_{50} values >1.0. Activity in PS when found occurred only at high dose levels. This category included the synthetic 12-aza analogue **29** and two water-soluble salts **24** and **26**.

Our present synthetic studies had two objectives in mind. The first was to prepare water-soluble analogues of camptothecin which might show better potency than **2**. As noted in Table I, compound **2** has only one-tenth the potency of **1**, although it is of the same order of activity as **1** at high dose levels. When **2** was administered by the intravenous (iv) route to mice, it was inactive in L-1210 leukemia, although it was of the same order of activity as **1** in L-1210 by the intraperitoneal (ip) route. These results suggest that **2** is inefficiently relactonized to **1** under ip conditions and at pH 7.2 (iv conditions) is not relactonized at all. Accordingly, we prepared the salts **24** and **26** which would not require relactonization for activity.

The selection of 10-hydroxycamptothecin (**3**) for this purpose was based on the strong activity of this compound. In the event the carboxy sodium salt **24** was totally inactive, the amine hydrochloride **26** had good activity at high dose levels but no cytotoxicity. From preliminary studies, we have reason to believe that the activity of **26** may be due to hydrolysis to **3**.[24] Hence, **26** may have some therapeutic possibilities as a *nontoxic, prodrug* for **3**. The inactivity of **24** and **26** may be due to steric or electronic interactions.

The availability of the tricyclic intermediate **15a** permitted us to survey other structure–function parameters, and this was a second objective of our studies. Although many syntheses of racemic camptothecin have been published, to the best of our knowledge none provided sufficient quantity for even minimal biological in vivo evaluation. *dl*-Camptothecin (**17**) showed cytotoxicity about one-tenth and P-388 leukemia activity about one-half that of **1**; i.e., maximal activity of **17** was observed at 8 mg/kg; for **1**, maximal activity was found at 4 mg/kg. Thus, it is evident that the *S* configuration of natural camptothecin is required for maximal antitumor activity. The lower 9KB cytotoxicity of **17** may indicate that the *S* configuration may be an *important factor* in DNA intercalation or binding also. The synthesis of compound **38** is the first approach to placing more polar substituents elsewhere in the molecule. The prior work of Sugasawa[8] had indicated that considerable modification of the alkyl substituent at C-20 was compatible with activity. The *dl*-18-methoxy analogue **38** was comparable in activity to *dl*-camptothecin **17** but somewhat more toxic. The 12-aza-analogue **29** was synthesized to determine the effect of replacing a benzene ring with a pyridine ring. We expected to find activity of **29** at least of the same order as **17**. Surprisingly, it was only of the order of one-eighth the activity of **17**.

We have subsequently been informed[25] that naphthyridine compounds which have ring AB systems similar to **29** complex with metals via the adjacent nitrogen atoms. If a similar metal complex were formed from **29**, steric or electronic interference of its binding with nucleic acid might occur. 10-Azacamptothecin, an isomer of **29**, is now in preparation to explore this hypothesis.

Finally, we should like to comment on the excellent predictability of the 9KB assay for in vivo activity in the camptothecin series. To be reliable it must be emphasized that new camptothecin analogues should always be tested against positive controls such as **1**, **2**, and **17**.

(24) In an aqueous solution, the amine hydrochloride **26** slowly undergoes hydrolysis to **3** as indicated by TLC. The sodium salt **24** is relatively stable under aqueous conditions.

(25) Private communication from Dr. S. G. Levine, whom we thank.

Experimental Section

Melting points (Kofler hot-stage microscope) are uncorrected. Infrared spectra were measured with a Perkin-Elmer 467 spectrophotometer. NMR spectra were recorded on a Varian Model HA-100 using Me_4Si as an internal standard. Chemical shifts are expressed in δ units. Only significant spectral data are reported. Mass spectra were recorded with an Associated Electrical Industries MS-902 instrument. Where analyses are indicated by mass spectra, the homogeneity of these compounds was established by TLC and other spectral properties. Microanalyses were carried out by Integral Microanalytical Laboratories, Inc., Raleigh, N.C. Each compound had IR and NMR spectra compatible with its structures. Analytical results were within ±0.4% of the theoretical values. All reactions were carried out under N_2 unless otherwise specified.

2-(Methoxycarbonyl)-6-cyano-7-methyl-1,5-dioxo-$\Delta^{6(8)}$-tetrahydroindolizine (7a). Anhydrous, powdered K_2CO_3 (12.6 g, 0.091 mol) was added to a solution of pyridinone **6**[15] (18.0 g, 0.87 mol) in DMF (500 mL), and the mixture was heated at 45 °C in a water bath. After the formation of a bright yellow suspension (typically 5–10 min), methyl acrylate (62 g, 0.73 mol) was added over a 10-min period and the reaction mixture was stirred vigorously at 45 °C for 40 h. The progress of the reaction was followed by TLC [SiO_2; toluene–dioxane–HOAc (90:25:4)]. The precipitate was filtered, and the residue was suspended in H_2O (500 mL) and acidified to pH 1.5 (concentrated HCl). The product was filtered and purified by crystallization from aqueous HOAc (15.9 g, 74%): mp 250 °C dec (lit.[13] 250 °C dec).

