Prodrug Analogues of the Antitumor Alkaloid Camptothecin

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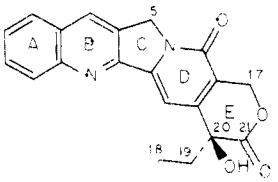
Two classes of (+)-camptothecin (1) derivatives are prepared by acylation of camptothecin-21-isopropylamide (**5a**). The 17-acetyl (**5b**) 17-hexanoyl (**5c**), and 17-acryloyl (**5d**) derivatives represent lipophilic substances with the potential for reconversion to 1 in vivo. A 17-pyrrolidinyl derivative **5e** is prepared from **5b** by a nucleophilic substitution reaction and represents a hydrophilic derivative of 1, which may not be able to be converted to 1 in vivo. It is reported also that **5b** undergoes C-17 substitution by other nucleophiles (isopropylamine, imidazole, cyanide, and ethanethiol). The ester amide derivatives **5b–d** are shown to have an antitumor activity in vivo (L1210, P388) less than 1 or its sodium salt **2a**, and the amine amide derivative **5e** is shown to be inactive in these assay systems. Both **5b** and **5c** exhibit apparent activity in vivo in the Lewis lung LL32 antitumor assay but no activity in the melanocytic melanoma B-16 assay. All of these derivatives have low or moderate in vitro biological activity (9KB, L1210, P388) relative to 1. In one preliminary in vitro assay, **5c** appeared to be nearly as active as **2a** in causing the fragmentation of DNA and inhibiting thymidine's incorporation into DNA in intact HeLa cells. Consequently, the intact α-hydroxy-δ-lactone ring of 1 may not be absolutely necessary for antitumor activity.

Camptothecin (1), an alkaloid isolated from the rare



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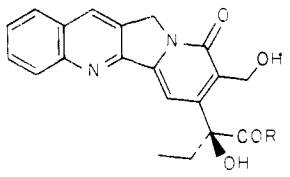
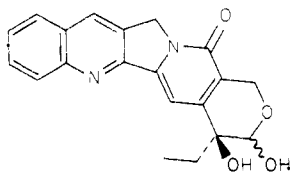


2a, R = O^-Na^+
b, R = $NHCH_3$



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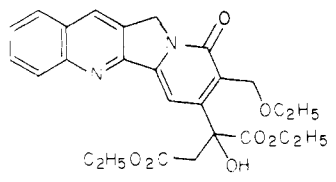

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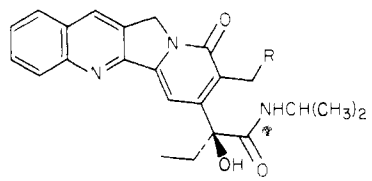

5a, R = OH
b, R = O_2CCH_3
c, R = $O_2CC_5H_{11}$
d, R = $O_2CCH{=}CH_2$
e, R = c-C_4H_8N-
f, R = c-(-NCHNCHCH)

Chinese tree *Camptotheca acuminata* Decne in 1967 by Wall and co-workers,[3] is a potent inhibitor of the growth of leukemia cells in vitro and shows good antitumor activity against murine L1210 and P388 leukemia in vivo.[3,4] However, despite initial encouraging reports that 1 was active clincally against solid tumors of the gi tract,[5] subsequent clinical evaluation of the drug led to discouraging results.[6] Today it is used in the clinical treatment of cancer only in the People's Republic of China, with apparent success.[7]

Many analogues of 1 have been prepared in the course of studies to define structure–activity relationships (SAR)[8] and to develop synthetic routes to this scarce compound.[9] It was shown in one of the early SAR studies that the intact α-hydroxy-δ-lactone (ring E) must be present for full expression of the in vivo antitumor activity.[8d,10] Thus, 20-deoxycamptothecin, 20-chlorocamptothecin, and camptothecin hemilactol (3) are essentially inactive in vivo.[8d] The C-21 methylamide **2b** has ca. three-fifths the activity of 1 in the L1210 life-prolongation assay,[8d] and the sodium salt **2a** has ca. one-third the activity of 1 in the P388 in vivo assay.[11] All analogues of 1 consisting of only rings C, D, and E, or D and E, with or without additional aliphatic or aromatic functionality,[8b] are inactive in vivo, despite some of them having moderate in vitro biological activity.[8a] The only analogue of 1 that does not have the intact ring E, but which has good in vivo antitumor activity, is 4. This compound and four other analogues of 1 (in which the C-20 ethyl group was replaced by $-CH_2CH{=}CH_2$, $-CH_2C{\equiv}CH$, $-CH_2C_6H_5$, or $-CH_2COC_6H_5$) prepared by Sugasawa et al.[12] are the only analogues reported to date possessing in vivo antitumor activity equal to or better than 1.

