Antitumor Agents, 130. Novel 4#-Arylamino Derivatives of 3',4'-Didemethoxy-3',4'-dioxo-4-deoxypodophyllotoxin as Potent Inhibitors of Human DNA Topoisomerase II

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ANTITUMOR AGENTS, 130.[1] NOVEL 4β-ARYLAMINO DERIVATIVES OF 3′,4′-DIDEMETHOXY-3′,4′-DIOXO-4-DEOXYPODOPHYLLOTOXIN AS POTENT INHIBITORS OF HUMAN DNA TOPOISOMERASE II

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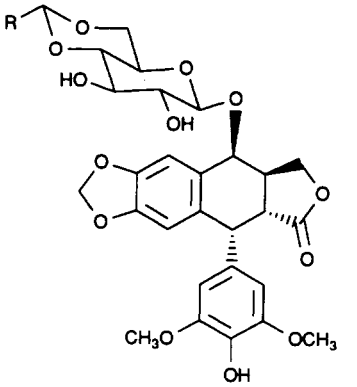
ABSTRACT.—A series of *ortho*-quinone analogues **1–28** of podophyllotoxin possessing various C-4 β-aniline moieties have been synthesized and evaluated for their inhibitory activity against human DNA topoisomerase II, their activity in causing cellular protein-linked DNA breakage, and their cytotoxicity against KB cells. Compounds **8–12**, **15**, **19**, and **23–28** are better than or comparable to etoposide in their inhibition of the human DNA topoisomerase II, while compounds **8–10** and **22** are comparable to or more potent than etoposide in causing DNA breakage. There is an apparent lack of correlation between cytotoxicity and the ability of these compounds to cause protein-linked DNA breaks or inhibit DNA topoisomerase II. This suggests that in addition to the mechanism of the topoisomerase II involving DNA breakages, other mechanisms of action, such as the radical mechanism or the direct adduct formation of the *ortho*-quinone with DNA or protein, may also involve and account for the apparent KB cytotoxicity. Two different modes of DNA topoisomerase II inhibition for **8–28** were proposed.

Etoposide [**1**] and teniposide [**2**] are epipodophyllotoxin derivatives used clinically as potent chemotherapeutic agents for a variety of tumors including small cell lung carcinoma, testicular cancer, and malignant lymphoma. The primary mechanism of action of these drugs is believed to be via their interaction with DNA (2). Dose-dependent



1 R=Me

2 R= 

[1]For part 129, see Kashiwada *et al.* (1).

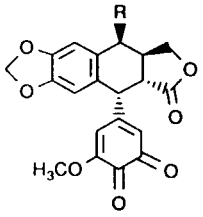
single- and double-stranded breaks in DNA caused by **1** or **2** have been widely reported (3–6), and these were suggested to be the initial event for a series of biological changes, including DNA protein cross-links (6) and chromosomal aberrations (7), which led to cytotoxicity. It was found that the presence of cellular components was essential for both DNA damage and the cytotoxicity (3). DNA topoisomerase II (Topo II) has been reported to be involved in the process of inducing DNA breakage (4,5,8), and **1** and its analogues are believed to inhibit the strand-rejoining activity of this enzyme by stabilizing the Topo II-DNA complex in a cleavable stage (5,9). However, the detailed mechanism by which this stimulation of cleavable complex formation leads to DNA breaks and cell death is not known.

On the other hand, a large amount of experimental evidence supports the contention that besides inhibiting DNA topoisomerase II, epipodophyllotoxins may also exert their cytotoxic effect through the metabolic activation of the dimethoxyphenol ring (E ring) to produce metabolites that can inactivate the DNA by forming chemical adducts. It has been shown that the 3′,4′-catechol derivative of **1** can be formed under cytochrome P-450-dependent oxidative *O*-demethylation of **1** (10), and the catechol can be further oxidized to the 3′,4′-*ortho*-quinone derivative in the presence of oxygen (11). The *ortho*-quinone derivatives of **1** can also be formed in vitro under the influence of horseradish peroxidase or prostaglandin E synthetase (12). In contrast to their parent compound **1**, which binds very weakly to purified DNA and does not inactivate biologically active DNA, both the *ortho*-quinone and the catechol derivatives of **1** bound strongly to purified calf thymus DNA and inactivated both single- and double-stranded biologically active FX174 DNA (11). During peroxidative activation, **1** had been shown to form a stable oxygen-centered free radical (13). Hydroxyl radicals have also been observed to be produced by the catechol of **1** in the presence of iron and H_2O_2 (14). In addition to these in vitro investigations, the importance of the oxidation-reduction processes has also been indicated in several cellular and in vivo studies. Dehydrogenase inhibitors or substrates and some free-radical scavengers were found to be capable of inhibiting **1**-induced DNA damage and cytotoxicity (15). Compound **1** was also found to be 30-fold more toxic to normally oxygenated EMT6 mouse mammary cells than to hypoxic cells (16). The in vivo antitumor activity of **1** against FS all C fibrosarcoma and Lewis lung carcinoma was increased considerably by oxygenation of the tumor tissue prior to administration of the drug (16,17). Because of the involvement of free radicals in these processes, a radical-mediated mechanism of action was suggested to account for the DNA damage by **1**. However, the importance of the direct reaction between **1** radicals and DNA was refuted by Kalyanaraman *et al.* (18) with the support of electron spin resonance (ESR) evidence. It was found that neither the primary nor the secondary phenoxyl radicals of **1** react with molecular oxygen or DNA at an appreciable rate. Instead of binding occurring through radicals, the observed activity between **1** semiquinone radical and DNA was suggested to be through the binding of the **1** quinone to DNA, thereby implying increased importance and direct involvement of the *ortho*-quinone in the mechanism of DNA adduct formation.

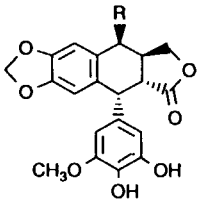
We have previously studied (19–22) the 4β-arylamino analogues related to **1**, such as the 3′,4′-*O*,*O*-didemethylepipodophyllotoxins (23) (Table 1, series B) and the 4′-*O*-demethylepipodophyllotoxins (19,20) (Table 1, series C), as potent inhibitors of human DNA topoisomerase II and as antitumor agents. In view of the importance of the *ortho*-quinone moiety with respect to its DNA breakage and antitumor activities described above, the synthesis of the 3′,4′-didemethoxy-3′,4′-dioxopodophyllotoxins (Table 1, series A) was initiated, and its biological results are reported herein.