2-(Methoxycarbonyl)-3-[2′-(methoxycarbonyl)ethyl]-6-cyano-7-methyl-1,5-dioxo-$\Delta^{6(8)}$-tetrahydroindolizine (7b). A solution of **7a** (100 mg, 0.406 mmol), anhydrous K_2CO_3 (84 mg, 0.609 mmol), and methyl acrylate (350 mg, 4.06 mmol) in DMF (2.5 mL) was heated on a steam bath for 10 h. The solution was cooled and then filtered. The precipitate was suspended in H_2O (5 mL) and acidified to pH 1.5 (concentrated HCl). The product was filtered and crystallized from MeOH (105 mg, 78%): mp 178–179 °C; IR ν_{max} ($CHCl_3$) 1730 (ester C=O), 1665 cm^{-1} (pyridinone); ^1H NMR ($CDCl_3$) δ 1.87 (m, 2, CH_2-COR), 2.52 (s, 3, CH_3), 3.0 (m, 2, $CHCH_2CH_2CO_2Me$), 3.53 (s, 3, CO_2CH_3), 3.90 (s, 3, CO_2CH_3), 5.30 (t, 1, J = 2 Hz, $CHCH_2$), 6.47 (s, 1, aromatic). Anal. ($C_{16}H_{16}N_2O_6$) C, H, N.

6-Cyano-7-methyl-1,5-dioxo-$\Delta^{6(8)}$-tetrahydroindolizine (8). A solution of the keto ester **7a** (22.5 g, 0.091 mol) in a mixture of acetic acid (225 mL) and concentrated HCl (225 mL) was refluxed for 2 h. The acid solution was extracted with CH_2Cl_2 until TLC [SiO_2; $CHCl_3$–acetone–MeOH (75:20:5)] showed the absence of ketone in the aqueous phase. The combined extracts were washed with brine, dried (Na_2SO_4), and evaporated. The resulting solid was crystallized from acetone to yield **8** (15.3 g, 89%): mp 219 °C (lit.[13b] 210 °C).

6-Cyano-1,1-(ethylenedioxy)-7-methyl-5-oxo-$\Delta^{6(8)}$-tetrahydroindolizine (9). A mixture of the ketone **8** (13.0 g, 0.069 mol), ethylene glycol (210 mL), and *p*-toluenesulfonic acid (1.1 g) in toluene (1.0 L) was refluxed using a Dean–Stark trap for 5 h. The toluene layer was decanted and another liter of toluene was added. The reaction mixture was refluxed for an additional 5 h and the toluene layer decanted as before. After repeating this procedure two more times, the toluene layers were combined, washed with brine, dried (Na_2SO_4), and evaporated to yield the crude product, which was crystallized from MeOH to give **9** (14.9 g, 93%): mp 191–192 °C (lit.[13b] 192 °C).

6-Cyano-1,1-(ethylenedioxy)-7-[(ethoxycarbonyl)-methyl]-5-oxo-$\Delta^{6(8)}$-tetrahydroindolizine (10). To a suspension of KH (11.9 g, 0.068 mol; the 23% dispersion of KH in mineral oil was washed twice with hexane and once with toluene before use) in toluene (40 mL) was added the ketal **9** (5.0 g, 0.022 mol).

The purity of the ketal was very critical for the success of the reaction. The reaction mixture was refluxed for 10 min before adding diethyl carbonate (6.79 g, 0.058 mol) and a catalytic amount (0.31 g, 6.7 mmol) of absolute ethanol over a period of 5 min. After refluxing the reaction mixture for 3 h, the hard, dark solid was crushed and the resulting suspension filtered. The dry potassium salt was decomposed by very cautious addition to cold aqueous acetic acid. It was then diluted with H_2O (100 mL) and the resulting solution was extracted with CH_2Cl_2 (3 × 100 mL). The organic phase was washed with brine, dried (Na_2SO_4), and evaporated to yield the crude product, which was purified by elution from silica gel with 2% MeOH in $CHCl_3$, followed by crystallization from MeOH (4.97 g, 76%): mp 172–173 °C (lit.[13b] 173 °C).

6-Cyano-1,1-(ethylenedioxy)-7-[1′-(ethoxycarbonyl)-propyl]-5-oxo-$\Delta^{6(8)}$-tetrahydroindolizine (11a). To a solution of ester **10** (4.01 g, 0.0132 mol) in anhydrous DME (70 mL) cooled to −78 °C was added potassium *tert*-butoxide (1.7 g, 15 mmol), and the resulting mixture was allowed to stir for 5 min before adding ethyl iodide (8.24 g, 0.053 mol) over a period of 5 min. The reaction mixture was stirred for 1.5 h at −78 °C, then allowed to warm to room temperature, and stirred overnight. After dilution of the mixture with H_2O (60 mL), the aqueous phase was extracted with CH_2Cl_2 (3 × 50 mL). The organic phase was washed with brine, dried (Na_2SO_4), and evaporated to yield the ester **11a** (4.3 g, 98%) of sufficient purity for the next step. A small sample was crystallized from $CHCl_3$: mp 126–127 °C (lit.[13b] 122–123 °C).

6-Cyano-1,1-(ethylenedioxy)-7-[1′-(ethoxycarbonyl)-3′-methoxypropyl]-5-oxo-$\Delta^{6(8)}$-tetrahydroindolizine (11b). A solution of ester ketal **10** (1.50 g, 4.93 mmol) in DMF (30 mL) was cooled in an ice bath and potassium *tert*-butoxide (0.656 g, 5.86 mmol) was added. After the solution was stirred for 10 min, 2-methoxyethyl bromide (4.02 g, 28.9 mmol) was added and the ice bath exchanged for an oil bath at 50 °C. The reaction mixture was stirred at 50 °C for 24 h, diluted with water (400 mL), and extracted with CH_2Cl_2 (4 × 100 mL). The combined CH_2Cl_2 extracts were washed with H_2O, dried (Na_2SO_4), and evaporated to give a crude solid, which was chromatographed (EM silica gel 60 prepacked column, 30% hexane–EtOAc) to give a white solid (1.09 g, 61%): mp 101–102 °C; IR ν_{max} ($CHCl_3$) 2230 (CN), 1730 (ester C=O), 1660 cm^{-1} (pyridinone); ^1H NMR ($CDCl_3$) δ 1.23 (t, 3, J = 7 Hz, CH_2CH_3), 1.90 (m, 2, $CHCH_2CH_3$), 2.37 (t, 2, J = 7 Hz, CH_2 α to ketal), 3.28 (s, 3, OCH_3), 3.35 (m, 2, CH_2OCH_3), 4.13 (s, 4, OCH_2CH_2O), 6.27 (s, 1, aromatic). Anal. ($C_{18}H_{22}N_2O_6$) C, H, N.

