2nd day after treatment with neomycin ended. The mass chromatogram is given in Fig. 1D. In peaks a, b, d, and e in Fig. 1D, the ratios of the peak intensities $[(M + 2)^+/M^+]$ were much smaller than those of the corresponding peaks in the mass chromatogram obtained when rutin-*d* was administered to untreated rats (Fig. 1B). In addition, peak c did not appear in Fig. 1D. Rutin-*d* was also administered to rats on the 14th day after neomycin treatment when the action of the intestinal microflora had been restored. In the mass chromatogram thus obtained (Fig. 1E), the $[(M + 2)^+/M^+]$ ratios were larger than those in the mass chromatogram obtained from neomycin-treated rats (Fig. 1D). The five metabolites were detected in the untreated rats. They were reduced or disappeared in the neomycin-treated rats and were increased on the 14th day after neomycin treatment. The results obtained from the intraperitoneal injection and the subsequent neomycin-treatment experiments showed that intestinal microflora greatly influence the metabolism of orally administered I.

Rutin-*d* was incubated with extracts of rat intestinal contents under anaerobic conditions. As shown in the mass chromatogram (Fig. 1F) thus obtained, three metabolites, *i.e.*, III (peak b), V (peak e), and VIII (peak c), appeared. Compound II and unchanged I were also found in the incubation mixture. The *in vitro* experiments suggested direct involvement of the intestinal microflora in the metabolism of I, even though all of the metabolites detected in the urine after oral administration of rutin-*d* were not found. The lack of formation of metabolites IV and VI in the mixture indicated that they were excreted in the urine *via* methylation or decarboxylation by enzymes in the intestinal walls or in other tissues after the absorption of orally administered I from the GI tract.

It has been reported that I was metabolized to give phenolic acids such as III, IV, VIII, *etc.* by the isolated perfused rat liver (19). These phenolic acids should be excreted in the urine *via* liver metabolism if I is absorbed as such from the GI tract after oral administration of rutin-*d* in neomycin-treated rats. However, these metabolites were not found in the urine from neomycin-treated rats (Fig. 1D), and neither I nor II was present. It is then reasonable to assume that orally administered I could not be absorbed from the GI tract *per se*. 3,4-Dihydroxyphenyl[carboxy-^{14}C]acetic acid (V), when administered to rats orally, was almost completely excreted in the urine in the form of III, IV, and V (20). Thus, I must be metabolized to the phenolic acids by intestinal microflora, with subsequent absorption from the GI tract.

In this study, five metabolites (III–VI and VIII) derived from orally administered rutin-*d* were differentiated from these compounds endogenously present in the urine and successfully identified by the mass chromatographic method. In addition, the involvement of intestinal microflora in the metabolism of I was investigated by this method (Table I). It is possible that the human intestinal microflora may also play a significant role in the formation of urinary metabolites of I (III–VII) in humans.

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Antitumor Agents LXII: Synthesis and Biological Evaluation of Podophyllotoxin Esters and Related Derivatives

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Abstract □ Synthetic esters of the C-4 hydroxyl group of podophyllotoxin (I) were prepared. In addition, esters were synthesized using the diol system of tetrahydropyranyl podophyllol (XV), produced by reducing the lactone ring of tetrahydropyranyl podophyllotoxin with lithium aluminum hydride. Six compounds, the acrylate (IV), 3,3-dimethyl acrylate (V), phenoxyacetate (IX), and ethyl adipate (XI) of I as well as podophyllol (XIV) and tetrahydropyranyl podophyllol dimesylate (XVIII), showed significant activity when tested using the P-388 lymphocytic leukemia screen at 3 mg/kg/day. None of the esters showed higher activity than that shown by the parent molecule I when tested at the same dosage level.

