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Antitumor Agents. I. DNA Topoisomerase II Inhibitory Activity and the Structural Relationship of Podophyllotoxin Derivatives as Antitumor Agents

Tadafumi TERADA,*[,a] Katsuhiko FUJIMOTO,[a] Makoto NOMURA,[a] Jun-ichi YAMASHITA,[a] Takashi KOBUNAI,[b] Setsuo TAKEDA,[b] Konstanty WIERZBA,[a] Yuji YAMADA,[a] and Hideo YAMAGUCHI[c]

Hanno Research Center, Taiho Pharmaceutical Co., Ltd.,[a] 216–1 Nakayashita Yaoroshi, Hanno, Saitama 357, Japan, Research Institute, Taiho Pharmaceutical Co., Ltd.,[b] Kawauchi-cho, Tokushima 771–01, Japan, and Osaka University of Pharmaceutical Sciences,[c] Kawai 2–10–65, Matsubara, Osaka 580, Japan. *Received March 9, 1992*

Various podophyllotoxin derivatives from desoxypodophyllotoxin (DPT) were synthesized to examine the structural relationships between the biological significance (cytotoxic effect, effects on DNA topoisomerase II and tubulin polymerization) *in vitro* and antitumor activity *in vivo* (L 1210).

An intact 6,7-methylenedioxy group of DPT is necessary to inhibit tubulin polymerization and topoisomerase II. 4′-Phenolic hydroxyl group of DPT is essential to inhibit DNA topoisomerase II and the inhibitory effect on DNA topoisomerase II contributes to a high cytotoxicity. The introduction of an aminoalkoxy group at 1-position of DPT enhances the inhibitory activity against DNA topoisomerase II and cytotoxic effect, causing the inhibitory activity against tubulin polymerization to disappear.

The results of antitumor test in mice bearing L 1210 on podophyllotoxin derivatives suggest the following: 1) the strong cytotoxic effect itself is not a good indication of antitumor activity *in vivo* as long as it is associated with inhibition of tubulin polymerization. DNA topoisomerase II inhibitory effect contributes to an antitumor activity *in vivo*; 2) detailed measurements of cytotoxicity and inhibition on DNA topoisomerase II and tubulin polymerization *in vitro* are necessary to evaluate podophyllotoxin derivatives.

Keywords antitumor agent; synthesis; structural relationship; *in vivo*; *in vitro*; DNA topoisomerase II; cytotoxicity; tubulin polymerization; podophyllotoxin derivative; desoxypodophyllotoxin

Podophyllotoxin, a natural product isolated from *Podophyllum peltatum* L. and *P. emodi* WALL., has been reported to be a potent inhibitor of mitosis.[1] Therefore, a large number of podophyllotoxin derivatives have been synthesized and examined as antitumor agents.[2]

Although several compounds (Fig. 1) showed considerable antitumor activity and thus had been well evaluated in clinical trials, the results of podophyllinic acid ethyl hydrazid (SP-1) and podophyllotoxin benzyliden-β-D-glucopyranoside (SP-G) had been unsatisfactory because of severe side effects.[3] These compounds are potent inhibitors of microtubule polymerization.[4] In contrast, the analogues etoposide (VP-16) and teniposide (VM-26), both widely used clinically, are potent inhibitors of DNA topoisomerase II and are devoid of microtubule polymerization.[5] These results suggest that DNA topoisomerase II, but not microtubules, is an important biochemical determinant of the biological activity of VP-16 and VM-26. Previous studies on the relationships between the structure of VP-16 congeners and their antitumor activity *in vitro*, on the other hand, have indicated some structural requirements.[6] Further, numerous C-1 substituted analogues of VP-16 have been synthesized and their inhibitory effects on DNA topoisomerase II and antitumor activities *in vitro* have been investigated.[7]

There is no report, however, systematically discussing the relationships between chemical structure, *in vitro* cytotoxicity, and inhibition of tubulin polymerization and DNA topoisomerase II, as predictive factors of *in vivo* antitumor activity of podophyllotoxin derivatives. Therefore, desoxypodophyllotoxin (DPT)[8] (Chart 1) was used as a starting material and subsequently modified on the 6,7-methylenedioxy moiety, 3′ or 4′-methoxy group, and C-1 position. The *in vitro* antitumor activities of these

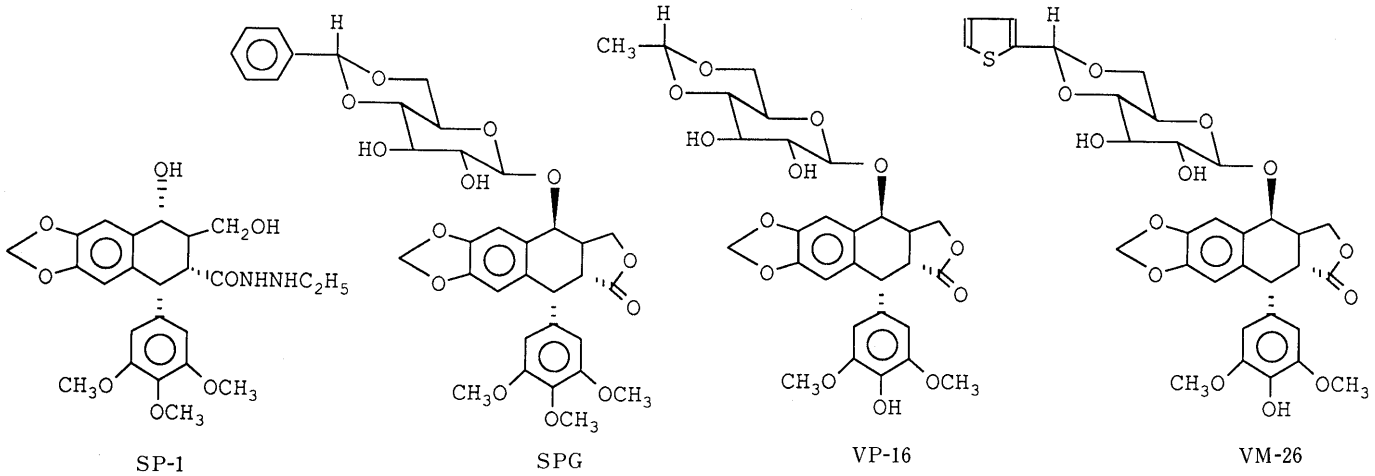
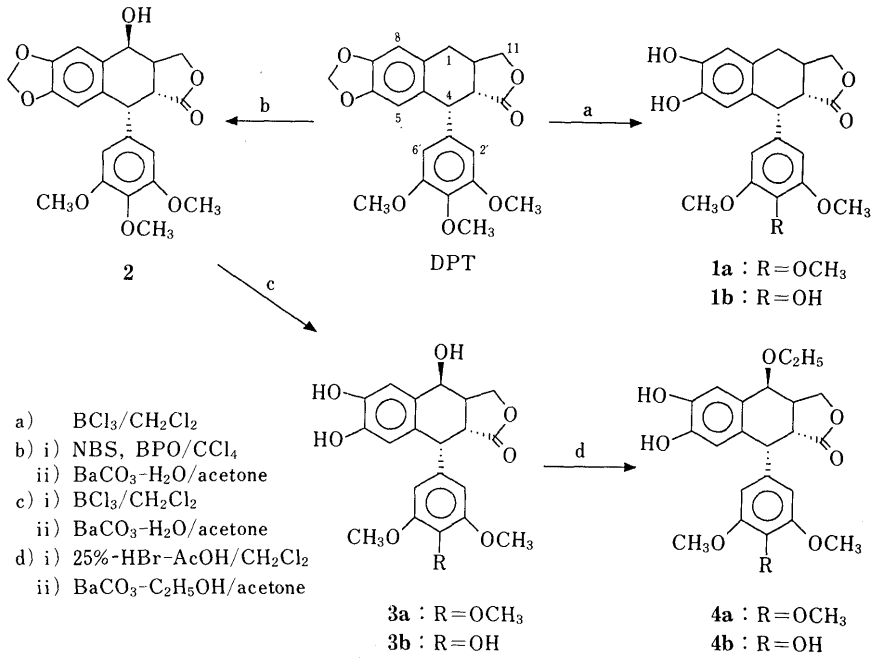