6-(Acetamidomethyl)-1,1-(ethylenedioxy)-7-[1′-(ethoxycarbonyl)propyl]-5-oxo-$\Delta^{6(8)}$-tetrahydroindolizine (12a). A solution of ester **11a** (2.0 g, 6.0 mmol) in a mixture of acetic anhydride (30 mL) and acetic acid (10 mL) was hydrogenated in the presence of Raney nickel (3 g; prewashed with acetic acid) during 6 h at 45 °C under 50 psi. The progress of the reaction was followed by TLC [SiO_2, $CHCl_3$–acetone–MeOH (75:20:5)]. The catalyst was removed by filtration, and the solvent was removed in vacuo to yield a crude oil (2.3 g, 100%), which was pure enough for the next step. A small sample was purified by column chromatography (silica gel 60, 2% MeOH–$CHCl_3$). Although homogeneous by TLC, attempted crystallization of **12a** was unsuccessful: IR ν_{max} ($CHCl_3$) 3440 (NH), 1725 (ester C=O), 1665 (amide C=O), 1655 cm^{-1} (pyridinone); ^1H NMR ($CDCl_3$) δ 0.90 (t, 3, J = 4 Hz, CH_2CH_3), 1.18 (t, 3, J = 7 Hz, OCH_2CH_3), 1.8 (m, 2, CH_2CH_3), 1.90 (s, 3, $COCH_3$), 2.35 (t, 2, J = 7 Hz, CH_2 α to ketal), 3.70 (t, 1, J = 7 Hz, $CHCH_2$), 4.10 (s, 4, OCH_2CH_2O), 6.26 (s, 1 aromatic), 6.8 (br s, NH). Anal. ($C_{19}H_{26}N_2O_6$) m/e 378.179.

6-(Acetamidomethyl)-1,1-(ethylenedioxy)-7-[1′-(ethoxycarbonyl)-3′-methoxypropyl]-5-oxo-$\Delta^{6(8)}$-tetrahydroindolizine (12b). The title compound was obtained from **11b** in exactly the same manner as **12a**. Although homogeneous by TLC, attempted crystallization of **12b** was unsuccessful: IR ν_{max} ($CHCl_3$) 1725 (ester C=O), 1665 (shoulder, amide C=O), 1655 cm^{-1} (pyridinone); ^1H NMR ($CDCl_3$) δ 1.17 (t, 3, J = 7 Hz, OCH_2CH_3), 1.90 (s, 3, $COCH_3$), 2.1 (m, 2, $CHCH_2CH_3$), 2.35 (t, 2, J = 7 Hz), 3.24 (s, 3, OCH_3), 3.28 (m, 2, CH_2OCH_3), 4.09 (s, 4, OCH_2CH_2O), 6.28 (s, 1, aromatic), 6.8 (br s, NH). Anal. ($C_{20}H_{28}N_2O_7$) m/e 408.190.

6-(Acetoxymethyl)-1,1′-(ethylenedioxy)-7-[1′-(ethoxycarbonyl)propyl]-5-oxo-$\Delta^{6(8)}$-tetrahydroindolizine (14a). To a cooled solution of the amide **12a** (2.3 g, 6.0 mmol) in acetic anhydride (30 mL) and acetic acid (10 mL) was added $NaNO_2$ (1.8 g, 26 mmol) and the reaction mixture stirred for 2 h at 0 °C. The inorganic salts were removed by filtration and the solvent was removed in vacuo at room temperature to leave the *N*-nitroso intermediate **13a** as an oil, which was converted directly to the acetate **14a** by refluxing overnight in CCl_4. The CCl_4 solution was washed with H_2O and dried (Na_2SO_4), and the solvent was removed in vacuo to give an oil (2.3 g, 100%) which was used without further purification: IR ν_{max} ($CHCl_3$) 1730 (ester C=O), 1665 cm^{-1} (pyridinone); ^1H NMR ($CDCl_3$) δ 0.88 (t, 3, J = 6 Hz, CH_2CH_3), 1.18 (t, 3, J = 7 Hz, OCH_2CH_3), 1.8 (m, 2, $CH_2CH_2CH_3$), 2.02 (s, 3, OAc), 2.32 (t, 2, J = 6 Hz, CH_2 α to ketal), 3.73 (t, 1, J = 7 Hz, $CHCH_2$), 4.08 (s, 4, OCH_2CH_2O), 5.18 (s, 2, $ArCH_2O$), 6.23 (s, 1, aromatic). Anal. ($C_{19}H_{25}NO_7$) m/e 379.163.

