Synthesis and Antimitotic Activity of Glycosidic Lignan Derivatives Related to Podophyllotoxin[1]

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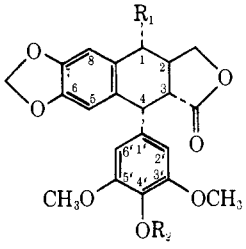
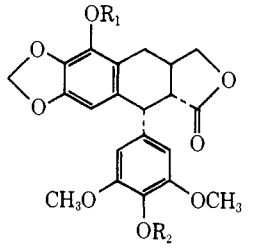
Epipodophyllotoxin β-D-glucopyranoside (XIII), 4′-demethylepipodophyllotoxin β-D-glucopyranoside (XV), and 4′-demethylepipodophyllotoxin β-D-galactopyranoside (XVI) react with aldehydes and ketones in the presence of acid catalysts to yield the corresponding cyclic acetals and ketals, resp. A number of 4′-demethylepipodophyllotoxin β-D-glucopyranoside derivatives exhibit a high cytostatic activity *in vitro* (P-815 mastocytoma cell culture) and give significant survival time increases in the mouse leukemia L-1210.

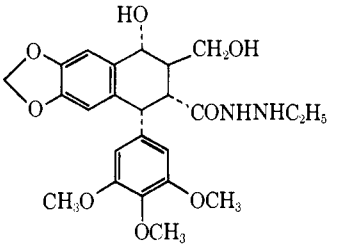
Podophyllotoxin (I) and some other structurally closely related lignans[2] and lignan glycosides (II-IX)[3] isolated from the roots and rhizomes of the American *Podophyllum peltatum* L. and the Indian species *P. emodi* Wall. exert a powerful and specific inhibition of mitosis.[4] Evaluated with systemic application as tumor-damaging agents these natural products failed to act satisfactorily in clinical trials due to nonspecific toxic side effects. Systematic chemical modification of the podophyllotoxin molecule, however, led to several therapeutically useful semisynthetic preparations, *e.g.*, podophyllinic acid ethylhydrazide (SP-I)[5-7] (X). In another approach natural lignan glucosides have been converted to cyclic acetals (SP-G)[6] by acid-catalyzed reaction with aldehydes. Such acetals (*e.g.*, XI) are well absorbed enterally and possess a favorable ratio between antimitotic activity and nonspecific toxicity.[7,8]



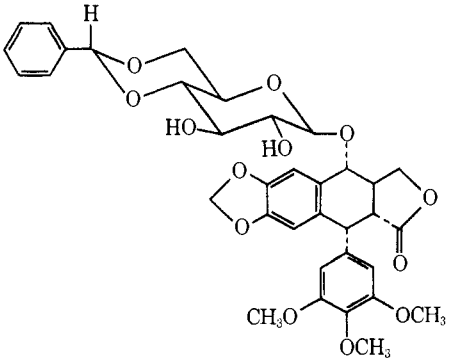
I, R_1 = OH; R_2 = CH_3; podophyllotoxin
II, R_1 = H; R_2 = CH_3; desoxypodophyllotoxin
III, R_1 = OH; R_2 = H; 4′-demethylpodophyllotoxin
VI, R_1 = β-D-glucopyranosido; R_2 = CH_3; podophyllotoxin β-D-glucopyranoside
VII, R_1 = β-D-glucopyranosido; R_2 = H; 4′-demethylpodophyllotoxin β-D-glucopyranoside



IV, R_1 = H; R_2 = H; α-peltatin
V, R_1 = H; R_2 = CH_3; β-peltatin
VIII, R_1 = β-D-glucopyranosyl; R_2 = H; α-peltatin β-D-glucopyranoside
IX, R_1 = β-D-glucopyranosyl; R_2 = CH_3; β-peltatin β-D-glucopyranoside



X, podophyllinic acid ethylhydrazide (SP-1)



XI, podophyllotoxin benzylidene-β-D-glucopyranoside

In the course of extensive studies which eventually led to the first synthesis of the genuine podophyllotoxin β-D-glucopyranoside (VI)[9] we developed a new glycosidation procedure for the hitherto unknown glycosides of the epipodophyllotoxin type. We could show that epipodophyllotoxin (XII) reacted stereoselectively with

(1) Mitosis-Inhibiting Natural Products. 24. For paper 23 of this series see M. Kuhn and A. von Wartburg, *Helv. Chim. Acta*, **52**, 948 (1969).