Keyphrases □ Podophyllotoxin—esters, synthesis, antileukemic activity in mice □ Synthesis—podophyllotoxin esters, antileukemic activity in mice □ Antileukemic agents—potential, podophyllotoxin esters, synthesis

The development of teniposide (VM-26) and etoposide (VP-16-213), two glucopyranosyl derivatives related to the lignan podophyllotoxin (I), as clinically effective anticancer drugs has been reviewed recently (1). An examination of the structural features of teniposide and etoposide indicated that a free hydroxyl group at C-4 in epipodophyllotoxin is not essential for potent activity. Thus, it was considered that further modification of the C-4 hydroxyl group of I or epipodophyllotoxin could yield additional potent antitumor agents. In view of the importance of an

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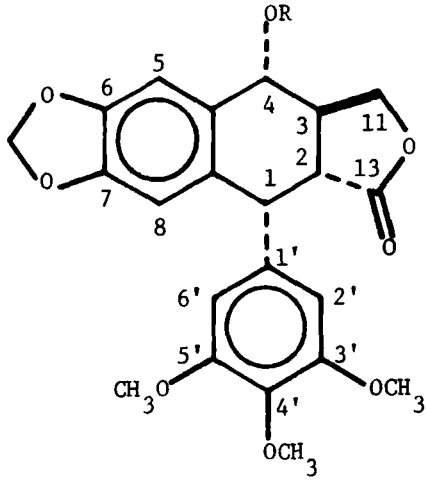
ester group in the cytotoxicity and antileukemic activity of compounds in certain classes (2–5), a series of C-4 hydroxyl esters of I were prepared and examined for their antileukemic activity against *in vivo* P-388 lymphocytic leukemia cell growth. The effect of the ester group on the antileukemic activity of I has not yet been studied (1). In addition, modification of the podophyllotoxin nucleus by substituting an ester moiety at the diol system of tetrahydropyranyl podophyllol (XV), as well as the antileukemic activity of the resulting products are reported.

RESULTS AND DISCUSSION

Several esters of podophyllotoxin (I) and tetrahydropyranyl podophyllol (XV) were prepared by a general method and assayed for *in vivo* antileukemic activity against P-388 lymphocytic leukemia growth in mice according to the standard National Cancer Institute procedures (6). The antileukemic activity of I–XIX are shown in Table I and are compared to podophyllotoxin (I) at the same dosage levels. The maximum observed T/C% value of 171 for I at 3 mg/kg/day compares favorably with that reported in the literature (7). This dosage was therefore chosen for a study of the structure–activity relationship of the various esters.

Generally, every compound tested showed a significant decrease in antileukemic activity compared with I when administered at 3 mg/kg/day. Compounds II, III, VI–VIII, X, XII, XIII, XV–XVII, and XIX were found to be inactive. Compounds which showed significant (T/C ≥ 120%) (6, 8) antileukemic activity included IV, V, IX, XI, XIV, and XVIII with respective T/C% values of 135, 121, 133, 120, 120, and 130 at 3 mg/kg/day. These data indicate that the introduction of an ester moiety into I and XV does not enhance the antileukemic activity (P-388), but in general causes a loss of activity.



I : R = H (podophyllotoxin)

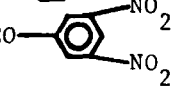
II : R = $COCH_3$

III : R = $COCH_2CH_2CH_3$

IV : R = $COCH{=}CH_2$

V : R = $COCH{=}C(CH_3)_2$

VI : R = CO– 

VII : R = CO– 

VIII : R = $COCH_2CH_2$– 

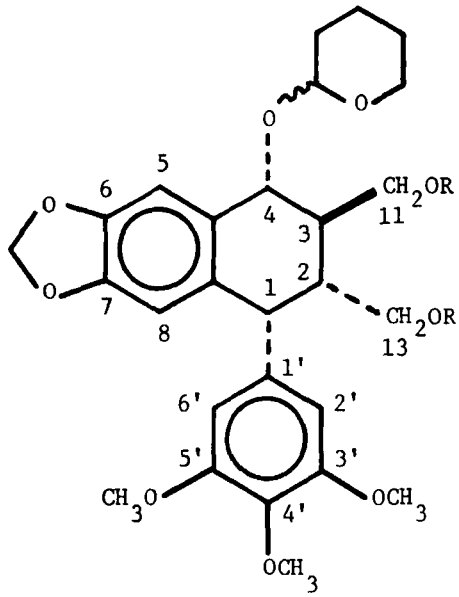
IX : R = $COCH_2O$– 

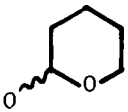
X : R = $COCH_2COOCH_2CH_3$

XI : R = $CO(CH_2)_4COOCH_2CH_3$

XII : R = $COCH_2CH_2COO$-podophyllotoxin

XIII : R = 



XIV : R = H, OH replaces 

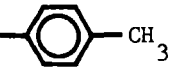
(podophyllol)

