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ANTITUMOR AGENTS, 107.[1] NEW CYTOTOXIC 4-ALKYLAMINO ANALOGUES OF 4′-DEMETHYL-EPIPODOPHYLLOTOXIN AS INHIBITORS OF HUMAN DNA TOPOISOMERASE II

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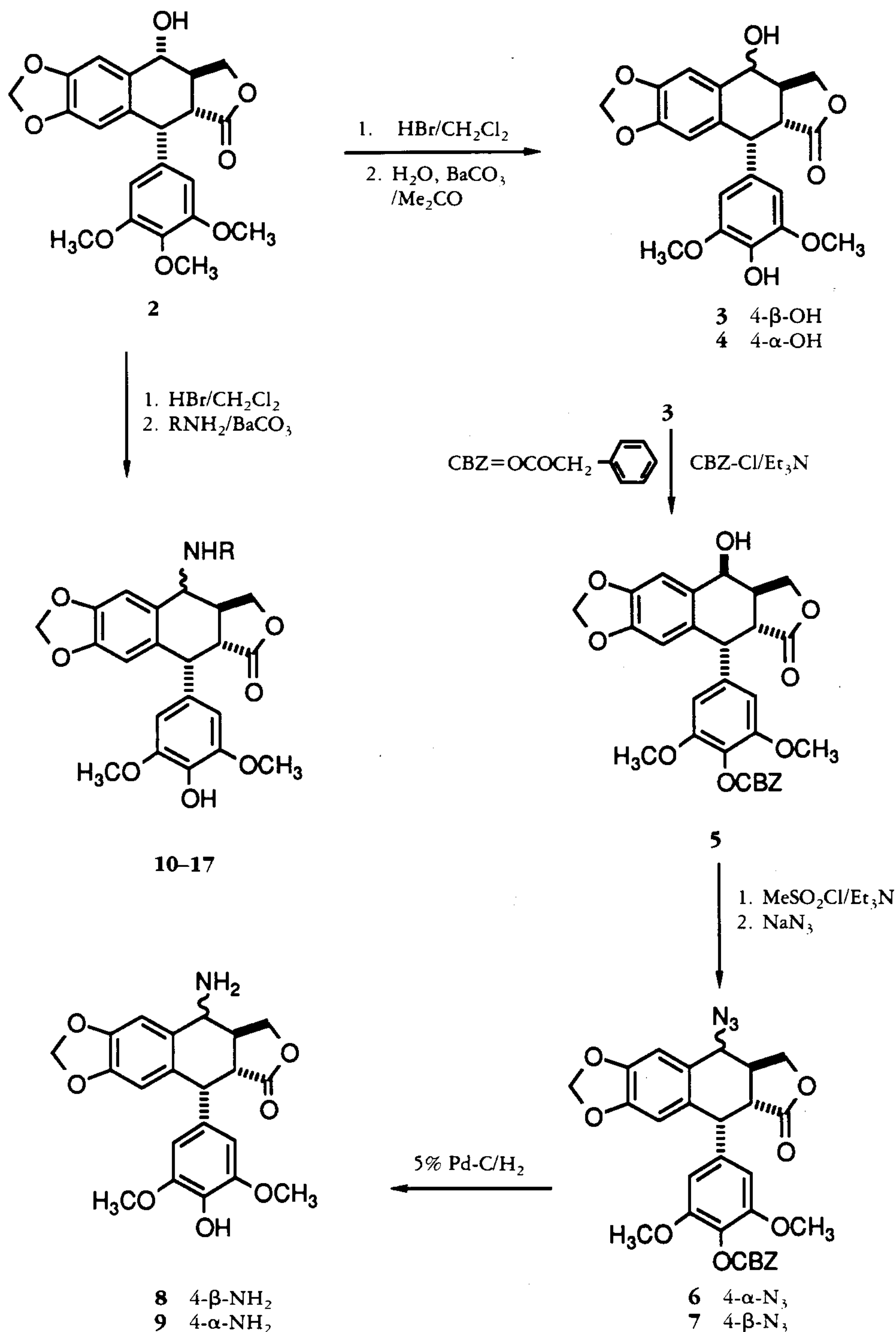
ABSTRACT.—A series of analogues of etoposide [**1**], the C-4 amino- and alkylamino-substituted 4′-demethyl-epipodophyllotoxins, have been synthesized and studied for their activity to inhibit type II human DNA topoisomerase as well as their activity in causing cellular protein-linked DNA breakage. Substitution of the glycosidic moiety of **1** by a 2″-hydroxyethylamino or 2″-methoxyethylamino chain at the C-4β position resulted in potent inhibitors (**10**, **13**, **15**, **16**) of the human DNA topoisomerase II. This inhibitory activity correlates reasonably well with their activity in causing protein-linked DNA breakage in KB cells. The in vitro cytotoxicity (KB) appears to have no correlation with the inhibitory activity of the human DNA topoisomerase II.

