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ANTITUMOR AGENTS, 99.[1] SYNTHETIC RING C AROMATIZED PODOPHYLLOTOXIN ANALOGUES AS POTENTIAL INHIBITORS OF HUMAN DNA TOPOISOMERASE II

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ABSTRACT.—Several ring C aromatized analogues of podophyllotoxin were synthesized for testing against human DNA topoisomerase II. The results indicate that aromatization of ring C gave rise to no inhibition of this enzyme at 200 μM. A comparison of the cytotoxicity among these compounds also demonstrates that a free hydroxyl group at C-4 contributes to significant cytotoxicity.

Semisynthetic analogues of the naturally occurring podophyllotoxin [**1**] have drawn much renewed interest in recent years as a result of the development of etoposide and teniposide as anticancer drugs (1). It is believed that such analogues of 4′-demethylepipodophyllotoxin exert their cytotoxic, antitumor activity through stabilization of a cleavable complex between DNA and type II DNA topoisomerase. This leads ultimately to inhibition of DNA catenation activity and produces single and double strand breaks (2,3).

As part of our ongoing research program aimed at the synthesis and biological evaluation of novel antitumor analogues related to etoposide (4,5), we report herein the synthesis and structure-activity relationships of several ring C aromatized podophyllotoxin analogues. These compounds were synthesized based upon the fact that justicidin A [**9**] and diphyllin [**10**], two ring C aromatized lignans isolated from *Justicia procumbens*, were found to show significant inhibitory activity in vivo against P-388 lymphocytic leukemia (T/C = 150% at 50 mg/kg/day) as well as in vitro cytotoxicity in the KB cell (ED_{50}<1.0 μg/ml each) culture assay (6). It would be of interest to compare these aromatized compounds with their corresponding non-aromatized substances for their ability in inhibiting human DNA topoisomerase II as well as for their cytotoxic, antitumor activity. Furthermore, such a study is of importance in defining the stereochemical requirement for the presence of three chiral centers located on ring C at C-2, C-3, and C-4 with regard to its effect upon biological activity.

The synthesis of target compounds **4**–**8** is shown in Scheme 1. The design of **7** and **8**, which bear a C-4′ OH group in the pendant aromatic ring as two key target substances, was based upon the fact that 4′-demethylpodophyllotoxin and etoposide are more potent antitumor agents than podophyllotoxin and related C-4′ *O*-methylated compounds (7). The introduction of a C-4 *O*-ethyl group in **8** was due to the ability of 4′-demethylepipodophyllotoxin ethyl ether to inhibit human DNA topoisomerase II as reported previously (4).

Picropodophyllone [**3**] was prepared by oxidation of the benzylic alcohol of picropodophyllotoxin [**2**] according to Gensler *et al.* (8). It is noteworthy that the lactone ring has to be epimerized before aromatizaion of **3** with SeO_2 can occur. Alkylation of

[1]For Part 98 see T. Yokoi, L.M. Yang, T. Yokoi, R.Y. Wu and K.H. Lee, *J. Antibiotics*, **41**, 494 (1988); for Part 97 see L.M. Yang, R.Y. Wu, A.T. McPhail, T. Yokoi, T. Yokoi and K.H. Lee, *J. Antibiotics*, **41**, 488 (1988).