(2) For leading references see (a) J. L. Hartwell and A. W. Schrecker, *Fortschr. Chem. Org. Naturst.*, **15**, 83 (1958); (b) W. M. Hearon and W. S. MacGregor, *Chem. Rev.*, **55**, 957 (1955).

(3) (a) A. Stoll, J. Renz, and A. von Wartburg, *Helv. Chim. Acta*, **37**, 1747 (1954); (b) A. Stoll, A. von Wartburg, E. Angliker, and J. Renz, *J. Amer. Chem. Soc.*, **76**, 5004 (1954); (c) A. von Wartburg, E. Angliker, and J. Renz, *Helv. Chim. Acta*, **40**, 1331 (1957).

(4) (a) I. W. Kaplan, *New Orleans Med. Surg. J.*, **94**, 388 (1942); (b) B. J. Sullivan and H. J. Wechsler, *Science*, **105**, 433 (1947); (c) M. G. Kelly and J. L. Hartwell, *J. Nat. Cancer Inst.*, **14**, 967 (1954).

(5) J. Rutschmann and J. Renz, *Helv. Chim. Acta*, **42**, 890 (1959).

(6) The product of the reaction of *P. emodi* glucosides with benzaldehyde is SP-G. The cytostatic drug Proresid comprises SP-G and podophyllinic acid ethylhydrazide (SP-I).

(7) H. Stähelin and A. Cerletti, *Schweiz. Med. Wochenschr.*, **94**, 1490 (1964).

(8) H. Emmenegger, H. Stähelin, J. Rutschmann, J. Renz, and A. von Wartburg, *Arzneim.-Forsch.*, **11**, 327, 459 (1961).

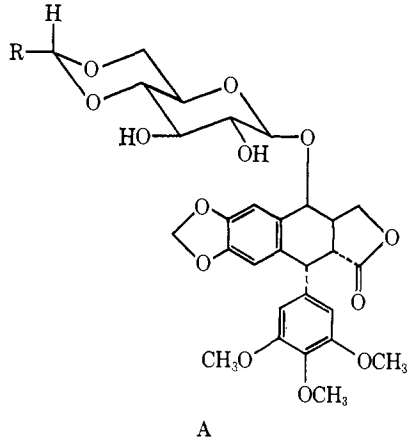
(9) (a) M. Kuhn and A. von Wartburg, *Helv. Chim. Acta*, **51**, 163 (1968); (b) the total syn of podophyllotoxin has been achieved by W. J. Gensler and C. D. Gatsonis, *J. Org. Chem.*, **31**, 4004 (1966).

TABLE I

CONDENSATION PRODUCTS OF EPIPODOPHYLLOTOXIN β-D-GLUCOPYRANOSIDE (A) WITH ALDEHYDES

No.	R	Formula	Mp, °C	[α]D, deg	Method	P-815 mastocytoma cells of the mouse, *in vitro*, ED_{50}, mg/l.	Mouse leukemia L-1210, % survival time increase
1	C_6H_5	$C_{35}H_{36}O_{13}$	166–170 ($CHCl_3$–hexane)	−101.2 ($CHCl_3$)	A	0.33	60
2	*p*-FC_6H_4	$C_{35}H_{35}FO_{13}$	169–172 (Me_2CO–hexane)	−97.2 ($CHCl_3$)	A	0.58	Not tested
3		$C_{33}H_{34}O_{14}$	172–176 (Me_2CO–hexane)	−95.8 ($CHCl_3$)	A	0.83	Not tested
4		$C_{33}H_{34}O_{13}S$	173–178 (Me_2CO–hexane)	−102.0 ($CHCl_3$)	A	0.37	Not tested

2,3,4,6-tetraacetyl-β-D-glucopyranose in the presence of BF_3–etherate to form the tetraacetate of epipodophyllotoxin β-D-glucopyranoside (XIII).[10] This useful reaction has been extended to a general synthesis for hexapyranosides of the epi isomers in the podophyllotoxin series, *e.g.*, 4′-demethylepipodophyllotoxin β-D-glucopyranoside (XV) and 4′-demethylepipodophyllotoxin β-D-galactopyranoside (XVI).[1] In the epi derivatives the O function at the C-1 position of the lignan skeleton possesses an inverse arrangement compared with the configuration present in podophyllotoxin. No significant effect of this structural variation on the biological activity was observed. Epipodophyllotoxin (XII), 4′-demethylepipodophyllotoxin (XIV), and the corresponding β-D-glucopyranosides did not show any striking peculiarity either with regard to the quality or the intensity of the antimitotic activity. As mentioned above, cyclic acetals of podophyllotoxin glycosides proved to be therapeutically interesting preparations. Accordingly we also produced analogous compounds in the epipodophyllotoxin series. The synthesis of the cyclic acetals and