Our initial attempt to finish the synthesis of target compounds was via the nucleophilic substitution on the C-4 position of 3′,4′-didemethoxy-3′,4′-dioxo-4β-

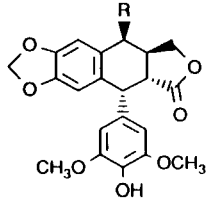
TABLE 1. Biological Evaluation of 4β-Arylamino Derivatives of 3′,4′-Didemethoxy-3′,4′-dioxo-4-deoxypodophyllotoxin.



A



B



C

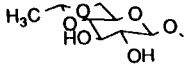
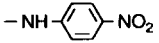
Compound	R	Cytotoxicity[a] ID_{50} KB (μM)			Inhibition of DNA Topoisomerase II Activity, ID_{50} (μM)[b]			Cellular protein-DNA complex Formation (%)[c]		
		A	B[d]	C[e]	A	B[d]	C[e]	A	B[d]	C[e]
1				0.20			50			100
5	=O	>4.0			5			56		
6	α-OH	1.9			10	50	>100	4	8	1.1
7	β-OH	2.8			<5	100	>100	41	50	0
8		1.3	1.3	0.5	10	10	10	128	200	323

TABLE 1. (Continued)

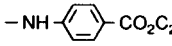
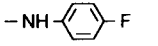
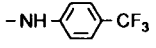
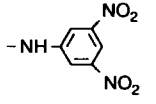
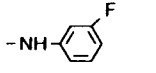
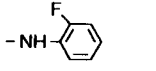
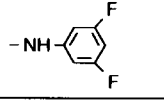
Compound	R	Cytotoxicity[a] ID_{50} KB (μM)			Inhibition of DNA Topoisomerase II Activity, ID_{50} (μM)[b]			Cellular protein-DNA complex Formation (%)[c]		
		A	B[d]	C[e]	A	B[d]	C[e]	A	B[d]	C[e]
9		1.4		0.84	25		5	110		207
10		1.3	1.5	0.2	25	10	5	92	117	213
11		6.5			25			51		
12		1.8			50			32		
13		1.6		0.23	100		50	37		158
14		1.2		0.25	100			29		121
15		2.0		1.08	50		50	57		115

TABLE 1. (Continued)

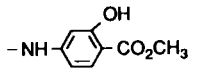
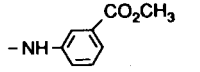
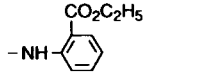
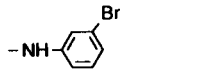
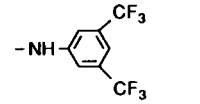
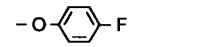
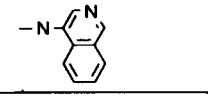
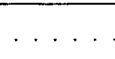
Compound	R	Cytotoxicity[a] ID_{50} KB (μM)			Inhibition of DNA Topoisomerase II Activity, ID_{50} (μM)[b]			Cellular protein-DNA complex Formation (%)[c]		
		A	B[d]	C[e]	A	B[d]	C[e]	A	B[d]	C[e]
16		1.9		5.8	100		50	47		129
17		1.2	2.3	2.7	100	25	50	67	94	129
18		7.0			100			4		
19		6.1		2.36	50			15		62
20		5.5		3.4	>100		>100	4		21
21		0.9		0.5	100		>100	30		57
22		1.5		<1.0	100		50	95		123

TABLE 1. (Continued)

Compound	R	Cytotoxicity[a] ID_{50} KB (μM)			Inhibition of DNA Topoisomerase II Activity, ID_{50} (μM)[b]			Cellular protein-DNA complex Formation (%)[c]		
		A	B[d]	C[e]	A	B[d]	C[e]	A	B[d]	C[e]
23		>2.0	1.8	0.7	25	25	25	32	128	243
24		>4.0	2.0	0.22	25	>50	50	27	77	99
25		>2.0	1.8	0.6	50	25		18	146	211
26		1.5	1.0		50	25		69	147	
27		2.0	1.2		25	25		41	109	
28		>4.0	2.0	1.0	50	50	50	33	75	230

[a] ID_{50} was the concentration of drug which affords 50% reduction in cell number after a 3-day incubation.

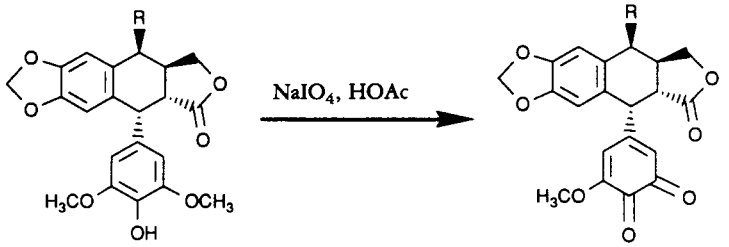
[b] Each compound was examined with five concentrations at 5, 10, 25, 50, and 100 μM. The ID_{50} value was established based on the degree of inhibition at these five concentrations.

[c] Concentration for series A and B: 20 μM; for series C: 10 μM.

[d] Data are from Wang *et al.* (23) (included for comparison).

[e] Data are from Lee *et al.* (19) and Wang *et al.* (20) (included for comparison).