XV : R = H

XVI : R = $COCH_2Br$

XVII : R = $COCH_2CH_2Br$

XVIII : R = SO_2CH_3

XIX : R = SO_2– 

EXPERIMENTAL[1]

Podophyllotoxin (I)—Podophyllotoxin was isolated from the chloroform extract of *Podophyllum peltatum*[2] according to literature methods (9, 10).

[1] Unless otherwise specified, microanalyses were performed by M-H. W. Laboratories, Phoenix, Ariz. or Integral Microlab, Inc., Raleigh, N.C. Mass spectral data were recorded using an A.E.I. MS-902 mass spectrometer. Melting points were determined on a Thomas-Hoover melting point apparatus. IR spectra were recorded in chloroform on a Perkin-Elmer 257 grating spectrophotometer. ^{1}H-NMR spectra were measured in deuterated chloroform either on a JEOL C-60HL or a JEOL FX-60 NMR spectrometer. All NMR data are given in parts per million and reported as downfield from the internal standard, tetramethylsilane. The abbreviations s, d, t, q, and m refer to single, doublet, triplet, quartet, and multiplet, respectively. Silica gel refers either to Mallinckrodt Silicar CC-7 (200–325 mesh) or Merck Silica gel 60 GF-254. TLC refers to precoated plates available from Analtech, which were developed in 15:1 chloroform–methanol. Visualization of TLC spots was performed in one or more of the following manners: (*a*) iodine vapor; (*b*) spraying with 40% sulfuric acid or 1% ceric sulfate in 10% sulfuric acid followed by heating; or (*c*) viewing under UV light.

[2] Purchased from Dr. Madis Laboratories Inc., South Hackensack, N.J.

Table I—Antileukemic Activity of Podophyllotoxinyl Esters and Related Compounds Against the P-388 Lymphocytic Leukemia in BDF_1 Male Mice (~22 g) Dosed on Days 1–9

Compound	Dose, mg/kg/day ip	Survival Treated/Control, days[a]	T/C %[b]
I	0.5	12.1/10.2	119
	1	15.0/10.2	147
	2	12.6/10.2	124
	3	17.4/10.2	171
	5	11.6/10.2	114
	10	7.1/10.2	70
II	3	11.5/10.2	113
	5	12.2/10.2	120
III	3	11.1/10.2	109
	5	10.2/10.2	100
IV	3	13.8/10.2	135
	5	13.6/10.2	133
V	3	12.3/10.2	121
VI	3	11.4/10.2	112
	5	11.3/10.2	111
VII	3	10.7/10.2	105
	5	9.6/10.2	98
VIII	3	11.8/10.2	116
IX	3	13.6/10.2	133
	5	11.5/10.2	113
X	3	9.7/10.2	99
XI	3	12.2/10.2	120
XII	3	11.6/10.2	118
	5	9.6/10.2	98
XIII	3	10.3/10.2	105
XIV	1.5	10.4/10.2	106
	3	11.8/10.2	120
	4.5	11.0/10.2	108
XV	3	10.4/10.2	106
	5	11.4/10.2	116
XVI	3	4.5/10.2	46[c]
XVII	3	11.4/10.2	116
XVIII	3	12.7/10.2	130
	5	10.5/10.2	103
XIX	3	11.2/10.2	114
	5	12.0/10.2	118
5-Fluorouracil	25	18.0/9.66	186
0.05% Polysorbate	—	10.2/10.2	100

[a] Average value. [b] A compound is active if it exhibits a T/C ≥ 120% (6, 8). [c] Toxic.