Since **2a**, **2b**, and 4 exhibit acceptable antitumor activity in vivo, we decided to prepare a series of camptothecin derivatives modeled after **2b**, which would be expected to have enhanced lipophilicity or hydrophilicity in vivo relative to 1. Our rationale was based on the well-established relationship of drug lipophilicity and/or basicity to antitumor activity,[13] e.g., the increased activity of MeCCNU over BCNU[14] and the excellent in vivo antitumor activity of *N*-(trifluoroacetyl)adriamycin 14-valerate (AD 32).[15] A second consideration was the potential for the reconversion of the derivatives to 1 in vivo. This would appear to be desirable for full expression of antitumor activity[8c] and may be why **2a**, **2b**, and 4 exhibit good antitumor activity. (It has never been proven if the latter three drugs' antitumor activity is intrinsic or due to their conversion to 1 in vivo.) We describe herein the preparation of one series of such camptothecin derivatives, which exhibit moderate in vivo antitumor activity, as well as an unexpected, novel aspect of the chemistry of 1.

Synthesis of Camptothecin Derivatives. (+)-Camptothecin formed a comparatively stable amide (**5a**) on reaction with excess isopropylamine at reflux temperature overnight. This amide was unstable in solution or to silica gel chromatography (→1) but could be acylated at C-17 to give the ester amides **5b–d** in excellent yield using standard methodology. The ester amides **5b** and **5c** are soluble in organic solvents of medium ($CHCl_3$, THF) to low (C_6H_6) polarity and thus represent lipophilic derivatives of 1. They also are rapidly transformed back to 1 in dilute aqueous–alcoholic acid or base at 25 °C, implying that they could be recoverted to 1 in vivo fairly easily. This is a very tenuous assumption, since in vivo the requisite hydrolysis may well be achieved only en-

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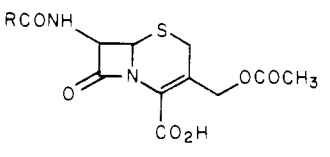
zymatically, which might not occur readily if appropriately unspecific amidases and esterases are not available.

It was anticipated that the acrylate amide **5d** would undergo Michael addition by secondary amines to give basic ester amide derivatives of 1. These should have been water soluble (as their salts) or at physiological pH should have shown enhanced binding to DNA, the presumed primary site of the biological action of 1.[8a] Surprisingly, reaction of **5d** with neat pyrrolidine at 25 °C gave **5e** (73%) by C-17 substitution rather than the expected 1,4-addition product. That **5e** had been formed was clearly evident from its positive response to Draggendorf's reagent (which 1 does not exhibit), its ability to be extracted into dilute aqueous acid, and its NMR and mass spectral characteristics. This amine amide derivative was not transformed to 1 in acid but was readily transformed in dilute aqueous bicarbonate at 25 °C. Consequently, it seems unlikely that **5e** would be reconverted to 1 in vivo as readily as **5b–d**.