6-(Acetoxymethyl)-1,1-(ethylenedioxy)-7-[1′-(ethoxycarbonyl)-3′-methoxypropyl]-5-oxo-$\Delta^{6(8)}$-tetrahydroindolizine (14b). The title compound was obtained from **12b** in exactly the same manner as **14a**. Although homogeneous by TLC, attempted crystallization of **14b** was unsuccessful: IR ν_{max} ($CHCl_3$) 1730 (ester and acetate C=O), 1660 cm^{-1} (pyridinone); ^1H NMR ($CDCl_3$) δ 1.17 (t, 3, J = 7 Hz, OCH_2CH_3), 2.00 (s, 3, OAc), 2.1 (m, 2, $CHCH_2CH_3$), 2.30 (t, 2, J = 7 Hz, CH_2 α to ketal), 3.23 (s, 3, OCH_3), 3.27 (m, 2, CH_2OCH_3), 4.07 (s, 4, OCH_2CH_2O), 5.17 (s, 2, $ArCH_2O$), 6.18 (s, 1, aromatic). Anal. ($C_{20}H_{27}NO_8$) m/e 409.173.

1,5-Dioxo(5′-ethyl-2′*H*,5′*H*,6′*H*-6-oxopyrano)[3′,4′-*f*]-$\Delta^{6(8)}$-tetrahydroindolizine (15a). The diester ketal **14a** (2.38 g, 6.0 mmol) was dissolved in DME (15 mL) and 2 N H_2SO_4 (15 mL) and stirred at 50 °C for 24 h. The reaction mixture was concentrated to approximately 10 mL in vacuo and then extracted with CH_2Cl_2 (3 × 20 mL). The organic phase was washed with H_2O, dried (Na_2SO_4), and evaporated to yield an oil, which was crystallized from EtOAc to yield **15a** (1.31 g, 88%): mp 162–163 °C; IR ν_{max} ($CHCl_3$) 1750 (shoulder, ketone), 1745 (lactone), 1660 cm^{-1} (pyridinone); ^1H NMR ($CDCl_3$) δ 1.00 (t, 3, J = 7 Hz, CH_3), 1.90 (m, 2, CH_2CH_3), 2.93 (t, 2, J = 7 Hz, CH_2CO), 3.50 (m, 1, $CHCH_2$), 4.32 (t, 2, J = 7 Hz, CH_2N), 5.33 (s, 2, $ArCH_2O$), 6.67 (s, 1, aromatic). Anal. ($C_{13}H_{13}NO_4$) C, H, N.

1,5-Dioxo[5′-(methoxyethyl)-2′*H*,5′*H*,6′*H*-6-oxopyrano]-[3′,4′-*f*]-$\Delta^{6(8)}$-tetrahydroindolizine (15b). The title compound was obtained from **14b** in 76% yield in exactly the same manner as **15a**. Although homogeneous by TLC, attempted crystallization of **15b** was unsuccessful: IR ν_{max} ($CHCl_3$) 1750 (shoulder, ketone C=O), 1740 (lactone), 1660 cm^{-1} (pyridinone); ^1H NMR ($CDCl_3$) δ 2.2 (m, 2, $CHCH_2CH_2$), 2.93 (t, 2, J = 7 Hz, CH_2CO), 3.30 (s, 3, OCH_3), 3.88 (t, 2, J = 7 Hz, CH_2OCH_3), 3.87 (m, 1, $CHCH_2$), 4.30 (t, 2, J = 7 Hz, CH_2N), 5.33 (AB q, 2, J = 5 Hz, $ArCH_2O$), 6.70 (s, 1, aromatic). Anal. ($C_{14}H_{15}NO_5$) m/e 277.096.

5-Methoxy-2-nitrobenzaldehyde Ethylene Acetal. A mixture of 5-methoxy-2-nitrobenzaldehyde (1.0 g, 5.5 mmol), ethylene glycol (2 mL), and *p*-toluenesulfonic acid (25 mg) in toluene (100 mL) was refluxed for 6 h using a Dean–Stark trap. The toluene solution was washed twice with H_2O, dried (Na_2SO_4), and evaporated in vacuo to give a brown oil (1.15 g, 92%). Although homogeneous by TLC, attempted crystallization of the acetal was unsuccessful: ^1H NMR ($CDCl_3$) δ 3.88 (s, OCH_3), 4.02 (s, 4, OCH_2CH_2O), 6.45 (s, 1, ArCH), 6.7–8 (m, 3, aromatic). Anal. ($C_{10}H_{11}NO_5$) m/e 225.064.

***dl*-20-Deoxycamptothecin (16).** A solution of *N*-(*o*-aminobenzylidene)-*p*-toluidine[16] (154 mg, 0.733 mmol) and tricyclic ketone **15a** (150 mg, 0.607 mmol) in toluene (9 mL) was refluxed using a Dean–Stark trap for 30 min. *p*-Toluenesulfonic acid (2 mg) was then added and refluxing was continued for an additional 3 h. The solvent was removed in vacuo and the residue chromatographed (SiO_2, 2% MeOH–$CHCl_3$) to give a yellow solid (159 mg, 79%), which was crystallized from $CHCl_3$–MeOH–Et_2O): mp 258–263 °C (lit.[16] 258–264 °C).

***dl*-Camptothecin (17).** Oxygen was bubbled through a solution of 20-deoxycamptothecin (**16**; 53 mg, 0.16 mmol), cupric chloride (54 mg, 0.32 mmol), and 40% aqueous dimethylamine (30 μL) in DMF (10 mL) until TLC [SiO_2; $CHCl_3$–acetone–MeOH (70:20:10)] showed all of the starting material had been consumed (ca. 20 h). The solvent was removed in vacuo and the residue was chromatographed (SiO_2, 5% MeOH–$CHCl_3$) to give a yellow solid (48 mg), which was further purified by crystallization from 13% MeOH–$CHCl_3$ and EtOAc (23 mg, 41%). This material was

shown to be identical with an authentic sample of natural **17** by NMR, IR, and TLC, mp 275–277 °C (lit.[16] 276–278 °C).