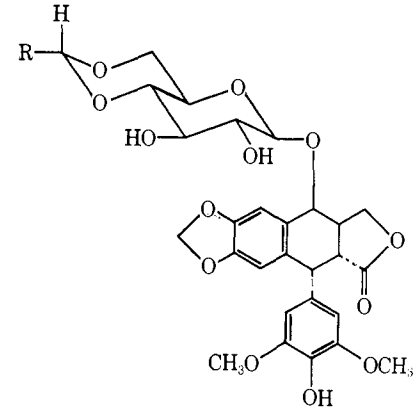


A

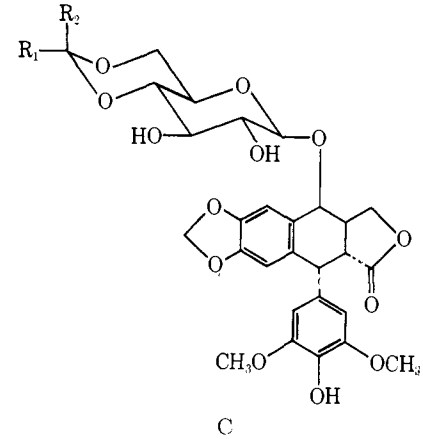
ketals shown in Tables I–IV was generally achieved by reaction of epipodophyllotoxin β-D-glucopyranoside (XIII), 4′-demethylepipodophyllotoxin β-D-glucopyranoside (XV), or β-D-galactopyranoside (XVI) with the corresponding carbonyl compds in the presence of acids or Lewis acids. The carbonyl compds may also be replaced by their acetals or ketals: this transacetalisation reaction is especially indicated for simple aliphatic carbonyl compds.

The condensation reaction took place generally with the OH groups at C-4 and C-6 of the hexapyranose moiety.

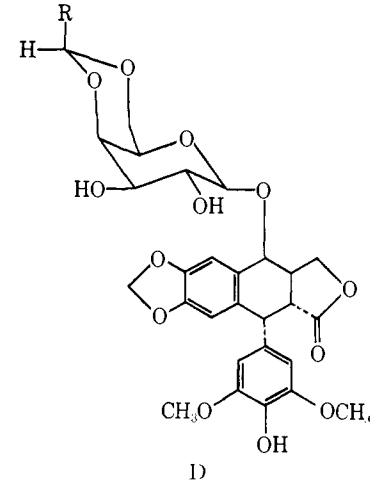
(10) M. Kuhn and A. von Wartburg, *Helv. Chim. Acta*, **51**, 1631 (1968).



B



C

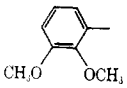
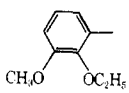
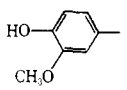
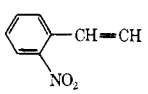


D

In the case of aldehydes the formation of two stereoisomers, differing from one another in the configuration at the newly introduced asymmetric C, is to be ex-