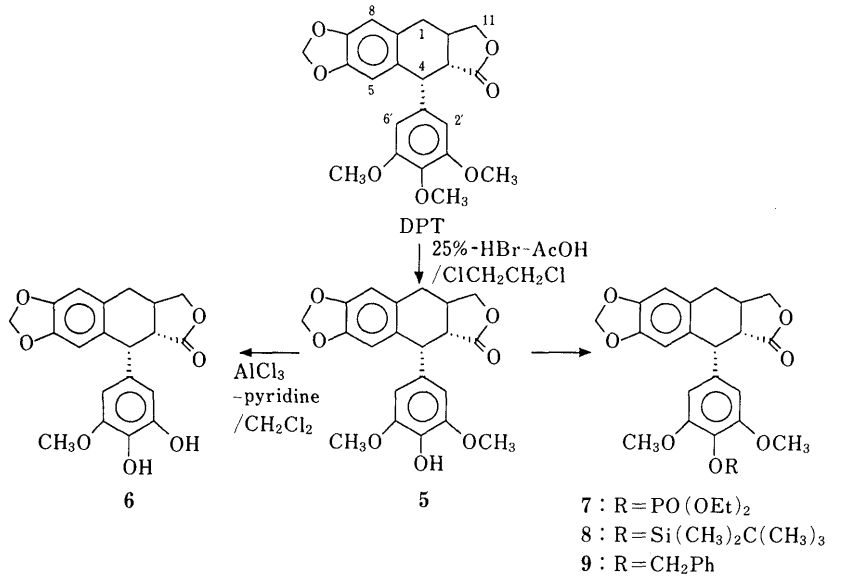
Fig. 1



2 DPT **1a** : R=OCH_3 **1b** : R=OH

3a : R=OCH_3 **3b** : R=OH **4a** : R=OCH_3 **4b** : R=OH

Chart 1



DPT **6** **5** **7** : R=$PO(OEt)_2$ **8** : R=$Si(CH_3)_2C(CH_3)_3$ **9** : R=CH_2Ph

Chart 2

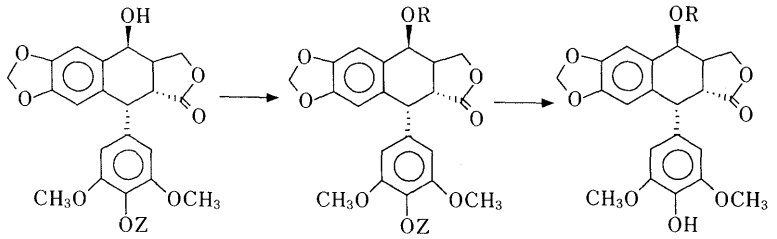
derivatives against sarcoma 180 and P 388 murine leukemia were examined and their inhibitory effects on mammalian DNA topoisomerase II and tubulin polymerization were determined. Several compounds showing significant biological activities *in vitro* were selected and their *in vivo* antitumor activity against L 1210 leukemia was evaluated.

This paper describes the syntheses of desoxypodophyllotoxin derivatives and the relationships between structure and biological activities *in vitro* and *in vivo*.

Chemistry

Syntheses of 6,7-catechol derivatives of DPT are shown in Chart 1. Treatment of DPT with boron trichloride at −78 or −20 °C gave the catechol **1a** or its 4′-demethyl compound **1b**, respectively.[9] Epipodophyllotoxin (EPT) (**2**) was prepared by bromination of DPT with *N*-bromosuccinimide, followed by hydrolysis with aqueous barium carbonate in acetone.[10] Cleavage of the methylenedioxy group of **2** under conditions similar to those of DPT gave the catechols **3a** and **3b**. The 1-ethoxy compounds **4a** and **4b** were prepared by bromination of 1-hydroxyl groups of **3a** and **3b** with 25% HBr–AcOH, and subsequently by ethanolysis with a mixture of barium carbonate and ethanol in acetone.

As shown in Chart 2, the 4′-methoxyl group of DPT was more easily cleaved than the other two methoxyl groups, affording 4′-demethylated DPT (**5**). Further demethylation of **5** with aluminum chloride in pyridine was necessary to obtain the 3′,4′-dihydroxy compound **6**. Three types of modification, diethylphosphorylation, *tert*-butyldimethylsilylation and benzylation were carried out on the 4′-hydroxyl group of **5** using appropriate alkyl halides and



10 11 12–15

Z=$COOCH_2Ph$

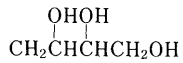
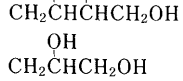
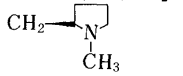
	R		
12a	$CH_2CH_2CH_2OH$		
12b	CH_2CH_3		
12c	$CH_2CH_2PO(OEt)_2$		
13a		16a	
13b		16b	
14a	$CH_2CH_2SCH_3$	17a	$CH_2CH_2\overset{+}{S}(CH_3)_2{}^-OTs$
14b	$CH_2CH_2CH_2SCH_3$	17b	$CH_2CH_2CH_2\overset{+}{S}(CH_3)_2{}^-OTs$
15a	$CH_2CH(OH)CH_2NH_2$	18a	$CH_2CH(OH)CH_2N(CH_3)_2$
15b		18b	

Chart 3

bases.

The 4′-*O*-benzyloxycarbonyl compound (**10**), derived from **2**,[11] was treated with alcohols in the presence of boron trifluoride etherate (BF_3–Et_2O) to give 1-alkoxy compounds (**11**) (Chart 3). 1-*O*-Alkyl-4′-demethylated derivatives of **2** (**12a—15b**) were obtained by hydrogenolysis of **11** over palladium on carbon in a mixture of methanol and ethyl acetate. The acetonide moieties of **13a** and **13b** were cleaved in dichloromethane–methanol in the presence of *p*-toluenesulfonic acid to give the trihydroxyalkyl and dihydroxyalkyl compounds **16a** and **16b**. Compounds **14a** and **14b**, possessing a methylthio group in their alkyl chain, were converted to sulfonium salts **17a** and **17b** by treatment with methyl *p*-toluenesulfonate in tetrahydrofuran. The *N*-methylated compounds **15a** and **15b** were obtained by hydrogenolysis of **18a** and **18b** over palladium on carbon in a mixture of methanol and ethyl acetate, followed by reductive amination with formaldehyde using sodium cyanoborohydride in acetic acid–methanol.

Biochemical Results and Discussion

DPT and 6,7-demethylene derivatives of DPT were evaluated for their ability to inhibit tubulin polymerization and DNA topoisomerase II, and for cytotoxicity, *in vitro*. The data are shown in Table I. DPT showed a high activity on tubulin polymerization and slightly lower inhibitory effect on DNA topoisomerase II, than that of VP-16. The IC_{50} values for DPT were 0.6 and 186.5 μM for the inhibition of tubulin polymerization and topoisomerase II, respectively. 6,7-Demethylene compounds (**1a** and **1b**) of DPT showed neither inhibition of tubulin polymerization at 15 and 30 μM, respectively, nor inhibition of DNA topoisomerase II. Although EPT (**2**) showed only an inhibitory effect on tubulin polymerization, its 6,7-demethylene compound (**3a**) was inactive. These results suggest that the presence of 6,7-methylenedioxy group is necessary for podophyllotoxin derivatives to inhibit tubulin polymerization and topoisomerase II.

TABLE I. Biological Evaluation of DPT Analogues Shown in Chart 1

Compound No.	Cytotoxicity[a] P-388 IC_{50} (M)	Cytotoxicity[a] S-180 IC_{50} (M)	Inhibition of tubulin[a] polymerization IC_{50} (μM)	Inhibition of Topo II[a] IC_{50} (μM)
DPT	1×10^{-8}	1×10^{-7}	0.6	186.5
1a	3×10^{-7}	5×10^{-7}	>15 (no effect)	>500
1b	3×10^{-6}	3×10^{-6}	>30 (no effect)	>500
EPT (2)	3×10^{-8}	3×10^{-7}	8.0	>250
3a	$>1\times10^{-5}$	$>1\times10^{-5}$	>100 (no effect)	>500
4a	$>1\times10^{-5}$	$>1\times10^{-5}$	>60 (no effect)	>125
4b	$>1\times10^{-5}$	$>1\times10^{-5}$	>90 (no effect)	>250
VP-16	1×10^{-8}	1×10^{-7}	>100 (no effect)	59.2

a) See Experimental section.

