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THE SYNTHESIS OF NEW PODOPHYLLOTOXIN DERIVATIVES

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ABSTRACT.—The preparation of a number of podophyllotoxin derivatives, including four new compounds, is described. Reaction of 4-bromo-4′-demethyl-4-deoxypodophyllotoxin with EtOH and glycerol afforded the corresponding 4-*O*-ethyl and 4-*O*-(2,3-dihydroxypropyl)-derivatives of 4′-demethylepipodophyllotoxin. Treatment of the 4-*O*-ethyl derivative with phenyliodonium diacetate in MeOH yielded a new quinone monoketal which underwent transketalization with ethylene glycol. Hydrolysis of the dimethyl ketal with aqueous acid afforded the corresponding *ortho*-quinone.

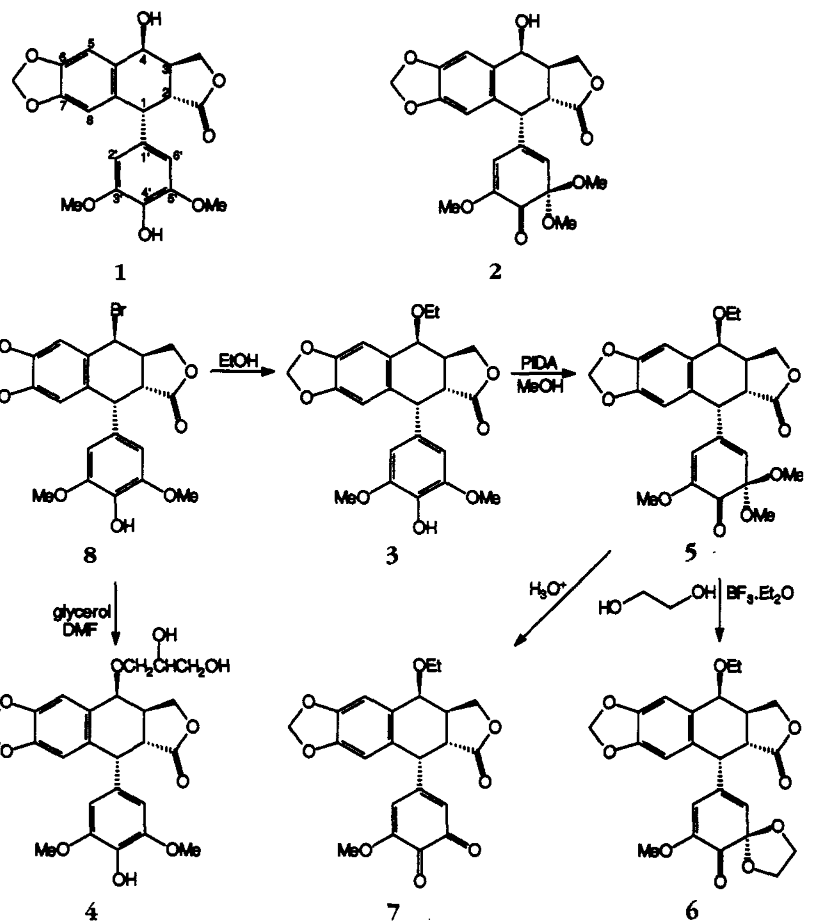
Podophyllotoxin derivatives are of current interest due to their use in cancer chemotherapy (1–3). This has resulted in several new approaches to the synthesis of podophyllotoxin derivatives (4) and extensive studies of their chemical modification (5–11). The possible involvement of quinone or quinone-methide intermediates in the biological mode of action of these compounds (12–14) has led us to investigate the preparation of reactive derivatives of this type (15,16).

We recently reported that oxidation of 4′-demethylepipodophyllotoxin [**1**] using phenyliodonium diacetate (PIDA) in MeOH gives the cyclohexa-2,4-dienone [**2**] (15). Addition of MeOH at the 2 position of the phenol is in contrast to our earlier work on simple phenols (17,18) and diarylbutanes (19) in which nucleophilic addition at the 4 position was observed. However, addition at the 2 position of an *ortho*-methoxyphenol has recently been reported (20). We now report the production of two 4-*O*-alkyl-4′-demethylepipodophyllotoxin derivatives ([**3**] and [**4**]) and conversion of one of these into a cyclohexa-2,4-dienone derivative [**5**]. This compound has in turn been converted into a spiroketal [**6**] with ethylene glycol, and into an *ortho*-quinone [**7**] with aqueous acid (Scheme 1).