TABLE II

CONDENSATION PRODUCTS OF 4′-DEMETHYLEPIPODOPHYLLOTOXIN β-D-GLUCOPYRANOSIDE (B) WITH ALDEHYDES

No.	R	Formula	Mp, °C	[α]D, deg	Method	P-815 mastocytoma cells of the mouse, *in vitro*, ED_{50}, mg/l.	Mouse leukemia L-1210, % survival time increase
5	CH_3	$C_{29}H_{32}O_{13}$	236–251 (MeOH)	−110.5 ($CHCl_3$)	B	0.031	167
6	C_2H_5	$C_{30}H_{34}O_{13}$	178–182 (MeOH)	−107.2 ($CHCl_3$)	B	0.0085	97
7	$CH_3CH{=}CH$	$C_{31}H_{34}O_{13}$	195–199 (EtOH)	−99.2 ($CHCl_3$)	C	0.016	121
8	$(CH_3)_2CH$	$C_{31}H_{36}O_{13}$	181–185 (EtOH)	−96.9 ($CHCl_3$)	B	0.0055	121
9	$CH_3(CH_2)_2$	$C_{31}H_{36}O_{13}$	170–176 (EtOH)	−100.5 ($CHCl_3$)	B	0.0071	67
10	$C_2H_5CHCH_3$	$C_{32}H_{38}O_{13}$	153–159 (Me_2CO–pentane)	−103.3 ($CHCl_3$)	B	0.0055	84
11	CH_3CHCH_2 (with CH_3 branch)	$C_{32}H_{38}O_{13}$	191–196 (Me_2CO–Et_2O)	−102.1 ($CHCl_3$)	B	0.0048	36
12	$(CH_3)_3C$	$C_{32}H_{38}O_{13}$	162–165; 173–177 (Me_2CO–pentane)	−96.5 ($CHCl_3$)	B	0.015	57
13	*n*-C_4H_9	$C_{32}H_{38}O_{13}$	234–251 (MeOH)	−101.9 ($CHCl_3$)	B	0.0062	85
14	C_5H_9	$C_{33}H_{38}O_{13}$	233–234 (EtOH–Et_2O)	−99.0 ($CHCl_3$)	B	0.0047	39
15	*n*-Am	$C_{33}H_{40}O_{13}$	219–238 (MeOH)	−158.3 (pyridine)	B	0.0092	65
16	C_6H_{11}	$C_{34}H_{40}O_{13}$	226–229 (EtOH)	−99.0 ($CHCl_3$)	B	0.011	42
17	$CH_3(CH_2)_2CH(CH_3)$	$C_{33}H_{40}O_{13}$	143–150 (Me_2CO–pentane)	−100.9 ($CHCl_3$)	B	0.012	68
18		$C_{32}H_{32}O_{14}$	267–269 (EtOH)	−107.2 ($CHCl_3$)	A	0.018	136
19		$C_{32}H_{32}O_{13}S$	246–255 (EtOH)	−108.6 ($CHCl_3$–MeOH, 9:1)	A	0.0048	121
20	C_6H_5	$C_{34}H_{34}O_{13}$	245–246 (EtOH)	−104.3 ($CHCl_3$)	A	0.0068	97
21	*o*-HOC_6H_4	$C_{34}H_{34}O_{14}$	182–188 (Me_2CO–pentane)	−103.7 ($CHCl_2$)	C	0.034	48
22	*o*-MeC_6H_4	$C_{35}H_{36}O_{13}$	174–180 (Me_2CO–pentane)	−95.5 ($CHCl_3$)	B	0.0086	61
23	*p*-$CH_3OC_6H_4$	$C_{35}H_{36}O_{14}$	248–250 (EtOH)	−92.5 ($CHCl_3$)	A	0.011	29
24	*o*-$MeOC_6H_4$	$C_{35}H_{36}O_{14}$	243–250 (EtOH)	−74.4 (Me_2CO)	B	0.012	46
25	*p*-FC_6H_4	$C_{34}H_{33}FO_{13}$	265–270 (EtOH)	−105.1 ($CHCl_3$)	A	0.027	64
26	*o*-ClC_6H_4	$C_{34}H_{33}ClO_{13}$	179–184 (Me_2CO–pentane)	−91.3 ($CHCl_3$)	C	0.0042	24
27	*m*-HOC_6H_4	$C_{34}H_{34}O_{14}$	189–194 (Me_2CO–Et_2O)	−99.9 (MeOH)	B	0.054	40
28	*m*-$MeOC_6H_4$	$C_{35}H_{36}O_{14}$	206–228 (MeOH)	−85.4 (Me_2CO)	C	0.0090	41
29	*m*-ClC_6H_4	$C_{34}H_{33}ClO_{13}$	173–176 (Me_2CO–pentane)	−101.7 ($CHCl_3$)	C	0.0086	33
30	*m*-$O_2NC_6H_4$	$C_{34}H_{33}NO_{15}$	178–185 (Me_2CO–pentane)	−77.5 (Me_2CO)	C	0.039	52
31	*p*-HOC_6H_4	$C_{34}H_{34}O_{14}$	196–201 (Me_2CO–pentane)	−96.2 (MeOH)	C	0.038	41
32	*p*-$H_3CC_6H_4$	$C_{35}H_{36}O_{13}$	248–265 (MeOH)	−166.8 (pyridine)	B	0.0086	64
33	*p*-*i*-PrC_6H_4	$C_{37}H_{40}O_{13}$	172–179 (Me_2CO–pentane)	−97.3 ($CHCl_3$)	A	0.034	33
34		$C_{36}H_{38}O_{15}$	174–177 (Me_2CO–pentane)	−86.1 ($CHCl_3$)	C	0.023	36
35		$C_{37}H_{40}O_{15}$	166–175 (Me_2CO–pentane)	−83.2 ($CHCl_3$)	A	0.022	27
36		$C_{35}H_{36}O_{15}$	243–250 (MeOH)	−169.9 (pyridine)	C	0.037	23
37	$C_6H_5CH_2$	$C_{35}H_{36}O_{13}$	165–170 (MeOH–$CHCl_3$)	−82.0 (Me_2CO)	C	0.012	46
38	$C_6H_5CH{=}CH$	$C_{36}H_{36}O_{13}$	239–246 (EtOH)	−86.1 (Me_2CO)	C	0.011	29
39	$C_6H_5(CH_2)_2$	$C_{36}H_{38}O_{13}$	193–199 (EtOH)	−103.9 ($CHCl_3$)	B	0.015	51
40		$C_{36}H_{35}NO_{15}$	244–254 (MeOH)	−76.9 (Me_2CO)	C	0.0093	29
41	1-Naphthyl	$C_{36}H_{36}O_{13}$	189–195 (Me_2CO–pentane)	−90.4 ($CHCl_3$)	A	0.013	95