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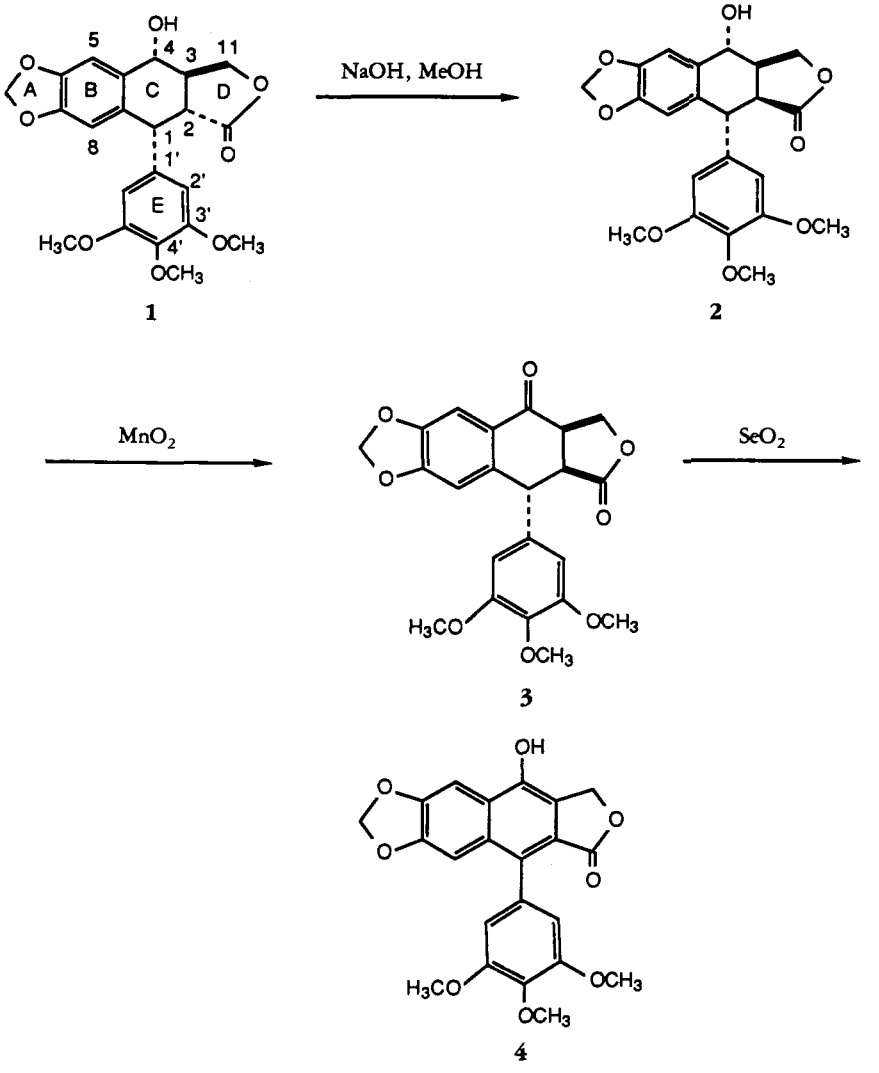