5-Methoxy-2-aminobenzaldehyde Ethylene Acetal (18). A solution of the preceding nitro acetal (1.00 g, 4.44 mmol) and technical grade (60%) sodium sulfide (2.13 g, 8.88 mmol) in ethanol (10 mL) and H_2O (2 mL) was refluxed for 30 min. Most of the solvent was removed in vacuo and the reaction mixture was diluted with H_2O (10 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 30 mL), dried (Na_2SO_4), and evaporated to yield a light colored oil (0.77 g, 89%). Although homogeneous by TLC, attempted crystallization of **18** was unsuccessful: IR ν_{max} ($CHCl_3$) 3440, 3360 cm^{-1} (NH_2); ^1H NMR ($CDCl_3$) δ 3.67 (s, 4, OCH_2CH_2O), 3.98 (s, 3, OCH_3), 5.73 (s, 1, ArCH), 6.2–7.3 (m, 3, aromatic). Anal. ($C_{10}H_{13}NO_3$) *m/e* 195.090.

***dl*-10-Methoxy-20-deoxycamptothecin (19).** A solution of amino acetal **18** (300 mg, 1.54 mmol) and tricyclic ketone **15a** (256 mg, 1.04 mmol) in toluene (40 mL) was refluxed using a Dean–Stark trap for 30 min. *p*-Toluenesulfonic acid (10 mg) was then added and refluxing was continued for an additional 2.5 h. The solvent was removed in vacuo and the residue chromatographed (SiO_2, 0.5% MeOH–$CHCl_3$) to give a yellow solid (318 mg, 85%), which was crystallized from $CHCl_3$–EtOAc, mp 266–267 °C (lit.[17] 264–265 °C).

***dl*-10-Methoxycamptothecin (20).** Oxygen was bubbled through a solution of 10-methoxy-20-deoxycamptothecin (**19**; 60 mg, 0.17 mmol), cupric chloride (100 mg, 0.743 mmol), and 40% aqueous dimethylamine (30 μL) in DMF (10 mL) until TLC [SiO_2; $CHCl_3$–acetone–MeOH (70:20:10)] showed all of the starting material had been consumed (ca. 20 h). The solvent was removed in vacuo and the residue passed through a clean-up column (SiO_2, 5% MeOH–$CHCl_3$). Further chromatography (SiO_2; 0.5% MeOH–$CHCl_3$) of this material gave a yellow solid (38 mg, 61%), which was crystallized from 13% MeOH–$CHCl_3$ and EtOAc. This material was shown to be identical with an authentic sample of natural **15** by NMR, IR, and TLC, mp 268–270 °C (lit.[17] 271–272 °C).

***dl*-10-Hydroxycamptothecin (21).** *dl*-10-Methoxycamptothecin (**20**; 30 mg, 0.079 mmol) was refluxed in 48% HBr (2 mL) for 1.5 h. The solvent was removed in vacuo and the residue chromatographed (silica gel 60, 2% MeOH–$CHCl_3$) to yield a yellow solid (14 mg, 48%), which was crystallized from 13% MeOH–$CHCl_3$ and EtOAc. This material was identical with an authentic sample of natural 10-hydroxy-20(*S*)-camptothecin by NMR, IR, and TLC, mp 265–268 °C (lit.[17] 263–264 °C).

10-[(Carbethoxymethyl)oxy]-20(*S*)-camptothecin (22). A suspension of 10-hydroxy-20(*S*)-camptothecin (**3**; 201 mg, 0.552 mmol), powdered K_2CO_3 (350 mg, 2.54 mmol), and ethyl bromoacetate (900 mg, 5.39 mmol) in anhydrous acetone (50 mL) was refluxed for 5 h. The reaction was monitored by TLC [SiO_2, $CHCl_3$–acetone-MeOH (75:20:5)] for the disappearance of starting material. The solvent was removed in vacuo and the residue acidified to pH 5–6 with 1 N HCl. The aqueous solution was extracted with CH_2Cl_2, dried (Na_2SO_4), and evaporated to leave a solid, which was chromatographed (silica gel 60, 1% MeOH–$CHCl_3$) to yield a yellow solid (154 mg, 62%). Crystallization from $CHCl_3$–EtOAc gave an analytical sample: mp 248–251 °C; IR ν_{max} (KBr) 1750 (shoulder, ester C=O), 1740 (lactone), 1660 cm^{-1} (pyridinone); ^1H NMR ($CDCl_3$–CD_3OD) δ 1.00 (t, 3, J = 7.5 Hz, CH_2CH_3), 1.31 (t, 3, J = 7 Hz, OCH_2CH_3), 1.90 (q, 2, J = 7.5 Hz, CH_2CH_3), 4.30 (q, 2, J = 7 Hz, OCH_2), 4.77 (s, 2, OCH_2CO_2Et), 5.21 (s, 2, $ArCH_2N$), 5.41 (AB q, 2, J = 8 Hz, $ArCH_2O$), 7.0–8.5 (m, 5, aromatic). Anal. ($C_{21}H_{22}N_2O_7$) C, H, N.

