and are uncorrected. The UV spectra were run in EtOH; IR spectra were recorded on a Beckman 5240 spectrophotometer using KBr pellets; NMR spectra were obtained on a Varian HA-100 spectrometer using $(CH_3)_4Si$ as an internal standard. All solid compounds were characterized by UV, IR, and NMR.

p-Nitrobenzyl 3-[(Diphenylphosphoryl)oxy]-6α-[1(R)-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (4). A solution of 1.75 g (4.85 mmol) of p-nitrobenzyl 6α-[1(R)-hydroxyethyl]-3,7-dioxo-4-methyl-1-azabicyclo[3.2.0]heptane-2-carboxylate[6,6] in 20 mL of acetonitrile is cooled to 0 °C under a nitrogen atmosphere. A solution of 726 mg (7.18 mmol) of diisopropylethylamine in 2 mL of acetonitrile is added followed by a dropwise addition of 1.51 g (5.60 mmol) diphenyl chlorophosphate in 12 mL of acetonitrile over a period of 3 min. The resulting solution is stirred at 0 °C for 20 min to provide the phosphonate 4 in situ, which was used for further chemical transformation without isolation.

p-Nitrobenzyl 3-[(Pyridin-4-ylmethyl)thio]-6α-[1(R)-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (5). To a cooled (−15 °C) solution of 1.2 g (2 mmol) of the phosphonate 4 in 10 mL of acetonitrile was added 390 mg (3 mmol) of diisopropylethylamine followed by 370 mg (3 mmol) of 2-(mercaptomethyl)pyridine under N_2. The reaction mixture was allowed to stir for 60 min at −15 °C and then an additional 60 min at 0 °C. The reaction was diluted with ethyl acetate, washed with ice water and brine, and dried ($MgSO_4$). Evaporation of solvents in vacuo gave a yellow oil, which was purified by SiO_2 column; elution of the column with 20% ethyl acetate in methylene chloride gave 375 mg (40% yield) of compound 5 as a white amorphous foam: IR (KBr) 3400, 1775, 1710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.20 (3 H, d, J = 6.7 Hz), 1.29 (3 H, d, J = 6.7 Hz), 3.14 (1 H, q, J = 6.2 and 2.0 Hz), 3.40 (1 H, m), 4.0 (1 H, d, J = 7.6 Hz), 4.12 (1 H, d, J = 7.6 Hz), 4.18 (1 H, q, J = 6.7 and 2.0 Hz), 4.25 (1 H, m), 5.25 (1 H, d, J = 11.3 Hz), 5.40 (1 H, d, J = 11.3 Hz), 7.15–8.2 (4 H, m).

3-[[(N-Methyl-4-pyridinio)methyl]thio]-6α-[1(R)-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (7). To a solution of 1.0 g (3 mmol) of compound 5 in 10 mL of methylene chloride was added 450 mg (3.3 mmol) of methyl trifluoromethanesulfonate and stirred at 23 °C for 90 min. Evaporation of methylene chloride in vacuo gave the quaternized pyridine as a foam, which was hydrogenated immediately without any further purification. The crude pyridinium salt was dissolved into tetrahydrofuran–ether–pH 7 buffer (1:1:1, 100 mL each) followed by 600 mg of 10% palladium on charcoal. The mixture was hydrogenated at 35 psi on the per shaker for 45 min. The mixture was filtered through a Celite pad, and the catalyst was washed with water (2 × 10 mL). The combined filtrate and washings were extracted with ether (2 × 100 mL) and lyophilized to give a yellow powder, which was purified on a C_{18} Bondapak reverse-phase column (10 g), eluting with 5% acetonitrile in water under 8 psi pressure. Each 15-mL fraction was assayed by high-pressure liquid chromatography, and fractions having an ultraviolet absorption at 300 nm max were collected and lyophilized to give 58 mg (11% yield) of the title compound as a pale yellow powder: IR (KBr) 3410, 1750, 1650 cm^{-1}; UV max (H_2O) 293 nm (ε 7295); ^1H NMR (D_2O) δ 1.15 (3 H, d, J = 6.5 Hz), 1.20 (3 H, d, J = 6.5 Hz), 3.20 (1 H, m), 3.45 (1 H, q, J = 6.0 and 2.0 Hz), 4.11 (1 H, q, J = 8.0 and 2.0 Hz), 4.20 (1 H, m), 4.35 (3 H, s), 7.95 (2 H, d, J = 5.2 Hz), 8.72 (2 H, d, J = 5.2 Hz). Anal. Calcd for $C_{17}N_{20}N_2O_4S_2 \cdot {}^1/_2H_2O$: C, 51.90; H, 6.36; N, 7.12. Found: C, 51.92; H, 5.71; N, 6.88.

3-[[(1,2-Dimethyl-3-pyridinio)methyl]thio]-6α-[1(R)-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (11). This compound was obtained as yellow powder in 14% yield from compound 4 in the same manner as that described for compound 7: IR (KBr) 3400, 1750, 1600 cm^{-1}; UV max (H_2O) 296 nm (ε 8500); ^1H NMR (D_2O) δ 1.25 (3 H, d, J = 6.5 Hz), 1.30 (3 H, d, J = 6.5 Hz), 2.95 (3 H, s), 3.40 (1 H, m), 3.50 (1 H, q, J = 6.2 and 1.8 Hz), 4.2–4.4 (4 H, m), 4.35 (3 H, s), 7.82 (1 H, t, J = 8.5 and 6.3 Hz), 8.40 (1 H, d, J = 8.5 Hz), 8.72 (1 H, d, J = 6.3 Hz).

3-[[(N-Methylimidazol-2-yl)methyl]thio]-6α-[1(R)-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (13). This compound was obtained as a yellow amorphous powder in 41% yield from compound 4 in the same manner as that described for compound 7: IR (KBr) 3400, 1750, 1620 cm^{-1}; UV max (H_2O) 293 nm (ε 8700); ^1H NMR (D_2O) δ 1.15 (3 H, d, J = 6.5 Hz), 1.25 (3 H, d, J = 6.5 Hz), 3.30 (1 H, m), 3.45 (1 H, q, J = 6.0 and 2.2 Hz), 3.55 (3 H, s), 4.2–4.6 (4 H, m), 7.25 (2 H, s).