Next, the 3′ or 4′-position of DPT was modified and the compounds were evaluated; the results are shown in Table II. The 4′-demethyl compound (**5**) showed a stronger inhibitory effect on DNA topoisomerase II than did DPT; its IC_{50} value was 24.5 μM. Although **5** had a slightly lower IC_{50} value on tubulin polymerization than DPT, its cytotoxic effect was stronger. 3′,4′-Dihydroxy compound (**6**) showed no inhibitory effect on DNA topoisomerase II at 62.5 μM and lower cytotoxicity than that of **5**. Analogous compounds (**7** and **8**) derived from **5** did not exhibit an inhibitory effect to DNA topoisomerase II at 62.5 and 31.3 μM, respectively; however, the inhibitory effect of **9** on tubulin polymerization was equal to that of DPT. The results indicate that a 4′-phenolic hydroxyl group is essential for DPT derivatives to inhibit DNA topoisomerase II, and that the inhibitory effect on DNA

topoisomerase II contributes to a high cytotoxicity.

Based on the above results and the specific activity of VP-16 on DNA topoisomerase II, various 1-alkoxy-4′-demethylated compounds of DPT were synthesized. These compounds were expected to show specificity for DNA topoisomerase II because of their structural similarity; the biological results are shown in Table III. All the compounds except **17b** showed a reduction or absence of inhibitory effect on tubulin polymerization. As expected, all except **12b** and **12c** inhibited DNA topoisomerase II. The IC_{50} values of **12a**, **16a**, **17a** and **17b** were higher than that of VP-16. Though introduction of glycerol group at 1-position of DPT (**16b**) is better for DNA topoisomerase II inhibitory activity than that of other hydroxyalkoxy groups (**12a** and **16a**) or alkoxy group (**12b**), its cytotoxicity is weaker than that of VP-16. Compounds with IC_{50} values of 60 μM or higher against DNA topoisomerase II showed unacceptable cytotoxicity. In particular, the sulfonium compound **17a** showed low cytotoxicity despite its inhibition of both tubulin polymerization and DNA topoisomerase II. In contrast, the introduction of an aminoalkoxy group at the 1-position raised the activity on DNA topoisomerase II and increased the cytotoxicity of the compounds. Two compounds with chains containing a dimethylamino group (**18a**), which replaced the hydroxy group at the end of the glycerol group of (**16b**) by amino group, or a methylpyrrolidino group (**18b**) showed the highest activities on DNA topoisomerase II (IC_{50}: 27.6 and 22.9 μM, respectively). With regard to cytotoxicity, these two compounds had IC_{50} values equal to or lower than that of DPT and VP-16. These results suggest that the strong inhibitory effect against DNA topoisomerase II contributes to a high cytotoxicity.

Representative compounds showing significant activity on tubulin polymerization, topoisomerase II and cytotoxicity were obtained and divided into three groups according to their activity. The first group consisting of **5**, **9** and DPT showed a high inhibitory effect on tubulin polymerization. It should be noted that among the studied compounds, **5** was not only a strong inhibitor of tubulin polymerization but also the strongest inhibitor of DNA topoisomerase II. Although compounds **1a** and **7**, the second group, showed neither activity on tubulin polymerization nor on DNA topoisomerase II, these compounds did exhibit cytotoxic effects. The third group of **16b**, **18a**, **18b** and VP-16 possessed a high activity only on DNA topoisomerase II. All compounds were tested *in vivo* for antitumor activity against L 1210 leukemia (Table IV). Compounds **5** and DPT showed a weak antitumor activity *in vivo* according to the life span, and compound **9** was inactive. The antitumor activity of **5** was surprisingly low considering its strong inhibitory effect on DNA topoisomerase II. Compounds **1a** and **7** in the second group showed no significant antitumor activity. In contrast, all the compounds in the third group showed high antitumor activity. These results suggest the following for podophyllotoxin

TABLE II. Biological Evaluation of DPT Analogues Shown in Chart 2

Compound No.	Cytotoxicity[a] P-388 IC_{50} (M)	Cytotoxicity[a] S-180 IC_{50} (M)	Inhibition of tubulin[a] polymerization IC_{50} (μM)	Inhibition of Topo II[a] IC_{50} (μM)
DPT	1×10^{-8}	1×10^{-7}	0.6	186.5
5	3×10^{-9}	3×10^{-8}	2.0	24.5
6	1×10^{-7}	3×10^{-7}	4.0	>62.5
7	4×10^{-7}	2×10^{-6}	>100 (no effect)	>62.5
8	3×10^{-7}	4×10^{-7}	—	>31.3
9	2×10^{-8}	2×10^{-8}	0.6	>62.5
VP-16	1×10^{-8}	1×10^{-7}	>100 (no effect)	59.2

a) See Experimental section.

TABLE III. Biological Evaluation of DPT Analogues Shown in Chart 3

Compound No.	Cytotoxicity[a] P-388 IC_{50} (M)	Cytotoxicity[a] S-180 IC_{50} (M)	Inhibition of tubulin[a] polymerization IC_{50} (μM)	Inhibition of Topo II[a] IC_{50} (μM)
12a	6×10^{-7}	4×10^{-6}	>100	211 (3.56)[b]
12b	3×10^{-6}	3×10^{-6}	>30	>500 (>8.44)
12c	5×10^{-6}	$>1\times10^{-5}$	>100	>125 (>2.11)
16a	3×10^{-6}	5×10^{-6}	>99 (33% at 99 μM)	102.5 (1.73)
16b	6×10^{-8}	2×10^{-6}	>60	57.9 (0.97)
17a	$>1\times10^{-5}$	$>1\times10^{-5}$	>100	147 (2.48)
17b	9×10^{-6}	$>1\times10^{-5}$	55.6	74.7 (1.26)
18a	2×10^{-8}	5×10^{-8}	>100	27.6 (0.46)
18b	6×10^{-9}	4×10^{-8}	>100	22.9 (0.38)
DPT	1×10^{-8}	1×10^{-7}	0.6	186.5 (3.15)
VP-16	1×10^{-8}	1×10^{-7}	>100	59.2 (1.00)

a) See Experimental section. *b*) Value in parentheses is the ratio of IC_{50} of individual compound/IC_{50} of VP-16.

TABLE IV. Dependence of *in Vivo* Antitumor Activity of DPT Analogues on Their Ability to Inhibit DNA Topoisomerase II and Microtubulin Polymerization

Compound	Cytotoxicity[a] S 180 cells IC_{50} (nM)	Microtubulin[a] polymerization inhibition IC_{50} (μM)	Topoisomerase II[a] inhibition IC_{50} (μM)	Antitumor effect[b] *in vivo* L 1210 ILS (%)	Comments
DPT	3	0.6	186.5	12	
5	30	2.0	24.5	20	Tubulin-strong
9	20	0.6	>62.5	−1	
1a	50	>15 (no effect)	>500	−1	Topo II-none
7	2000	>100 (no effect)	>62.5	−1	Tubulin-none
VP-16	100	>50	59.2	106	
16b	2000	>60	57.9	52	Topo II-strong
18a	50	>100	27.6	70	Tubulin-none
18b	40	>100	22.9	71	

a) See Experimental section. *b*) 1×10^5 L 1210 cells were inoculated i.p. to male CDF mice (5 weeks old) on day 0, and each compound was administered i.p. on day 1 to 5. The percentage increase of life span (ILS) was max., which was calculated from the mean survival period of the treated group compared with that of the control group.

derivatives: 1) the strong cytotoxic effect itself is not a good indication of antitumor activity *in vivo* as long as it is associated with inhibition of tubulin polymerization. DNA topoisomerase II inhibitory effect contributes to an antitumor activity *in vivo*. 2) detailed measurements of cytotoxicity and inhibitory effect on DNA topoisomerase II and tubulin polymerization are necessary to evaluate podophyllotoxin derivatives.

Experimental

All melting points were determined on a Yanagimoto MP-3 micro melting point apparatus and are uncorrected. The instruments used in this study were as follows: elemental analyses, Yanagimoto MT-3, IR spectra, Hitachi I-3000 IR spectrometer; specific rotation, Horiba SEPA-200 spectropolarimeter; FAB-MS spectra, JMS-DX303; and ^{1}H-NMR spectra, FT NMR JNM-FX90Q spectrometer. Chemical shifts were reported in ppm (δ) downfield from tetramethylsilane as internal standard; coupling constants are given in Hz. Column chromatography was carried out on Merck silica gel (Kieselgel 60, 70—230 mesh). Preparative thin layer chromatography (PTLC) was carried out on precoated silica gel plates (Merck Kieselgel $60F_{254}$, 0.5 mm thickness).