Topoisomerases are enzymes which control the topological state of DNA (1,2). Type II topoisomerases catalyze DNA strand passage through transient double strand breaks in the DNA. The resulting change in linking number of DNA allows these enzymes to mediate DNA interconversions, such as supercoiling and relaxation of supercoiling, catenation and decatenation, knotting and unknotting (1,2). These enzymes have been implicated in a number of vital cellular processes, including DNA replication and transcription and chromosomal segregation (3). The prime importance of this enzyme makes it a critical target for the action of a wide variety of anticancer drugs (4), including the clinically useful 4′-demethylated epipodophyllotoxins, exemplified by etoposide [**1**] and teniposide. There exists ample evidence suggesting that these drugs block the catalytic activity of DNA topoisomerase II by stabilizing an enzyme-DNA complex in which the DNA is cleaved and covalently linked to the enzyme (5–7). This may be the key step leading to cell death. Structure-activity studies have demonstrated a direct correlation between cytotoxicity, DNA breakage, and mice-derived topoisomerase II inhibition activities among the podophyllotoxin analogues (8). The recent isolation and purification of human type II topoisomerase from lymphocytic leukemia cells has provided this laboratory with the means to use this enzyme as a target to investigate the structure-activity relationships among **1** and related congeners.

In our previous studies (9), we found that substitution of the glycosidic moiety in etoposide by an 4-alkoxy group, as in 4′-demethyl-epipodophyllotoxin ethyl ether, retains the inhibitory activity on DNA topoisomerase II at higher concentration. Furthermore, a series of 4-acyl congeners were prepared and found to be less active, although some of them possessed potent cytotoxicity (10). As part of our continuing efforts along this line, we describe in this paper the preparation (Scheme 1) and structure-activity relationships of 4-alkylamino congeners, which showed potent inhibitory activity on human DNA topoisomerase II.

The synthesis of the 4-hydroxyl series followed a previous procedure (10), a modifi-

[1]For Part 106 see X.Z. Yan, Y.H. Kuo, T.J. Lee, T.S. Shih, C.H. Chen, D.R. McPhail, A.T. McPhail, and K.H. Lee, *Phytochemistry*, in press.



SCHEME 1.

cation of the method of Kuhn *et al.* (11) that achieves regioselective 4′-*O*-demethylation of podophyllotoxin [**2**]. The major product, 4′-demethyl-epipodophyllotoxin [**3**], resulted from inversion of the C-4 hydroxyl group, which was accompanied by the retention product, 4′-demethyl-podophyllotoxin [**4**].

The synthesis of the 4-amino series of analogues started from **3**, which required an initial protection of the phenolic group with carbobenzyloxy chloride to afford **5**. After conversion of **5** to the corresponding alpha chloride, it was treated with NaN_3 to yield a mixture of C-4-azido isomers. The 4β-azido isomer **6** can be purified by crystallization. Further reduction of the crude azides led to a mixture of 4-amino-4-demethyl-podophyllotoxins **8** and **9**, which were easily separated by chromatography.

Compounds **10–17** of the 4-alkylamino series were synthesized by direct nucleophilic substitution with appropriate alkylamines on the C-4-bromo intermediate, resulting from hydrobromide demethylation (10). The bulky C-1α pendant aromatic ring dictates the substitution to be stereoselective in yielding the C-4β alkylamino isomer as the major product. In some cases, the C-4α isomers of 4′-demethyl or 4′-methoxyl compounds were also observed.

The assignment of the configuration at C-4 for compounds **7–17** was based on the difference of $J_{3,4}$ coupling constants. The C-4 β-substituted compounds **7**, **8**, **10**, and **12–17** have a $J_{3,4} = 4.0$ Hz as seen in **1**, due to a *cis* relationship between H-3 and H-4. The C-4 α-substituted derivatives **8** and **11**, however, like **2**, have a $J_{3,4} > 10.0$ Hz as H-3 is trans to H-4.