10-[(Carboxymethyl)oxy]-20(*S*)-camptothecin (23). A solution of ester **22** (70 mg, 0.156 mmol) and K_2CO_3 (87 mg, 0.63 mmol) in EtOH (4 mL) and H_2O (4 mL) was stirred at room temperature for 4 h, at which time TLC [SiO_2, $CHCl_3$–acetone–MeOH (75:20:5)] showed the presence of only trace amounts of starting material. The volume of the reaction mixture was reduced to approximately 0.5 mL. It was then transferred to a centrifuge tube and acidified to pH 2–3 with 1 N HCl. After the mixture was centrifuged, the supernatant was decanted, more H_2O was added to the yellow precipitate, and the process repeated twice more. The remaining H_2O was removed in vacuo, and the residue was dissolved in 13% MeOH–$CHCl_3$, filtered, and evaporated to yield a yellow solid (62.5 mg, 95%): mp 210–230 °C dec; IR ν_{max} (KBr) 1740 (br with shoulders, lactone and carboxylic acid), 1655 cm^{-1} (pyridindone); ^1H NMR (Me_2SO-d_6) δ 0.89 (t, 3, J = 7 Hz, CH_2CH_3), 1.83 (m, 2, CH_2CH_3), 4.85 (s, 2, OCH_2CO_2H), 5.18 (s, 2, $ArCH_2N$), 5.38 (s, 2, $ArCH_2O$), 7.2–8.6 (m, 5, aromatic). Anal. ($C_{22}H_{18}N_2O_7$) C, H, N.

Sodium Salt of 10-[(Carboxymethyl)oxy]-20(*S*)-camptothecin (24). Carboxylic acid **23** (62.5 mg, 0.148 mmol) was suspended in MeOH (4 mL) and $NaHCO_3$ (12.4 mg, 0.148 mmol) in H_2O (1 mL) was added. The acid dissolved almost instantaneously. The solvent was removed in vacuo, and the residue was taken up in water (3 mL) and washed once with CH_2Cl_2. The aqueous solution was lyophilized to yield a yellow solid (62.7 mg, 95%): mp gradual dec; IR ν_{max} (KBr) 1740 (lactone), 1660 cm^{-1} (pyridinone); ^1H NMR (Me_2SO-d_6) δ 0.87 (t, 3, J = 6 Hz, CH_2CH_3), 1.83 (m, 2, CH_2CH_3), 4.34 (s, 2, OCH_2CO_2Na), 5.16 (m, 2, $ArCH_2N$), 5.38 (s, 2, $ArCH_2O$), 7.1–8.5 (m, 5, aromatic). Anal. ($C_{22}H_{17}N_2O_7Na\cdot H_2O$) C, H, N.

10-[2′-(Diethylamino)ethoxy]-20(*S*)-camptothecin (25). A suspension of 10-hydroxy-20(*S*)-camptothecin (**3**; 100 mg, 0.275 mmol), 2-(diethylamino)ethyl chloride hydrochloride (77 mg, 0.448 mmol), and anhydrous K_2CO_3 (190 mg, 1.38 mmol) in dry DMF (2.5 mL) was stirred at 50 °C for 1 h. TLC [SiO_2, $CHCl_3$–acetone–MeOH (75:20:5)] showed no starting material. The solvent was removed in vacuo, and the residue was taken up in MeOH–$CHCl_3$, filtered, and chromatographed (silica gel 60, 20% MeOH–$CHCl_3$) to give a yellow solid (107 mg, 84%): mp 206–208 °C; IR ν_{max} (KBr) 1745 (lactone), 1655 cm^{-1} (pyridinone); ^1H NMR ($CDCl_3$–CD_3OD) δ 1.01 (t, 3, J = 6 Hz, CH_2CH_3), 1.11 (t, 6, J = 8 Hz, NCH_2CH_3), 1.94 (m, 2, CH_2CH_3), 2.72 (q, 4, J = 7 Hz, NCH_2CH_3), 3.0 (t, 2, J = 6 Hz, CH_2CH_2N), 5.24 (s, 2, $ArCH_2N$), 5.46 (AB q, 2, J = 16 Hz, $ArCH_2O$), 7.2–8.4 (m, 5, aromatic). Anal. ($C_{26}H_{29}N_3O_5\cdot H_2O$) C, H, N.

10-[2′-(Diethylamino)ethoxy]-20(*S*)-camptothecin Hydrochloride (26). The compound **25** (54 mg, 0.117 mmol) was dissolved in a mixture of absolute ethanol (4 mL) and chloroform (1 mL). The solution was cooled in a dry ice–CCl_4 bath and HCl gas was bubbled through the solution at a moderate rate for several minutes before flushing the system with a stream of N_2. The hydrochloride was precipitated by dilution with ether (10 mL), and the precipitate was filtered and dried under N_2 to yield **26** (58 mg, 100%): mp 167–174 °C; IR ν_{max} (KBr) 1745 (lactone), 1655 (pyridinone), 1620 and 1595 cm^{-1} (aromatic). Anal. ($C_{26}H_{29}N_3O_5\cdot HCl\cdot 1.5H_2O$) C, H, N.

***dl*-12-Aza-20-deoxycamptothecin (28).** Tricyclic ketone **15a** (222 mg, 0.899 mmol) and 2-amino-3-formylpyridine[18] (121 mg, 1.00 mmol) were heated neat at 100 °C for 2 h. The residue was chromatographed (silica gel 60, 2% MeOH–$CHCl_3$) to give a solid, which was crystallized from $CHCl_3$–EtOAc to yield **28** (156 mg, 52%): mp 280–285 °C dec; IR ν_{max} (KBr) 1740 (lactone), 1655 cm^{-1} (pyridinone); ^1H NMR (TFA-d_1) δ 1.17 (t, 3, J = 7 Hz, CH_2CH_3), 2.23 (m, 2, $CHCH_2CH_3$), 3.97 (m, 1, $CHCH_2$), 5.67 (s, 2, $ArCH_2O$ or CH_2N), 5.70 (s, 2, $ArCH_2O$ or $ArCH_2N$) 7.7–9.6 (m, 5, aromatic). Anal. ($C_{19}H_{15}N_3O_3$) C, H, N.