3-[[(1,3-Dimethyl-2-imidazolio)methyl]thio]-6α-[1(R)-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (14). This compound was obtained as a yellow powder in 32% yield from compound 4 in the same manner as that described for compound 7: IR (KBr) 3400, 1758, 1600 cm^{-1}; UV max (H_2O) 294 nm (ε 7194); ^1H NMR (D_2O) δ 1.10 (3 H, d, J = 6.3 Hz), 1.25 (3 H, d, J = 6.3 Hz), 3.30 (1 H, m), 3.42 (1 H, q, J = 6.0 and 2.2 Hz), 3.85 (6 H, s), 4.2–4.6 (4 H, m), 7.40 (2 H, s).

Acknowledgment. We thank the Analytical Research Department for the elemental analyses and the IR, UV, and NMR. We also thank the Microbiology Department for supplying the antibacterial data.

Antitumor Agents. 100.[1] Inhibition of Human DNA Topoisomerase II by Cytotoxic Ether and Ester Derivatives of Podophyllotoxin and α-Peltatin

Lee S. Thurston,[†] Yasuhiro Imakura,[†] Mitsumasa Haruna,[†] De-Hua Li,[‡] Zong-Chao Liu,[‡] Su-Ying Liu,[‡] Yung-Chi Cheng,[‡] and Kuo-Hsiung Lee*,[†]

Natural Products Laboratory, Division of Medicinal Chemistry and Natural Products, School of Pharmacy, and Department of Pharmacology, School of Medicine, University of North Carolina, Chapel Hill, North Carolina 27599. *Received June 10, 1988*

A principal mechanism of action of the clinical antitumor drugs etoposide (1) and teniposide (2) is the inhibition of catalytic activity of type II DNA topoisomerase and concurrent enzyme-mediated production of lethal DNA strand breaks. Substitution of the glycosidic moiety of 1 or 2 by ester and ethers, as well as the esterification and etherification of α-peltatin (4) including its glucosidic ethylidene and thenylidene cyclic acetals (25 and 26), has afforded compounds of much less activity than that of 1. The in vitro cytotoxicity (KB) appears to have no correlation with the inhibitory activity of the human DNA topoisomerase II.

The clinically useful nonintercalative antitumor drugs etoposide (1) and teniposide (2) most likely produce their therapeutic effect by inducing DNA strand breaks which lead to tumor cell death.[2] This cleavage reaction is brought about by the formation of a "cleavable complex"

[†] Natural Products Laboratory, Division of Medicinal Chemistry and Natural Products.

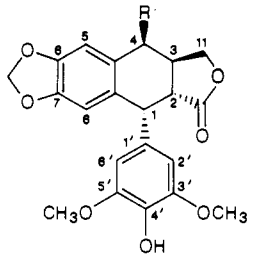
[‡] Department of Pharmacology.

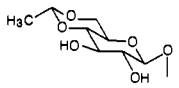
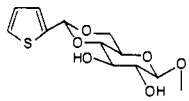
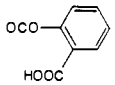
(1) Part 99: Beers, S. A.; Imakura, Y.; Dai, H. J.; Li, D. H.; Cheng, Y. C.; Lee, K. H. *J. Nat. Prod.* **1988**, *51*, 901.

(2) Ross, W.; Rowe, T.; Glisson, B.; Yalowich, J.; Liu, L. *J. Antibiot.* in press.

0022-2623/89/1832-0604$01.50/0

Table I. Biological Evaluation of Podophyllotoxin Analogues



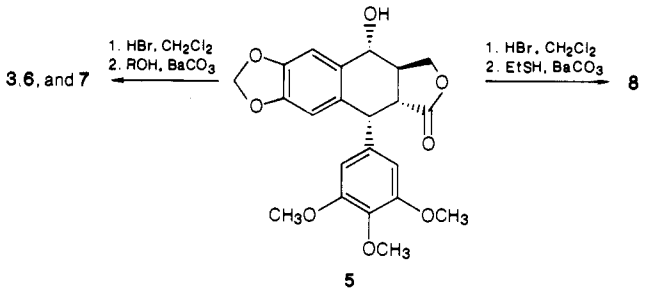
compd	R	cytotoxicity: ED_{50} KB (μg/mL)	DNA topoisomerase II act: % inhib[a]
1		0.12	++++[b]
2			
3	OCH_2CH_3	1.1	+
6	OCH_3	1.1	+
7	OH	0.06	++
8	SCH_2CH_3	1.1	+
10	$OCOCH_3$	0.2	+
11	$OCOCH_2CH_3$	1.0	−
12	$OCOCH_2CH_2COOH$	1.0	−
13		0.8	ND[c]
14	$OCOCH{=}CHCH_3$	0.9	ND[c]
15	$OCOCH_2C_6H_5$	0.00028	ND[c]

[a] Preliminary results. ED_{50} is the concentration of drug that affords 50% reduction in cell number after 3 days of incubation. [b] +, ++, ++++, and − denotes 25%, 50% >88%, and 0% inhibition. [c] Not determined.