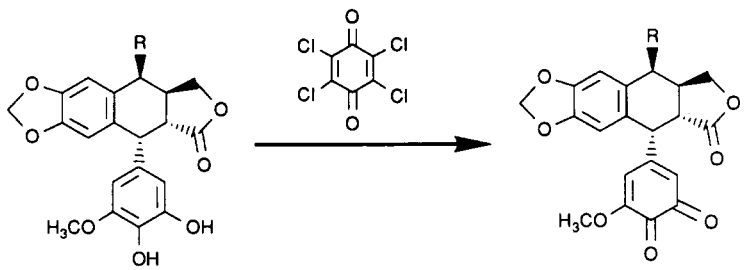
bromo-4-deoxypodophyllotoxin with an appropriate arylamine, which has been used successfully for the preparation of similar compounds in our previous work (19,20). Unfortunately, the preparation of 3′,4′-didemethoxy-3′,4′-dioxo-4β-bromo-4-deoxypodophyllotoxin failed, since HBr as a strong reductive agent turned the *ortho*-quinone into its catechol. As a variety of 4β-arylamino-4′-*O*-demethyl-4-deoxypodophyllotoxins and 4β-arylamino-3′,4′-*O*-didemethyl-4-deoxypodophyllotoxins are in hand (23), we used them as starting material instead. Target compounds **8–22** were synthesized from the corresponding 4′-*O*-demethylpodophyllotoxin analogues [**3**] using a modified method of Nemec (24) (Scheme 1). The yields of the oxidation



3 **8–22**

SCHEME 1

were in a range of 67–99%, while compounds **23–28** were prepared from their corresponding 3′,4′-didemethylpodophyllotoxin analogue **4** using the tetrachloro-1,2-benzoquinone as an oxidant (Scheme 2) with a yield of 90–100%. The *ortho*-benzoquinone group was characterized by typical quinonoid peaks in the ir spectrum around 1685 and 1650 cm^{-1} (25).



4 **23–28**

SCHEME 2

RESULTS AND DISCUSSION

Table 1 shows the activities of Topo II inhibition, protein-linked DNA complex formation, and the cytotoxicity toward the KB-ATCC cell line of the 4β-arylamino derivatives **8–28** of 3′,4′-didemethoxy-3′,4′-dioxo-4-deoxypodophyllotoxin (series A). For Topo II inhibitory activity, compounds **8–12**, **15**, **19**, and **23–28** are better than or comparable to **1**. However, only **8–10** and **22** have activity comparable to **1** for protein-linked DNA complex formation ability. All others are less active. As for KB-ATCC cell toxicity, all compounds have larger ID_{50} values compared to **1**. Comparing

with compounds **5–7**, introduction of the 4β-arylamino group gives some increase to the protein-linked DNA complex formation ability in some compounds (e.g., **8** and **9**) but not as large as with the compounds of series B or C. In contrast to the protein-DNA complex formation ability, introducing the 4β-arylamino side chain decreased the Topo II inhibitory activity. There was still a lack of correlation between the ability of compounds in causing protein-linked DNA breaks and cytotoxicity, as observed formerly within compounds of series B and C. This further suggests that in addition to the mechanism of the Topo II involved DNA damages, other mechanisms of action, such as the mechanism of chemical adduct formation, may also involve and account for the apparent KB cytotoxicity. Comparing series A, B, and C, it is clear that in most cases, the *ortho*-quinone compounds (series A) are less potent than their congeners of series B, which in turn are less potent than those of series C (A<B<C). However, the activity order is different in some cases, especially for the cytotoxicity (e.g., **8**, **10**, **16**, **17**). In the case of compounds **16** and **17**, the *ortho*-quinones are most cytotoxic compared with their congeners of series B and C.

Maanen *et al.* (11) reported that the *ortho*-quinone of etoposide can bind covalently to microsomal proteins upon incubation. Sinha and his group (12,13,26) also suggested the binding of the etoposide *ortho*-quinone and/or the corresponding semiquinone radical with both protein and DNA. Since the inhibition of Topo II does not parallel well with the DNA-protein complex formation in the *ortho*-quinone series (series A, e.g., compounds **5–7**, **11**, **12**, **15**, and **19** inhibit Topo II strongly, but are weak in their ability to form protein-DNA complexes), we propose that the *ortho*-quinones of epipodophyllotoxin inhibit the DNA topoisomerase II by two different modes. One of these modes is to inhibit the strand-rejoining activity of the enzyme by forming a non-covalent DNA-Topo II-drug complex, which causes protein-associated DNA breakage just as etoposide does. The other mode is to form a covalent bond with the enzyme, such as the direct adduct formation with *ortho*-quinone, to cause enzyme inactivation. Since introducing the 4β-arylamino moiety to the *ortho*-quinone nucleus decreases the Topo II inhibitory activity, while in some cases, increasing the ability of the protein-DNA complex formation (e.g., **8A**, **9A**), these two modes may have different structural preferences. The apparent inhibiting activity towards the enzyme is contributed by both modes. Covalent binding of the *ortho*-quinone compounds with Topo II might inactivate the enzyme, and therefore destroy its ability to form the protein-DNA complex.

On the other hand, the radical mechanism or the direct adduct-formation of the *ortho*-quinones with either DNA or other important functional proteins might also be responsible for the cytotoxicity of these etoposide derivatives. The apparent cytotoxicity may be contributed from both the non-covalent Topo II involved mechanism (protein-DNA complex formation) and the covalent chemical adduct formation mechanism described above. The covalent mechanism may contribute more to the apparent cytotoxicity in the *ortho*-quinone series (series A) than in the other two (series B and C), which may need metabolic conversion to the *ortho*-quinones as suggested by the literature (10,11,18). When the importance of the covalent mode exceeds that of the non-covalent one, which might happen with the structure variation, the *ortho*-quinone compounds might be more cytotoxic than their congeners of series B or C. This may explain the reversed cytotoxicity order mentioned above in cases of **16** and **17**. However, in most cases, compounds with better protein-DNA complex formation ability show better cytotoxicity, suggesting that the non-covalent Topo-II-involved mechanism of action is more important in most cases.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All mp's were taken on a Fischer-Johns mp apparatus

and were uncorrected. Ir spectra were recorded on a Perkin-Elmer 1320 spectrophotometer, and ^{1}H-nmr spectra were obtained by using a Bruker AC-300 nmr spectrometer with TMS as the internal standard. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA. 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. All new target compounds were characterized by mp, ^{1}H-nmr, and ir spectral analyses, as well as elemental analyses or ms analyses. Specific rotations were measured with a Rudolph Research Autopol III polarimeter.

SYNTHESIS OF COMPOUNDS **8–22**.—A solution containing sodium periodate (56 mg, 0.26 mmol) in H_2O (1 ml) was added to a solution containing an appropriate 4β-arylamino-4′-demethyl-4-deoxypodophyllotoxin (**3**, 100 mg, 0.20 mmol) in 1,4-dioxane (2 ml). After adding 0.4 ml of glacial HOAc, the solution was stirred overnight at room temperature. The mixture was then evaporated and resolved in $CHCl_3$ or EtOAc, washed with H_2O, dried ($MgSO_4$), and evaporated in vacuo. The crude product was purified by recrystallization.