***dl*-12-Azacamptothecin (29).** Through a solution of **28** (103 mg, 0.309 mmol), cupric nitrate trihydrate (284 mg, 1.18 mmol), and 25% aqueous dimethylamine (80 μL) in DMF (25 mL) was bubbled oxygen for approximately 1 h. The reaction was monitored by TLC [silica gel, $CHCl_3$–acetone–MeOH (70:20:10)]. The solvent was removed in vacuo and the residue chromatographed on a short silica gel 60 column (10% MeOH–$CHCl_3$). The residue was taken up in a large amount of CH_2Cl_2 and washed with H_2O; the CH_2Cl_2 extract was dried (Na_2SO_4) and evaporated to yield a yellow solid, which was further purified by chromatography (silica gel 60, 5% MeOH–$CHCl_3$) and crystallized from 13% MeOH–$CHCl_3$ and EtOAc to yield **29** (26 mg, 24%): mp 300–305 °C dec; IR ν_{max} (KBr) 1740 (lactone), 1655 cm^{-1} (pyridinone); ^1H NMR (TFA-d_1) δ 1.15 (t, 3, J = 7 Hz, CH_2CH_3), 2.18 (q, 2, J = 7 Hz, CH_2CH_3), 5.76 (AB q, 2, J = 17 Hz, $ArCH_2O$), 5.79 (s, 2, CH_2N), 8–9.5 (m, 5, aromatic). Anal. ($C_{19}H_{15}N_3O_4$) C, H, N.

3-Nitro-2-naphthalenemethanol (31). A solution of diborane in THF (38 mL, 0.98 M, 37.2 mmol) was added dropwise to a solution of 3-nitro-2-naphthoic acid[19] (**30**; 2.72 g, 12.6 mmol) in anhydrous THF (15 mL). The solution was allowed to stir overnight at room temperature. The reaction was quenched with 50% aqueous THF (20 mL), and K_2CO_3 was added until the two phases separated. The aqueous layer was extracted with Et_2O (30 mL). The combined organic layers were dried ($MgSO_4$),

filtered, and evaporated to yield a solid, which was crystallized from $CHCl_3$–hexane to give **31** (2.42 g, 95%): mp 123–124 °C; ^1H NMR ($CDCl_3$–CD_3OD) δ 5.10 (s, 2, $ArCH_2O$), 7.3–8.8 (m, 6, aromatic). Anal. ($C_{11}H_9NO_3$) C, H, N.

3-Nitro-2-naphthaldehyde (32). MnO_2 (5 g, 57.5 mmol) was added to a solution of **31** (2.74 g, 13.5 mmol) in $CHCl_3$ (60 mL), and the solution was stirred at room temperature for 3 h. Additional MnO_2 was added in 5-g portions every 3 h until TLC (SiO_2, 5% MeOH–$CHCl_3$) showed the reaction was complete (a total of 25 g of MnO_2 was used). The reaction mixture was filtered and the solvent evaporated to give a brown solid, which was crystallized from $CHCl_3$–hexane to give **32** (1.80 g, 66%): mp 124 °C; IR ν_{max} ($CHCl_3$) 1695 cm^{-1} (aldehyde C=O); ^1H NMR ($CDCl_3$) δ 7.4–8.8 (m, 6, aromatic), 10.50 (s, 1, CHO). Anal. ($C_{11}H_7NO_3$) C, H, N.

3-Nitro-2-naphthaldehyde Ethylene Acetal (33). A solution of **32** (1.75 g, 8.71 mmol), ethylene glycol (3.5 mL), and *p*-toluenesulfonic acid (20 mg) in toluene (100 mL) was refluxed in a Dean-Stark apparatus for 4 h. The solvent was removed in vacuo and the residue taken up in CH_2Cl_2 (100 mL), washed with H_2O (2 × 100 mL), dried (Na_2SO_4), and evaporated to give a brown solid, which was crystallized from $CHCl_3$–hexane to give **33** (2.08 g, 97%): mp 112–114 °C; ^1H NMR ($CDCl_3$) δ 6.68 (s, 1, ArCH), 7.4–8.8 (m, 6, aromatic). Anal. ($C_{13}H_{11}NO_4$) C, H, N.

3-Amino-2-naphthaldehyde Ethylene Acetal (34). A solution of **33** (300 mg, 1.22 mmol) in ethanol (30 mL) was shaken in a Paar shaker in the presence of PtO_2 (200 mg) for 15 min at room temperature under hydrogen (40 psi). The reaction mixture was filtered and the solvent removed in vacuo to give an oil (236 mg, 100%), which was homogeneous by TLC: ^1H NMR ($CDCl_3$) δ 5.90 (s, 1, ArCH), 6.8–7.9 (m, 6, aromatic). Anal. ($C_{13}H_{13}NO_2$) *m/e* 215.095.

***dl*-Benz[*j*]deoxycamptothecin (35).** A solution of **34** (238 mg, 1.11 mmol) and the tricyclic ketone **15a** (211 mg, 0.854 mmol) in anhydrous toluene (50 mL) was refluxed for 30 min in a Dean–Stark apparatus. *p*-Toluenesulfonic acid (10 mg) was then added and the reaction mixture was refluxed for 3 h. The solvent was removed in vacuo and the residue chromatographed (silica gel, 2% MeOH–$CHCl_3$) to give a yellow solid, which was crystallized from 13% MeOH–$CHCl_3$ to give **35** (148 mg, 41%): mp 306 °C dec; IR ν_{max} (KBr) 1735 (lactone), 1660 cm^{-1} (pyridinone); ^1H NMR (TFA-d_1) δ 1.19 (t, 3, J = 7 Hz, CH_2CH_3), 2.26 (m, 2, CH_2CH_3), 4.04 (m, 1, ArCH), 5.72 (AB q, 2, J = 18 Hz, $ArCH_2O$), 5.82 (s, 2, CH_2N), 7.8–9.6 (m, 8, aromatic). Anal. ($C_{24}H_{18}N_2O_3$) C, H, N.