pected. However the isomer with an equatorial bond of the aldehyde residue predominates almost exclusively; the isomer with an axial substitutent is produced only in minimal amounts and is usually lost during the purification of the main reaction product. The actual configuration of sugar acetals of this type may often be recognised, especially with acetals of aromatic aldehydes, by a characteristic chemical shift of the axial proton in the nmr spectrum.[11]

As indicated in Table I, no dramatic increase in *biological activity* was observed with the acetals of epipodophyllotoxin β-D-glucopyranoside. It was there-

(11) N. Bagget, J. M. Duxbury, A. B. Foster, and J. M. Webber, *Chem. Ind. (London)*, 1832 (1964).

TABLE III

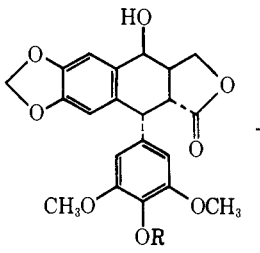
CONDENSATION PRODUCTS OF 4′-DEMETHYLEPIPODOPHYLLOTOXIN β-D-GLUCOPYRANOSIDE (C) WITH KETONES

No.	R_1	R_2	Formula	Mp, °C	$[\alpha]_D$, deg	Method	P-815 mastocytoma cells of the mouse, *in vitro*, ED_{50}, mg/l.	Mouse leukemia L-1210, % survival time increase
42	Me	Me	$C_{30}H_{34}O_{13}$	210–212 (MeOH)	−108.0 ($CHCl_3$)	A	0.015	106
43	Et	Me	$C_{31}H_{36}O_{13}$	166–176 (Me_2CO–pentane)	−98.2 ($CHCl_3$)	A	0.0060	69
44	$CH_2(CH_2)_2CH_2$		$C_{32}H_{36}O_{13}$	176–182 (EtOH–Et_2O)	−105.8 ($CHCl_3$)	C	0.019	51
45	$CH_2(CH_2)_3CH_2$		$C_{33}H_{38}O_{13}$	188–190 (EtOH)	−103.0 ($CHCl_3$)	A	0.015	97