By subsequent experimentation, it was revealed that the acetate amide **5b** also underwent substitution by pyrrolidine to give **5e**, as well as by several other nucleophiles (isopropylamine, imidazole, cyanide, and sulfide). These latter substitution products of **5b** were formed in a moderate to good yield and were only partially characterized due to their instability to chromatography and recrystallization (Experimental Section). Nevertheless, it was clear that **5b** has an unusually electrophilic carbon at C-17, whose ease of nucleophilic substitution is precedented by the chemistry of cephalosporin antibiotics (**6**) and



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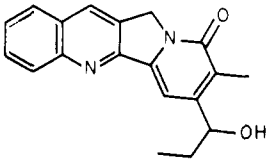

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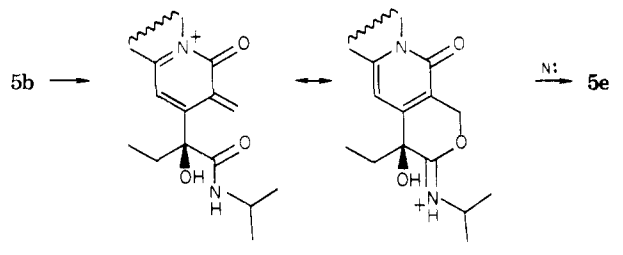
the synthesis of mappicine (**7**) from 1. In the case of **6**, many derivatives of the antibiotic have been made by substitution at the carbon bearing the acetoxy group despite the fact that the enamine center ($N^1C^2C^3$), which could assist in displacement of the acetate group, is deactivated by electron-withdrawing substituents.[16] Mappicine (**7**) is prepared via sodium borohydride reduction of an intermediate 17-formyl-20-keto degradation product of 1, which underscores the electrophilicity of the C-17 carbon atom.[17] It may be that substitution at C-17 of **5b** is facilitated by the following mechanistic rationale:



Biological Activity. The antitumor activity of **5b–f** was compared to that of 1 and **2a** at equimolar dosage levels in vivo (mice) in the P388 and L1210 assays; **5b** and **5c** were also assayed in the melanocytic melanoma B-16 and Lewis lung carcinoma test systems but without reference to 1 or **2a** as a control. These testing results are shown in Table I as percentage increase in life span vs. dosage, along with the comparative in vitro cytotoxic activity of 1 and **5b–f**. It is immediately clear that none of these camptothecin derivatives have an antitumor activity equal to or greater than 1 or **2a**. The potency of the two most active compounds, **5b** and **5d**, is not greater than 85% of that of 1 at the optimal 9.5 μmol/kg dosage level. The pyrrolidinamide **5e** is essentially inactive in vivo but seems to have significant in vitro activity in the L12110 cell cytotoxicity assay, whereas the imidazolamide **5f** is inactive in both assay systems. Both **5b** and **5c** appear to have moderate activity in the Lewis lung carcinoma assay but not against melanocytic melanoma B-16 in which 1 exhibits high activity (2a, somewhat lower activity).[11] However, we caution that these particular data may not be significant and *must* be confirmed by retesting with appropriate controls (of 1 or **2a**), since both 1 and **2a** are known to give erratic results in the Lewis lung assay.[18]

We cannot assess at this time if any of our camptothecin derivatives have intrinsic antitumor activity in vivo. From our observations during the preparation of **5b–f**, it was clear that these substances are not highly stable to chromatography on silica gel or recrystallization. Trace amounts of 1 would reappear during such manipulations, even though crystalline samples could be obtained with sharp melting points. Consequently, it is very likely that a small amount of 1 was formed from these derivatives in vivo. Reversion of **5e** and **5f** to 1 in vivo seems least likely based on their chemical properties, which could account for their inactivity in vivo.

On the other hand, **5c** had an activity identical to 1 and **5b** ca. one-half that of 1 in their effects on Hela cell DNA sedmentation in vitro; **5c** was as active as **2a** in inhibiting the incorporation of radioactive thymidine into acid-insoluble material in HeLa cells.[19] Although these results are preliminary, subject to retesting, they indicate that either **5b** and **5c** may have intrinsic antitumor activity. To the extent that the in vitro activity of camptothecin analogues is meaningful vis-a-vis their antitumor effects in vivo,[8a,c] this latter conclusion is consistent with the conclusion made by Sugasawa et al.[12] regarding the antitumor activity of **4**, whose conversion to 1 in vivo would not occur as readily as **5b–d**. Recently, analogous observations have been reported about AD-32, i.e., that it may have an intrinsic antitumor activity in vivo without transformation to adriamycin.[15b]

We acknowledge that it remains to be proven clearly if the intact E ring of 1 is absolutely essential for antitumor activity.