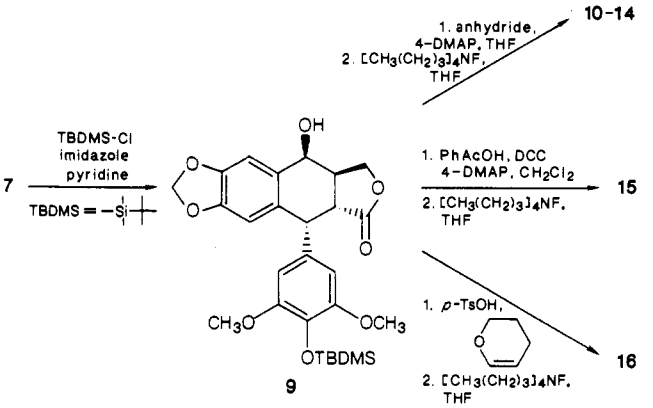
between drug, DNA topoisomerase II, and DNA.[3,4] The enzyme's catalytic activity (DNA strand passing) is inhibited by the drug as well.[4] Structure–activity studies involving podophyllotoxin congeners related to 1 demonstrated inhibitory activity of a calf thymus type II topoisomerase that paralleled cytotoxicity to cultured human lung adenocarcinoma cells.[5] The recent isolation and purification of human type II topoisomerase from the cells of patients with lymphocytic leukemia has provided this laboratory with the means to use this enzyme as a target to investigate the structure–activity relationships among 1 and related compounds.

In a previous report[6] we have discovered that substitution of etoposide's glycosidic moiety by an ethoxy group as in 4′-demethylepipodophyllotoxin ethyl ether (3) does not eliminate inhibition of DNA topoisomerase II activity. Equivalent inhibition of unknotting activity of DNA topoisomerase II relative to 1 was reported at a 0.4 mM level of 3. We now find that 3 is much less potent than 1 in inhibiting the knotting activity of this enzyme. The initial discovery that etoposide's glucosidic moiety was not essential for inhibition of enzyme activity prompted us to continue substitution with various ether and ester groups.

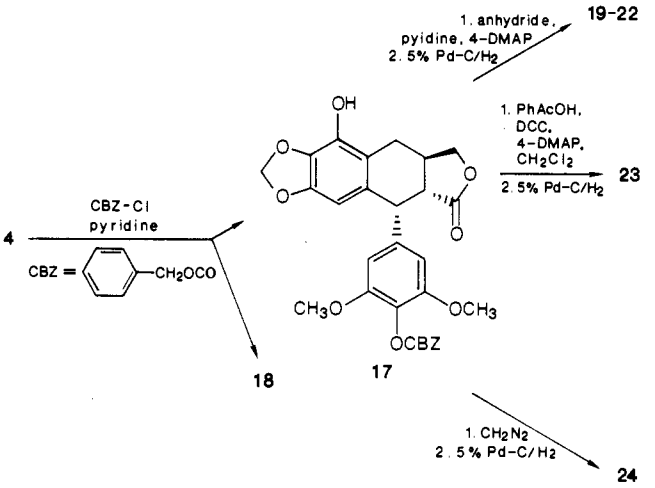
Scheme I



Scheme II



Scheme III



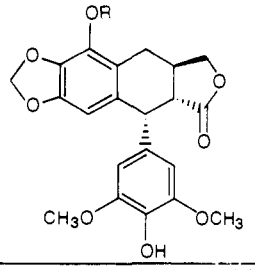
Analogous substitutions at the C-5 position of α-peltatin (4) with ether and esters were also made.

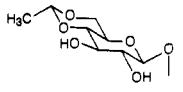
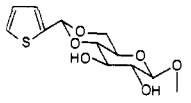
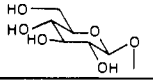
Chemistry

Podophyllotoxin (5) served as starting material for all the C-4-substituted ethers and esters (see Table I). A modified version of Kuhn's method[7] employing hydrogen bromide was used to simultaneously O-demethylate at the para position and to invert the stereochemistry at C-4 from α to β orientation. The ester series was synthesized by esterification of the resultant β-hydroxyl, while the ethers (Scheme I) were made (except for THP ether) by direct nucleophilic substitution by the appropriate alcohol or thiol instead of water. Substitution was also stereoselective due to the steric hindrance imposed by the bulky pendant aromatic ring. The highly acidic character of this one-pot ether synthesis generally limits the choice to only neutral, acid-stable nucleophiles.

(3) Nelson, E.; Tewey, K.; Liu, L. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 1361.
(4) Chen, G.; Yang, L.; Rowe, T.; Halligan, B.; Tewey, K.; Liu, L. *J. Biol. Chem.* **1984**, *259*, 13560.
(5) Minocha, A.; Long, B. *Biochem. Biophys. Res. Commun.* **1984**, *122*, 165.
(6) Thurston, L.; Irie, H.; Tani, S.; Han, F. S.; Liu, Z. C.; Cheng, Y. C.; Lee, K. H. *J. Med. Chem.* **1986**, *29*, 1547.
(7) Kuhn, M.; Keller-Juslen, C.; von Wartburg, A. *Helv. Chim. Acta* **1969**, *52*, 944.

Table II. Biological Evaluation of α-Peltatin Analogues



compd	R	cytotoxicity: ED_{50} KB (μg/mL)	DNA topoisomerase II act: % inhibn[a]
1 (etoposide)		0.12	++++[b]
4	H	0.09	-
18	$COOCH_2C_6H_5$	0.01	+
19	$COCH_3$	0.01	-
20	$COCH_2CH_2COOH$	0.01	-
21	$COCH_2CH_2CH_3$	0.004	+
22	$COCH_2CH_3$	0.005	+
23	$COCH_2C_6H_5$	0.01	ND[c]
24	CH_3	0.001	+
25		2.0	
26		2.0	+
27		1.1	+

[a-c] Same as footnotes a–c found in Table I.

The synthesis of the C-4 ester class (Scheme II) required a slightly more circuitous route involving a protection of the C-4′ phenol before esterification at the C-4 alcoholic position. The acetate (**10**), propionate (**11**), succinate (**12**), phthalate (**13**), and crotonate (**14**) esters were made by reaction of intermediate **9** with the appropriate anhydride in pyridine followed by deprotection. The phenylacetate (**15**) was made by using Hassner's coupling method.[8]

Synthesis of the C-5 esters of α-peltatin (Scheme III) also required initial protection of the C-4′ hydroxyl. This was accomplished with benzyl chloroformate in pyridine. Esterification at the C-5 phenol proceeded in the same manner as that used for the C-4 position. Hydrogenolytic deprotection afforded **19–23**.