3′,4′-Didemethoxy-3′,4′-dioxo-4β-(4″-nitroanilino)-4-deoxypodophyllotoxin [**8**].—Yield 67%; crystals from MeOH; mp 234–237°; ir (KBr) 3370, 2920, 1760, 1650, 1592, 1555, 1495, 1300 cm^{-1}; ^{1}H nmr (DMSO-d_6) δ 8.03 (d, J = 8.6 Hz, 2H, H-3″, -5″), 7.55 (d, J = 8.6 Hz, 1H, exchangeable, NH), 6.81 (d, 3H, H-2″, -6″, -5), 6.73 (s, 1H, H-8), 6.32 (s, 1H, H-6′), 6.04 (s, 1H, OCH_2O), 6.03 (s, 1H, OCH_2O), 5.14 (s, 1H, H-2′), 5.06 (dd, J = 8.5, 4.2 Hz, 1H, H-4), 4.48 (t, 2H, H-1, -11), 3.70 (t, 4H, H-11 and 5′-OMe), 3.44 (dd, J = 14.4, 5.5 Hz, 1H, H-2), 3.11 (m, 1H, H-3). *Anal.* calcd for $C_{26}H_{20}N_2O_3 \cdot \frac{1}{2}H_2O$, C 60.81, H 4.13, N 5.46; found C 61.07, H 4.34, N 5.10.

3′,4′-Didemethoxy-3′,4′-dioxo-4β-(4″-ethoxycarbonylanilino)-4-deoxypodophyllotoxin [**9**].—Yield 75%; crystals from MeOH; mp 203–205°; ir (KBr) 3360, 2900, 1765, 1680, 1650, 1600, 1555, 1515 cm^{-1}; ^{1}H nmr (DMSO-d_6) δ 7.72 (d, J = 8.4 Hz, 2H, H-3″, -5″), 6.87 (d, J = 8.3 Hz, 1H, exchangeable, NH), 6.78 (s, 1H, H-5), 6.75 (d, J = 8.4 Hz, 2H, H-2″, -6″), 6.71 (s, 1H, H-8), 6.32 (s, 1H, H-6′), 6.03 (s, 1H, OCH_2O), 6.02 (s, 1H, OCH_2O), 5.16 (s, 1H, H-2′), 4.96 (dd, J = 8.3, 3.7 Hz, 1H, H-4), 4.45 (t, 2H, H-1, -11), 4.22 (q, J = 7.0 Hz, 2H, CO_2CH_2Me), 3.70 (t, 4H, H-11 and 5′-OMe), 3.46 (dd, J = 14.4, 5.5 Hz, 1H, H-2), 3.07 (m, 1H, H-3), 1.28 (t, J = 7.0 Hz, 3H, CO_2CH_2Me). *Anal.* calcd for $C_{29}H_{25}NO_9 \cdot 2H_2O$, C 61.36, H 5.16, N 2.47; found C 60.96, H 5.29, N 2.31.

3′,4′-Didemethoxy-3′,4′-dioxo-4β-(4″-fluoroanilino)-4-deoxypodophyllotoxin [**10**].—Yield 97%; mp 175–177°; ir (KBr) 3380, 2910, 1765, 1650, 1620, 1552, 1500 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 6.96 (t, J = 8.6 Hz, 2H, H-3″, -5″), 6.72 (s, 1H, H-5), 6.54 (s, 1H, H-6′), 6.53 (s, 1H, H-8), 6.48 (m, 2H, H-2″, -6″), 6.01 and 5.99 (ABq, J = 1.2 Hz, 2H, OCH_2O), 5.29 (s, 1H, H-2′), 4.55 (m, 2H, H-4, -11), 4.28 (4.28 d, J = 5.6 Hz, 1H, H-1), 4.11 (dd, J = 10.5, 8.5 Hz, 1H, H-11), 3.85 (s, 3H, 5′-OMe), 3.36 (dd, J = 14.1, 5.6 Hz, 1H, H-2), 2.99 (m, 1H, H-3). *Anal.* calcd for $C_{26}H_{20}NO_7F \cdot H_2O$, C 63.02, H 4.48, N 2.83; found C 62.94, H 4.66, N 2.42.

3′,4′-Didemethoxy-3′,4′-dioxo-4β-(4″-trifluoromethylanilino)-4-deoxypodophyllotoxin [**11**].—Yield 94%; mp 201–203°; ir (KBr) 3380, 2900, 1760, 1650, 1608, 1520 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 7.48 (d, J = 8.4 Hz, 2H, H-3″, -5″), 6.72 (s, 1H, H-5), 6.59 (d, J = 8.7 Hz, 2H, H-2″, -6″), 6.54 (s, 1H, H-8), 6.53 (d, J = 1.6 Hz, 1H, H-6′), 6.48 (m, 2H, H-2″, -6″), 6.01 and 6.00 (ABq, J = 1.2 Hz, 2H, OCH_2O), 5.28 (d, J = 1.6 Hz, 1H, H-2′), 4.70 (t, J = 4.5 Hz, 1H, H-4), 4.56 (t, J = 8.2 Hz, 1H, H-11), 4.29 (d, J = 5.6 Hz, 1H, H-1), 4.11 (d, J = 5.9 Hz, 1H, exchangeable, NH), 4.04 (dd, J = 10.2, 8.2 Hz, 1H, H-11), 3.85 (s, 3H, 5′-OMe), 3.30 (dd, J = 14.3, 5.6 Hz, 1H, H-2), 3.03 (m, 1H, H-3). *Anal.* calcd for $C_{27}H_{20}NO_7F_3 \cdot H_2O$, C 59.44, H 4.07, N 2.57; found C 59.02, H 4.01, N 2.60.