RESULTS AND DISCUSSION

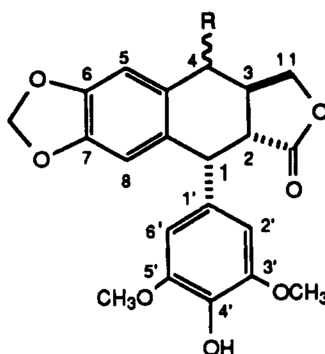
The degree of inhibition of different drugs on human type II DNA topoisomerase was estimated by observing the relative density of unknotted and knotted P4 DNA on agarose gel in comparison with the results obtained with different dilutions of enzyme alone. The effect of different drugs on the formation of protein-linked DNA breakage in drug-treated KB cells was determined by K-SDS assay (12). This assay provides a quick and sensitive means for monitoring the cellular level of protein-linked DNA complexes, which reflects the activity of drug-induced DNA breakage.

The results of enzyme inhibition and protein-linked DNA breakage are assembled in Table 1 along with the cytotoxicity (KB) data. In the enzyme inhibition assay, all compounds were screened at 100 μM concentration, and the protein-linked DNA breakage assay was performed at 10 μM concentrations.

For all compounds tested, the inhibition of enzyme activity correlates quite well with their ability to cause DNA strand breakage (Table 1). The fact that the C-4 hydroxyl (**3**,**4**), amino (**8**,**9**), and alkylamino (**10**, **12–17**) derivatives are all active indicates that the glucosidic ethylidene cyclic acetal moiety is not required for inhibitory activity. The stereochemistry of C-4 substituents is also quite important in determining the inhibitory potency, with the β-isomers being much more potent than the α-isomers (**3** vs. **4**, **8** vs. **9**, and **10** vs. **11**). Among the alkylamino series, those compounds with a β-hydroxyethylene chain (**10**, **15**, and **16**) demonstrated potent inhibition on enzyme as well as DNA strand breakage. Prolongation of the ethylene side chain, as in propyl (**12**), allyl (**14**), and hydroxypropyl (**17**) analogues, all led to reduction in potency.

The in vitro cytotoxicity of all the compounds tested apparently does not correlate with their enzyme inhibitory activity and DNA strand breakage activity. Thus, although the alkylamino analogues **10** and **13–17** are potent enzyme inhibitors, they showed reduction in cytotoxicity in comparison with etoposide [**1**]. This is in agreement with our previous findings from C-4β ester congeners (10) and those of others from podophyllin analogues (13). It should be pointed out that the mechanism of cytotoxicity of **1** and podophyllotoxin is very different. The primary action of **1** is through DNA topoisomerase II inhibition, and that of podophyllotoxin is through the inhibition of microtubule polymerization. The compounds synthesized have structural features of both **1** and podophyllotoxin; therefore, their action could be the same as that of either **1** or podophyllotoxin.

TABLE 1. Biological Evaluation of 4-Alkylamino-epipodophyllotoxin Analogues.



Compound	R	Cytotoxicity[a] ED_{50} KB (μg/ml)	Inhibition of DNA Topoisomerase II Activity[b]	Cellular Protein-DNA Complex Formation[c] (%)	
				10 μM	5 μM
1	β-	0.16	++++ (50μM)	100.0	56.2
3	β-OH	0.34	++	42.2	
4	α-OH	0.045	+	3.3	
8	β-NH_2	0.19	++	36.4	
9	α-NH_2	0.25	+	8.0	
10	β-$NHCH_2CH_2OH$	1.6	++++ (50μM)	121.4	70.1
11	α-$NHCH_2CH_2OH$	>1.0	—	0.0	
12	β-$NHCH_2CH_2CH_3$	<0.4	++	69.7	
13	β-$NHCH_2CH_2OCH_3$	>4.0	+++	110.8	
14	β-$NHCH_2CH{=}CH_2$	3.4	+++	84.1	
15	β-$NHCH_2CH(OH)CH_3$	3.6	++++ (25μM)	167.2	83.9
16	β-$NHCH(CH_3)CH_2OH$	2.3	++++ (25μM)	161.7	89.3
17	β-$NHCH_2CH_2CH_2OH$	4.0	++	89.2	

[a]ED_{50} is the concentration of drug which affords 50% reduction in cell number after 3 days incubation.

[b]Inhibition: +, 25%; ++, 50%; +++, 75%; ++++, >75%; —, 0%. Estimated ID_{50} of the drugs is in parentheses.

[c]Relative activities of cellular protein–DNA complex formation in KB ATCC tissue culture cells measured at 5 μM and 10 μM drug concentration as compared to the complex formed by 10 μM of etoposide.