6,7-Demethylene-DPT (1a) A solution of DPT (1.0 g, 2.5 mmol) in dry CH_2Cl_2 (15 ml) was added to a solution of BCl_3 (1.17 g, 10 mmol) in dry CH_2Cl_2 (4 ml) at −78 °C with stirring. After 0.5 h, the reaction mixture was added to a mixture of saturated $NaHCO_3$ solution (10 ml) and ice (20 g) under vigorous stirring. After 30 min, the whole was acidified with dil. HCl and extracted with AcOEt. The extract was washed with water, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was recrystallized from EtOH to give 760 mg of **1a** (78.8% yield), mp 238—240 °C, $[\alpha]_D^{24}$ −116° (c=0.5, EtOH). *Anal.* Calcd for $C_{21}H_{22}O_7$: C, 65.27; H, 5.74. Found: C, 65.43; H, 5.86. IR (KBr): 3420, 3340 (OH), 1770 (C=O) cm^{-1}. FAB-MS m/z: 387 (M^++H). ^{1}H-NMR (DMSO-d_6) δ: 8.60 (2H, br, OH), 6.53 (1H, s, 8-H), 6.30 (1H, s, 5-H), 6.25 (2H, s, 2′-, 6′-H), 4.35 (1H, m, 4-H), 3.80—4.60 (2H, m, 11α,β-H), 3.60 (9H, s, 3′-, 4′-, 5′-OCH_3), 2.65—3.14 (4H, m, 1-, 2-, 3-H).

4′-Demethyl-6,7-demethylene-1-DPT (1b) **1b** was synthesized from DPT in a similar manner to that described for **1a**, except that the reaction was carried out at −20°C. Recrystallization from petro ether gave 600 mg of **1b** (71% yield), mp 244—245 °C, $[\alpha]_D^{20}$ −73.77° (c=0.61, DMF). *Anal.* Calcd for $C_{20}H_{20}O_7$: C, 64.51; H, 5.41. Found: C, 64.47; H, 5.51. IR (KBr): 3340 (OH), 1765 (CO) cm^{-1}. FAB-MS m/z: 373 (M^++H). ^{1}H-NMR (DMSO-d_6) δ: 8.77 (1H, s, 6- or 7-OH), 8.72 (1H, s, 6- or 7-OH), 8.21 (1H, s, 4′-OH), 6.57 (1H, s, 8-H), 6.33 (1H, s, 5-H), 6.24 (2H, s, 2′-, 6′-H), 4.20—4.44 (2H, m, 4-, 11β-H), 3.68—4.04 (1H, m, 11α-H), 3.60 (6H, s, 3′-, 5′-OCH_3), 2.60—3.08 (4H, m, 1-, 2-, 3-H).

1-Epipodophyllotoxin (EPT) (2) A mixture of DPT (2 g, 5 mmol), *N*-bromosuccinimide (NBS) (894 mg, 5 mmol) and benzoic peroxide (200 mg, 1.44 mmol) in CCl_4 (130 ml) was refluxed for 2 h under dry N_2. After cooling, the reaction mixture was poured into water and extracted with $CHCl_3$. The extract was washed with brine and dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo*. The mixture of $BaCO_3$ (2 g), H_2O (10 ml) and acetone (20 ml) was added to the residue and the mixture was refluxed for 2 h with stirring. After cooling, the whole was acidified with dil. HCl and extracted with AcOEt. The extract was washed with water, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography with $CHCl_3$–MeOH (30:1) and recrystallized from Et_2O–petro ether to give **2** (765 mg, 36.8% yield), mp 169—170 °C, $[\alpha]_D^{20}$ −89.79° (c=0.84, DMSO). *Anal.* Calcd for $C_{22}H_{22}O_8$: C, 63.76; H, 5.35. Found: C, 63.42; H, 5.51. IR (KBr): 3340 (OH), 1770 (CO) cm^{-1}. FAB-MS m/z: 415 (M^++H). ^{1}H-NMR (DMSO-d_6) δ: 6.94 (1H, s, 8-H), 6.50 (1H, s, 5-H), 6.25 (2H, s, 2′-, 6′-H), 5.99 (2H, s, OCH_2O), 5.45 (1H, d, J=5.73, OH), 4.60—4.80 (1H, br, 1-H), 4.53 (1H, d, J=5.27, 4-H), 4.00—4.40 (2H, m, 11α,β-H), 3.63 (6H, s, 3′-, 5′-OCH_3), 3.61 (3H, s, 4′-OCH_3), 3.29 (1H, dd, J=9.67, 4.61, H-3), 2.60—3.00 (1H, m, 2-H).

6,7-Demethylene-1-epipodophyllotoxin (3a) A solution of EPT (**2**) (500 mg, 1.2 mmol) in dry CH_2Cl_2 (25 ml) was added to a solution of BCl_3 (0.58 g) in dry CH_2Cl_2 (2 ml) at −78 °C with stirring. After 0.5 h, the reaction mixture was added to a mixture of saturated $NaHCO_3$ solution (10 ml) and ice (20 g) under vigorous stirring. After 30 min, the mixture was extracted with AcOEt. The extract was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo* below 30 °C. A mixture of acetone (15 ml), water (10 ml) and $BaCO_3$ (800 mg) was added to the residue and the mixture was refluxed for 2 h with stirring. After cooling, the whole was acidified with dil. HCl and extracted with AcOEt. The extract was washed with water, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography with AcOEt–hexane (2:1) and recrystallized from Et_2O to give **3a** (155 mg, 32.1% yield), mp 225—227 °C, $[\alpha]_D^{20}$ −127.92° (c=0.50, DMSO). *Anal.* Calcd for $C_{21}H_{22}O_8$: C, 62.68; H, 5.51. Found: C, 62.47; H, 5.51. IR (KBr): 3488, 3328 (OH), 1742 (CO) cm^{-1}. FAB-MS m/z: 403 (M^++H). ^{1}H-NMR (DMSO-d_6) δ: 8.88 (2H, br, 6-, 7-OH), 6.79 (1H, s, 8-H), 6.33 (1H, s, 5-H), 6.25 (2H, s, 2′-, 6′-H), 5.24 (1H, d, J=3.6, 1-OH), 4.72—4.80 (1H, br, 1-H), 4.40 (1H, d, J=5.2, 4-H), 4.00—4.40 (2H, m, 11α,β-H), 3.61 (6H, s, 3′-, 5′-OCH_3), 3.60 (3H, s, 4′-OCH_3), 3.26 (1H, dd, J=14.0, 4.0, 3-H), 2.60—3.00 (1H, m, 2-H).

4′-Demethyl-6,7-demethylene-EPT (3b) **3b** was synthesized from **2** in a similar manner to that described for **3a**, except that the reaction was carried out at −20°C. Recrystallization from petro ether gave 300 mg of **3b** (64.4% yield), mp 176—178 °C, $[\alpha]_D^{20}$ −127.92° (c=0.50, DMSO). *Anal.* Calcd for $C_{20}H_{20}O_8$: C, 61.85; H, 5.19. Found: C, 61.67; H, 5.31. IR (KBr): 3340 (OH), 1765 (CO) cm^{-1}. FAB-MS m/z: 389 (M^++H). ^{1}H-NMR (DMSO-d_6) δ: 8.87 (2H, s, 6-, 7-OH), 8.20 (1H, s, 4′-OH), 6.78 (1H, s, 8-H), 6.33 (1H, s, 5-H), 6.20 (2H, s, 2′-, 6′-H), 5.22 (1H, s, 1-OH), 3.96—4.24 (3H, m, 4-, 11α,β-H), 3.60 (6H, s, 3′-, 5′-OCH_3), 3.00—3.20 (1H, m, 3-H), 2.60—3.08 (1H, m, 2-H).