Synthesis of Esters—Except for bis(podophyllotoxinyl) succinate (XII), all compounds were synthesized by the following general procedures: To 200 mg (0.48 mmole) of podophyllotoxin in 10 ml of dry benzene and 5 ml of pyridine was added 2.4 mmoles of the appropriate acid chloride. The mixture was stirred at room temperature until TLC indicated that the reaction was complete. At that time, 5 ml of 5% sulfuric acid was added in a dropwise manner and the mixture was stirred for an additional hour. The organic layer was then removed, and the aqueous layer was extracted twice with chloroform. The organic layers were combined, dried over magnesium sulfate, filtered, and then evaporated *in vacuo*. The residue was column chromatographed on silica gel eluted with chloroform. The first fraction collected contained the desired compound with analytical data as specified.

Podophyllotoxinyl Acetate (II)—Colorless needles (175 mg, 80% from EtOH), mp 177–179° [lit. mp 179–181° (11), 204° (12), and 209–210° (13)]; IR (nujol): 1775 (γ-lactone), 1715 (ester), and 1230 (acetate) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.98 (3, s, $OCOCH_3$), 2.70–2.90 (2, m, H-2 and H-3), 3.78 (6, s, OCH_3-3′ and OCH_3-5′), 3.81 (3, s, OCH_3-4′), 4.00–4.70 (4, unresolved m, H-1, H-4, and H-11), 5.98 (2, s, OCH_2O), 6.38 (2, s, H-2′ and H-6′), 6.52 (1, s, H-8), and 6.77 ppm (1, s, H-5).

Anal.—Calc. for $C_{24}H_{24}O_9$: *m/z* 456.1418 (M^+). Found: *m/z* 456.1424.

Podophyllotoxinyl Butyrate (III)—White amorphous solid (217 mg, 93% yield), mp 57–61° (sintered at 44–46°); R_f 0.62; IR: 1770 (γ-lactone), 1735, and 1235 (ester) cm^{-1}; ^{1}H-NMR: δ 1.01 (3, t, J = 7.0 Hz, CH_2CH_3), 1.40–2.00 (2, m, $CH_2CH_2CH_3$), 2.44 (2, t, J = 7.0 Hz, $CH_2CH_2CH_3$), 2.75–3.05 (2, m, H-2 and H-3), 3.78 (6, s, OCH_3-3′ and OCH_3-5′), 3.83 (3, s, OCH_3-4′), 4.00–4.70 (4, m, H-1, H-4 and H-11), 5.98 (2, s, OCH_2O), 6.39 (2, s, H-2′ and H-6′), 6.53 (1, s, H-8), and 6.75 ppm (1, s, H-5).

Anal.—Calc. for $C_{26}H_{28}O_9$: *m/z* 484.1732 (M^+). Found: *m/z* 484.1739.

Podophyllotoxinyl Acrylate (IV)—White amorphous powder (150 mg, 66% yield), mp 86–90° (sintered at 68°); R_f 0.64; IR: 1770 (γ-lactone), 1720 (ester), 1410, 1290, 995, and 935 (terminal olefin) cm^{-1}; ^{1}H-NMR: δ 2.80–3.10 (2, m, H-2 and H-3), 3.78 (6, s, OCH_3-3′ and OCH_3-5′), 3.81 (3, s, OCH_3-4′), 4.00–4.70 (4, unresolved m, H-1, H-4, and H-11), 6.04 (2, s, OCH_2O), 6.53 (1, s, H-8), 6.80 (1, s, H-5), 5.98 (2, m, $C{=}CH_2$), 6.41 (2, s, H-2′ and H-6′), and 6.15–6.65 ppm (1, overlapped m, $OCH{=}CH_2$).

Anal.—Calc. for $C_{25}H_{24}O_9$: C, 64.10; H, 5.13. Found: C, 64.11; H, 5.45.

Podophyllotoxinyl 3,3-Dimethyl Acrylate (V)—Colorless oil (66 mg, 27% yield); R_f 0.64; IR: 1765 (γ-lactone), 1700, 1230, and 1120 (acrylate) cm^{-1}; ^{1}H-NMR: δ 1.95 (3, d, J = 1.2 Hz, $=C{-}CH_3$), 2.22 (3, d, J = 1.2 Hz, $=C{-}CH_3$), 2.70–3.05 (2, m, H-2 and H-3), 3.78 (6, s, OCH_3-3′ and OCH_3-5′), 3.82 (3, s, OCH_3-4′), 4.15–4.70 (4, m, H-1, H-4, and H-11), 5.75 [1, m, $CH{=}C(CH_3)_2$], 5.98 (2, s, OCH_2O), 6.39 (2, s, H-2′ and H-6′), 6.53 (1, s, H-8), and 7.78 ppm (1, s, H-5).