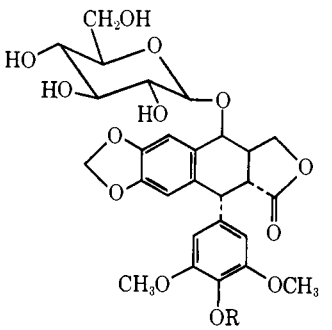
TABLE IV

CONDENSATION PRODUCTS OF 4′-DEMETHYLEPIPODOPHYLLOTOXIN β-D-GALACTOPYRANOSIDE (D) WITH ALDEHYDES

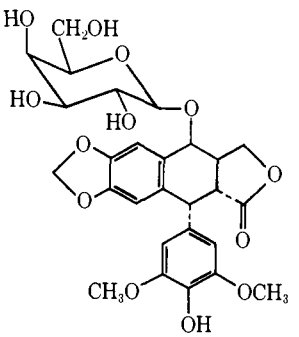
No.	R	Formula	Mp, °C	$[\alpha]_D$, deg	Method	P-815 mastocytoma cells in the mouse, *in vitro*, ED_{50}, mg/l.	Mouse leukemia L-1210, % survival time increase
46	C_2H_5	$C_{30}H_{34}O_{13}$	177–179 (EtOH)	−87.6 ($CHCl_3$)	B	0.046	30
47	C_6H_5	$C_{34}H_{34}O_{13}$	184–185 (MeOH)	−99.7 ($CHCl_3$)	A	0.048	12
48		$C_{32}H_{32}O_{13}S$	189–190 (MeOH)	−99.7 ($CHCl_3$)	A	0.039	23



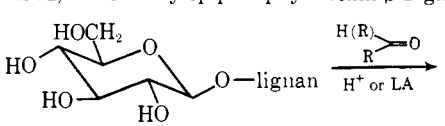
XII, R = CH_3; epipodophyllotoxin
XIV, R = H; 4′-dimethylepipodophyllotoxin

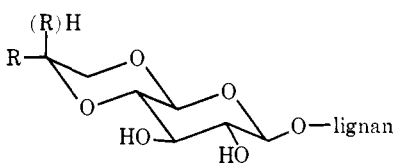


XIII, R = CH_3; epipodophyllotoxin β-D-glucopyranoside
XV, R = H; 4′demethylepipodophyllotoxin β-D-glucopyranoside



XVI, 4′-demethylepipodophyllotoxin β-D-galactopyranoside





fore surprising that some cyclic acetals and ketals of 4′-demethylepipodophyllotoxin β-D-glucopyranoside not only exhibit high activity in the *in vitro* tests (P-815 mastocytoma cell culture) but also give a significant survival time increase in the lymphocytic leukemia L-1210 test (Tables II and III), which can be considered as the most reliable animal predicting system for the clinical use of cytostatic drugs.[12] Some outstanding preparations are now in clinical trials.

The biological activity, especially the effect on mouse leukemia L-1210, appears to be structurally specific and correlated also with the nature of the sugar moiety. Cyclic acetals of 4′-demethylepipodophyllotoxin β-D-galactopyranoside[1] formed in analogous manner did not give comparable biological results (Table IV).

Experimental Section

Melting points were detd on a Kofler app and are corrected. Tlc were carried out on silica gel G plates, using in most cases $CHCl_3$–MeOH, 94:6, or 96:4 as solvent systems. The spots were visualized by spraying with 0.2% soln of cerium(IV) sulfate in 50% H_2SO_4 and subsequent heating at 120–130°. Column chromatog were carried out using kieselgel Merck (70–375 mesh ASTM). Nmr spectra were obtained on a Varian A-60 instrument (Me_4Si). Chemical shifts are recorded in ppm, coupling constants (J) are given in Hz; s = singlet, d = doublet, m = multiplet. Ir spectra were recorded on a Perkin-Elmer spectrophotometer, Model 21; γ_{max} are given in cm^{-1}. All compds listed in Tables I–IV had appropriate ir and nmr spectra and gave combustion values within 0.3% of theoretical.

General Preparation of Cyclic Acetals and Ketals.—Using *liquid* arom or heterocyclic aldehydes and ketones the glycosides XIII, XV, and XVI were suspended in an excess of the carbonyl compd and, after addn of the catalyst, were stirred at 20° with exclusion of moisture. Anhyd $ZnCl_2$ (method A), or, Dowex cation-exchange resin (Dowex 50-WX2 dried *in vacuo* at 120°) (method C), were used as catalyst. The course of the reaction was followed by tlc. With *solid* carbonyl compds or aliph aldehydes, the reactants were dissolved or suspended in CH_3NO_2 and the condensation was catalyzed by addn of *p*-TsOH (method B) or Dowex cation-exchange resin (method C). For transacetalization, the dimethylacetal, *e.g.*, of acetaldehyde or of phenylacetaldehyde, or the dimethylketal, *e.g.*, of acetone, was added to a suspension of the lignan glycoside in CH_3NO_2 and one of the above mentioned catalysts was introduced. For the

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isolation of the resulting lignan glycoside acetals, the reaction mix was dild with $CHCl_3$ and the catalyst was removed by filtration or washing out with H_2O. After removal of excess carbonyl compd the reaction product was obtd in pure form after column chromatogr or by direct crystn. Noncryst products were purified by pptn from a suitable solvent.