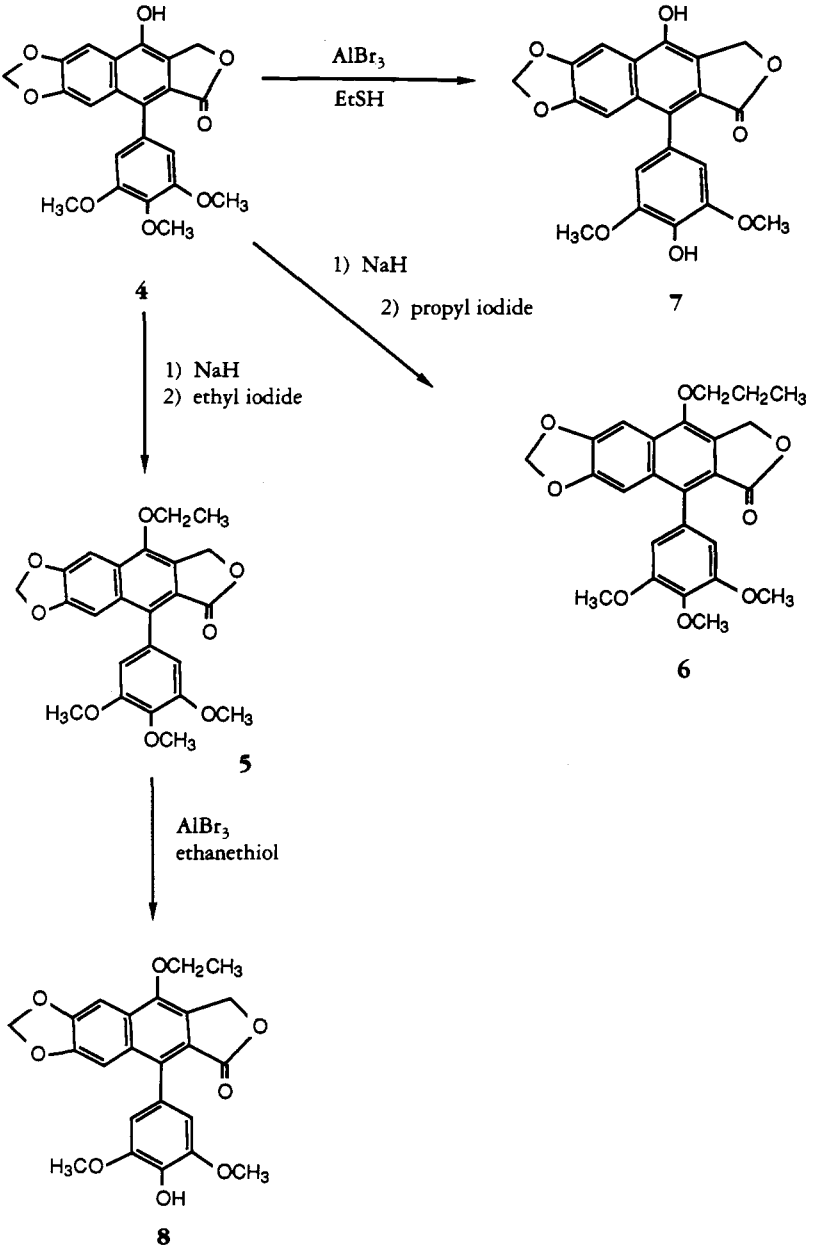
SCHEME 1

the resulting phenol **4** with NaH and an alkyl iodide gave **5** and **6** in 55–60% yield. Subsequent selective 4′-demethylation was achieved by use of $AlBr_3$ and ethanethiol in 50–55% yield.

The cytotoxicity test was carried out in KB tissue culture cells. The screening on human DNA topoisomerase II was done at 200 μM concentration. As seen in Table 1, none of the ring C aromatized compounds **4–8** were shown to have any inhibitory activity against DNA topoisomerase II, indicating that a non-aromatized ring C is structurally required for the potent inhibitory activity against this target enzyme as compounds **11**, **12**, and etoposide show 60–85% inhibition at 200–400 μM. A comparison of the cytotoxicity among the aromatized compounds **4–8** indicates that a free OH at C-4, such as in **4** and **7**, contributes to significant (ED_{50}<4.0 μg/ml) cytotoxicity as the C-4 *O*-substituted derivatives (**5**, **6**, and **8**) are inactive.

EXPERIMENTAL

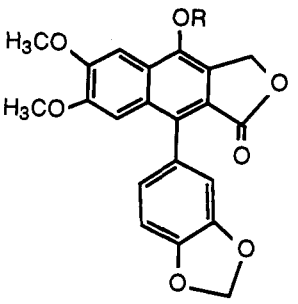
GENERAL EXPERIMENTAL PROCEDURES.—All melting points were taken on a Fisher-Johns melt-



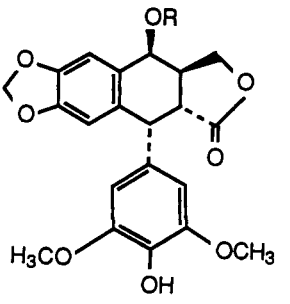
SCHEME 1. Continued.

ing point apparatus and are uncorrected. Ir spectra were recorded on a Perkin-Elmer 1320 spectrophotometer, and ^{1}H-nmr spectra were obtained from a Bruker-250 NMR spectrometer; all chemical shifts are reported in ppm from TMS. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. Mass spectral analyses were determined on a V.G. Micromass 70-70 instrument at 70 eV with a direct inlet system. Analytical tlc was carried out on Merck precoated Si gel 60 F-254. EM Kieselgel 60 (230–400 mesh ASTM) was used for cc. All new compounds were characterized by melting point, ^{1}H-nmr, ir, mass spectral, and elemental analyses.