Anal.—Calc. for $C_{27}H_{28}O_9$: C, 65.32; H, 5.64. Found: C, 65.68; H, 5.71.

Podophyllotoxinyl Benzoate (VI)—White amorphous solid (197 mg, 70% yield), mp 72–74° (sintered at 55–57°) [lit. (14) mp 113–117° as needles from EtOH]; R_f 0.65; IR:1770 (γ-lactone), 1710, 1250, 1120 (benzoate), 750, and 710 (monosubstituted aromatic ring) cm^{-1}; ^{1}H-NMR: δ 2.90–3.10 (2, m, H-2 and H-3), 3.69 (6, s, OCH_3-3′ and OCH_3-5′), 3.81 (3, s, OCH_3-4′), 4.25–4.75 (4, m, H-1, H-4, and H-11), 6.00 (1, s, H-8), 6.88 (1, s, H-5), and 7.30–8.40 ppm (5, m, COC_6H_5).

Anal.—Calc. for $C_{29}H_{26}O_9$: *m/z* 518.1576 (M^+). Found: *m/z* 518.1572.

Podophyllotoxinyl 3,5-Dinitrobenzoate (VII)—Yellow powder (110 mg, 37% yield), mp 153–156° (sintered at 149°); R_f 0.64; IR: 1775 (γ-lactone), 1730 (ester), 1540, and 1340 (aromatic nitro group) cm^{-1}; ^{1}H-NMR: δ 2.90–3.20 (2, m, H-2 and H-3), 3.83 (9, s, OCH_3-3′, OCH_3-4′, and OCH_3-5′), 4.20–4.80 (4, m, H-1, H-4, and H-11), 6.04 (2, s, OCH_2O), 6.45 (2, s, H-2′ and H-6′), 6.63 (1, s, H-8), 6.80 (1, s, H-5), and 9.01–9.41 ppm [3, m, $C_6H_3(NO_2)_2$].

Anal.—Calc. for $C_{29}H_{24}N_2O_{13}$: *m/z* 608.1275 (M^+). Found: *m/z* 608.1269.

Podophyllotoxinyl Hydrocinnamate (VIII)—Colorless oil (171 mg, 65% yield); R_f 0.62; IR: 1770 (γ-lactone), 1725, and 1235 (ester) cm^{-1}; ^{1}H-NMR: δ 2.70–3.10 (6, m, $COCH_2CH_2$-Ph, H-2, and H-3), 3.75 (6, s, OCH_3-3′ and OCH_3-5′), 3.81 (3, s, OCH_3-4′), 3.90–4.70 (4, m, H-1, H-4, and H-11), 5.94 (2, s, OCH_2O), 6.35 (2, s, H-2′ and H-6′), 6.50 (2, br s, H-5 and H-8), and 7.22 ppm (5, s, C_6H_5).

Anal.—Calc. for $C_{31}H_{30}O_9$: *m/z* 546.1889 (M^+). Found: *m/z* 546.1897.

Podophyllotoxinyl Phenoxyacetate (IX)—White amorphous solid (252 mg, 95% yield), mp 71–74° (sintered at 59–62°); R_f 0.65; IR: 1765 (γ-lactone), 1730, 1240 (ester), and 1040 (aromatic ether) cm^{-1}; ^{1}H-NMR: δ 2.80–3.10 (2, m, H-2 and H-3), 3.75 (6, s, OCH_3-3′ and OCH_3-5′), 3.82 (3, s, OCH_3-4′), 4.00–4.70 (4, m, H-1, H-4, and H-11), 4.76 (2, s, $COCH_2O$), 5.98 (2, s, OCH_2O), 6.36 (2, s, H-2′ and H-6′), 6.52 (1, s, H-8), 6.62 (1, s, H-5), and 6.70–7.50 ppm (5, m, OC_6H_5).