3′,4′-Didemethoxy-3′,4′-dioxo-4β-(3″,5″-dinitroanilino)-deoxypodophyllotoxin [**12**].—Yield 76%; crystals from toluene; mp 232–233°; ir (KBr) 3380, 3100, 2900, 1760, 1650, 1620, 1530 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 8.44 (t, J = 1.6 Hz, 1H, H-4″), 7.71 (d, J = 1.6 Hz, 2H, H-2″, -6″), 6.70 (s, 1H, H-5), 6.58 (s, 1H, H-8), 6.52 (d, J = 1.5 Hz, 1H, H-6′), 6.04 and 6.03 (ABq, J = 1.1 Hz, 2H, OCH_2O), 5.28 (d, J = 1.5 Hz, 1H, H-2′), 4.82 (dd, J = 6.4, 4.8 Hz, 1H, H-4), 4.67 (t, J = 8.6 Hz, 1H, H-11), 4.63 (d, J = 6.0 Hz, 1H, H-1), 3.99 (dd, J = 10.0, 8.6 Hz, 1H, H-11), 3.86 (s, 3H, 5′-OMe), 3.29 (dd, J = 14.8, 6.0 Hz, 1H, H-2), 3.16 (m, 1H, H-3); fabms *m/z* (rel. int.) 552 (3), 523 (2), 247 (24), 185 (69), 93 (100).

3′,4′-Didemethoxy-3′,4′-dioxo-4β-(3″-fluoroanilino)-4-deoxypodophyllotoxin [**13**].—Yield 92%; crystals from toluene; mp 203–204°; ir (KBr) 3360, 2900, 1760, 1650, 1605, 1550 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 7.17 (dt, J = 6.7, 8.0 Hz, 1H, H-5″), 6.73 (s, 1H, H-5), 6.32 (m, 3H, H-4″, -6′, -8), 6.31 (dd, J = 8.1, 1.8 Hz, 1H, H-6″), 6.24 (dt, J = 11.8, 1.8 Hz, 1H, H-2″), 6.01 and 5.99 (ABq, J = 1.0 Hz, 2H, OCH_2O), 5.28 (s, 1H, H-2′), 4.61 (br, 1H, H-4), 4.57 (t, J = 8.2 Hz, 1H, H-11), 4.28 (d, J = 5.6, 1H, H-1), 4.09 (dd, J = 10.6, 9.0 Hz, 1H, H-11), 3.90 (br, 1H, exchangeable, NH), 3.85 (s, 3H, 5′-OMe),

3.31 (dd, J = 14.0, 5.6 Hz, 1H, H-2), 3.00 (m, 1H, H-3). *Anal.* calcd for $C_{26}H_{20}NO_7F \cdot 1.5\ H_2O$, C 61.89, H 4.60, N 2.78; found C 61.58, H 4.21, N 2.64.

3′,4′-Didemethoxy-3′,4′-dioxo-4β-(2″-fluoroanilino)-4-deoxypodophyllotoxin [**14**].—Yield 94%; mp 163–169°; ir (KBr) 3380, 2900, 1755, 1650, 1610, 1550, 1495 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.05 (m, 2H, H-4″, -5″), 6.76 (m, 1H, H-3″), 6.72 (s, 1H, H-5), 6.58 (t, J = 7.8 Hz, 1H, H-6″), 6.54 (s, 2H, H-8, -6′), 6.01 and 5.99 (ABq, J = 1.2 Hz, 2H, OCH_2O), 5.29 (s, 1H, H-2′), 4.64 (br, 1H, H-4), 4.56 (dd, J = 8.6, 7.5 Hz, 1H, H-11), 4.30 (d, J = 5.5 Hz, 1H, H-1), 4.10 (dd, J = 10.7, 8.6 Hz, 1H, H-11), 4.02 (br, 1H, exchangeable, NH), 3.85 (s, 3H, 5′-OMe), 3.37 (dd, J = 14.1, 5.6 Hz, 1H, H-2), 3.02 (m, 1H, H-3). *Anal.* calcd for $C_{26}H_{20}NO_7F \cdot H_2O$, C 63.02, H 4.48, N 2.83; found C 63.36, H 4.40, N 2.70.

3′,4′-Didemethoxy-3′,4′-dioxo-4β-(3″,5″-difluoroanilino)-4-deoxypodophyllotoxin [**15**].—Yield 80%; crystals from toluene; mp 204–208°; ir (KBr) 3360, 2900, 1760, 1650, 1615, 1500 cm^{-1}; ^1H nmr ($CDCl_3$) δ 6.72 (s, 1H, H-5), 6.53 (s, 1H, H-8), 6.52 (d, J = 1.7 Hz, 1H, H-6′), 6.26 (tt, J = 9.0, 2.1 Hz, 1H, H-4″), 6.06 (dd, J = 9.3, 2.0 Hz, 2H, H-2″, -6″), 6.02 and 6.00 (ABq, J = 1.0 Hz, 2H, OCH_2O), 5.26 (d, J = 1.7 Hz, 1H, H-2″), 4.58 (m, 2H, H-4, -11), 4.29 (d, J = 5.5 Hz, 1H, H-1), 4.06 (dd, J = 11.0, 9.0 Hz, 1H, H-11), 4.04 (d, J = 5.7 Hz, 1H, exchangeable, NH), 3.85 (s, 3H, 5′-OMe), 3.27 (dd, J = 14.2, 5.5 Hz, 1H, H-2), 2.99 (m, 1H, H-3); fabms *m/z* (rel. int.) 498 (15), 369 (16), 277 (8), 247 (10), 185 (83), 119 (16), 93 (100).

3′,4′-Didemethoxy-3′,4′-dioxo-4β-(3″-hydroxy-4″-methoxycarbonylanilino)-4-deoxypodophyllotoxin [**16**].—Yield 94%; crystals from toluene; mp 215–218°; ir (KBr) 3360, 2900, 1760, 1645, 1615, 1555 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.67 (d, J = 8.5 Hz, 1H, H-5″), 6.73 (s, 1H, H-5), 6.53 (s, 1H, H-8), 6.52 (d, J = 1.5 Hz, 1H, H-6′), 6.05 (m, 2H, H-2″, -6″), 6.01 and 6.00 (ABq, J = 1.2 Hz, 2H, OCH_2O), 5.28 (d, J = 1.5 Hz, 1H, H-2′), 4.71 (dd, J = 6.5, 5.0 Hz, 1H, H-4), 4.58 (t, J = 8.0 Hz, 1H, H-11), 4.29 (d, J = 5.5 Hz, 1H, H-1), 4.23 (d, J = 6.5 Hz, 1H, exchangeable, NH), 4.05 (dd, J = 9.6, 8.0 Hz, 1H, H-11), 3.91 (s, 3H, CO_2Me), 3.85 (s, 3H, 5′-OMe), 3.26 (dd, J = 14.0, 5.5 Hz, 1H, H-2), 3.01 (m, 1H, H-3). *Anal.* calcd for $C_{28}H_{23}NO_{10} \cdot \frac{1}{2}H_2O$, C 61.97, H 4.47, N 2.58; found C 61.85, H 4.47, N 2.39.