Method A. 4′-Demethyl-1-*O*-[4,6-*O*-(2-thenylidene)-β-D-glucopyranosyl]epipodophyllotoxin (19).—Dried 4′-demethylepipodophyllotoxin β-D-glucopyranoside[1] (6.8 g) was suspended in 68 ml of freshly distd 2-thiophenecarboxaldehyde, 3.4 g of anhyd $ZnCl_2$ was added, and the mixt was shaken under N_2. The course of the condensation was followed by tlc. After 3–4 hr, the yellow sol was dild with 300 ml of $CHCl_3$ and 300 ml of H_2O. The org layer was sepd and the H_2O phase was extd twice with $CHCl_3$. The combined $CHCl_3$ exts were washed several times with H_2O and then concd *in vacuo* to 50–70 ml. The remaining colorless oil was dropped into 1000 ml of pentane with stirring, whereby an oily ppt formed. After decanting the solvent the ppt was taken up in 40 ml of $CHCl_3$, and this soln was added dropwise to 1500 ml of pentane. The ppt was filtered off (6.5 g) and crystd from EtOH: mp 246–255°; $[\alpha]^{20}D$ −108.6° (*c* 0.5, $CHCl_3$–MeOH, 9:1); ir (Nujol) 3490, 3375 (OH); 1780 (γ-lactone); 1605, 1514, 1504, 1487 (arom C=C); nmr (DMSO-d_6) δ 8.24 (1 H, s), H, phenol OH, 7.7–6.8 (4 H, m), H of thiophene ring and H at C-8, 6.56 (1 H, s), H at C-5, 6.22 (2 H, s), H at C-2′ and H at C-6′, 6.04 (2 H, s) CH_2O_2, 5.88 (1 H, s), acetal H of the thenylidene group, 3.64 (6 H, s), 2 CH_3O.

4′-Demethyl-1-*O*-[4,6-*O*-(isopropylidene)-β-D-glucopyranosyl]epipodophyllotoxin (42).—To a suspension of 8 g of 4′-demethylepipodophyllotoxin β-D-glucopyranoside in 160 ml of CH_3NO_2 was added 4 g of anhyd $ZnCl_2$ and 32 ml of acetone dimethyl ketal, and the mixt was stirred for 0.5 hr at 20° with exclusion of moisture. The clear sol was then dild with 500 ml of $CHCl_3$ and washed 3 times, with 50-ml portions of H_2O. The org layer was dried (Na_2SO_4) and evapd to dryness. The residue was chromatographed on 150 g of kieselgel using $CHCl_3$–MeOH (98:2) as eluant. The tlc-pure fractions were crystd from MeOH yielding 5.11 g: mp 210–212°; $[\alpha]^{20}D$ −108° (*c* 1, $CHCl_3$); ir (CH_2Cl_2) 3580, 3527 (OH); 1775 (γ-lactone); 1618, 1515, 1503, 1484 (arom C=C); nmr ($CDCl_3$) δ 6.85 (1 H, s), 6.56 (1 H, s), 6.27 (2 H, s), H, C-8, H, C-5, and 2 H, C-2′, C-6′, 5.99 (2 H, s), CH_2O_2, 5.52 (1 H, s), H, phenol OH, 3.76 (6 H, s), 2 CH_3O, 1.52 (3 H, s) and 1.44 (3 H, s) H isopropylidene.

Method B. 4′-Demethyl-1-*O*-[4,6-*O*-(ethylidene)-β-D-glucopyranosyl]epipodophyllotoxin (5).—To a suspension of 1.5 g of 4′-demethylepipodophyllotoxin β-D-glucopyranoside in 30 ml of CH_3NO_2 was added 6 ml of acetaldehyde dimethyl acetal and 150 mg of *p*-TsOH, and the mixt was stirred under N_2 for 1 hr at 20°. The soln was dild with 400 ml of $CHCl_3$ and washed 3 times with H_2O (25-ml portions). The org phase was dried (Na_2SO_4) and evapd *in vacuo* yielding 1.74 g of crude product. Chromatog on 100 g of kieselgel using $CHCl_3$–MeOH (95:5) as eluant afforded 1.24 g of pure material which was crystd from MeOH: mp 236–251°; $[\alpha]^{20}D$ −110.5° (*c* 0.6, $CHCl_3$); ir (CH_2Cl_2) 3578, 3527 (OH), 1776 (γ-lactone), 1610, 1515, 1503, 1484 (arom C=C); nmr (DMSO-d_6) δ 8.23 (1 H, s), H of the phenolic OH, 7.03 (1 H, s), 6.55 (1 H, s), 6.21 (2 H, s), H at C-8, C-5, C-2′ and C-6′, 6.04 (2 H, s) H CH_2O_2, 3.62 (6 H, s), 2 CH_3O, 1.25 (3 H, d, J = 5 Hz) CH_3CH.