6,7-Demethylene-1-*O*-ethyl-1-epipodophyllotoxin (4a) A solution of 25% HBr–AcOH (2 ml) was added to a stirred solution of **3a** (494 mg, 1.2 mmol) in CH_2Cl_2 (20 ml) at room temperature and the mixture was stirred for 14 h. After concentration *in vacuo*, a mixture of $BaCO_3$ (2 g), C_2H_5OH (5 ml) and acetone (20 ml) was added, and the mixture was refluxed for 2 h with stirring. After cooling, the whole was acidified with dil. HCl and extracted with AcOEt. The extract was washed with water, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography with $CHCl_3$–CH_3COCH_3 (5:1). Recrystallization from MeOH gave **4a** (300 mg, 58.0% yield), mp 209—211 °C, $[\alpha]_D^{20}$ −82.96° (c=0.40, DMF). *Anal.* Calcd for $C_{23}H_{26}O_8$: C, 64.17; H, 6.09. Found: C, 64.11; H, 6.02. IR (KBr): 3360 (OH), 1774 (C=O) cm^{-1}. FAB-MS m/z: 431 (M^++H). ^{1}H-NMR (DMSO-d_6) δ: 9.00 (2H, br, 6-, 7-OH), 6.78 (1H, s, 8-H), 6.35 (1H, s, 5-H), 6.21 (2H, s, 2′-, 6′-H), 4.41 (2H, m, 1-, 4-H), 3.80—4.32 (2H, m, 11α,β-H), 3.61 (9H, s, 3′-, 4′-, 5′-OCH_3), 3.00—3.40 (1H, m, 2-H), 2.60—3.00 (1H, m, 3-H), 1.12 (3H, t, J=7.0, CH_2CH_3).

6,7-Demethylene-4′-demethyl-1-*O*-ethyl-1-epipodophyllotoxin (4b) **4b** was synthesized from **3b** in a similar manner to that described for **4a**. Recrystallization from acetone–hexane gave 110 mg of **4b** (51.3% yield), mp 217—219 °C, $[\alpha]_D^{20}$ −93.19° (c=0.48, DMF). *Anal.* Calcd for $C_{22}H_{24}O_8$: C, 63.45; H, 5.81. Found: C, 63.34; H, 5.76. IR (KBr): 3440 (OH), 1774 (C=O) cm^{-1}. FAB-MS m/z: 417 (M^++H). ^{1}H-NMR (DMSO-d_6) δ: 8.87 (1H, br, 6- or 7-OH), 8.20 (1H, br, 6- or 7-OH), 6.77 (1H, s, 8-H), 6.35 (1H, s, 5-H), 6.17 (2H, s, 2′-, 6′-H), 4.41 (3H, m, 1-, 4-, 11β-H), 3.92—4.20 (1H, m, 11α-H), 3.59 (6H, s, 3′-, 5′-OCH_3), 3.20—3.80 (2H, q, J=7.0, OCH_2CH_3), 3.00—3.20 (1H, m, 3-H), 2.60—3.00 (1H, m, 2-H), 1.12 (3H, t, J=7.0, OCH_2CH_3).

4′-Demethyl-1-DPT (5) A mixture of DPT (1 g, 2.51 mmol) and 25% HBr–AcOH (4 ml) in $ClCH_2CH_2Cl$ (50 ml) was stirred for 8 h at room temperature. The reaction mixture was added to a mixture of saturated $NaHCO_3$ solution (30 ml) and ice (50 g), then extracted with AcOEt. The extract was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo* below 30 °C. The residue was purified by silica gel column chromatography with $CHCl_3$–MeOH (30:1). Recrystallization from THF–MeOH gave 819 mg of **5** (84.9% yield), mp 244—245 °C, $[\alpha]_D^{20}$ −120.60° (c=0.35, DMSO). *Anal.* Calcd for $C_{21}H_{20}O_7$: C, 65.61; H, 5.24. Found: C, 65.65; H, 5.27. IR (KBr): 3396 (OH), 1764 (CO) cm^{-1}. FAB-MS m/z: 385 (M^++H). ^{1}H-NMR (DMSO-d_6) δ: 8.24 (1H, s, OH), 6.74 (1H, s, 8-H), 6.49 (1H, s, 5-H), 6.24 (2H, s, 2′-, 6′-H), 5.96 (1H, d, J=1.3, OCH_2O), 5.94 (1H, d, J=1.3, OCH_2O), 4.45 (1H, d, J=4.62, 4-H), 4.20—4.48 (1H, m, 11β-H), 3.72—4.04 (1H, m, 11α-H), 3.61 (6H, s, 3′-, 5′-OCH_3), 2.52—3.20 (4H, m, 1-, 2-, 3-H).

3′,4′-Didemethyl-1-DPT (6) Pyridine (2 ml) was added dropwise to a mixture of **5** (440 mg, 1.15 mmol) and $AlCl_3$ (540 mg, 4.04 mmol) in CH_2Cl_2. The mixture was refluxed for 18 h at 46 °C with stirring. The reaction mixture was added to 5% HCl (50 ml) and extracted with AcOEt. The extract was washed with water, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography with $CHCl_3$–MeOH (50:1). Recrystallization from benzene gave 248 mg of **6** (58.2% yield), mp 228—229 °C, $[\alpha]_D^{20}$ −123.75° (c=0.50, DMF). *Anal.* Calcd for $C_{20}H_{18}O_7$: C, 64.86; H, 4.90.

Found: C, 64.65; H, 4.90. IR (KBr): 3500 (OH), 1758 (CO) cm^{-1}. FAB-MS: 371 ($M^+ + H$). ^{1}H-NMR (DMSO-d_6) δ: 8.66 (1H, s, 3′- or 4′-OH), 8.09 (1H, s, 3′- or 4′-OH), 6.79 (1H, s, 8-H), 6.49 (1H, s, 5-H), 6.34 (1H, d, J=1.78, 6′-H), 5.95 (2H, s, OCH_2O), 5.88 (1H, d, J=1.98, 2′-H), 4.20—4.48 (2H, m, 4-, 11β-H), 3.76—4.04 (1H, m, 11α-H), 3.65 (3H, s, 5′-OCH_3), 2.32—3.08 (4H, m, 1-, 2-, 3-H).

4′-*O*-(Diethylphosphoryl)-4′-demethyl-1-DPT (7) A mixture of **5** (202 mg, 0.52 mmol) and $(EtO)_2POCl$ (0.4 ml, d=1.194, 2.77 mmol) in pyridine (1.3 ml) was stirred for 8 h at room temperature. The reaction mixture was added to 5% HCl (50 ml) and extracted with benzene. The extract was washed with water, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography with $CHCl_3$. Recrystallization from benzene–hexane gave 120 mg of **7** (43.8% yield), mp 168—171 °C, $[\alpha]_D^{20}$ −50.76° (c=0.45, $CHCl_3$). *Anal.* Calcd for $C_{25}H_{29}O_{10}P$: C, 57.69; H, 5.62. Found: C, 57.49; H, 5.74. IR (KBr): 3470 (OH), 1585 (CO), 1300 (P=O), 1240 (POPh) cm^{-1}. FAB-MS m/z: 521 ($M^+ + H$). ^{1}H-NMR (DMSO-d_6) δ: 6.66 (1H, s, 8-H), 6.49 (1H, s, 5-H), 6.35 (2H, s, 2′-, 6′-H), 5.95 (1H, d, J=1.3, OCH_2O), 5.92 (1H, d, J=1.3, OCH_2O), 4.56 (1H, m, 4-H), 4.28—4.52 (1H, m, 11β-H), 4.32 (2H, q, J=7.2, $-OCH_2CH_3$), 4.31 (2H, q, J=7.2, $-OCH_2CH_3$), 3.80—4.00 (1H, m, 11α-H), 3.73 (6H, s, 3′-, 5′-OCH_3), 2.46—3.20 (4H, m, 1-, 2-, 3-H), 1.37 (3H, t, J=7.2, $-OCH_2CH_3$), 1.36 (3H, t, J=7.2, $-OCH_2CH_3$).

4′-Demethyl-4′-*O*-*tert*-butyldimethylsilyl-1-DPT (8) A mixture of **5** (100 mg, 0.26 mmol), *tert*-butyldimethylsilyl chloride (87 mg, 0.58 mmol) and imidazole (54 mg, 0.79 mmol) in DMF (7 ml) was stirred for 4 d at room temperature. The reaction mixture was added to a mixture of saturated $NaHCO_3$ solution (50 ml) and ice (10 g), and extracted with Et_2O. The extract was washed with water, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography with $CHCl_3$. Recrystallization from $CHCl_3$–hexane gave 100 mg of **8** (77.1% yield), mp 209—210 °C, $[\alpha]_D^{20}$ −87.18° (c=0.523, $CHCl_3$). *Anal.* Calcd for $C_{27}H_{34}O_7Si$: C, 65.16; H, 6.99. Found: C, 65.03; H, 6.87. IR (KBr): 1767 (CO) cm^{-1}. FAB-MS m/z: 499 ($M^+ + H$). ^{1}H-NMR (DMSO-d_6) δ: 6.65 (1H, s, 8-H), 6.52 (1H, s, 5-H), 6.29 (2H, s, 2′-, 6′-H), 5.93 (2H, s, OCH_2O), 4.60 (1H, m, 4-H), 4.28—4.52 (1H, m, 11β-H), 3.72—4.00 (1H, m, 11α-H), 3.67 (6H, s, 3′-, 5′-OCH_3), 2.52—3.20 (4H, m, 1-, 2-, 3-H), 0.98 (9H, s, *-tert*-Bu), 0.10 (6H, s, $-Si(CH_3)_2$).