***dl*-Benz[*j*]camptothecin (36).** Oxygen was bubbled through a solution of **35** (280 mg, 0.733 mmol), cupric acetate (150 mg), and 25% aqueous dimethylamine (1.5 mL) in DMF (150 mL) until TLC [SiO_2, MeOH–acetone–$CHCl_3$ (5:20:75)] showed the disappearance of starting material (approximately 2 h). The solvent was removed in vacuo and the residue chromatographed (silica gel 60, 4% MeOH–$CHCl_3$) to give a yellow solid, which was crystallized from 13% MeOH–$CHCl_3$ and then HOAc to yield yellow needles (68 mg, 23%): mp 285 °C dec; IR ν_{max} (KBr) 1740 (lactone), 1655 cm^{-1} (pyridinone); ^1H NMR (TFA-d_1) δ 1.14 (t, 3, J = 7 Hz, CH_2CH_3), 2.16 (m, 2, CH_2CH_3), 5.71 (AB q, 2, J = 16 Hz, $ArCH_2O$), 5.81 (s, 2, CH_2N), 7.8–9.6 (m, 8, aromatic). Anal. ($C_{24}H_{18}N_2O_4 \cdot 0.5H_2O$) C, H, N.

18-Methoxydeoxycamptothecin (37). A solution of ketone **15b** (459 mg, 1.66 mmol) and *N*-(*o*-aminobenzylidene)-*p*-toluidine[16] (419 mg, 2.00 mmol) in toluene (40 mL) was refluxed for 30 min. The mixture was cooled, *p*-toluenesulfonic acid (5 mg) was added, and then the mixture was refluxed for an additional 3 h. The solvent was removed in vacuo and the residue chromatographed (silica gel 60, 0.5% MeOH–$CHCl_3$) to give a solid, which was crystallized from $CHCl_3$–EtOAc to yield **37** (477 mg, 79%): mp 260–261 °C; IR ν_{max} ($CHCl_3$), 1740 (lactone), 1660 cm^{-1} (pyridinone); ^1H NMR ($CDCl_3$–CD_3OD) δ 2.36 (m, 2, $CHCH_2CH_2$), 3.31 (s, 3, OCH_3), 3.50 (t, 2, J = 6 Hz, CH_2OCH_3), 3.90 (m, 1, $CHCH_2$), 5.28 (s, 2, CH_2N), 5.44 (AB q, 2, J = 6 Hz, $ArCH_2O$), 7.3–8.4 (m, 6, aromatic). Anal. ($C_{21}H_{18}N_2O_4$) C, H, N.

***dl*-18-Methoxycamptothecin (38).** Oxygen was bubbled through a solution of deoxycamptothecin (**37**; 90 mg, 0.249 mmol), cupric nitrate trihydrate (230 mg, 0.954 mmol), and 25% aqueous dimethylamine (65 μL) in DMF (20 mL) until no more starting material remained (approximately 1 h). The reaction was monitored by TLC [silica gel, $CHCl_3$–acetone–methanol (70:20:10)]. The solvent was removed in vacuo, and the residue was taken up in CH_2Cl_2 and washed with H_2O. The organic phase was dried (Na_2SO_4) and evaporated to yield a solid, which was chromatographed (silica gel 60, 1% MeOH–$CHCl_3$) to give a yellow product which was crystallized from $CHCl_3$ to yield **38** (47 mg, 50%): mp 244–245 °C; IR ν_{max} (KBr) 1740 (lactone), 1655 (pyridinone) cm^{-1}; ^1H NMR ($CDCl_3$–CD_3OD) δ 2.16 (m, 2, $CHCH_2CH_2$), 3.36 (s, 3, OCH_3), 3.56 (m, 2, CH_2OCH_3), 5.24 (s, 2, CH_2N), 5.44 (AB q, 2, J = 16 Hz, $ArCH_2O$), 7.5–8.5 (m, 6, aromatic). Anal. ($C_{21}H_{18}N_2O_5$) C, H, N.

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Accumulation of Drugs by Guinea Pig Isolated Atria. Quantitative Correlations

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The time course of the tissue accumulation of 16 neutral, cationic, and anionic drugs by resting and 2-Hz stimulated atria of the guinea pig was measured. The accumulation of the substances was quantified by means of their tissue to medium ratios (T/M). Auricles driven with 2 Hz accumulated the drugs faster and during a long period of time to a greater extent than resting atria. By extrapolation of the binding characteristics, the final equilibrium T/M values were estimated. The variance in these accumulation data at equilibrium (log T/M) could be best described by a linear combination of log P (octanol/water) and the ability of the drugs to bind to atrial homogenate (log percent bound/percent free). A parameter calculated from protein binding appeared less significant. Comparable results were obtained for the accumulation data measured in resting and 2-Hz stimulated atrial muscles. It is suggested that the degree of accumulation of drugs into atrial tissue is determined by the facility of their penetration of the plasma membrane and the extent of their intracellular binding.

Uptake studies of drugs by tissues on which they exert their pharmacological effect have frequently been made. These experiments have usually been designed to obtain information on the affinity of drugs for their receptors in the effector organ. Many of these investigations were hampered by a considerable degree of accumulation, which cannot be satisfactorily related to the amount of drug bound by the receptors. The rate of binding of atropine, for instance, appeared to be much slower than the rate of the antagonistic action.[2-4] At equilibrium only a fraction

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