Anal.—Calc. for $C_{30}H_{28}O_{10}$: *m/z* 548.1683 (M^+). Found: *m/z* 548.1685.

Podophyllotoxinyl Ethyl Malonate (X)—White amorphous compound (169 mg, 64% yield), mp 68–70° (sintered at 54°); IR: 1775 (γ-lactone), 1730, and 1745 (slight shoulder) (esters) cm^{-1}; ^{1}H-NMR: δ 1.20 (3, t, J = 7.0 Hz, OCH_2CH_3), 2.70–3.00 (2, m, H-2 and H-3), 3.43 (2, s, $COCH_2CO$), 3.67 (6, s, OCH_3-3′ and OCH_3-5′), 3.43 (3, s, OCH_3-4′), 4.15 (2, overlapped q, J = 7.0 Hz, OCH_2CH_3), 4.00–4.65 [4, m (overlapped by q), H-1, H-4, and H-11], 5.90 (2, s, OCH_2O), 6.30 (2, s, H-2′ and H-6′), 6.45 (1, s, H-8), and 6.76 ppm (1, s, H-5).

Anal.—Calc. for $C_{27}H_{28}O_{11}$: *m/z* 528.1629 (M^+). Found: *m/z* 528.1625.

Podophyllotoxinyl Ethyl Adipate (XI)—Colorless oil (72 mg, 26% yield); IR:1770 (γ-lactone), 1720, and 1235 (ester) cm^{-1}; ^{1}H-NMR: δ 1.28 (3, t, J = 7.0 Hz, OCH_2CH_3), 1.50–2.00 (4, m, $CH_2CH_2CH_2CH_2$), 2.00–2.65 (4, m, $COCH_2(CH_2)_2CH_2CO$), 2.75–3.00 (2, m, H-2 and H-3), 3.78 (6, s, OCH_3-3′ and OCH_3-5′), 3.83 (3, s, OCH_3-4′), 4.15 (2, q, J = 7.0 Hz, OCH_2CH_3), 4.00–4.70 (4, m, H-1, H-4, and H-11), 6.00 (2, s, OCH_2O), 6.40 (2, s, H-2′ and H-6′), 6.54 (1, s, H-8), and 6.75 ppm (1, s, H-5).

Anal.—Calc. for $C_{30}H_{34}O_{11}$: *m/z* 570.2101 (M^+). Found: *m/z* 570.2106.

Bis(podophyllotoxinyl) Succinate (XII)—To 200 mg (0.48 mmole)

of podophyllotoxin (I) dissolved in 10 ml of dry benzene was added 100 mg (0.65 mmole) of succinyl chloride in 10 ml of benzene. The mixture was refluxed until TLC indicated the reaction was complete. Ten milliliters of water was added and the reaction was stirred for an additional hour, after which time the organic layer was removed, washed twice with saturated sodium bicarbonate solution, dried over magnesium sulfate, filtered, and then evaporated *in vacuo*. The oily residue was dried under high vacuum to give 97 mg (44%) of XII, mp 154–157°; IR:1765 (γ-lactone) and 1735 (ester) cm^{-1}; ^{1}H-NMR: δ 2.80 (4, br s, $COCH_2CH_2CO$), 2.70–2.95 (4, m, two H-2 and H-3), 3.77 (12, s, two OCH_3 at C-3′ and C-5′), 3.82 (6, s, two OCH_3 at C-4′), 4.20–4.70 (8, m, two H-1, H-4, and H-11), 5.98 (4, br s, two OCH_2O), 6.39 (4, s, two H-2′ and H-6′), 6.53 (2, two H-8), and 6.81 ppm (2, s, two H-5). Reactions attempted using a small amount of pyridine to improve the yield of XII were unsuccessful.

Anal.—Calc. for $C_{48}H_{46}O_{18}$: *m/z* 910.2680 (M^+). Found: *m/z* 910.2691.