3′,4′-Didemethoxy-3′,4′-dioxo-4β-(3″-methoxycarbonylanilino)-4-deoxypodophyllotoxin [**17**].—Yield 95%; mp 181–182°; ir (KBr) 3360, 2900, 1755, 1695, 1650, 1600, 1555 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.48 (d, J = 7.8 Hz, 1H, H-4″), 7.28 (t, J = 8.0 Hz, 1H, H-5″), 7.22 (d, J = 1.7 Hz, 1H, H-2″), 6.73 (dd, J = 8.1, 2.3 Hz, 1H, H-6″), 6.72 (s, 1H, H-5), 6.54 (s, 2H, H-8, -6′), 6.01 and 5.99 (ABq, J = 1.2 Hz, 2H, OCH_2O), 5.30 (s, 1H, H-2′), 4.69 (br, 1H, H-4), 4.59 (dd, J = 9.2, 8.2 Hz, 1H, H-11), 4.30 (d, J = 5.5 Hz, 1H, H-1), 4.07 (dd, J = 10.5, 9.2, Hz, 1H, H-11), 3.91 (s, 3H, CO_2Me), 3.86 (s, 3H, 5′-OMe), 3.34 (dd, J = 14.2, 5.5 Hz, 1H, H-2), 3.04 (m, 1H, H-3). *Anal.* calcd for $C_{28}H_{23}NO_9 \cdot H_2O$, C 62.79, H 4.71, N 2.62; found C 62.47, H 5.07, N 2.58.

3′,4′-Didemethoxy-3′,4′-dioxo-4β-(2″-ethoxycarbonylanilino)-4-deoxypodophyllotoxin [**18**].—Yield 90%; mp 172–175°; ir (KBr) 3340, 2900, 1760, 1650, 1620, 1600, 1575, 1555, 1495 cm^{-1}; ^1H nmr ($CDCl_3$) δ 8.08 (d, J = 6.9 Hz, 1H, exchangeable, NH), 8.00 (dd, J = 8.0, 1.6 Hz, 1H, H-3″), 7.44 (dt, J = 1.6, 8.0 Hz, 1H, H-5″), 6.74 (t, J = 8.0 Hz, 1H, H-4″), 6.72 (s, 1H, H-5), 6.62 (d, J = 8.0 Hz, 1H, H-6″), 6.54 (d, J = 1.4 Hz, 1H, H-6′), 6.51 (s, 1H, H-8), 5.99 and 5.98 (ABq, J = 1.2 Hz, 2H, OCH_2O), 5.32 (d, J = 1.4 Hz, 1H, H-2′), 4.80 (dd, J = 8.9, 7.5 Hz, 1H, H-4), 4.52 (t, J = 7.7 Hz, 1H, H-11), 4.30 (d, J = 5.7 Hz, 1H, H-1), 4.27 (dq, J = 2.1, 7.2 Hz, 2H, CO_2CH_2Me), 3.98 (dd, J = 10.8, 8.9 Hz, 1H, H-11), 3.86 (s, 3H, 5′-OMe), 3.35 (dd, J = 14.0, 5.7 Hz, 1H, H-2), 3.04 (m, 1H, H-3), 1.59 (t, J = 7.2 Hz, 3H, CO_2CH_2Me). *Anal.* calcd for $C_{29}H_{25}NO_9 \cdot H_2O$, C 63.37, H 4.96, N 2.55; found C 63.50, H 4.91, N 2.47.

3′,4′-Didemethoxy-3′,4′-dioxo-4β-(3″-bromoanilino)-4-deoxypodophyllotoxin [**19**].—Yield 99%; crystals from toluene; mp 198–200°; ir (KBr) 3360, 2900, 1758, 1650, 1585, 1555 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.09 (t, J = 8.0 Hz, 1H, H-5″), 6.94 (d, J = 8.0, 1H, H-4″), 6.72 (s, 1H, H-5), 6.69 (s, 1H, H-2″), 6.53 (s, 2H, H-8, -6′), 6.46 (d, J = 8.0 Hz, 1H, H-6″), 6.02 and 6.00 (ABq, J = 1.0 Hz, 2H, OCH_2O), 5.28 (s, 1H, H-2′), 4.61 (br, 1H, H-4), 4.57 (t, J = 8.5 Hz, 1H, H-11), 4.28 (d, J = 5.5 Hz, 1H, H-1), 4.07 (t, J = 10.0 Hz, 1H, H-11), 3.85 (s, 3H, 5′-OMe), 3.30 (dd, J = 14.0, 5.5 Hz, 1H, H-2), 3.00 (m, 1H, H-3). *Anal.* calcd for $C_{26}H_{20}NO_7Br \cdot H_2O$, C 56.12, H 3.99, N 2.52; found C 56.12, H 4.02, N 2.43.

3′,4′-Didemethoxy-3′,4′-dioxo-4β-[3″,5″-di(trifluoromethyl)anilino]-4-deoxypodophyllotoxin [**20**].—Yield 96%; mp 173–178°; ir (KBr) 3360, 2900, 1760, 1650, 1615, 1550 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.29 (s, 1H, H-4″), 6.92 (s, 2H, H-2″, -6″), 6.73 (s, 1H, H-5), 6.56 (s, 1H, H-8), 6.52 (s, 1H, H-6′), 6.03 and 6.01 (ABq, J = 1.1 Hz, 2H, OCH_2O), 5.28 (s, 1H, H-2′), 4.73 (dd, J = 7.5, 5.5 Hz, 1H, H-4), 4.57 (t, J = 8.5 Hz, 1H, H-11), 4.32 (d, J = 5.7 Hz, 1H, H-1), 4.27 (d, J = 7.5 Hz, 1H, exchangeable, NH), 3.99 (dd, J = 10.5, 8.5 Hz, 1H, H-11), 3.86 (s, 3H, 5′-OMe), 3.30 (dd, J = 14.0, 5.7 Hz, 1H, H-2),

3.10 (m, 1H, H-3). *Anal.* calcd for $C_{28}H_{19}NO_7F_6 \cdot H_2O$, C 54.81, H 3.46, N 2.28; found C 54.61, H 3.67, N 2.12.