4′-Demethyl-4′-*O*-benzyl-1-DPT (9) A mixture of **5** (100 mg, 0.26 mmol), benzyl chloride (50 mg, 0.4 mmol) and K_2CO_3 (100 mg, 0.72 mmol) in DMF (5 ml) was stirred for 8 h at room temperature. The reaction mixture was added to 5% HCl (100 ml), and extracted with benzene. The extract was washed with water, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography with CH_2Cl_2. Recrystallization from hexane gave 80 mg of **9** (64.8% yield), mp 88—91 °C, $[\alpha]_D^{20}$ −69.87° (c=0.47, $CHCl_3$). *Anal.* Calcd for $C_{28}H_{26}O_7$: C, 70.87; H, 5.52. Found: C, 70.75; H, 5.63. IR (KBr): 1770 (CO) cm^{-1}. FAB-MS m/z: 475 ($M^+ + H$). ^{1}H-NMR (DMSO-d_6) δ: 7.20—7.48 (5H, m, $PhCH_2$), 6.80 (1H, s, 8-H), 6.52 (1H, s, 5-H), 6.33 (2H, s, 2′-, 6′-H), 5.96 (2H, s, OCH_2O), 4.83 (2H, s, $PhCH_2$), 4.53 (1H, d, J=4.4, 4-H), 4.20—4.44 (1H, m, 11β-H), 3.94 (1H, t, J=9.0, 11α-H), 3.65 (6H, s, 3′-, 5′-OCH_3), 2.52—3.12 (4H, m, 1-, 2-, 3-H).

4′-Demethyl-1-*O*-(3-hydroxypropyl)-1-epipodophyllotoxin (12a) BF_3–Et_2O (0.06 ml, 0.48 mmol) was added at −15 to −10 °C to a stirred mixture of **10** (130 mg, 0.24 mmol) and 1,3-propanediol (105 mg, 1.38 mmol) in CH_2Cl_2 (2 ml). After 1.5 h, the reaction mixture was quenched with pyridine (0.1 ml) and CH_2Cl_2 (40 ml) and extracted with CH_2Cl_2. The extract was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography with benzene–AcOEt (7:3). The main spot was recovered and concentrated *in vacuo*. The residue was reduced for 3 h on 5% Pd–C (50 mg) with H_2 (1 atm) in MeOH–AcOEt (1:1) (10 ml). The reaction mixture was filtered, washed with AcOEt, and the filtrate evaporated under reduced pressure. The residue was purified by silica gel column chromatography with MeOH–$CHCl_3$ (1:50). Recrystallization from Et_2O gave 70 mg of **12a** (63.6% yield), mp 179—181 °C, $[\alpha]_D^{20}$ −88.46° (c=0.26, $CHCl_3$). *Anal.* Calcd for $C_{24}H_{26}O_9$: C, 63.87; H, 5.71. Found: C, 63.72; H, 5.51. IR (KBr): 3516 (OH), 1756 (CO) cm^{-1}. FAB-MS m/z: 459 ($M^+ + H$). ^{1}H-NMR (DMSO-d_6) δ: 6.82 (1H, s, 8-H), 6.54 (1H, s, 5-H), 6.26 (2H, s, 2′-, 6′-H), 5.98 (2H, s, OCH_2O), 5.42 (1H, br, 4′-OH), 4.60 (1H, d, J=5.2, 1-H), 4.44 (1H, d, J=3.3, 4-H), 4.33 (2H, m, 11α,β-H), 3.64—3.91 (4H, m, $-OCH_2CH_2CH_2O$), 3.76 (6H, s, 3′-, 5′-OCH_3), 3.35 (1H, dd, J=5.2, 6.9, 3-H), 2.92 (1H, m, 2-H), 1.85 (2H, m, $-OCH_2CH_2CH_2O$).

4′-Demethyl-1-*O*-ethyl-1-epipodophyllotoxin (12b) **12b** was synthesized from **10** (100 mg, 0.187 mmol) and C_2H_5OH (46 mg, 0.4 mmol) in a similar manner to that described for **12a**. Recrystallization from MeOH gave **12b** (72 mg, 89.9% yield), mp 230—231 °C, $[\alpha]_D^{20}$ −118.21° (c=0.56, DMSO). *Anal.* Calcd for $C_{23}H_{24}O_8$: C, 64.48; H, 5.65. Found: C, 64.34; H, 5.66. IR (KBr): 3370 (OH), 1760 (C=O) cm^{-1}. FAB-MS m/z: 429 ($M^+ + H$). ^{1}H-NMR (acetone-d_6) δ: 7.10 (1H, s, 4′-OH), 6.95 (1H, s, 8-H), 6.54 (1H, s, 5-H), 6.31 (2H, s, 2′-, 6′-H), 5.99 (2H, s, OCH_2O), 4.18—4.30 (4H, m, 1-, 4-, 11α,β-H), 3.67 (6H, s, 3′-, 5′-OCH_3), 3.48—3.88 (2H, q, J=6.8, OCH_2CH_3), 2.92—3.40 (2H, m, 2-, 3-H), 1.18 (3H, t, J=6.8, OCH_2CH_3).

4′-Demethyl-1-*O*-(diethylethylphosphonyl)-1-epipodophyllotoxin (12c) **12c** was synthesized from **10** (100 mg, 0.187 mmol) and diethyl-2-hydroxyethyl phosphonate (74 mg, 0.40 mmol) in a similar manner to that described for **12a**. Recrystallization from Et_2O gave **12c** (82 mg, 77.7% yield), mp 165.5—166.5 °C, $[\alpha]_D^{20}$ −59.00° (c=0.5, $CHCl_3$). *Anal.* Calcd for $C_{27}H_{33}O_{11}P$: C, 57.45; H, 5.89. Found: C, 57.54; H, 5.66. IR (KBr): 3400 (OH), 1760 (C=O), 1228 (P=O), 1036 (P–O) cm^{-1}. FAB-MS m/z: 565 ($M^+ + H$). ^{1}H-NMR (DMSO-d_6) δ: 8.29 (1H, br, 4′-OH), 7.05 (1H, s, 8-H), 6.52 (1H, s, 5-H), 6.17 (2H, s, 2′-, 6′-H), 6.02, 6.00 (each 1H, d, J=1.0, OCH_2O), 4.20—4.64 (4H, m, 1-, 4-, 11α,β-H), 3.92 (2H, dd, J=14.4, 7.0, $-OCH_2CH_2PO$), 3.96 (4H, q, J=7.0, $-POOCH_2CH_3$), 3.61 (6H, s, 3′-, 5′-OCH_3), 3.08—3.44 (1H, m, 3-H), 2.68—3.08 (1H, m, 2-H), 2.08 (2H, dt, J=18.0, 7.2, $-CH_2CH_2PO$), 1.19 (6H, t, J=7.0, $PO(OCH_2CH_3)_2$).