4-*O*-Ethyl-4′-demethylepipodophyllotoxin [**3**] (8), mp 156–8°, was prepared from 4-bromo-4′-demethyl-4-deoxypodophyllotoxin [**8**] (21) by reaction with EtOH. As would be expected, this reacton proceeds through an S_N1 mechanism and the relative configuration at C-4 is dictated by the presence of the bulky pseudo-axial substituent at C-1. Because both of the clinically useful derivatives of podophyllotoxin contain a hydrophilic glycoside unit at C-4, we next chose to prepare a more hydrophilic analogue of **3**. Thus, treatment of the 4-bromo compound **8** with glycerol gave the dihydroxypropyl derivative **4**, in which one of the primary hydroxyl groups is attached to the podophyllotoxin skeleton.

We have also prepared a reactive dienone derivative from the ethoxy compound **3**. Treatment of **3** with PIDA in MeOH gave the dienone **5**, $\nu_{C=O}$ 1696 and 1776 cm^{-1}, as a golden-yellow powder. Hydrolysis of **5** under acidic conditions in aqueous THF gave the quinone **7** as a red powder. The quinone was characterized on the basis of the appearance of two carbonyl signals at δ 174.79 and 178.23 ppm in its ^{13}C-nmr spectrum in addition to the lactone carbonyl at 175.35 ppm.

Finally, transketalization of the quinone ketal **5** with ethylene glycol in the presence of BF_3 etherate gave the related dienone **6**. The ^1H- and ^{13}C-nmr



SCHEME 1

spectra of the dienone derivatives **2**, **5**, and **6** and the quinone **7** are listed in Tables 1 and 2, respectively. The biological activity of these compounds is under investigation and indicates that their level of activity is similar to that of the parent compounds. Full details will be reported separately in due course.

The conversion of **8** into **4** demonstrates the possibility of introducing a hydrophilic substituent at C-4. Oxidation of **1** and **3** to give **2** and **5**, respectively, demonstrates the possibility of introducing a hydrophilic group into ring C. In particular, compounds of type **5** and **6** are masked quinones closely related to intermediates implicated in anticancer activity. Thus, these processes delineate simple methods by which hydrophilic groups can be introduced into strategic positions on the podophyllotoxin structure and offer promise for the preparation of second generation anticancer agents based on podophyllotoxin.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker 250WM instrument operating at 250 and 62.5 MHz, respectively. TMS was used as the internal standard and spectra were recorded in $CDCl_3$ unless otherwise indicated. Mass spectra were obtained using a VG-12-250 spectrometer, with hrms being obtained on a ZAB-E double focussing instrument. Ir spectra were recorded on a Pye Unicam SP1050 spectrometer. Uv spectra were

TABLE 1. ^{1}H-Nmr Spectra of Podophyllotoxin Derivatives.[a]

Proton	Compound						
	1	2	3	4	5	6	7
H-1	4.60 d (5.1)	4.17 d (5.2)	4.60 d (5.3)	4.35 d (5.6)	4.19 d (5.4)	4.15 d (5.3)	4.36 d (3.2)
H-2	3.26 dd (5.1,14.1)	3.29 dd (5.2,14.1)	3.38 dd (5.2,14.0)	3.3 m	3.42 dd (5.4,14.0)	3.38 dd (5.3,13.9)	3.60 dd (5.9,14.0)
H-3	2.85 m	2.85 m	2.85 m	2.8 m	2.88 m	2.90 m	2.85 m
H-4	4.87 d (3.3)	4.82 d (3.2)	4.43 d (3.3)	4.48 d (4.0)	4.42 s	4.35 d (3.4)	4.28 d (5.8)
H-5	6.88 s	6.84 s	6.81 s	7.04 s	6.77 s	6.75 s	6.77 s
H-8	6.55 s	6.60 s	6.53 s	6.50 s	6.61 s	6.59 s	6.54 s
H-2′	6.29 s	6.30 d (1.6)	6.26 s	6.18 s	6.27 d (1.6)	6.35 d (1.6)	6.50 d (1.6)
H-6′		5.08 t (1.4)			5.07 d (1.6)	4.74 d (1.6)	5.17 t (1.3)
OCH_2O	5.97 ABq (1.2)	5.99 ABq (1.2)	5.97 ABq (1.3)	5.98 ABq (1.2)	5.98 ABq (1.1)	5.98 ABq (1.3)	6.00 s
OMe	3.77 s	3.71 s	3.75 s	3.59 s	3.72 s	3.73 s	3.85 s
5′-OR	—	3.26 s	—	—	3.26 s	4.01 m	—
5′-OR	—	3.17 s	—	—	3.17 s	4.25 m	—
CH_2	4.39 m	4.43 m	4.34 m	4.2–4.8 m	4.37 m	4.37 dd (8.3,10.7)	4.42 dd (8.4,10.5)
	4.34 m	4.49 m	4.30 m		4.49 t (8.0)	4.49 t (8.0)	4.50 t (7.9)
4-OCH_2	—	—	3.54 dq (8.9,7.0)	3.52 m	3.53 dq (8.9,7.0)	3.52 dq (8.9,7.0)	3.5 dq (7.0,8.8)
	—	—	3.75 m	—	3.72 m	3.72 m	3.72 dq (7.0,8.8)
OCH_2CH_3	—	—	1.23 t (7.0)	—	1.21 t (7.0)	1.21 t (7.0)	1.21 t (7.0)
OH	5.42 s	2.4 br	5.5 br	8.24 s	—	—	—
CH(OH)	—	—	—	4.54 m	—	—	—
CH_2OH	—	—	—	3.46 m	—	—	—