3',4'-Didemethoxy-3',4'-dioxo-4β-(4"-fluorophenyl)-4-deoxypodophyllotoxin [**21**].—Yield 90%; mp 133–135°; ir (KBr) 3400, 3100, 2900, 1760, 1650, 1620, 1550 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.05 (m, 5H, H-2", -3", -5", -6", -5), 6.54 (s, 1H, H-8), 6.46 (s, 1H, H-6'), 6.03 (s, 1H, OCH_2O), 6.01 (s, 1H, OCH_2O), 5.53 (s, 1H, H-2'), 5.30 (d, J = 10.0 Hz, 1H, H-4), 4.28 (m, 2H, H-1, -11), 3.91 (t, J = 10.5 Hz, 1H, H-11), 3.83 (s, 3H, 5'-OMe), 3.05 (dd, J = 14.0, 5.5 Hz, 1H, H-2), 2.94 (m, 1H, H-3). *Anal.* calcd for $C_{26}H_{19}FO_8 \cdot H_2O$, C 62.89, H 4.27; found C 62.82, H 4.60.

3',4'-Didemethoxy-3',4'-dioxo-4β-(3"-quinolylamino)-4-deoxypodophyllotoxin [**22**].—Yield 90%; mp 157–160°; ir (KBr) 3360, 2900, 1750, 1650, 1600, 1550 cm^{-1}; ^1H nmr ($CDCl_3$) δ 8.45 (d, J = 3.0 Hz, 1H, H-2"), 7.99 (m, 1H, H-4"), 7.67 (m, 1H, H-7"), 7.51 (m, 2H, H-5", -6"), 7.00 (d, J = 3.0 Hz, 1H, H-8"), 6.73 (s, 1H, H-5), 6.57 (s, 1H, H-8), 6.55 (d, J = 1.0 Hz, 1H, H-6'), 6.03 and 6.01 (ABq, J = 1.1 Hz, 2H, OCH_2O), 5.31 (d, J = 1.0 Hz, 1H, H-2'), 4.76 (dd, J = 7.0, 5.0 Hz, 1H, H-4), 4.63 (t, J = 8.5 Hz, 1H, H-11), 4.34 (d, J = 5.7 Hz, 1H, H-1), 4.15 (d, J = 7.0 Hz, 1H, exchangeable, NH), 4.09 (dd, J = 10.5, 8.5 Hz, 1H, H-11), 3.87 (s, 3H, 5'-OMe), 3.39 (dd, J = 14.0, 5.7 Hz, 1H, H-2), 3.12 (m, 1H, H-3); fabms *m/z* (rel. int.) 513 (14), 247 (12), 185 (57), 93 (100).

SYNTHESIS OF COMPOUNDS **23–28**.—To a solution of an appropriate 3',4'-*O*-didemethyl-4β-arylamino-4-deoxypodophyllotoxin (**4**, 0.1 mmol) in Et_2O (0.5 ml) was added tetrachloro-1,2-benzoquinone (0.15 mmol) in Et_2O (0.5 ml) at room temperature. After stirring for 10 min, the reaction mixture was filtered, and the solid was collected, washed with Et_2O, and dried to provide compounds **25–30** with a yield of 90–100%.

3',4'-Didemethoxy-3',4'-dioxo-4β-anilino-4-deoxypodophyllotoxin [**23**].—Crystals from Et_2O; mp 194–197° (dec); $[\alpha]^{25}D$ −144° (c = 0.05, C_2H_6CO); ir (KBr) 3380, 1760, 1685, 1650, 1615, 1590, 1550, 1490, 1475 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.22 (t, J = 7.6 Hz, 2H, H-3", -5"), 6.80 (t, J = 7.6 Hz, 1H, H-4"), 6.50 (s, 1H, H-6'), 6.72 (s, 1H, H-5), 6.52 (overlap, 2H, H-2", -6"), 6.50 (s, 1H, H-8), 5.98 and 5.96 (s, 2H, OCH_2O), 5.27 (s, 1H, H-2'), 4.61 (d, J = 4.0 Hz, 1H, H-4), 4.26 (d, J = 5.6 Hz, 1H, H-1), 4.54 (dd, J = 8.1, 8.0 Hz, 1H, H-11), 4.09 (dd, J = 10.0 , 8.2 Hz, 1H, H-11), 3.83 (s, 3H, 5'-OMe), 3.33 (dd, J = 14.2, 5.8 Hz, 1H, H-2), 2.99 (m, 1H, H-3); fabms *m/z* (rel. int.) 460 (4), 369 (21), 309 (15), 229 (7), 185 (10), 155 (54), 119 (100).

3',4'-Didemethoxy-3',4'-dioxo-4β-chloroanilino-4-deoxypodophyllotoxin [**24**].—Crystals from Et_2O; mp 194–197° (dec); $[\alpha]^{25}D$ −108° (c = 0.03, C_2H_6CO); ir (KBr) 3370, 1760, 1685, 1650, 1615, 1590, 1550, 1475 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.16 (d, J = 8.6 Hz, 2H, H-3", -5"), 6.68 (s, 1H, H-5), 6.50 (s, 1H, H-8), 6.49 (s, 1H, H-6'), 6.45 (d, J = 8.6 Hz, 2H, H-2", -6"), 5.98 and 5.96 (s, 2H, OCH_2O), 5.25 (s, 1H, H-2'), 4.56 (d, J = 4.3 Hz, 1H, H-4), 4.52 (dd, J = 8.1, 7.8 Hz, 1H, H-11), 4.26 (d, J = 5.6 Hz, 1H, H-1), 4.04 (dd, J = 10.7, 8.6 Hz, 1H, H-11), 3.82 (s, 3H, 5'-OMe), 3.30 (dd, J = 14.0, 5.5 Hz, 1H, H-2), 2.98 (m, 1H, H-3); fabms *m/z* (rel. int.) 495 (13), 494 (5), 369 (52), 309 (30), 275 (8), 229 (10), 185 (12), 155 (68), 119 (100).