Furthermore, the uptake of **1** could also play a key role in its potency (19). Mechanisms other than topoisomerase II inhibition, such as inhibition of microtubule polymerization, may have to be considered for the cytotoxic activity of C-4 α-substituted isomers **4** and **9**. Also, different abilities to transport across the cell membrane for all C-4 alkylamino analogues may account for their reduced cytotoxicity in comparison with **1**. It is not surprising to observe the lack of correlation between the DNA topoisomerase inhibitory activity and the cytotoxicity. Their potential antitumor activity will require further examination.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All melting points were taken on a Fischer-Johns melt-

ing point apparatus and are uncorrected. Ir spectra were recorded on a Perkin-Elmer 1320 spectrophotometer, and ^{1}H-nmr spectra were obtained by using either a JEOL FX-60 or a Bruker 250 nmr spectrometer; all chemical shifts are reported in ppm from TMS. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tennessee. Mass spectral analyses were determined on a V.G. Micromass 70-70 instrument at 70 eV with a direct inlet system. Analytical tlc was carried out on Merck precoated Si gel 60 F-254. EM Kieselgel 60 (230–400 mesh ASTM) was used for cc. Preparative tlc was performed on Analtech precoated Si gel GF (500 μm, 20 × 20 cm). All new target compounds were characterized by mp, ^{1}H nmr, and ir spectral analyses, as well as elemental analysis.

4'DEMETHYL-EPIPODOPHYLLOTOXIN [**3**].—Podophyllotoxin (5 g, 12.10 mmol) was dissolved in 75 ml of anhydrous CH_2Cl_2. Dry HBr gas was bubbled through the solution to saturation. The reaction mixture was capped and allowed to stand at room temperature for 48 h. Removal of the solvent yielded a residue which was treated with H_2O (25 ml), Me_2CO (50 ml), and $BaCO_3$ (5 g) and heated at reflux for 1 h. The reaction mixture was extracted with $CHCl_3$ and chromatographed on a Si gel column. The product was obtained by elution with $CHCl_3$-MeOH (30:1) and recrystallized from CH_2Cl_2/Et_2O to give 2.5 g (52%) of **3**. Spectral data, specific rotation, and mp agree with those in the literature (11).

4'-DEMETHYL-PODOPHYLLOTOXIN [**4**].—This compound was obtained from the aforementioned column by further elution with $CHCl_3$-MeOH (30:1) and crystallized from Me_2CO in 5% (0.5 g) yield. Spectral data, specific rotation and mp agree with those in the literature (14).

4'-*O*-CARBOBENZOXY-EPIPODOPHYLLOTOXIN [**5**].—A solution of 2 g of **3** (5 mmol) in 200 ml of anhydrous CH_2Cl_2 was cooled in an ice bath and treated with 2.5 ml of triethylamine (18 mmol) and 2.5 ml of carbobenzoxychloride (17.5 mmol). The reaction mixture was allowed to stir at room temperature for 2 h, after which time H_2O (100 ml) was added. The organic layer was dried ($MgSO_4$), concentrated, and purified using Si gel cc. The product was obtained upon elution with $CHCl_3$ and recrystallized from $CHCl_3$/EtOH to give 2.4 g (89%) of **5**. Spectral data, specific rotation and mp agree with those in the literature (15).

4'-*O*-CARBOBENZOXY-4-AZIDO-EPIPODOPHYLLOTOXIN [**6**].—A solution of 3 g (5.6 mmol) of **5** in 100 ml of anhydrous CH_2Cl_2 was cooled in an ice bath and treated successively with 1.5 ml (10.8 mmol) of triethylamine and 1.2 ml (15.5 mmol) of methanesulfonylchloride. The ice bath was removed and the reaction mixture stirred at room temperature for 1 h. This was evaporated in vacuo to dryness, and 40 ml of anhydrous DMF was added along with 3 g (46 mmol) of NaN_3. The reaction mixture was stirred overnight at room temperature and then partitioned between H_2O (100 ml) and EtOAc. The organic layer was washed with H_2O, dried ($MgSO_4$), and concentrated to yield a crude residue, which was checked by tlc and nmr analyses to be a mixture of 4α- and 4β-azido isomers (ca. 1:3). Crystallization from $CHCl_3$/EtOH provided the pure β-isomer **7** (2.3 g, 73%): mp 202–204°; ms *m/z* $[M]^+$ 559, 424, 382; ir (KBr) 2950, 2900, 2100 (azide), 1770 (carbonate C=O), 1745 (lactone C=O), 1600, 1475 (aromatic C=C) cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 7.40 (m, 5H, cbz aromatic), 6.82 (s, 1H, H-5), 6.58 (s, 1H, H-8), 6.27 (s, 2H, H-2',6'), 6.03 (ABq, J= 1.1 and 4.0 Hz, 2H, O-CH_2-O), 5.25 (s, 2H, OCH_2Ph), 4.77 (d, J= 4 Hz, 1H, H-4), 4.65 (d, J= 5 Hz, 1H, H-1), 4.31 (d, J= 9 Hz, 2H, H-11, 11'), 3.66 (s, 6H, 3',5'-OMe), 3.2 (dd, J= 5, 14 Hz, 1H, H-2), 2.90 (m, 1H, H-3). *Anal.* calcd for $C_{29}H_{25}O_9N_3 \cdot 1/2\ H_2O$, C 61.27, H 4.58; found C 61.21, H 4.52.