Experimental Section

All solvents and liquid reagents were redistilled prior to use. Evaporation in vacuo was done at ≤40 °C on a rotary evaporator. Thin-layer (TLC) and preparative-layer (PLC) chromatography was done using EM silica gel purchased from Brinkmann Inc., New York. IR spectra were determined with a Perkin-Elmer 257 infrared spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker HX-90E nuclear magnetic resonance spectrometer at 90 or 22.63 MHz, respectively. Mass spectra were run on an AEI-MS-9 mass spectrometer interfaced to a Nova 2 data system or on a Finnegan quadrupole 1015 GC–mass spectrometer interfaced to a Finnegan M6000 data system. Optical rotations were taken on a Perkin-Elmer 241 polarimeter. Elemental analyses were done by Galbraith Laboratories, Knoxville, Tenn. Melting points were determined on a Koefler hot stage and are uncorrected.

Isopropylamide of Camptothecin (5a). (+)-Camptothecin (116 mg, 0.33 mmol, NSC 94600, supplied by the NCI, lot no. 69-12-16-01) was mixed with excess dry isopropylamine (20–25 mL), and the resulting slurry was refluxed for 18 h with magnetic stirring under N_2 to suppress the C-5 oxidation of 1.[20] The final reaction mixture was a clear, light-amber solution, TLC analysis of which ($CHCl_3$–MeOH, 95:5) showed that complete conversion to **5a** had been achieved (R_f 0.55 relative to 1). Removal of excess isopropylamine in vacuo gave **5a** as a light-amber solid glass

Table I. Cytotoxicity and Antitumor Activity of Camptothecin and its Analogues

assay system: in vitro[d]	ED_{50}, μmol/mL (relative activity)						
	1	2a	5b	5c	5d	5e	5f
9KB	$<2.8 \times 10^{-6}$ (100)	*b*	7.8×10^{-4} (2.8)	8.1×10^{-5} (29)	6.9×10^{-5} (25)	2.2×10^{-5} (7.8)	*b*
P388	8.6×10^{-6} (100)	*b*	7.4×10^{-3} (0.08)	1.2×10^{-3} (0.01)	3.9×10^{-4} (0.45)	1.1×10^{-4} (0.13)	>200
L1210	2.8×10^{-6} (100)	*b*	*b*	5.5×10^{-4} (2.0)	7.6×10^{-4} (2.7)	6.5×10^{-5} (23)	>200
	T/C, %[e] (mg/kg; μmol/kg)						
in vivo[c]	1	2a	5b	5c	5d	5e	5f
P388, 10.3–11.2[d]	81 (10.0; 29)	67 (11.1; 29)	97 (12.8; 28.5)	155 (14.3; 28.3)	155 (6.5; 14.1)	125 (62.5; 136)[g]	92 (45.0; 100)
	204 (3.3; 9.5)	85 (3.7; 9.5)	173 (4.3; 9.6)	139 (4.8; 9.5)	165 (2.5; 5.4)[f]	126 (18.9; 41)	89 (23.0; 50)
	147 (1.1; 3.2)	168 (1.2; 3.1)	126 (1.4; 3.1)	107 (1.6; 3.2)	*i*	111 (5.1; 11)	105 (6.4; 14)
	137 (0.4; 1.1)	139 (0.4; 1.0)	126 (0.5; 1.1)	107 (0.5; 1.0)			
L1210, 8.8[d]	85 (*h*)	74 (*h*)	105 (*h*)	133 (*h*)	125 (6.5; 14.1)	113 (*h*)	*b*
	168	114	147	116	*i*	101	
	131	147	116	105		109	
	114	116	110	105			
B16, 20.2[d]	*b*	*b*	114 (43.5; 97)	109 (46; 91)	*b*	*b*	*b*
			105 (14.8; 33)	109 (14.6; 29)			
			100 (4.9; 11)	110 (4.0; 8)			
Lewis lung, 20.4[d]	*b*	*b*	156 (48.0; 107)	134 (44.4; 88)	*b*	*b*	*b*
			166 (15.7; 35)	147 (14.6; 29)			
			147 (4.0; 9)	163 (4.0; 8)			