3',4'-Didemethoxy-3',4'-dioxo-4β-(4"-cyanoanilino)-4-deoxypodophyllotoxin [**25**].—Crystals from Et_2O; mp 228–230° (dec); $[\alpha]^{25}D$ −150° (c = 0.053, C_2H_6CO); ir (KBr) 2200, 1760, 1685, 1650, 1600, 1550, 1510, 1475 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.50 (d, J = 8.7 Hz, 2H, H-3", -5"), 6.68 (s, 1H, H-5), 6.54 (d, J = 8.7 Hz, 2H, H-2", -6"), 6.52 (s, 1H, H-8), 6.50 (s, 1H, H-6'), 6.00 and 5.98 (s, 2H, OCH_2O), 5.25 (s, 1H, H-2'), 4.68 (dd, J = 5.2, 5.1 Hz, 1H, H-4), 4.53 (dd, J = 8.2, 8.0 Hz, 1H, H-11), 4.28 (d, J = 5.4 Hz, 1H, H-1), 4.25 (overlap, 1H, NH), 3.98 (dd, J = 10.5, 8.8 Hz, 1H, H-11), 3.83 (s, 3H, 5'-OMe), 3.25 (dd, J = 14.4, 5.6 Hz, 1H, H-2), 3.02 (m, 1H, H-3); fabms *m/z* (rel. int.) 391 (7), 309 (36), 275 (10), 155 (76), 119 (100).

3,',4'-Didemethoxy-3',4'-dioxo-4β-(4"-Acetylanilino)-4-deoxypodophyllotoxin [**26**].—Crystals from Et_2O; mp 237–239° (dec); $[\alpha]^{25}D$ −161° (c = 0.067, C_2H_6CO); ir (KBr) 3360, 1760, 1686, 1640, 1580, 1510, 1495, 1475 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.86 (d, J = 8.5 Hz, 2H, H-3", -5"), 6.70 (s, 1H, H-5), 6.53 (d, J = 8.5 Hz, 2H, H-2", -6"), 6.52 (s, 1H, H-8), 6.50 (s, 1H, H-6'), 5.99 and 5.97 (s, 2H, OCH_2O), 5.26 (s, 1H, H-2'), 4.74 (brs, 1H, H-4), 4.54 (dd, J = 8.0, 7.7 Hz, 1H, H-11), 4.28 (d, J = 5.3 Hz, 1H, H-1), 4.27 (overlap, 1H, NH), 4.01 (dd, J = 10.4, 9.0 Hz, 1H, H-11), 3.83 (s, 3H, 5'-OMe), 3.27 (dd, J = 14.3, 5.5 Hz, 1H, H-2), 3.03 (m, 1H, H-3), 2.51 (s, 3H, Ac); fabms *m/z* (rel. int.) 369 (37), 309 (20), 285 (7), 229 (9), 185 (14), 155 (60), 119 (100).

3',4'-Didemethoxy-3',4'-dioxo-4β-(4"-cyanomethyleneanilino)-4-deoxypodophyllotoxin [**27**].—Crystals from Et_2O; mp >300° (dec); $[\alpha]^{25}D$ −191° (c = 0.035, C_2H_6CO); ir (KBr) 3370, 2240, 1760, 1685, 1660, 1605, 1550, 1510, 1470 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.16 (d, J = 8.4 Hz, 2H, H-3", -5"), 6.69 (s, 1H, H-5), 6.52 (d, J = 8.4 Hz, 2H, H-2", -6"), 6.50 (s, 2H, H-8, -6'), 5.99 and 5.97 (s, 2H, OCH_2O), 5.26 (s, 1H, H-2'), 4.60 (d, J = 3.7 Hz, 1H, H-4), 4.53 (dd, J = 8.0, 7.8 Hz, 1H, H-11), 4.26 (d,

J = 5.6 Hz, 1H, H-1), 4.04 (dd, J = 9.0, 10.3 Hz, 1H, H-11), 3.83 (s, 3H, 5′-OMe), 3.64 (s, 2H, CH_2CN-4″), 3.31 (dd, J = 14.3, 5.6 Hz, 1H, H-2), 3.02 (m, 1H, H-3); fabms *m/z* (rel. int.) 499 (1.3), 391 (8), 369 (8), 309 (34), 275 (8), 155 (72), 119 (100).

3′,4′-Didemethoxy-3′,4′-dioxo-4β-(3″-nitroanilino)-4-deoxypodophyllotoxin [**28**].—Crystals from Et_2O; mp 208–210° (dec); $[\alpha]^{25}D$ −178° (c = 0.04, C_2H_6CO); ir (KBr) 3380, 1760, 1680, 1650, 1610, 1550, 1510, 1475 cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.63 (dd, J = 8.2, 1.9 Hz, 1H, H-4″), 7.36 (t, J = 8.2 Hz, 1H, H-5″), 7.36 (s, 1H, H-2″), 6.84 (dd, J = 8.1, 2.4 Hz, 1H, H-6″), 6.69 (s, 1H, H-5), 6.52 (s, 1H, H-8), 6.50 (brs, 1H, H-6′), 6.00 and 5.98 (s, 2H, OCH_2O), 5.26 (s, 1H, H-2′), 4.70 (brs, 1H, H-4), 4.60 (dd, J = 8.1, 7.9 Hz, 1H, H-11), 4.29 (d, J = 5.4 Hz, 1H, H-1), 4.16 (d, J = 5.5 Hz, 1H, NH), 4.01 (dd, J = 10.4, 8.9 Hz, 1H, H-11), 3.83 (s, 3H, 5′-OMe), 3.30 (dd, J = 14.2, 5.6 Hz, 1H, H-2), 3.04 (m, 1H, H-3); fabms *m/z* (rel. int.) 505 (4.2), 369 (33), 309 (26), 275 (8), 229 (8), 185 (10), 155 (69), 119 (100).

BIOLOGICAL ASSAY.—Assays for the inhibition of human DNA topoisomerase II and the cellular protein-linked DNA breaks as well as the cytotoxicity in KB cells were carried out according to the procedures described previously (27).

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