[a]All spectra recorded in $CDCl_3$ solution, except where indicated.
[b]Spectrum recorded in DMSO-d_6.

TABLE 2. ^{13}C-Nmr Spectra of Podophyllotoxin Derivatives.[a,b]

Carbon	Compound						
	1	**2**	**3**	**4**[c]	**5**	**6**	**7**
C-1	43.73	43.99	43.77	42.89	43.99	44.11	45.29
C-2	40.58	39.29	41.30	40.54	40.26	40.14	40.23
C-3	38.20	38.35	38.26	37.97	38.37	38.02	38.87
C-4	66.78	66.23	74.36	70.99	73.99	74.07	73.67
C-5	110.54	109.86	110.67	108.31	109.57	109.42	109.78
C-8	108.92	109.22	109.44	109.67	110.16	110.37	110.19
C-6	148.56	148.59	148.28	147.40	148.45	148.27	147.62
C-7	147.47	147.80	146.75	147.05	147.15	147.21	148.71
C-3′	146.41	149.18	146.41	145.96	149.18	148.41	157.85
C-5′		93.07			93.10	98.75	178.23
C-4′	134.01	190.37	134.02	134.64	190.33	193.26	174.79
C-1′	132.15	137.50	132.35	130.28	137.76	139.63	151.65
C-4a	131.83	132.09	130.97	132.17	130.22	130.03	129.77
C-8a	130.51	129.92	129.79	129.96	129.89	129.89	128.71
C-2′	107.80	127.71	107.95	109.80	127.72	127.95	126.36
C-6′		113.54			113.59	114.19	113.39
CH_2	67.60	68.02	67.60	167.35	67.87	67.87	67.99
CO	175.09	175.33	175.16	174.75	175.21	175.28	175.38
OCH_2O	101.57	101.71	101.43	101.16	101.60	101.57	101.78
OMe	56.46	55.73	56.45	55.92	55.70	55.69	56.19
5′-OR	—	50.34	—	—	50.37	65.46	—
5′-OR	—	50.14	—	—	50.11	65.08	—
4-OCH_2	—	—	66.06	72.28	66.05	66.14	65.87
OCH_2CH_3	—	—	15.44	—	15.38	15.40	15.35
*C*H(OH)	—	—	—	73.90	—	—	—
CH_2OH	—	—	—	71.71	—	—	—

[a]All spectra recorded in $CDCl_3$ solution, except where indicated.
[b]All assignments in accord with DEPT spectra.
[c]Spectrum recorded in DMSO-d_6.

recorded on a Phillips PU8720 scanning spectrometer. Mps were recorded on an Electrothermal digital melting-point apparatus and are uncorrected. Optical rotation values were obtained on a Perkin Elmer 141 polarimeter using a sodium lamp at 589 nm and values are recorded in units of 10^{-1} deg cm^2 g^{-1}.

Analytical hplc was carried out using an LDC 3100 Spectromonitor, 3000 Constametric pump, CI-4100 integrator, and an Apex ODS II 5 μm column. Tlc was carried out on Merck 5735 Kieselgel $60F_{254}$ fluorescent plates. Flash chromatography was performed with Si gel (Merck 9385, Kieselgel 60, 230–400 mesh). THF was dried by stirring overnight over calcium hydride, passing down a dry alumina column, and distillation from Na wire and benzophenone.

Preparation of 4-O-ethyl-4′-demethylepipodophyllotoxin [**3**].—4-Bromo-4′-demethyldeoxypodophyllotoxin [**8**] (21) (2.0 g, 4.3 mmol) in anhydrous EtOH (200 ml) was stirred at 50–60° for 3 h, when **8** had almost completely disappeared (tlc). The excess EtOH was evaporated under reduced pressure and the yellow-brown residue was crystallized from EtOH to give **3** (1.3 g, 70%): mp 156–8°; $[\alpha]^{20}$D −89.1° (c=0.55, $CHCl_3$); ir (KBr) ν max 3363 (OH), 1760 (C=O) cm^{-1}; eims (70 eV) m/z 428 (100, M^+), 399 (7, M−C_2H_5), 382 (17, M−C_2H_5OH); cims (70 eV) m/z 429 (37, M+H), 446 (100, M+NH_4); hrms m/z 428.1471 ($C_{23}H_{24}O_8$ requires 428.1470).