The synthesis of the ethylidene cyclic acetal (**25**) of α-pelatatin β-D-glucoside (**27**) was accomplished by using Keller-Juslen's procedure.[9] The analogous thenylidene cyclic acetal (**26**) was made in the same manner.

Results and Discussion

As demonstrated by the agarose gels, the degree of the human type II DNA topoisomerase inhibition is quantitatively estimated by observing the relative density of unknotted P4 DNA and the length of the knotted P4 DNA and then comparing to the enzyme dose response to get the percentage of inhibition. These results together with the cytotoxicity (KB) data for the ether and ester derivatives of podophyllotoxin and α-peltatin are shown in Tables I and II, respectively. The KB cell assay was used as it is a human tumor cell line. The enzyme is also derived from human source. In the enzyme assay, all compounds were screened at 0.4 mM concentrations and were found to be less active than 1.

The fact that **25** and **26** were much less active than **1** indicates the requirement of a glucosidic ethylidene or thenylidene cyclic acetal moiety to be placed at a C-4 β-position instead of an aromatic C-5 position as seen in the α-peltatin skeleton. The in vitro cytotoxicity obviously does not correlate with the human type II DNA topoisomerase inhibitory activity as many compounds including **4**, **7**, **18**, **19**, **21–24**, which are more cytotoxic than **1**, were less active than **1** in their DNA topoisomerase II inhibitory activity. This may be related to their potential inhibitory activity against microtubule polymerization. Podophyllotoxin (**5**) itself is not an inhibitor of DNA topoisomerase II, but it could inhibit the microtubule polymerization. Thus, podophyllotoxin-type compounds may exert their cytotoxicity through the inhibition of microtubule polymerization. On the other hand, the etoposide (**1**) type compounds are potent inhibitors of DNA topoisomerase II, but do not inhibit microtubule polymerization. These **1**-type compounds exert cytotoxicity through their interaction with DNA topoisomerase II. The group of compounds synthesized in this study as described above share common structural characteristics with both **5**- and **1**-type compounds. Compounds that do not inhibit DNA topoisomerase II may still have potent cytotoxicity through their inhibition of microtubule polymerization as seen in **5**. Therefore, it is not unexpected that there is a lack of correlation between cytotoxicity and DNA topoisomerase II inhibiting activity for synthetic compounds mentioned above.

Experimental Section

All melting points were taken on a Fisher-Johns melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 1320 spectrophotometer, UV spectra were taken on a Varian 2200 UV–vis spectrophotometer, and ^1H NMR spectra were obtained by using either a JEOL FX-60 or a Bruker-250 NMR spectrometer; all chemical shifts are reported in parts per million from $(CH_3)_4Si$. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. Mass spectral analyses were determined on a V. G. Micromass 70-70 instrument at 70 eV with a direct inlet system. Analytical thin-layer chromatography (TLC) was carried out on Merck precoated silica gel 60 F-254. EM Kieselgel 60 (230–400 mesh ASTM) was used for column chromatography. Preparative TLC was performed on Analtech precoated silica gel GF (500 μm, 20 × 20 cm). Preparative, centrifugal TLC was performed with a Chromatotron (Harrison Research) on radial plates coated (2 mm) in our laboratory with Merck PF-254 silica gel. All new target compounds were characterized by melting point, ^1H NMR and IR spectral, and elemental or mass spectrometric analysis.

Natural Product Isolation. Podophyllotoxin (**5**), α-peltatin (**4**), and α-peltatin β-D-glucoside (**27**) were isolated by using modified literature methods[6,10] which include fractionation of the ethanolic extract of the roots and rhizomes of *Podophyllum peltatum* on a silica gel column with a 3% MeOH in $CHCl_3$ mobile phase. The glucoside **27** was isolated by silica gel column chromatography of the $CHCl_3$-insoluble plant extract with a 15% MeOH in $CHCl_3$ eluant. TLC demonstrated positive $FeCl_3$ test and an identical R_f value with that of an authentic sample.[11] Compound **4** was formed upon acidic hydrolysis of **27**.

Synthesis of Ether Analogues 3, 6, and 8. One gram (2.42 mmol) of podophyllotoxin was suspended into about 10 mL of anhydrous CH_2Cl_2. Hydrogen bromide gas was introduced at 0 °C until the solution became saturated. The reaction mixture was capped and allowed to stand for 20 h at 0 °C. Excess amount of absolute methanol, ethanol, or freshly distilled ethanethiol was then added along with anhydrous $BaCO_3$ upon which a vigorous evolution of gas commenced. After 2 h of stirring, the mixture was diluted with CH_2Cl_2. It was filtered and partitioned with

(8) Hassner, A.; Alexanian, V. *Tetrahedron Lett.* **1978**, *46*, 4475.
(9) Keller-Juslen, C.; Kuhn, M.; von Wartburg, A. *J. Med. Chem.* **1971**, *14*, 936.
(10) Hartwell, J.; Detty, W. *J. Am. Chem. Soc.* **1950**, *72*, 247.
(11) Stoll, A.; von Wartburg, A.; Renz, J. *J. Am. Chem. Soc.* **1955**, *77*, 1711.

water. The organic phase was dried ($MgSO_4$), concentrated, and flash chromatographed on silica gel with $CHCl_3$–MeOH 39:1 as an eluant. The major product was the desired ether derivative.