4′-Demethyl-1-*O*-(2,3,4-trihydroxybutyl)-1-epipodophyllotoxin (16a) BF_3–Et_2O (0.24 ml, 1.92 mmol) was added at −15 to −10 °C to a stirred mixture of **10** (400 mg, 0.74 mmol) and 2,3-*O*-isopropylidene-1,4-butanediol (240 mg, 1.48 mmol) in CH_2Cl_2 (6 ml). After 2.0 h, the reaction mixture was quenched with pyridine (0.24 ml) and $CHCl_3$ (100 ml) and extracted with $CHCl_3$. The extract was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by PTLC with $CHCl_3$–acetone (5:1) to give a major product containing **13a**, which was reduced for 1.5 h on 5% Pd–C (50 mg) with H_2 (1 atm) in MeOH–AcOEt (1:1) (10 ml). The reaction mixture was filtered, washed with AcOEt, and concentrated *in vacuo*. The residue was stirred for 4 h at room temperature with *p*-toluenesulfonic acid (100 mg) in CH_2Cl_2–MeOH (1:1) (10 ml). The reaction mixture was added to a mixture saturated with $NaHCO_3$ solution (10 ml) and ice (20 g) under vigorous stirring and extracted with AcOEt. The extract was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo* below 30 °C. The residue was purified by silica gel column chromatography with MeOH–$CHCl_3$ (1:20). Recrystallization from Et_2O gave 200 mg of **16a** (52.9% yield from **10**), mp 153—155 °C, $[\alpha]_D^{20}$ −51.28° (c=0.39, DMF). *Anal.* Calcd for $C_{25}H_{28}O_{11}$: C, 59.52; H, 5.59. Found: C, 59.32; H, 5.72. IR (KBr): 3430 (OH), 1775 (CO) cm^{-1}. FAB-MS m/z: 505 ($M^+ + H$). ^{1}H-NMR (CD_3OD) δ: 6.98 (1H, s, 8-H), 6.51 (1H, s, 5-H), 6.28 (2H, s, 2′-, 6′-H), 5.96, 5.95 (each 1H, d, J=1.0, OCH_2O), 4.60 (1H, d, J=3.5, 1-H), 4.57 (1H, d, J=5.5, 4-H), 4.42 (1H, dd, J=10.5, 8.5, 11β-H), 4.39 (1H, t, J=8.5, 11α-H), 3.70 (6H, s, 3′-, 5′-OCH_3), 3.5—3.8 (6H, m, $-OCH_2CH(OH)CH(OH)CH_2O$), 3.47 (1H, dd, J=14.0, 5.5, 3-H), 2.95 (1H, dddd, J=14.0, 10.5, 8.5, 3.5, 2-H).

4′-Demethyl-1-*O*-(2,3-dihydroxypropyl)-1-epipodophyllotoxin (16b) **16b** was synthesized from **10** (400 mg, 0.74 mmol) and 2,3-*O*-isopropylidene-propanol (98 mg, 0.74 mmol) in similar manner to that described for **16a**. Recrystallization from MeOH gave 210 mg of **16b** (59.8% yield from **10**), mp 183—184.5 °C, $[\alpha]_D^{20}$ −62.31° (c=0.5, $CHCl_3$). *Anal.* Calcd for $C_{24}H_{26}O_{10}$: C, 60.76; H, 5.52. Found: C, 60.46; H, 5.66. IR (KBr): 3604, 3532 (OH), 1770 (C=O) cm^{-1}. FAB-MS m/z: 475 ($M^+ + H$). ^{1}H-NMR ($CDCl_3$) δ: 6.84 (1H, s, 8-H), 6.55 (1H, s, 5-H), 6.26 (2H, s, 2′-, 6′-H), 5.99, 5.97 (each 1H, d, J=1.5, OCH_2O), 5.40 (1H, s, 4′-OH), 4.60 (1H, d, J=5.0, 1-H), 4.55 (1H, d, J=3.5, 1-H), 4.29—4.38 (2H, m, 11α,β-H), 3.89 (1H, m, $-OCH_2CH(OH)CH_2OH$), 3.77 (6H, s, 3′-, 5′-OCH_3), 3.58—3.80 (4H, m, $-OCH_2CH(OH)CH_2OH$), 3.33 (1H, d, J=14.0, 5.5, 3-H), 2.88 (1H, dddd, J=14.0, 10.5, 8.5, 3.5, 2-H).

2-*O*-(4′-Demethyl-1-epipodophyllotoxinyl)ethyldimethylsulfonium *p*-Toluenesulfonate (17a) BF_3–Et_2O (0.24 ml, 1.92 mmol) was added at −15 to −10 °C to a stirred mixture of **10** (400 mg, 0.74 mmol) and 2-methylthioethanol (838 mg, 9.10 mmol) in CH_2Cl_2 (8 ml). After 9 h, the reaction mixture was quenched with pyridine (0.24 ml) and CH_2Cl_2 (40 ml) and extracted with CH_2Cl_2. The extract was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography with benzene–AcOEt (4:1). The main fraction was recovered and concentrated *in vacuo*. The residue was reduced for 8 h on 5% Pd–C (50 mg) with H_2 (1 atm) in MeOH–AcOEt

(1 : 1) (30 ml). The reaction mixture was filtered, washed with AcOEt, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography with benzene–AcOEt (4 : 1). The main fraction containing **14a** was recovered and concentrated *in vacuo*. The residue was stirred with methyl *p*-toluenesulfonate (1 ml) for 19 d in tetrahydrofuran (THF) (3 ml). The precipitated crystals were filtered, washed with THF, and then recrystallized from AcOEt to give **17a** (293 mg, 59.9% from **10**), mp 181.5—182 °C, $[\alpha]_D^{20}$ −33.50° (c=0.40, DMF). *Anal.* Calcd for $C_{32}H_{36}O_{11}S_2 \cdot 2H_2O$: C, 55.16; H, 5.79. Found: C, 54.88; H, 5, 5.89. IR (KBr): 3428 (OH), 1768 (CO), 1428, 1386, 1334 (S=O) cm^{-1}. FAB-MS m/z: 489 ($M^+ - TsOH + H$). ^{1}H-NMR (DMSO-d_6) δ: 8.32 (1h, br, 4′-OH), 7.46, 7.12 (each 2H, d, J=8.1, $CH_3C_6\underline{H}_4SO_3$), 7.13 (1H, s, 8-H), 6.55 (1H, s, 5-H), 6.16 (2H, s, 2′-, 6′-H), 6.04, 6.01 (each 1H, d, J=1.0, OCH_2O), 4.66 (1H, d, J=2.8, 4-H), 3.80—4.60 (7H, m, 1-, 11α,β-H, $-OC\underline{H}_2C\underline{H}_2S$), 3.61 (6H, s, 3′-, 5′-$OCH_3$), 3.08—3.24 (1H, m, 3-H), 2.86 (6H, s, $-S(C\underline{H}_3)_2$), 2.60—3.08 (1H, m, 2-H), 2.28 (3H, s, $C\underline{H}_3C_6H_4$).

3-*O*-(4′-Demethyl-1-epipodophyllotoxinyl)propyldimethylsulfonium *p*-Toluenesulfonate (17b) **17b** was synthesized from **10** (400 mg, 0.74 mmol) and 3-methylthiopropanol (966 mg, 9.10 mmol) in a similar manner as described for **17a**. Recrystallization from AcOEt gave **17b** (268 mg, 53.6% yield from **10**), mp 163—166 °C, $[\alpha]_D^{20}$ −34.89° (c=0.74, DMF). *Anal.* Calcd for $C_{33}H_{38}O_{11}S_2 \cdot H_2O$: C, 57.21; H, 5.82. Found: C, 57.21; H, 5.88. IR (KBr): 3440 (OH), 1770 (C=O), 1428, 1384 (S=O), cm^{-1}. FAB-MS m/z: 503 ($M^+ - TsOH + H$). ^{1}H-NMR (DMSO-d_6) δ: 8.29 (1H, s, 4′-OH), 7.47, 7.13 (each 2H, d, J=8.1, $CH_3C_6\underline{H}_4SO_3$), 7.05 (1H, s, 8-H), 6.53 (1H, s, 5-H), 6.16 (2H, s, 2′-, 6′-H), 6.03, 6.00 (each 1H, d, J=1.0, OCH_2O), 4.08—4.66 (4H, m, 1-, 4-, 11α,β-H), 3.48—3.88 (2H, m, $-OC\underline{H}_2CH_2CH_2S$), 3.61 (6H, s, 3′, 5′-$OCH_3$), 3.12—3.44 (3H, m, 3-, $-C\underline{H}_2S(CH_3)_2$), 2.83, 2.82 (each 3H, s, $-S(C\underline{H}_3)_2$), 2.28 (3H, s, $C\underline{H}_3C_6H_4$), 1.84—2.20 (2H, m, $-OCH_2C\underline{H}_2CH_2S$).