Preparation of 4-O-(2,3-dihydroxypropyl)-4′-demethylepipodophyllotoxin [**4**].—4-Bromo-4′-demethyl-4-deoxypodophyllotoxin [**8**] (21) (0.5 g, 1.2 mmol), dry glycerol (15 ml) and DMF (15 ml) were stirred vigorously at 60° for 4 h. The mixture was diluted with $CHCl_2$ (50 ml) and H_2O (50 ml) and then dried over anhydrous Na_2SO_4. After removal of the solvent *in vacuo* the yellow residue was recrystallized from MeOH/$CHCl_3$ to give **4** as a white crystalline solid (240 mg, 44%): mp 163–7°; $[\alpha]^{22}$D −79.9° (c=0.39, $CHCl_3$); ir (KBr) ν max 3384 (OH), 1758 (CO), 1612 cm^{-1}; eims (70 eV) m/z 474 (2, M^+), 382 (100, M−$HOCH_2CH(OH)CH_2OH$); cims (70 eV) m/z 492 (100, M+NH_4), 416 (40), 399 (70), 383 (95);

hrms *m/z* 492.1870 ($C_{24}H_{26}O_{10}+NH_4$ requires 492.1869).

Reaction of **3** *with PIDA in MeOH to give* **5**.—To a suspension of **3** (428 mg, 1.0 mmol) in anhydrous MeOH (25 ml) was added PIDA (324 mg, 1 equivalent) in portions at room temperature with stirring. After 3 h, $NaHCO_3$ (400 mg) was added and the mixture concentrated *in vacuo* to give a yellow residue which was extracted with EtOAc (2×60 ml). The combined EtOAc extracts were dried and evaporated and the yellow residual syrup was purified by cc on Si gel (CH_2Cl_2-Et_2O, 1:1) to give **5** (254 mg, 55%) as a golden-yellow amorphous powder: $[\alpha]^{20}D$ −15.1° (c=0.60, $CHCl_3$); ir (KBr) ν max 1776 (lactone), 1696 (C=O) cm^{-1}; eims (70 eV) *m/z* 458 (14, M^+), 428 (100, M−MeOH); cims (70 eV) *m/z* 446 (100, M−MeOH+NH_4), 427 (37, M−MeOH+H); fabms *m/z* 458 (61, M^+), 481 (6, M+Na), 427 (100); hrms *m/z* 458.1582 ($C_{24}H_{26}O_9$ requires 458.1577).

Hydrolysis of quinone monoketal **5** *to give* ortho-*quinone* **7**.—To a solution of **5** (92 mg, 0.2 mmol) in THF (20 ml) was added dropwise 0.1 M HCl (0.35 ml) at room temperature. The mixture was stirred for 4 h, after which **5** had disappeared (tlc). After dilution with CH_2Cl_2 (50 ml) and washing with aqueous $NaHCO_3$ and H_2O, the solution was dried ($MgSO_4$), filtered, and evaporated to give a red residue which was purified by cc on Si gel (CH_2Cl_2-Et_2O, 1:1), to give **7** as a red amorphous solid (40 mg, 48%): eims (70 eV) *m/z* 414 (100, M+2); cims (70 eV) *m/z* 415 (17), 432 (27); fabms *m/z* 413 (100, M+H); hrms *m/z* 414.1315 ($C_{22}H_{22}O_8$ requires 414.1315).

Preparation of ethylene ketal derivative **6**.—To a solution of **5** (230 mg, 0.5 mmol) and ethylene glycol (1.0 ml) in dry THF (10 ml) was added freshly distilled BF_3 OEt_2 (0.1 ml). The mixture was stirred at 60° for 4 h before being cooled again to room temperature and diluted with CH_2Cl_2 (50 ml). The CH_2Cl_2 solution was extracted with H_2O (20 ml), dried, and evaporated to give a yellow solid which was purified by cc on Si gel (CH_2Cl_2/Et_2O) to give **6** as an amorphous yellow powder (69 mg, 30%): ir (KBr) ν max 1778 (lactone), 1689 (C=O) cm^{-1}; fabms *m/z* 456 (M^+, 75), 457 (M+H, 100), 458 (M+2, 63), 479 (M+Na, 27); hrms *m/z* 456.1423 ($C_{24}H_{24}O_9$ requires 456.1420), 457.1498 ($C_{24}H_{24}O_9$+H requires 457.1498).

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