4'-DEMETHYL-4β-AMINO-4-DESOXYPODOPHYLLOTOXIN [**8**].—To a solution of the crude 4'-demethyl-4-azidopodophyllotoxin (2.3 g, 4.1 mmol) [**6** + **7**] in 200 ml of EtOAc was added 500 mg of 10% palladium on carbon. This mixture was shaken under 40 psi of H_2 for 4 h. The reaction mixture was filtered through celite and the filtrate evaporated in vacuo. The residue was chromatographed on a Si gel column and eluted first with a $CHCl_3$-EtOAc (2:1) solvent system to remove nonpolar products. Further elution with a $CHCl_3$-MeOH (19:1) mobile phase yielded pure **8** (0.85 g, 52%), crystallized from CH_2Cl_2/Et_2O: mp 132–135°; ms *m/z* $[M]^+$ 399; ir (KBr) 3360 (OH), 3290 (primary amine), 2900, 1745 (lactone), 1590 (aromatic C-H) cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 6.81 (s, 1H, H-5), 6.49 (s, 1H, H-8), 6.30 (s, 2H, H-2',6'), 5.96 (ABq, J= 1 Hz, 2H, OCH_2O), 5.3 (s, 1H, OH, D_2O exchangeable), 4.55 (d, J= 5.2 Hz, 1H, H-1), 4.28 (d, J= 9.5 Hz, 2H, H-11 and H-11'), 4.17 (d, J= 4.1 Hz, 1H, H-4), 3.77 (s, 6H, 3',5'-OMe), 3.28 (dd, J= 5.2, 14 Hz, 1H, H-2), 2.85 (m, 1H, H-3). *Anal.* calcd for $C_{21}H_{21}O_7N \cdot H_2O$, C 60.43, H 5.55; found C 60.25, H 5.35.

4'-DEMETHYL-4α-AMINO-4-DESOXYPODOPHYLLOTOXIN [**9**].—This compound was obtained from the aforementioned column by further elution with a $CHCl_3$-MeOH (19:1) mobile phase. The pure product **9** (0.34 g, 20%) was crystallized from CH_2Cl_2/Et_2O: mp 133–135°; ms *m/z* $[M]^+$ 399; ir (KBr) 3360 (OH), 3295 (NH_2), 2900, 1743 (lactone), 1590 (aromatic C-H) cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 7.14 (s, 1H, H-5), 6.54 (s, 1H, H-8), 6.20 (s, 2H, H-2',6'), 6.00 (ABq, J= 1 Hz, 2H, OCH_2O), 4.63 (d,

$J = 5.1$ Hz, 1H, H-1), 4.61 (d, $J = 9.0$ Hz, 1H, H-11α), 4.07 (dd, $J = 9.0$, 10.4 Hz, 1H, H-11β), 3.83 (d, $J = 10.3$ Hz, 1H, H-4), 3.81 (s, 6H, 3′,5′-OMe), 2.85 (dd, $J = 5.1$, 14.1 Hz, 1H, H-2), 2.57 (m, 1H, H-3). *Anal.* calcd for $C_{21}H_{21}O_7N \cdot H_2O$, C 60.43, H 5.55; found C 60.31, H 5.64.

SYNTHESIS OF 4-ALKYLAMINO-4-DESOXYPODOPHYLLOTOXIN [**10**–**17**].—A solution of podophyllotoxin [**2**] (5 g, 12.1 mmol) in 50 ml of anhydrous CH_2Cl_2 was kept at room temperature, and dry HBr gas was bubbled through the solution until saturation was achieved. The flask was then capped and allowed to stand for 48 h, after which time dry N_2 was bubbled through the solution to drive off excess HBr. $BaCO_3$ (2 g) and 2 ml of the appropriate amine were added, resulting in vigorous evolution of gas. This mixture was allowed to stand for 5 h at room temperature, after which the reaction mixture was filtered, washed with H_2O, dried, and purified via cc. Yields ranged from 5 to 10%.