4′-Demethyl-1-*O*-(2-hydroxy-3-dimethylaminopropyl)-1-epipodophyllotoxin (18a) BF_3–Et_2O (0.24 ml, 1.92 mmol) was added at −15 to −10 °C to a stirred mixture of **10** (500 mg, 0.93 mmol) and *N*-benzyloxycarbonyl-1-amino-2,3-propanediol (232 mg, 1.03 mmol) in CH_2Cl_2 (10 ml). After 1 h, the reaction mixture was quenched with pyridine (0.24 ml) and AcOEt (100 ml) and extracted with AcOEt. The extract was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography with $CHCl_3$–MeOH (30 : 1) to give the major product, which was reduced for 3 h on 5% Pd–C (800 mg) with H_2 (1 atm) in MeOH–AcOEt (1 : 1) (10 ml). The reaction mixture was filtered, washed with AcOEt, and concentrated *in vacuo*. The residue containing **15a** was stirred for 1 h with $NaCNBH_3$ (60 mg, 0.95 mmol) and 37% HCHO (0.1 ml) in MeOH (6 ml) at room temperature. The residue was purified by silica gel column chromatography with MeOH–$CHCl_3$ (1 : 5). Recrystallization from Et_2O gave 252 mg of **18a** (53.6% yield from **10**), mp 201—203 °C, $[\alpha]_D^{20}$ −75.39° (c=0.19, DMSO). *Anal.* Calcd for $C_{26}H_{31}NO_9$: C, 62.26; H, 6.23; N, 2.79. Found: C, 62.12; H, 6.51; N, 2.69. IR (KBr): 3392 (OH), 1766 (CO) cm^{-1}. FAB-MS m/z: 502 ($M^+ + H$). ^{1}H-NMR ($CDCl_3$) δ: 6.91 (1H, s, 8-H), 6.54 (1H, s, 5-H), 6.26 (2H, s, 2′-, 6′-H), 5.99, 5.96 (each 1H, d, J=1.0, OCH_2O), 5.40 (1H, s, 4′-OH), 4.59 (1H, d, J=5.5, 1-H), 4.58 (1H, d, J=3.5, 4-H), 4.33—4.43 (2H, m, 11α,β-H), 3.86 (1H, m, $-OCH_2C\underline{H}(OH)CH_2N$), 3.76 (6H, s, 3′-, 5′-$OCH_3$), 3.67 (1H, dd, J=10.0, 4.0, $-OC\underline{H}_2CH(OH)CH_2N$), 3.55 (1H, dd, J=10.0, 6.0, $-OC\underline{H}_2CH(OH)CH_2N$), 3.36 (1H, dd, J=14, 5.5, 3-H), 2.86 (1H, m, 2-H), 2.45 (1H, dd, J=12.0, 4.0, $-OCH_2CH(OH)C\underline{H}_2N$), 2.26 (1H, dd, J=12.0, 3.5, $-OCH_2CH(OH)C\underline{H}_2N$), 2.16 (6H, s, $N(CH_3)_2$).

4′-Demethyl-1-*O*-((*R*)-(−)-2-pyrrolidinemethyl)-1-epipodophyllotoxin (18b) **18b** was synthesized from **10** (400 mg, 0.75 mmol) and *N*-benzyloxycarbonyl-(*R*)-(−)-2-pyrrolidinemethanol (356 mg, 1.51 mmol) in a similar manner to that described for **18a**. Recrystallization from Et_2O gave 174 mg of **18b** (46.6% yield from **10**), mp 211—212.5 °C, $[\alpha]_D^{20}$ −37.89° (c=0.57, DMF). *Anal.* Calcd for $C_{27}H_{31}NO_8$: C, 65.18; H, 6.28; N, 2.82. Found: C, 65.15; H, 6.30; N, 2.69. IR (KBr): 3390 (OH), 1765 (CO) cm^{-1}. FAB-MS m/z: 498 ($M^+ + H$). ^{1}H-NMR ($CDCl_3$) δ: 6.86 (1H, s, 8-H), 6.54 (1H, s, 5-H), 6.25 (2H, s, 2′-, 6′-H), 5.98, 5.96 (each 1H, d, J=1.5, OCH_2O), 5.40 (1H, s, 4′-OH), 4.59 (1H, d, J=5.5, 1-H), 4.46 (1H, br, 4-H), 4.34 (2H, m, 11α,β-H), 3.83 (1H, br, 1″- or 2″-H), 3.76 (6H, s, 3′-, 5′-OCH_3), 3.44 (1H, br, 1″- or 2-H″), 3.38 (1H, dd, J=14.0, 5.0, 3-H), 3.13 (1H, br, 1″- or 2″-H), 2.88 (1H, m, 2-H), 2.51 (1H, br, 5″-H), 2.41 (3H, s, N–CH_3), 2.31 (1H, brs, 5″-H), 1.99 (1H, m, 3″- or 4″-H), 1.78 (3H, br, 3″-, 4″-H).

Biological Screening Cell Lines and Cytotechnology Test: P-388 (a murine leukemic cell line) and S-180 (a murine sarcoma cell line) were kept in continuous culture in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS). Cell lines were plated in 24-well flatbottom plates (Corning, type 25820), and incubated for 96 h with individual test compounds. On day 4, cell numbers were counted using a hemocytometer. The IC_{50} value was defined as the drug concentration needed to produce a 50% reduction in cell number relative to the control.

Preparation of Crude Nuclear Extracts: Crude nuclear extracts were prepared by modification of a previously reported method.[12] Exponentially growing cells were collected by centrifugation and washed in ice-cold NB (NB consists of 2 mM K_2HPO_4, 5 mM $MgCl_2$, 150 mM NaCl, 1 mM EGTA, and 0.1 mM dithiothreitol, pH adjusted to 6.5). The washed cells were resuspended in NB, and a solution of 9 mM of NB supplemented with 0.35% Triton X-100 and 1 mM phenylmethylsulfonyl fluoride was added slowly through the side of the tube. The cell suspension was mixed by rotation for 5 min at 4 °C, centrifuged at 1000 × *g* for 10 min, and then nuclear pellet washed in Triton-free NB. Nuclear protein was extracted from the nuclei for 30 min at 4 °C with ice-cold NB containing 0.35 M NaCl. DNA and nuclear debris were pelleted by centrifugation at 17000 × *g* for 10 min and the supernatant decanted. Protein concentration in the supernatant was determined by the method of Bradford.[13]

Topo II Catalytic Activity Assay: Topo II catalytic activity was assayed using the decatenation assay.[14] The standard reaction mixture for this assay was 50 mM Tris–HCl (pH 7.5), 8.5 mM KCl, 10 mM $MgCl_2$ 0.5 mM dithiothreitol, 0.5 mM EDTA, bovine serum albumin (0.03 mg/ml), and 1 mM ATP. Decatenation of kinetoplast DNA was carried out by incubating 4 μl nuclear extract (0.05 μg protein) with 1 μg of kinetoplast DNA and standard reaction mixture for 30 min at 30 °C. Reactions were terminated by the addition of 5 μl of 5% sodium dodecyl sulfate (SDS), 0.13% bromophenol blue, and 50% glycerol. Samples were then electrophoresed in 1% agarose with 40 mM Tris, 2 mM EDTA, 19 mM acetic acid, pH 8.1 at 50 V for 1 h. Gels were stained with ethidium bromide (1.0 μg/ml) for 30 min and destained for 1 h in H_2O. DNA bands were visualized by transillumination with UV and photographed using Polaroid type 665 positive/negative film. Inhibitory activity was calculated from the densitometer scanning of gel negatives. The IC_{50} value was defined as drug concentration needed to produce a 50% reduction of amount of mini-circle DNA relative to the control.

Tubulin Preparation and Anti-microtubular Activity Test: Bovine brain tubulin was prepared as described previously.[15] Purification was carried out in a buffer composed of 100 mg Mes (2-(*N*-morpholino)-ethanesulfonic acid), 1 mM ethyleneglycol-bis-*N*,*N*-tetraacetic acid (EGTA), 1 mM $MgSO_4$, 5 mM NaH_2PO_4 and 0.02% NaN_3, pH 6.75 (MEM buffer). After one cycle of polymerization–depolymerization, the final pellets were stored at −80 °C. Tubulin polymerization was carried out by incubating 50 μl of tubulin preparation (200 μg protein) with 250 μl of MEM buffer containing 1 mM GTP for 15 min at 37 °C. For assembly measurements, turbidity was monitored spectrophotometrically at 350 nm with a temperature controlled Hitachi U3210 spectrophotometer. The IC_{50} value was defined as drug concentration needed to produce a 50% reduction of polymerization relative to the control.

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