[a] Determined at the School of Pharmacy under the auspices of an NCI contract (CM 67076, to D. Perlman) using the protocols described in *Cancer Chemother. Rep.*, **25**, 1 (1962). [b] Not assayed. [c] Determined at WARF, Inc., Madison, Wis., under NCI protocols for 3LE21 and 3PS31 (six mice were used per test and the drug was injected daily for 9 days); 3B131 and 3LL32 (ten mice per test; drug injected every 4 days). 20–30 control animals receiving no injection were used. [d] Mean/median survival time of control animals in days. [e] Mean/median survival time of test group divided by mean/median survival of control group × 100. [f] Drug injected daily for only 6 days. [g] Tested as the hydrochloride salt. [h] Same dosages used as in the P388 assay. [i] Insufficient drug for assays at lower level.

(100%): IR (KBr) ν 3300, 1648, 1580–1510 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.10 [d + t, H-18 + $(CH_3)_2$], 2.28 (m, H-19), 4.08 [m, $(CH_3)_2CHN$], 4.85 (s, H-17), 5.03 (s, H-5), 6.73 (d, J = 8 Hz, NH), 7.51–8.11 (6 arom H).

17-Acetylcamptothecin-21-isopropylamide (5b). A sample of crude **5a** (from 0.33 mmol of 1) was dissolved in $CHCl_3$–CH_2Cl_2 (1:1, 2–3 mL) and mixed with 1 mL each of Ac_2O and dry pyridine. This reaction mixture was stirred magnetically for ca. 3.5 h at room temperature and then the solvents and excess reagents were removed in vacuo (oil pump) by a MeOH–$C_6H_5CH_3$ azeotrope. (Longer acetylation times resulted in the formation of a C-20 acetate byproduct.) The resulting solid residue was purified by PLC in $CHCl_3$–MeOH (100:6) to give **5b** (150 mg, 100%): yellow needles, mp 204–206 °C (isopropyl alcohol); IR ($CHCl_3$) ν 3410, 3300, 1735, 1660, 1600, 1585, 1520, 1460, 1240 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.15 (t, J = 6.5 Hz, H-18), 1.21 [d, J = 6.1 Hz, $(CH_3)_2CH$], 2.06 (s, $OCOCH_3$), 2.42 (m, H-19), 3.47 (br d, $J \approx 3$ Hz, OH), 4.07 [m, $(CH_3)_2CHN$], 5.13 (s, H-5), 5.48 (q, J_{AB} = 11.8 Hz, H-17), 6.76 (d, J = 8.3 Hz, NH), 7.45–8.11 (6 arom H); MS, m/e (rel intensity), 390 (M^+ · – 59) (5), 373 (15), 303 (35); $[\alpha]^{24}_D$ +87° (c 0.06, $CHCl_3$). Anal. ($C_{25}H_{27}N_3O_5$·i-PrOH) C, H, N.

17-Hexanoylcamptothecin-21-isopropylamide (5c). A sample of crude **5a** (from 0.33 mmol of 1) was dissolved in $CHCl_3$–CH_2Cl_2 by magnetic stirring. Hexanoyl chloride (0.1 mL, 0.6 mmol) and ethyldiisopropylamine (0.1 mL, 0.6 mmol) were added to this solution, and the reaction mixture was stirred for 4 h at room temperature. The solvents and excess reagents were removed in vacuo (oil pump), and the resulting residue was purified by PLC in $CHCl_3$–EtOH (100:6), twice developed, to give **5c** (122 mg, 71%): pale-yellow needles, mp 187–187.5 °C (EtOH–H_2O); IR ($CHCl_3$) ν 3405, 3300, 1730, 1661, 1600, 1585, 1520, 1460, 1240, 1165 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.10 (t, H-18), 1.20 (d + m), 2.35 (m, H-19), 3.48 (d, OH), 5.48 (q, J_{AB} = 12.7 Hz, H-17), 6.82 (d, J = 8.0, NH); MS, m/e (rel intensity), 360 (M^+ · – 145) (15), 303 (5), 77 (100); $[\alpha]^{24}_D$ +81° (c 0.06, $CHCl_3$). Anal. ($C_{29}H_{35}N_3O_5$) C, H, N.