4′-DEMETHYL-4β-[2″-HYDROXYETHYLAMINO]-4-DESOXYPODOPHYLLOTOXIN [**10**].—Amorphous powder from CH_2Cl_2/Et_2O: mp 120°; ms *m/z* $[M]^+$ 443; ir (KBr) 3420 (NH, OH), 2900 (aliphatic C-H), 1755 (lactone), 1600, 1475 (aromatic C=C) cm^{-1}; ^1H nmr ($CDCl_3$) δ 6.82 (s, 1H, H-5), 6.49 (s, 1H, H-8), 6.29 (s, 2H, H-2′,6′), 5.97 (ABq, $J = 1.0$, 4.4 Hz, 2H, OCH_2O), 4.57 (d, $J = 5.0$ Hz, 1H, H-1), 4.35 (m, 2H, H-11,11′), 3.93 (d, $J = 4.0$ Hz, 1H, H-4), 3.79 (s, 6H, 3′,5′-OMe), 3.76 (m, 2H, H-2″), 3.3 (dd, $J = 5.0$, 13.5 Hz, 1H, H-2), 3.09 (m, 1H, H-3), 2.75 (m, 2H, H-1″). *Anal.* calcd for $C_{23}H_{25}O_8N \cdot 1/2\ H_2O$, C 61.06, H 5.75; found C 60.67, H 5.75.

4′-DEMETHYL-4α-[2″-HYDROXYETHYLAMINO]-4-DESOXYPODOPHYLLOTOXIN [**11**].—Crystals from CH_2Cl_2/Et_2O: mp 230–234°; ms *m/z* $[M]^+$ 443; ir (KBr) 3425 (NH, OH), 2900 (aliphatic C-H), 1753 (lactone), 1600, 1475 (aromatic C=C) cm^{-1}; ^1H nmr (CD_3OD) δ 6.83 (s, 1H, H-5), 6.47 (s, 1H, H-8), 6.38 (s, 2H, H-2′,6′), 5.92 (ABq, $J = 1.0$, 4.3 Hz, 2H, OCH_2O), 4.40 (s, 1H, H-11), 4.25 (s, 1H, H-11′), 3.75 (s, 6H, 3′,5′-OMe), 3.65 (m, 4H, H-1″,2″), 3.56 (m, 1H, H-1), 3.49 (dd, $J = 6.1$, 11.3 Hz, 1H, H-4), 2.87 (ddd, $J = 5.1$, 6.3, 13.5, 1H, H-3), 2.67 (dd, $J =$ 6.1, 8.2, 1H, H-2). *Anal.* calcd for $C_{23}H_{25}O_8N \cdot 1/2\ H_2O$, C 61.06, H 5.75; found C 60.83, H 5.70.

4′-DEMETHYL-4β-PROPYLAMINO-4-DESOXYPODOPHYLLOTOXIN [**12**].—Crystals from CH_2Cl_2/Et_2O: mp 153–156°; ms *m/z* $[M]^+$ 441; ir (KBr) 3470 (OH), 3320 (NH), 1750 (lactone), 1600, 1475 (aromatic C=C) cm^{-1}; ^1H nmr ($CDCl_3$) δ 6.77 (s, 1H, H-5), 6.47 (s, 1H, H-8), 6.28 (s, 2H, H-2′,6′), 5.95 (ABq, $J = 1.2$, 5.0 Hz, 2H, OCH_2O), 4.30 (d, $J = 5.0$ Hz, 1H, H-1), 4.30 (d, $J = 4.0$ Hz, 1H, H-4), 4.28 (m, 2H, H-11,11′), 3.78 (s, 6H, 3′,5′-OMe), 3.30 (dd, $J = 5.0$, 13.9 Hz, 1H, H-2), 2.83 (m, 2H, H-1″), 2.52 (m, 1H, H-3), 1.55 (m, 2H, H-2″), 0.95 (t, $J = 7.6$ Hz, 3H, H-3″). *Anal.* calcd for $C_{24}H_{27}O_7N \cdot 1/2\ H_2O$, C 64.00, H 6.22; found C 63.72, H 5.86.