4′-Demethylepipodophyllotoxin 4-Ethyl Ether (3). Physical properties were identical with those of the known compound.[6]

4′-Demethylepipodophyllotoxin 4-Methyl Ether (6). Crystals were obtained from MeOH–CH_2Cl_2 in 35% yield: mp 183 °C; IR ($CHCl_3$) 3350 (phenol), 1772 (γ-lactone), 1618 (arom C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.82 (s, 1 H, 5-H), 6.56 (s, 1 H, 8-H), 6.27 (s, 2 H, 2′,6′-H), 5.98 (q, 2 H, OCH_2O), 5.4 (s, 1 H, 4′-OH), 4.60 (d, $J_{1,2}$ = 5.6 Hz, 1 H, 1-H), 4.30–4.37 (m, 3 H, 1-H, 11αβ-H), 3.77 (s, 6 H, 3′,5′-OCH_3), 3.47 (s, 3 H, 4-OCH_3), 3.38 (dd, $J_{1,2}$ = 5.6 Hz, $J_{2,3}$ = 14 Hz, 2-H), 2.87 (m, 1 H, 3-H); MS, *m/z* 414 (M^+). Anal. ($C_{22}H_{22}O_8$) C, H.

4′-Demethylepipodophyllotoxin 4-ethyl thioether (8) was crystallized from MeOH–CH_2Cl_2 in 30% yield: mp 230 °C; IR ($CHCl_3$) 3540 (phenol), 1770 (γ-lactone), 1610 (arom C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.92 (s, 1 H, 5-H), 6.43 (s, 1 H, 8-H), 6.30 (s, 2 H, 2′,6′-H), 5.96 (q, 2 H, OCH_2O), 5.41 (s, 1 H, 4′-OH), 3.78 (s, 6 H, OCH_3), 2.58 (q, 2 H, *J* = 7 Hz, SCH_2CH_3), 1.36 (t, 3 H, *J* = 7 Hz, SCH_2CH_3); MS, *m/z* 444 (M^+).

4′-Demethylepipodophyllotoxin (7). The preceding ether synthetic route was used except that an excess of 50% H_2O–acetone solution was added along with $BaCO_3$.[7] Desired product was obtained in about 25% yield. Spectral data and melting point match those reported in the literature.[6] Anal. ($C_{21}H_{20}O_8 \cdot {}^3/_4H_2O$) C, H.

4′-(*tert*-Butyldimethylsilyl)-4′-demethylepipodophyllotoxin (9). To a solution of 0.514 g (1 mmol) of **7** and 0.170 g (2.5 mmol) of imidazole in anhydrous THF was added a solution of 0.188 g (1.25 mmol) of TBDMS-Cl in THF according to Corey's method.[12] The desired monoprotected derivative **9** was isolated by TLC ($CHCl_3$–acetone 6:1) and crystallized from MeOH in 60% yield: mp 212 °C; IR ($CHCl_3$) 1770 (γ-lactone), 1584, 1500, 1480, 1414 (arom C=C, 931 (Si–O–Ar) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.86 (s, 1 H, 5-H), 6.56 (s, 1 H, 8-H), 6.24 (s, 2 H, 2′,6′-H), 5.99 (brs, 2 H, OCH_2O), 3.68 (s, 6 H, OCH_3), 0.98 (s, 9 H, *t*-Bu), 0.10 (s, 6 H, $Si(Me)_2$). Anal. ($C_{27}H_{34}O_8Si$) C, H.

4′-Carbobenzoxy-α-peltatin (17). To a solution of 3.0 g (7.5 mmol) of **4** in pyridine–CH_2Cl_2 (5:1) at 0 °C under argon was added 2.14 mL (15 mmol) of benzyl chloroformate with stirring. After 9 h at room temperature, the mixture was partitioned between $CHCl_3$ and 2 N H_2SO_4 four times. The organic fraction was washed with H_2O, dried ($MgSO_4$), concentrated, and separated on a column of silica gel ($CHCl_3$–EtOAc 5:1). The desired product **17** was crystallized in 35% yield. The diprotected derivative and **18** were isolated in 38 and 8% yield, respectively. Starting material was recovered in 25% yield. Compound **17**: mp 218 °C; IR ($CHCl_3$) 1765 (γ-lactone), 1603, 1484, 1460 (arom C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.39 (s, 5 H, arom CBZ), 6.39 (s, 2 H, 2′,6′-H), 6.22 (s, 1 H, 8-H), 5.26 (s, 2 H, benzyl), 3.7 (s, 6 H, OCH_3). Anal. ($C_{29}H_{26}O_{10}$) C, H.

5-Carbobenzoxy-α-peltatin (18). Synthesized from the previous reaction and crystallized in 8% yield: mp 141–2 °C; IR ($CHCl_3$) 3700 (phenol), 1769 (γ-lactone), 1705 (ester carbonyl), 1480, 1460 (arom C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.42 (s, 5 H, CBZ arom), 6.51 (s, 1 H, 8-H), 6.34 (s, 2 H, 2′,6′-H), 6.01 (s, 2 H, OCH_2O), 5.32 (s, 2 H, benzyl), 3.77 (s, 6 H, 3′,5′-OCH_3). Anal. ($C_{29}H_{26}O_{10} \cdot {}^1/_4H_2O$) C, H.

Esterification of 9 and 17. To a solution of **9** or **17** in absolute pyridine was added 2 equiv of the appropriate anhydride (and 0.2 equiv of 4-(dimethylamino)pyridine (4-DMAP) in the case of succinic and phthalic anhydride). The reaction was stirred for 12–24 h at room temperature under argon and monitored by TLC. Upon completion, the reaction mixture was diluted with $CHCl_3$ and washed four times with 3% HCl and once with H_2O. The organic phase was dried ($MgSO_4$), filtered, and flash evaporated to a semicrystalline residue and was used without further purification.

Synthesis of 10–14. Removal of the TBDMS ether protecting group was accomplished by adding 3 equiv of tetrabutylammonium fluoride to a solution of protected **10–14** in anhydrous THF.[12] The reaction generally gave nearly quantitative yields and was complete within 15 min. Purification by preparative TLC ($CHCl_3$:MeOH 20:1) followed. Yields are reported from **9**.