NATURAL PRODUCT ORIGIN.—Podophyllotoxin [**1**] was isolated from the EtOH extract of the roots and rhizomes of *Podophyllum peltatum* and *Podophyllum hexandrum* using Si gel cc and eluting with a $CHCl_3$-MeOH (20:1) mobile phase. Fractions containing the product were evaporated in vacuo and the podophyllotoxin recrystallized from C_6H_6.



9 R=Me
10 R=H



11 R=H
12 R=Et

PICROPODOPHYLLOTOXIN [**2**].—A modified version of the method used by Thurston *et al.* (4) was used to invert the stereochemistry of the lactone ring. Five grams of **1** (12.1 mmol) was dissolved in 200 ml of MeOH. One gram of NaOH (25 mmol) was added. This was stirred for 5 min during which time a white precipitate formed. The mixture was then acidified to pH 4 with dilute HCl. The excess MeOH was evaporated, and the white solid was collected on a Buchner funnel and washed with cool H_2O. The dried crystals weighed 4.2 grams (84%). Spectral data for **2** are identical to those reported by Jardine and Cassady (7).

PICROPODOPHYLLONE [**3**].—Picropodophyllone was prepared using the method of Gensler *et al.* (8).

DEHYDROPODOPHYLLOTOXIN [**4**].—Dehydropodophyllotoxin was prepared using the method of Gensler *et al.* (8).

DEHYDROPODOPHYLLOTOXIN ETHYL ETHER [**5**].—Dehydropodophyllotoxin [**4**] (1 g) (2.44 mmol) was dissolved in 100 ml of DMSO. NaH (70 mg, 2.9 mmol) was added. This was stirred for 5 min, after which time 1.5 ml of ethyl iodide (18.6 mmol) was added and the mix stirred at room temperature for 30 min before being poured into 250 ml of H_2O. This was extracted with EtOAc and applied to a Si gel column. The product was eluted with a $CHCl_3$ mobile phase and was recrystallized from $CHCl_3$/EtOH to yield 650 mg (60.7%) of **5**: mp 247–250°; ir (KBr) 3050 (C=C-H), 2900 (C-H), 1760 (lactone), 1575, 1450 (aromatic C=C), 1260, 1025 (C-O) cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.58 (s, 1H, H-8), 7.24 (s, $CHCl_3$), 7.07 (s, 1H, H-5), 6.5 (s, 2H, H-2′,6′), 6.1 (s, 2H, OCH_2O), 5.48 (s, 2H, H-11), 4.26 (q, 2H, CH_2), 3.97 (s, 3H, 4′-OMe), 3.85 (s, 6H, 3′,5′-OMe), and 1.55 (t, 3H, Me); ms *m/z* 438. *Anal.* calcd for $C_{24}H_{22}O_8 \cdot \frac{1}{2} H_2O$, C 64.43, H 5.15; found C 64.53, H 5.37.

TABLE 1. Inhibition of DNA Topoisomerase II by Ring C Aromatized Podophyllotoxin Analogues and Related Compounds.

Compounds	Cytotoxicity ED_{50} (KB), 5 μg/ml	% Inhibition of DNA[a] Topoisomerase II
4	4.0	[b]
5	4.0	[b]
6	4.0	[b]
7	2.2	[b]
8	4.0	[b]
11	0.1	50[c]
12	1.1	25[c]
Etoposide	0.1	80[b], 85[c]

[a]Equal to $100-(D_{drug}-D_{-E})/(D_{+E}-D_{-E})$, where D = density of P_4 unknotted DNA form.

[b]No inhibition at 200 μM.

[c]Measured at 400 μM by Thurston *et al.* (4).