4′-DEMETHL-4β-[2″-METHOXYETHYLAMINO]-4-DESOXYPODOPHYLLOTOXIN [**13**].—Crystals from CH_2Cl_2/Et_2O: mp 202–204°; ms *m/z* $[M]^+$ 457; ir (KBr) 3440 (OH, NH), 1750 (lactone), 1600, 1475 (aromatic C=C) cm^{-1}; ^1H nmr ($CDCl_3$) δ 6.80 (s, 1H, H-5), 6.44 (s, 1H, H-8), 6.25 (s, 2H, H-2′,6′), 5.92 (ABq, $J = 1.0$, 5.0 Hz, 2H, OCH_2O), 4.50 (d, $J = 5.4$ Hz, 1H, H-1), 4.28 (m, 2H, H-11,11′), 3.88 (d, $J = 4.0$ Hz, 1H, H-4), 3.75 (s, 6H, 3′,5′-OMe), 352 (m, 2H, H-2″), 3.37 (s, 3H, H-3″), 3.38 (dd, $J =$ 14.4, 5.4 Hz, 1H, H-2), 3.05 (m, 1H, H-3), 2.75 (m, 2H, H-1″). *Anal.* calcd for $C_{24}H_{27}O_8N \cdot 1/4\ H_2O$, C 62.40, H 5.96; found C 62.48, H 5.89.

4′-DEMETHYL-4β-ALLYLAMINO-4-DESOXYPODOPHYLLOTOXIN [**14**].—Amorphous powder from CH_2Cl_2/Et_2O: mp 225–228°; ms *m/z* $[M]^+$ 439; ir (KBr) 3340 (OH, NH), 2885 (aliphatic C-H), 1745 (lactone), 1600, 1475 (aromatic C=C) cm^{-1}; ^1H nmr ($CDCl_3$) δ 6.77 (s, 1H, H-5), 6.49 (s, 1H, H-8), 6.28 (s, 2H, H-2′,6′), 5.95 (ABq, $J = 1.0$, 4.5 Hz, 2H, OCH_2O), 5.90 (m, 1H, H-2″), 5.4 (m, 1H, N-H, D_2O exchangeable), 5.22 (dd, $J = 4.0$, 17.5 Hz, 2H, H-3″), 4.53 (d, $J = 5.5$ Hz, 1H, H-1), 4.30 (m, 2H, H-11,11′), 3.88 (d, $J = 3.6$ Hz, 1H, H-4), 3.75 (s, 6H, 3′,5′-OMe), 3.30 (dd, $J = 5.4$, 14.4 Hz, 1H, H-2), 3.30 (m, 1H, H-1″), 2.80 (m, 1H, H-3). *Anal.* calcd for $C_{24}H_{25}O_7N \cdot 2.2\ H_2O$, C 60.17, H 6.14; found C 60.05, H 5.78.

4′-DEMETHYL-4β-[2″-HYDROXYPROPYLAMINO]-4-DESOXYPODOPHYLLOTOXIN [**15**].—Crystals from CH_2Cl_2/Et_2O: mp 145–150°; ms *m/z* $[M]^+$ 457; ir (KBr) 3330 (OH, NH), 2890 (aliphatic C-H), 1750 (lactone), 1600, 1475 (aromatic C=C) cm^{-1}; ^1H nmr ($CDCl_3$) δ 6.83 (s, 1H, H-5), 6.47 (s, 1H, H-8), 6.27 (s, 2H, H-2′,6′), 5.95 (ABq, $J = 1.0$, 6.3 Hz, 2H, OCH_2O), 5.40 (m, 1H, N-H, D_2O exchangeable), 4.54 (d, $J = 4.6$ Hz, 1H, H-1), 4.30 (m, 2H, H-11,11′), 3.85 (m, 1H, H-2″), 3.85 (d, $J = 3.8$ Hz, 1H, H-4), 3.75 (s, 6H, 3′,5′-OMe), 3.25 (dd, $J = 4.6$, 13.8 Hz, 1H, H-2), 2.85 (dd, $J = 6.8$, 12.5 Hz, 1H, H-1″), 2.82 (m, 1H, H-3), 2.63 (dd, $J = 3.8$, 12.5 Hz, 1H, H-1″), 1.20 (d, $J = 6.3$ Hz, 3H, H-3″). *Anal.* calcd for $C_{24}H_{25}O_8N \cdot 1/2\ H_2O$, C 61.80, H 6.01; found C 61.61, H 6.00.