TLC analysis of crystalline **5c** in the above solvent system showed six trace contaminants having pale-blue fluorescence under short-wave UV light: R_f 0.18, 0.48, 0.67, 1.27, 1.43, 1.70 relative to **5c**. These also appeared after elution of **5c** (clearly a single band) from PLC adsorbents with EtOH. Similar impurities appeared in crystalline or PLC pure **5b**. These may be forming during TLC analysis on silica gel, since they were absent or appeared in lesser amounts in TLC analysis on Al_2O_3 (same solvents), on cellulose (toluene–EtOAc, 7:2), or on silica gel (acetone or EtOAc).

17-Acryloylcamptothecin-21-isopropylamide (5d). A sample of crude **5a** (122 mg, 0.3 mmol) was acylated with acryloyl chloride (0.8 mmol), by the same procedures as used for the preparation of **5c**, to give **5d** (57 mg, 41%): mp 188–194.5 °C (isopropyl alcohol); IR ($CHCl_3$) ν 3500, 3408, 1725, 1660, 1655, 1585, 1405 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.16 (d + t, H-18 + 6 H), 2.47 (m, H-19), 3.99 (m, NCH), 5.07 (q, J_{AB} = 19.5 Hz, H-5), 5.58 (q, J_{AB} = 12.0 Hz, H-17), 5.71–6.53 (m, J = 2, 10, and 18 Hz, $CH{=}CH_2$), 6.87 (d, J = 7.8 Hz, NH), 7.35–8.01 (6 arom H); MS, m/e (rel intensity), 443 (M^+ · – 18) (5), 402 (5), 390, 373 [M^+ · – ($O_2CCH{=}CH_2$ + OH)] (20), 360 (25), 304 (65), 219 (85), 44 (100). Anal. ($C_{26}H_{27}O_5N_3$·i-PrOH) C, H, N.

17-Pyrrolidinylcamptothecin-21-isopropylamide (5e). Acetate amide **5b** (90 mg, 0.2 mmol) was dissolved in pyrrolidine (ca. 1 mL), and the mixture was stirred magnetically for 30 min at room temperature. The excess pyrrolidine was removed in vacuo, and the residue was purified by PLC in $CHCl_3$–EtOH (90:10). Several blue fluorescing bands (short-wave UV light) were seen; the major band (R_f 0.4) that reacted with Draggendorf's reagent was eluted from the adsorbent with EtOH to give **5e** (70 mg, 76%) as a pale-yellow solid: mp 203–209 °C dec (EtOH–H_2O); IR (film) ν 3400, 1650, 1590 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.25 [m, $(CH_3)_2$ + H-18], 1.92 (m, β-CH_2 of pyrrolidine), 2.38 (m, H-19), 2.98 (m, α-CH_2 of pyrrolidine), 4.18 [m + q, J_{AB} = 11 Hz, $(CH_3)_2CH$– + H-17], 5.17 (s, H-5), 7.33 (d, J = 8 Hz, NH), 7.48–8.28 (6 arom H); ^{13}C NMR ($CDCl_3$) δ 8.1 (C-18), 22.8 and 23.6 [$(CH_3)_2$ + β-CH_2 of pyrrolidine], 32.8 (C-19), 41.5 [NCH-$(CH_3)_2$], 48.8 (C-17), 50.4 (C-5), 52.5 (α-CH_2 of pyrrolidine), 79.8 (C-20), 102.1 (C-14), 125.9–131.1 (C-6 to -12), 143.8 (C-3), 148.8 (C-15), 151.6 (C-13), 157.5 (C-2), 161.7 (C-16a), 173.8 (C-21) (these assignments were made by reference to the ^{13}C NMR spectrum of compounds 1[21] and **5b** and SFOR decoupling experiments); UV (MeOH) 370, 293 (sh), 254 nm; UV (MeOH + 0.1 N HCl) 365, 254 nm; MS, m/e (rel intensity), 460.2449 ($C_{25}H_{32}N_4O_3$, calcd: 460.2473) (10), 391 (15), 303 (100); $[\alpha]^{24}_D$ +108° (c 0.01, $CHCl_3$).