4′-Demethylepipodophyllotoxin 4-Acetate (10). Crystals were obtained from ethyl ether–acetone in 79% yield: mp 192 °C; IR ($CHCl_3$) 3615 (phenol), 1773 (br, γ-lactone), 1587, 1504, 1482 (arom C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.88 (s, 1 H, 5-H), 6.56 (s, 1 H, 8-H), 6.30 (s, 2 H, 2′,6′-H), 6.00 (s, 2 H, OCH_2O), 4.67 (d, 1 H), 3.78 (s, 6 H, OCH_3), 2.13 (s, 3 H, $COCH_3$). Anal. ($C_{23}H_{22}O_9$) C, H.

4′-Demethylepipodophyllotoxin 4-Propionate (11). Crystals were obtained from ethyl ether–acetone in 74% yield: mp 213 °C; IR ($CHCl_3$) 1775 (γ-lactone), 1728 (ester carbonyl), 1586, 1505, 1482, 1461 (arom C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.88 (s, 1 H, 5-H), 6.57 (s, 1 H, 8-H), 6.30 (s, 2 H, 2′,6′-H), 6.0 (s, 2 H, OCH_2O), 4.67 (d, 1 H), 3.79 (s, 6 H, OCH_3), 1.4 (q, 2 H, $COCH_2CH_3$), 1.18 (t, 3 H, $COCH_2CH_3$). Anal. ($C_{24}H_{24}O_9$) C, H.

4′-Demethylepipodophyllotoxin 4-Succinate (12). Crystals were obtained in 52% yield from ether–MeOH: mp 234–5 °C; IR (Nujol) 3400–2500 (br, carboxylic OH), 1761 (γ-lactone), 1720 (ester carbonyl), 1680 (carboxylic carbonyl) cm^{-1}; ^1H NMR (pyridine-d_5) δ 6.78 (s, 2 H, 2′,6′-H), 6.49 (s, 1 H, 8-H), 5.97 (brs, 2 H, OCH_2O), 3.74 (s, 6 H, OCH_3), 2.94 (brs, 4 H, CH_2CH_2). Anal. ($C_{25}H_{24}O_{11}$) C, H.

4′-Demethylepipodophyllotoxin 4-Phthalate (13). Crystals were obtained in 36% yield from MeOH: mp 202 °C; IR ($CHCl_3$) 3020 (br, carboxylic acid), 1770 (γ-lactone), 1720, 1690 (ester, carboxyl), 1611 (arom C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.61 (brs, 4 H, phthalate), 7.04 (s, 1 H, 5-H), 6.53 (s, 1 H, 8-H), 6.31 (s, 2 H, 2′,6′-H), 5.94 (brd, 2 H, OCH_2O), 3.79 (s, 6 H, OCH_3). Anal. ($C_{29}H_{24}O_{11} \cdot {}^1/_2H_2O$) C, H.

4′-Demethylepipodophyllotoxin 4-Crotonate (14). Crystalline plates were obtained in 80% yield: mp 176–7 °C; IR ($CHCl_3$) 1770 (γ-lactone), 1705 (ester), 1650 (enone C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.89 (s, 1 H, 5-H), 6.57 (s, 1 H, 8-H), 6.32 (s, 2 H, 2′,6′-H), 6.00 (brs, 2 H, OCH_2O), 6.00 (d, 1 H, overlapped 2″-H), 5.73 (d, *J* = 2 Hz, 1 H, 2″-H), 3.79 (s, 6 H, OCH_3), 1.93 (dd, *J* = 7 and 2 Hz, 3 H, 4″-CH_3); MS, *m/z* 468 (M^+).

4′-Demethylepipodophyllotoxin 4(*R*,*S*)-Tetrahydropyranyl Ether (16). To a solution of 1 equiv each of **9** and *p*-TsOH in absolute CH_2Cl_2 at 0 °C and under nitrogen atmosphere was added 10 equiv of dihydropyran.[13] The reaction was allowed to come to room temperature and TLC ($CHCl_3$:acetone 6:1) indicated completion at 2 h. The mixture was diluted with CH_2Cl_2 and washed three times with saturated $NaHCO_3$ and once with H_2O. The organic phase was dried ($MgSO_4$), concentrated, and separated on TLC. Two unresolved compounds collected from TLC were dissolved in absolute THF, and 2.5 equiv of $[CH_3(CH_2)_3]_4NF$ was added.[12] The reaction mixture was stirred for 15 min at 0 °C under argon, reuslting in quantitative desilylation. Purification by preparative TLC ($CHCl_3$:MeOH 15:1) afforded crystals from hexane in 65% yield: mp 213–6 °C; IR ($CHCl_3$) 3530 (phenol), 1773 (γ-lactone), 1610 (br), 1481 (arom C=C), 1130 (aliphatic ether) cm^{-1}; ^1H NMR ($CDCl_3$), a diastereomeric ratio of 1.65:1 was indicated by integration of peak pairs of H-5, H-8, H-2′ and H-6′, 6.86 and 7.07 (s, 1 H, 5-H), 6.55 and 6.49 (s, 1 H, 8-H), 6.26 and 6.29 (s, 2 H, 2′,6′-H), 5.97 (m, 2 H, OCH_2), 5.40 (s, 1 H, OH), 3.77 (s, 6 H, OCH_3), 1.57 (brs, $C(CH_2)_3C$, overlapped with H_2O). Anal. ($C_{26}H_{28}O_9 \cdot {}^1/_4H_2O$) C, H.

Synthesis of 19–22. Removal of the CBZ protecting group by catalytic hydrogenolysis with H_2 and 5% Pd–C in EtOH–EtOAc was complete within 1 h. Filtration and flash evaporation generally afforded product in nearly quantitative yield. Yields are reported from **17**.

α-Peltatin 5-Acetate (19). Crystals were obtained in 92% yield from acetone–ethanol: mp 130–4 °C; IR ($CHCl_3$) 3545 (phenol), 1772 (γ-lactone and acetate overlap), 1616, 1501, 1477, 1460 (arom C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.42 (s, 1 H, 8-H), 6.27 (s, 2 H, 2′,6′-H), 5.92 (s, 2 H, OCH_2O), 3.72 (s, 6 H, OCH_3), 2.30 (s, 3 H, $COCH_3$). Anal. ($C_{23}H_{22}O_9$) C, H.