Tetrahydropyranyl Podophyllotoxin (XIII)—Podophyllotoxin (200 mg, 0.48 mmole) was suspended in 10 ml of anhydrous ether and stirred at room temperature. *p*-Toluenesulfonic acid monohydrate (8 mg) and 530 mg (6 mmoles) of dihydropyran were added. As the reaction continued, the product dissolved in the ether, leaving a colorless solution. When TLC showed that the reaction was complete, the ether solution was washed twice with 5 ml of saturated sodium bicarbonate followed by water, dried over magnesium sulfate, filtered, and then evaporated *in vacuo*. Petroleum ether caused XIII, a white amorphous compound (185 mg 77% yield), to precipitate out of an ether solution of the resulting residue, observed mp 93–95° (effervescing from 65°) [lit. (15) mp 90–100°]; R_f 0.51; IR: 1775 (γ-lactone), 1130, and 1075 (aliphatic ether) cm^{-1}; ^{1}H-NMR: δ 1.65 [6, m, $C(CH_2)_3C$ (THP)], 2.83 (2, m, H-2 and H-3), 3.75 (6, s, OCH_3-3′ and OCH_3-5′), 3.80 (3, s, OCH_3-4′), 3.30–5.00 [7, overlapped br m, H-1, H-4, H-11, $—OCH_2$ (THP) and O—CH—O (THP)], 5.96 (2, s, OCH_2O), 6.40 (2, s, H-2′ and H-6′), 6.84 (1, s, H-8), and 7.12 ppm (1, s, H-5).

Anal.—Calc. for $C_{27}H_{30}O_9$: *m/z* 498.1889 (M^+). Found: *m/z* 498.1892.

Podophyllol (XIV)—This was prepared from podophyllotoxin by lithium aluminum hydride reduction according to a literature method (16).

Tetrahydropyranyl Podophyllol (XV)—Tetrahydropyranyl podophyllotoxin (XIII) (440 mg, 0.88 mmole) was dissolved in 10 ml of dry ether. This solution was added dropwise to an ice-cold suspension of 44 mg (1.1 mmoles) of 95% lithium aluminum hydride and stirred at room temperature. When TLC indicated completion, the reaction was cooled to 0° with ice, and a few drops of water were added slowly. After the initial vigorous reaction subsided, an additional 10 ml of water was added slowly, and the entire mixture was stirred for 1 hr. The mixture was filtered through a pad of diatomaceous earth[3] to remove the solids, and the aqueous layer was separated from the organic layer. The aqueous layer was extracted twice with ether and the organic phase was washed with water, dried over magnesium sulfate, filtered, and evaporated *in vacuo*, to give 416 mg (93%) of a white amorphous solid (XV), mp 61–63° (sintered at 41–42°) [lit. (15) mp 75–90°]; R_f 0.67; IR: 3200–3600 (OH), 1130 (ether), and 1040 (primary OH) cm^{-1}; ^{1}H-NMR: δ 1.45–2.50 [8, m, H-2, H-3, and $—C(CH_2)_3C—$ (THP)], 3.75 (6, s, OCH_3-3′ and OCH_3-5′), 3.82 (3, s, OCH_3-4′), 5.90 (2, s, OCH_2O), 6.33 (2, s, H-2′ and H-6′), 6.40 (1, s, H-8), 6.78 (1, s, H-5), and 3.00–5.00 ppm [9, overlapped m, H-1, H-4, H-11, H-13, $—OCH_2$ (THP), and —OCHO— (THP)].

Anal.—Calc. for $C_{27}H_{34}O_9$: *m/z* 502.2201 (M^+). Found: *m/z* 502.2206.

Tetrahydropyranyl Podophyllol Di-(2-bromoacetate) (XVI)—White oily gummy solid (157 mg, 53% yield); R_f 0.66; IR: 1740 (ester) and 1235 (CH_2Br) cm^{-1}; ^{1}H-NMR: δ 1.65 [6, m, $C(CH_2)_3C$ (THP)], 2.20–2.70 (2, m, H-2 and H-3), 3.78 (6, s, OCH_3-3′ and OCH_3-5′), 3.83 (3, s, OCH_3-4′), 3.76 (4, br s, $COCH_2Br$), 3.00–5.08 [9 (exclusive of above), m, H-1, H-4, CH_2OCO, CH_2O (THP), and OCHO (THP)], 5.91 (2, s, OCH_2O), and 6.10–6.80 ppm (4, m, H-5, H-8, H-2′, and H-6′).