4′-DEMETHYL-4β-[1″-METHYL-2″-HYDROXYETHYLAMINO]-4-DESOXYPODOPHYLLOTOXIN [**16**].—Amorphous powder from CH_2Cl_2/Et_2O: mp 220–225°; ms *m/z* $[M]^+$ 457; ^1H nmr ($CDCl_3$) δ 6.89 (s, 1H, H-5), 6.47 (s, 1H, H-8), 6.27 (s, 2H, H-2′,6′), 5.95 (ABq, $J = 1.0$, 4.5 Hz, 2H, OCH_2O),

5.40 (m, 1H, N-H, D_2O exchangeable), 4.52 (d, J=4.8 Hz, 1H, H-1), 4.30 (d, J=9.0 Hz, 2H, H-11,11′), 4.00 (d, J=4.0 Hz, 1H, H-4), 3.74 (s, 6H, 3′,5′-OMe), 3.50 (m, 2H, H-2″), 3.22 (dd, J=4.8, 13.5 Hz, 1H, H-2), 2.85 (m, 1H, H-3), 2.82 (m, 1H, H-1″), 1.05 (d, J=6.3 Hz, 3H, 1″-Me). *Anal.* calcd for $C_{24}H_{25}O_8N\cdot 1/2\ H_2O$, C 61.80, H 6.01; found C 61.69, H 5.98.

4′-DEMETHYL-4β-[3″-HYDROXYPROPYLAMINO]-4-DESOXYPODOPHYLLOTOXIN [**17**].—Crystals from CH_2Cl_2/Et_2O: mp 193–196°; ms *m/z* $[M]^+$ 457; ir (KBr) 3460 (OH), 3320 (NH), 2900 (aliphatic C-H), 1740 (lactone), 1600, 1475 (aromatic C=C) cm^{-1}; ^1H nmr ($CDCl_3$) δ 6.75 (s, 1H, H-5), 6.46 (s, 1H, H-8), 6.24 (s, 2H, H-2′,6′), 5.94 (ABq, J=1.0, 4.4 Hz, 2H, OCH_2O), 4.52 (d, J=5.3 Hz, 1H, H-1), 4.33 (dd, J=7.9, 8.0 Hz, 1H, H-11), 4.23 (dd, J=8.0, 10.7 Hz, 1H, H-11′), 3.78 (d, J=4.0 Hz, 1H, H-4), 3.73 (s, 6H, 3′,5′-OMe), 3.72 (t, 2H, H-3″), 3.21 (dd, J=5.3, 14.0 Hz, 1H, H-2), 3.11 (dd, J=5.9, 11.4 Hz, 1H, H-1″), 2.64 (ddd, J=14.0, 7.0, 11.0 Hz, 1H, H-1″), 1.75 (m, 2H, H-2″). *Anal.* calcd for $C_{24}H_{27}O_8N\cdot 3/4\ H_2O$, C 61.21, H 6.06; found C 61.31, H 5.76.

ISOLATION OF HUMAN DNA TOPOISOMERASE II.—Human DNA topoisomerase II was isolated from peripheral blast cells of a patient with acute leukemia. The isolation procedure is a partial combination of the procedures of Goto *et al.* (16) and Halligan *et al.* (17) which has been detailed in our previous work (10).

PREPARATION OF DRUGS.—Drugs were dissolved in Me_2SO at a concentration of 20 mM as the stock solution and diluted before use with H_2O to the desired concentration of each drug.

DNA TOPOISOMERASE II ASSAY.—The P4 unknotting reaction was a modification of the procedure reported by Hsieh (18).

The reaction mixture (20 μl), which contained 50 mM HEPES (pH 7.0), 50 mM KCl, 100 mM NaCl, 0.1 mM EDTA, 10 mM $MgCl_2$, 1.0 mM ATP, 50 μg/ml bovine serum albumin, 0.4 μg P4 knotted DNA, and enzyme, was incubated with or without drugs.

The reaction mixture was incubated at 37° for 30 min, and the reaction was terminated by adding 5.0 μl of a stop solution (2% sodium dodecyl sulfate, 20% glycerol, 0.05% bromophenol blue). These samples were loaded onto a 1% agarose gel and electrophoresed at 55 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. A photograph was taken of the DNA bands visualized with fluorescence induced by a long-wavelength uv lamp. The data reported in Table 1 reflect a 100 μM drug concentration.

K-SDS PRECIPITATION ASSAY FOR PROTEIN-DNA COMPLEXES.—The intracellular formation of covalent topoisomerase II–DNA complexes was quantitated using the potassium SDS precipitation assay, a procedure adapted from the method of Rowe *et al.* (12). KB cells were obtained from the American Type Culture Collection (ATCC) and were prelabeled with 0.05 mCi/ml [^{14}C] thymidine (specific activity 50.5 mCi/mmol) for 18 h. A final concentration of 5×10^5 cells/sample was treated with 10 μM of the drugs at 37° for 1 h, and the procedure described in Rowe *et al.* (12) was used to detect the protein-linked DNA levels.

CYTOTOXICITY ASSAY.—The cytotoxicity (KB) assay was carried out according to a procedure described in Ferguson *et al.* (19).

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