Method C. 4′-Demethyl-1-*O*-[4,6-*O*-(cyclopentylidene)-β-D-glucopyranosyl]epipodophyllotoxin (44).—A mixt of 2 g of dried 4′-demethylepipodophyllotoxin β-D-glucopyranoside in 40 ml of cyclopentanone and 4 g of Dowex WX2 ion exchanger was stirred for 2 hr at 20° with exclusion of moisture. The catalyst was filtered, and the filtrate was dild with 500 ml of $CHCl_3$. The soln was washed several times with H_2O, dried (Na_2SO_4), and evapd. The residue was chromatographed on 120 g of kieselgel using $CHCl_3$–MeOH (96:4) as eluant. A pure product was obtd after repeated chromatog of the main fractions. The anal. sample (400 mg) was crystd from EtOH–Et_2O: mp 176–182°; $[\alpha]^{20}D$ −105.8° (*c* 0.8, $CHCl_3$); ir (CH_2Cl_2) 3575, 3525 (OH), 1775 (γ-lactone), 1620, 1518, 1504, 1485 (arom C=C); nmr ($CDCl_3$) δ 6.83 (1 H, s), 6.53 (1 H, s), 6.24 (2 H, s), H at C-8, C-5, C-2′, and C-6′, 5.96 (2 H, s), CH_2O_2, 5.6–5.4 (1 H, m), H of phenolic OH, 3.74 (6 H, s), 2 CH_3O, 2.25–1.4 (8 H, m), H cyclopentylidene.

Carcinogenic and Adrenocorticolytic Derivatives of Benz[*a*]anthracene[1]

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A series of dimethyl, trimethyl, hydroxymethyl, and formyl derivatives of benz[*a*]anthracene structurally related to the carcinogenic 7-methyl-, 12-methyl-, and 7,12-dimethylbenz[*a*]anthracene and to the adrenocorticolytic 7-hydroxymethyl-12-methylbenz[*a*]anthracene were synthesized. Me substitution outside the "*critical*" region (*i.e.*, the 6, 7, 8, and 12 positions) was shown to block sarcomagenic activity when in the 1, 2, 3, 4, and 5 positions (one exception noted), whereas introduction of Me groups elsewhere in the molecule was without apparent effect on the biological action.

Introduction of one, two, or three Me groups into the 6, 7, 8, or 12 positions of benz[*a*]anthracene dramatically transforms this biologically inert hydrocarbon into a highly potent carcinogen.[2] The position of substitution is critical in that Me groups in other sites fail to elicit these effects. 7,12-Dimethylbenz[*a*]anthracene (7,12-DMBA) appears unique in this series in its ability to also destroy the adrenal cortex of the rat.[3] 7-Hydroxymethyl-12-methylbenz[*a*]anthracene, formed *in vivo*, has been shown to be the active intermediate species,[4] and several other 7-hydroxyalkyl (and potential 7-hydroxyalkyl) derivatives of benz[*a*]anthracene have also been found active.[3b]

In connection with our continuing investigations of the relation between the structure and the carcinogenic[2] and andrenocorticolytic[3] activity of derivatives of benz[*a*]anthracene, we prepared a series of dimethyl, trimethyl, hydroxymethyl, and formyl derivatives of benz[*a*]anthracene including several active new compounds, whose synthesis we now report.

The trimethylbenz[*a*]anthracene (TMBA) isomers synthesized,† except for 6,8,12-TMBA, were members

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† There are theoretically possible a total of 220 trimethylbenz[*a*]anthracene and 66 dimethylbenz[*a*]anthracene isomers; of the latter, 21 have one or both Me groups in the 7 or 12 positions.