Substitution Products of 5b. The following compounds were prepared only once and partially characterized.

17-Isopropylaminocamptothecin-21-isopropylamide. A small sample of **5b** was stirred in neat isopropylamine for 20 min at room temperature. One major product was formed, which reacted positively with Draggendorf's reagent: IR (film) ν 3400, 1650, 1590 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.03 (m, 15 H), 2.27 (m, H-19), 3.35 (m, 1 H), 4.03 (m + q, 3 H), 5.13 (s, H-5), 6.02 (d, J = 10 Hz, NH); MS, m/e (rel intensity), 448 (M^+ ·) (5), 390 (M^+ · – 58) (50), 303 (85).

17-Imidazolylcamptothecin-21-isopropylamide (5f). A sample of **5b** (18 mg, 0.04 mmol) and imidazole (10 mg) were dissolved in Me_2SO (1 mL), and the reaction was stirred magnetically for 30 h at 50 °C. The reaction solution was poured into H_2O (15 mL) and extracted with $CHCl_3$ (3 × 5 mL), and the combined $CHCl_3$ extracts were dried (Na_2SO_4) and evaporated in vacuo. The resulting residue was purified by PLC in $CHCl_3$–MeOH (90:10) to give a single, Draggendorf-positive substance (18 mg, 100%): mp 189–194 °C; IR (film) ν 3200, 1650, 1600 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 0.92 (6, 3 H), 1.01, 1.09 (dd, 6 H), 2.18 (m, H-19), 3.9 (m, 1 H), 5.25 (br s, H-5), 5.39 (br s, H-17), 6.39–8.65 (9 arom H + NH); MS, m/e (rel intensity), 457.2124 ($C_{26}H_{27}N_5O_3$, calcd: 457.2114] (7), 390 (9), 389 (9), 371 (21), 360 (20), 303 (100), 68 (58).

17-Cyanocamptothecin-21-isopropylamide. A solution of **5b** (34 mg, 0.075 mmol) and KCN (100 mg, 1.53 mmol) in Me_2SO (1 mL) was stirred for 5 min at room temperature. The Me_2SO was removed in vacuo (oil pump), and the resulting residue was purified by PLC in $CHCl_3$–MeOH (95:5) to give a single product (27 mg, 90%): IR (film) ν 3250, 1670, 1600 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.3 (m, 9 H), 2.4 (m, H-19), 3.02 (q, J_{AB} = 18 Hz, 2 H), 3.94 (m, 1 H), 5.30 (br s, H-5), 7.30–8.42 (6 arom H); MS, m/e (rel intensity), 390 [M^+ · – CN] (6), 373 [M^+ · – (CN + OH)] (2), 360 (21), 303 (10), 44 (100).

17-Ethylmercaptocamptothecin-21-isopropylamide. A sample of **5b** (20 mg, 0.044 mmol) was dissolved in Me_2SO (1 mL) containing EtSH (0.1 mL) and solid K_2CO_3 (5 mg). The reaction mixture was stirred magnetically for 1 h at room temperature; then the Me_2SO and excess EtSH were evaporated in vacuo (oil pump). The resulting solid residue was extracted with $CHCl_3$–MeOH (4:1), and the soluble material was purified by PLC in $CHCl_3$–MeOH (95:5) to yield one major product: IR (film) ν 3280, 1650, 1600 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.2 (m, 12 H), 2.40 (m, 2 H), 2.80 (m, 2 H), 4.06 [m, $(CH_3)_2CH$), 4.08 (q, J_{AB} = 13.5 Hz, 2 H), 5.07 (br s, H-5), 6.85 (d, J = 8 Hz, NH), 7.5–8.4 (6 arom H); MS, m/e (rel intensity), 451 [M^+·] (4), 390 (25), 372 [M^+ · – (EtSH + OH)] (35), 360 (50), 303 (100).