α-Peltatin 5-Succinate (20). Crystals were obtained in 43% yield: mp 205 °C; IR ($CHCl_3$) 3545 (phenol), 3500–2500 (carboxylic acid), 1771 (γ-lactone), 1735, 1728 (succinate), 1620, 1501, 1481,

(12) Corey, E. J. *J. Am. Chem. Soc.* **1972**, *94*, 6190.

(13) Bernady, K.; Floyd, M.; Poletto, J.; Weiss, M. *J. Org. Chem.* **1979**, *19*, 1438.

1461 (arom C=C) cm^{-1}; ^1H NMR (pyridine-d_5) δ 6.88 (s, 2 H, 2',6'-H), 6.48 (s, 1 H, 8-H), 5.97 (s, 2 H, OCH_2O), 4.84 (d, 1 H), 3.71 (s, 6 H, OCH_3), 2.85 (brs, 4 H, succinate). Anal. ($C_{25}H_{24}O_{11}$) C, H.

α-Peltatin 5-Butyrate (21). Amorphous solid was isolated in 69% yield: IR ($CHCl_3$) 1768 (γ-lactone and ester overlap), 1615 (arom C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.49 (s, 1 H, 8-H), 6.35 (s, 2 H, 2',6'-H), 5.99 (s, 2 H, OCH_2O), 3.78 (s, 6 H, OCH_3), 2.62 (t, 2 H, 2''-H), 1.86 (m, 2 H, 3''-H), 1.07 (t, 3 H, 4''-H). Anal. ($C_{25}H_{26}O_9$) C, H.

α-Peltatin 5-Propionate (22). Amorphous crystals were obtained in 88% yield: mp 140 °C; IR ($CHCl_3$) 1764 (γ-lactone and ester overlap), 1612 (arom C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.49 (s, 1 H, 8-H), 6.36 (s, 2 H, 2',6'-H), 6.00 (s, 2 H, OCH_2O), 3.78 (s, 6 H, OCH_3), 2.68 (q, J = 8 Hz, 2 H overlapped, 2''-H), 1.3 (t, J = 8 Hz, 3 H, 3''-H). Anal. ($C_{24}H_{24}O_9 \cdot {}^1/_6H_2O$) C, H.

Synthesis of 15 and 23. To a solution of **9** or **17** in anhydrous CH_2Cl_2 was added 2 equiv of DCC, 0.2 equiv of 4-DMAP, and 2 equiv of phenylacetic acid. The reaction mixture was stirred at room temperature under argon for 2 h and upon complete esterification it was diluted 10-fold with ethyl ether. After filtration of the insoluble dicyclohexylurea, the filtrate was concentrated and separated by preparative TLC ($CHCl_3$:MeOH 30:1). The major product was isolated and used without further purification. Removal of the CBZ group by catalytic hydrogenolysis near atmospheric pressure with 5% Pd–C in EtOH–EtOAc afforded a quantitative yield within 1 h. The product was separated and purified by filtration, concentration, and crystallization from EtOH. Yields are reported from **9** or **17**.

4'-Demethylepipodophyllotoxin 4-Phenylacetate (15). Crystalline plates were obtained from EtOH in 80% yield: mp 204 °C; IR ($CHCl_3$) 1771 (γ-lactone), 1723 (ester), 1612 (arom C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.32 (s, 5 H, phenyl), 6.85 (s, 1 H, 5-H), 6.56 (s, 1 H, 8-H), 6.27 (s, 2 H, 2',6'-H), 6.00 (s, 2 H, OCH_2O), 5.43 (s, 1 H, phenol), 3.78 (s, 6 H, OCH_3), 3.67 (s, 2 H, benzyl). Anal. ($C_{29}H_{26}O_9$) C, H.

α-Peltatin 5-Phenylacetate (23). Amorphous solid was isolated in 71% yield: mp 115 °C; IR ($CHCl_3$) 3540 (phenol), 1770 (γ-lactone), 1611 (arom C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.39 (s, 5 H, phenyl), 6.47 (s, 1 H, 5-H), 6.32 (s, 2 H, 2',6'-H), 5.99 (s, 2 H, OCH_2O), 3.94 (s, 2 H, benzyl), 3.77 (s, 6 H, OCH_3); MS, m/z 518 (M^+). Anal. ($C_{29}H_{26}O_9 \cdot 1.5H_2O$) C, H.

α-Peltatin 5-Methyl Ether (24). Excess diazomethane was added to an ice-cooled solution of 0.107 g (0.2 mmol) of **17** in 10 mL of Et_2O–$CHCl_3$. After 1 h the solvent was evaporated to an oil which was used without further purification. The foregoing method of hydrogenolytic cleavage of the CBZ group was performed to afford 70 mg of amorphous crystals in 85% yield from **17**: mp 226–231 °C; IR ($CHCl_3$) 3540 (phenol), 1770 (γ-lactone), 1606, 1520, 1462 (br), 1419 (arom C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.36 (s, 2 H, 2',6'-H), 6.27 (s, 1 H, 8-H), 5.91 (s, 2 H, OCH_2O), 4.07 (s, 3 H, 5-OCH_3), 3.79 (s, 6 H, 3',5'-OCH_3). Anal. ($C_{22}H_{22}O_8$) C, H.

α-Peltatin 5-[4,6-*O*-(*R*)-Ethylidene-β-D-glucopyranoside] (25). One equivalent of **15** and 0.25 equiv of *p*-TsOH were suspended in an excess of acetaldehyde diethyl acetal, and the mixture was vigorously stirred under argon for 3 h. Then the reaction mixture was diluted with $CHCl_3$ and washed four times with H_2O. The organic phase was dried ($MgSO_4$) and concentrated. Chromatography on TLC ($CHCl_3$:MeOH 15:1) afforded crystalline **25** in 74% yield: mp 233–5 °C; IR (KBr) 3600–3100 (hydroxyls), 1776 (γ-lactone), 1606, 1510, 1463, 1424 (arom C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.38 (s, 1 H, 8-H), 6.35 (s, 2 H, 2',6'-H), 5.97 (q, 2 H, OCH_2O), 5.42 (s, 1 H, phenol), 4.76 (q, 1 H, ethylidene acetal), 3.78 (s, 6 H, OCH_3), 1.4 (d, 3 H, CH_3). Anal. ($C_{29}H_{32}O_{13} \cdot H_2O$) C, H.