Anal.—Calc. for $C_{31}H_{36}Br_2O_{11}$: *m/z* 742.0624 (M^+). Found: *m/z* 586 (corresponds to $[M - 2\,Br]^+$). M^+ was not observed, but M+2 and M+4 peaks were both observed indicating the presence of two bromine atoms.

Tetrahydropyranyl Podophyllol Di-(3-bromopropionate) (XVII)—Colorless oil (85 mg, 39% yield); R_f 0.72; IR: 1735 (ester) and 1215 (alkyl bromide) cm^{-1}; ^{1}H-NMR: δ 1.65 [6, m, $(CH_2)_3C$ (THP)], 2.80 (4, m, two $OCOCH_2CH_2$), 2.30–3.10 (2, m, H-2 and H-3), 3.58 (4, overlapped t, two CH_2Br), 3.79 (6, s, OCH_3-3′ and OCH_3-5′), 3.83 (3, s, OCH_3-4′), 3.00–5.00 [9 (exclusive of above), m, H-1, H-4, CH_2OCO, CH_2O (THP), and OCHO (THP)], 5.91 (2, s, OCH_2O), and 6.10–6.85 ppm (4, m, H-5, H-8, H-2′, and H-6′).

Anal.—Calc. for $C_{33}H_{40}Br_2O_{11}$: *m/z* 770.0937 (M^+). Found: *m/z* 691 (corresponds to $[M - Br]^+$). Although M^+ was not observed, M+2 and M+4 were also detected indicating the presence of two bromine atoms.

Tetrahydropyranyl Podophyllol Dimesylate (XVIII)—White amorphous solid (90 mg, 79.5% yield), mp 47–50° (dec.); R_f 0.72; IR: 1370, 1245, and 1175 (SO_2) cm^{-1}; ^{1}H-NMR: δ 1.65 [6, m, $C—(CH_2)_3—C$ (THP)], 3.01 (3, s, $SO_2—CH_3$), 3.19 (3, s, SO_2CH_3), 2.00–3.00 (2, m, H-2 and H-3), 3.78 (6, s, OCH_3-3′ and OCH_3-5′), 3.82 (3, s, OCH_3-4′), and 5.92 ppm (2, s, OCH_2O).

Anal.—Calc. for $C_{29}H_{38}O_{13}S_2$: C, 52.88; H, 5.81; S, 9.75. Found: C, 52.97; H, 5.85; S, 9.85.

Tetrahydropyranyl Podophyllol Ditosylate (XIX)—White amorphous solid (384 mg, 57% yield), mp 70–72° (sintered at 56–58°) [lit. (15) mp 76–88°]; R_f 0.77; IR: 1360, 1235, and 1187 (SO_2) cm^{-1}; ^{1}H-NMR: δ 1.58 [6, m, $C—(CH_2)_3—C$ (THP)], 2.45 (6, s, $ArCH_3$), 2.00–3.00 (2, m, H-2 and H-3), 3.00–5.00 [9 (exclusive of below), m, H-1, H-4, $CH_2O—S$, $—OCH_2$ (THP), and OCHO (THP)], 3.75 (6, s, OCH_3-3′ and OCH_3-5′), 3.82 (3, s, OCH_3-4′), 5.88 (2, s, OCH_2O), 6.30 (2, s, H-2′ and H-6′), 6.20–7.00 [2 (exclusive of above), m, H-5 and H-8], and 6.50 ppm (4, q, J = 8.5 Hz, $SO_2C_6H_4CH_3$).

Anal.—Calc. for $C_{41}H_{46}O_{13}S_2$: *m/z* 810.2381 (M^+). Found: *m/z* 810.2376.

Biological Assay—The antileukemic activity was assessed against the P-388 lymphocytic leukemia growth in BDF_1 male mice (~22 g). In this screen, 10^6 cells were implanted on day 0. The test compounds were administered intraperitoneally from day 1 to day 9. T/C values were calculated according to the protocol of the National Institutes of Health (14). 5-Fluorouracil was used as the internal standard in the screen.

[3] Celite.

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