Acknowledgment. We thank Mr. Gary Girdaukas, School of Pharmacy, and Mr. Mel Micke, University of Wisconsin Biochemistry Department, for low-resolution mass spectral determinations; Mr. Jim Blackbourn, School of Pharmacy, for NMR spectral determinations; and the National Institutes of Health (CA 17127) for partial research support. We are particularly indebted to Mr. Brent Hasse, University of Wisconsin School of Pharmacy, and Professor S. Horwitz, Albert Einstein College of Medicine, New York City, for in vitro assays.

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(2) Career Development Awardee of the National Cancer Institute (CA 00253), 1976–1981.
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Synthesis and Antitumor Activity of Water-Soluble (2-Chloroethyl)nitrosoureas

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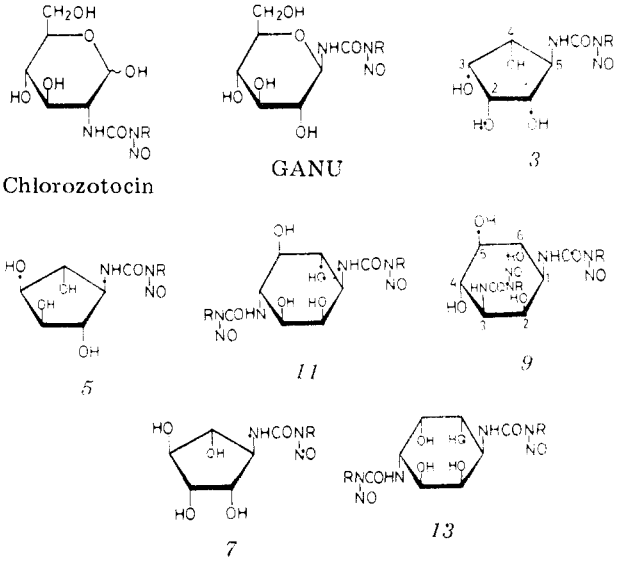
Six water-soluble (2-chloroethyl)nitrosoureido derivatives of cyclopentanetetrols and cyclohexanetetrols have been prepared. Their antitumor activities were determined against leukemia L1210 in mice.

Steptozotocin is a naturally occurring antitumor antibiotic[1-4] and has a unique structure: 2-(3-methyl-3-nitrosoureido)-2-deoxy-D-glucopyranose.[5,6] In this antibiotic, the methylnitrosoureido group seems to be an essential functional group for biological activity, and the carbohydrate moiety might play an important role as a carrier of the functional group. This has been verified by the fact that the introduction of the functional group into a polyhydroxycyclohexane system did not result in loss of activity against leukemia L1210.[7] Also, O-methylation of an anomeric hydroxyl group of streptozotocin[8] and its analogues[9] was not detrimental for activity.

Montgomery and his co-workers showed in their systematic studies on *N*-nitrosoureas[10,11] that a replacement of the methyl group in this functional group with a 2-chloroethyl group markedly enhanced the activity of the *N*-nitrosoureas against leukemia L1210. Accordingly, the clinically useful BCNU,[10] CCNU,[11] and MeCCNU[11] were synthesized. More recently, 2-[3-(2-chloroethyl)-3-nitrosoureido]-2-deoxy-D-glucopyranose (chlorozotocin)[12] exhibited strong antileukemic activity,[13-15] and its positional isomer, 1-(2-chloroethyl)-3-(β-D-glucopyranosyl)-1-nitrosourea (GANU)[16] showed marked activity against leukemia L1210.[17]

Now we report a synthesis of six water-soluble (2-chloroethyl)nitrosoureido derivatives of cyclopentanetetrols and cyclohexanetetrols and their antileukemic activities against leukemia L1210. The melting points and yields of compounds **2-13** are listed in Tables I and II.



Chemistry. A synthesis of 5-[3-(2-chloroethyl)-3-nitrosoureido]-1,2,3,4-cyclopentanetetrols was exemplified in the case of its (1,2,3,4/5) stereoisomer.[18] When tetra-*O*-acetyl-(1,2,3,4/5)-5-acetamido-1,2,3,4-cyclo-

0022-2623/79/1822-0314$01.00/0