α-Peltatin 5-[4,6-*O*-(*R*)-Thenylidene-β-D-glucopyranoside] (26). An adaptation of Keller-Juslen's method was used.[9] One equivalent of **27** and 2 equiv of $ZnCl_2$ were dissolved in excess thiophenecarboxaldehyde, and the mixture was stirred at room temperature under argon for 5 h. TLC indicated 75% conversion to one upper R_f product. The reaction mixture was partitioned between $CHCl_3$ and H_2O, followed by drying ($MgSO_4$) and filtering of organic fraction. Dilution with hexane produced a colorless precipitate, which was filtered. The filtrate was evaporated and separated by TLC ($CHCl_3$:MeOH 15:1). The product was crystallized from ethyl ether:acetone in 55% yield: mp 191 °C; IR ($CHCl_3$) 3600 (alcoholic OH), 3545 (phenolic OH), 1773 (γ-lactone), 1615, 1461 (arom C=C) cm^{-1}; ^1H NMR ($CHCl_3$) δ 7.45–6.92 (m, 3 H, thiophene), 6.37 (s, 1 H, 8-H), 6.34 (s, 2 H, 2',6'-H), 5.96 (s, 2 H, OCH_2O), 5.81 (s, 1 H, acetal of thenylidene), 5.43 (brs, 1 H, phenol), 3.77 (s, 6 H, OCH_3). Anal. ($C_{32}H_{32}O_{13}S \cdot {}^3/_4H_2O$) C, H, S.

Isolation of Human Topoisomerase II. Human DNA topoisomerase II was isolated from peripheral blast cells of a patient with chronic lymphocytic leukemia. This enzyme was purified by polyethylene glycol (PEG) 8000, precipitation, hydroxylapatite column, heparin-Sepharose column, and hexylamine agarose affinity chromatography, to near homogeneity. Three bands (142 000, 132 000, and 114 000 daltons) could be seen with Coomassie blue staining. The detailed procedure, which is a partial combination of Wang's[14] and Liu's[15] procedures, will be published elsewhere.

Preparation of Drugs. Drugs were dissolved in 1/10 or 2/10 volume of Me_2SO, and 9/10 or 8/10 volume of 0.1 M HEPES buffer, pH 6.7, was added to make a 0.4 mM final concentration of each drug.

DNA Topoisomerase II Assay. The P4 knotted DNA unknotting reaction was a modification of the Hsieh[16] procedure.

The reaction mixture (20 μL), which contained 50 mM HEPES, pH 6.7, 50 mM KCl, 100 mM NaCl, 0.1 mM EDTA, 10 mM $HgCl_2$, 0.1 mP ATP, 50 μg/mL bovine serum albumin, 0.26 μg P4 knotted DNA, and enzyme, was incubated with or without drugs.

The reaction mixture was incubated at 37 °C for 30 min and terminated by adding a stop solution (2% sodium dodecyl sulfate, 20% glycerol, 0.05% bromphenol blue). These samples were loaded onto a 1% agarose gel and electrophoresed at 50 V overnight with an electrophoresis buffer that contained 90 mM Tris–boric acid, pH 8.3, and 2.5 mM EDTA. At completion, the gel was stained in 0.5 μg/mL of ethidium bromide. Then a photograph was taken of the DNA bands visualized with fluorescence induced by a long-wavelength UV lamp. The data reported in Tables I and II reflect a 0.4 mM drug concentration.

Acknowledgment. We thank Dr. David Millington of Duke University and Dr. David Harris of the Chemistry Department, UNC—Chapel Hill for mass spectral and NMR spectral analyses, respectively. Thanks also to Mike Fisher of the Pharmacology Department, UNC—Chapel Hill for KB cell culture assay and to Dr. A. von Wartburg of Sandoz, Ltd. for an authentic sample of α-peltatin β-D-glucoside. This work was supported by grants from the American Cancer Society (No. CH-370, K-H.L.) and the NIH (No. CA-44358, Y.C.C.).

Registry No. **3**, 102306-95-6; **5**, 518-28-5; **6**, 118356-05-1; **7**, 6559-91-7; **8**, 118356-06-2; **9**, 118356-07-3; **10**, 111712-27-7; **11**, 118356-08-4; **12**, 118356-09-5; **13**, 118356-10-8; **14**, 118356-11-9; **15**, 118356-12-0; **16**, 118356-13-1; **17**, 118356-14-2; **18**, 118397-69-6; **19**, 118417-81-5; **20**, 118397-70-9; **21**, 118356-15-3; **22**, 118397-68-5; **23**, 118417-82-6; **24**, 118490-57-6; **25**, 33419-42-0; **26**, 53956-10-8; **27**, 11024-58-1; DNA topoisomerase, 80449-01-0; methanol, 67-56-1; ethanol, 64-17-5; ethanethiol, 75-08-1; benzyl chloroformate, 501-53-1; acetic anhydride, 108-24-7; propionic anhydride, 123-62-6; succinic anhydride, 108-30-5; phthalic anhydride, 85-44-9; crotonic anhydride, 623-68-7; dihydropyran, 110-87-2; phenylacetic acid, 103-82-2; acetaldehyde diethyl acetal, 105-57-7.

(14) Goto, T.; Laipia, P.; Wang, J. *J. Biol. Chem.* **1984**, 259, 10422.
(15) Halligan, B.; Edwards, K.; Liu, L. *J. Biol. Chem.* **1985**, *260*, 2475.
(16) Hsieh, T. *J. Biol. Chem.* **1983**, *258*, 8413.