DEHYDROPODOPHYLLOTOXIN PROPYL ETHER [**6**].—Compound **4** (1 g) (2.44 mmol) was dissolved in 150 ml of Me_2CO. NaH (90 mg, 3.7 mmol) was added, and the mixture was stirred for 5 min. Next, 1.5 ml of propyl iodide (15.4 mmol) was added and the mixture was stirred at room temperature for 30 min. The mixture was then evaporated in vacuo and chromatographed on a Si gel column. The product was eluted with $CHCl_3$ and was recrystallized from $CHCl_3$/EtOH to give 610 mg (55%) of **6**: mp 220–225°; ir (KBr) 3050 (C=C-H), 2900 (C-H), 1740 (lactone), 1575, 1450 (aromatic C=C), 1225, 1020 (C-O) cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.63 (s, 1H, H-8), 7.25 ($CHCl_3$), 7.07 (s, 1H, H-5), 6.55 (s, 2H, H-2′,6′), 6.1 (s, 2H, OCH_2O), 5.5 (s, 2H, H-11), 4.17 (t, 2H, $OCH_2CH_2CH_3$), 3.95 (s, 3H, 4′-OMe), 1.95 (m, 2H, $OCH_2CH_2CH_3$), 1.25 (t, 3H, $OCH_2CH_2CH_3$); ms *m/z* 452. *Anal.* calcd for $C_{25}H_{24}O_8 \cdot ½$ EtOH, C 65.08, H 5.42; found C 65.24, H 5.43.

4′-DEMETHYLDEHYDROPODOPHYLLOTOXIN [**7**].—$AlBr_3$ (690 mg, 2.58 mmol) was added to 3.5 ml of anhydrous CH_2Cl_2 and 1.5 ml of ethanethiol (20.3 mmol). This was cooled to −10°, and 295 mg of **4** (0.72 mmol) was added. This was stirred for 40 min while the temperature remained at −10°. Next, 20 ml of H_2O was added, and the product was extracted with EtOAc. This was evaporated in vacuo, and the product was recrystallized from EtOH/H_2O to give 92 mg (32%) of **7**: mp 285°; ir (KBr) 3440 (phenol), 3080 (C=C-H), 2900 (C-H), 1755 (lactone), 1600, 1450 (aromatic C=C) cm^{-1}; ^1H nmr (DMSO-d_6) δ 8.45 (s, phenol), 7.6 (s, 1H, H-8), 6.9 (s, 1H, H-5), 6.45 (s, 2H, H-2′,6′), 6.15 (s, 2H, OCH_2O), 5.35 (s, 2H, H-11), 3.7 (s, 6H, 3,5-OMe), and 2.49 (m, CH_3SOCH_3); ms *m/z* 396. *Anal.* calcd for $C_{21}H_{16}O_8$ H_2O, C 60.87, H 4.35; found C 60.68, H 4.50.

4′-DEMETHYLDEHYDROPODOPHYLLOTOXIN ETHYL ETHER [**8**].—Dehydropodophyllotoxin ethyl ether (500 mg, 1.14 mmol) was added to 10 ml of anhydrous CH_2Cl_2, 761 mg of $AlBr_3$ (2.85 mmol), and 1 ml of ethanethiol (13.6 mmol) at −10°. This was stirred at −10° for 40 min, at which time 25 ml of H_2O was added. The product was extracted with $CHCl_3$ and chromatographed on a Si gel column. The product was crystallized from $CHCl_3$/EtOH to yield 265 mg (53%) of **8**: mp 258–260°; ir (KBr) 3350 (phenol), 3070 (C=C-H), 2900 (C-H), 1745 (lactone), 1590, 1450 (aromatic C=C), 1225, 1025 (C-O) cm^{-1}; ^1H nmr ($CDCl_3$) δ 7.58 (s, 1H, H-8), 7.1 (s, 1H, H-5), 6.55 (s, 2H, H-2′,6′), 6.1 (s, 2H, OCH_2O), 5.62 (s, phenol), 5.45 (s, 2H, H-11), 4.25 (q, 2H), 3.85 (s, 6H, 3′,5′-OMe), 1.55 (t, 3H, Me); ms *m/z* 424. *Anal.* calcd for $C_{23}H_{20}O_8$, C 65.09, H 4.72; found C 65.20, H 4.79.

ACKNOWLEDGMENTS

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