Journal of Natural Products
Vol. 58, No. 6, pp. 870-877, June 1995

METHYL ETHERS OF PODOPHYLLOTOXIN-RELATED CYCLOLIGNANS

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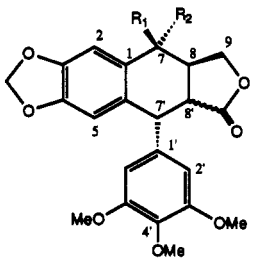
ABSTRACT.—Methyl ethers of podophyllotoxin [**1**] and its epimers at positions 7 and 8′ were obtained through the corresponding chlorinated or brominated derivatives at position 7. Additionally, the corresponding diol methyl ethers were obtained by reducing the lactone with $LiAlH_4$. 7-*O*-Methylepipicropodophyllotoxin [**12**], 7-*O*-methylpicropodophyllotoxin [**13**], the diol methyl ethers **15–18** and the corresponding diacetates are described here for the first time. Most of the cyclolignans obtained were evaluated for their cytotoxic activity.

Lignans are natural products biosynthesized through the shikimic acid-cinnamic acid pathway with a diverse spectrum of biological properties. Podophyllotoxin [**1**], first isolated from several species of *Podophyllum* (Berberidaceae), has applications in clinical medicine for its antineoplastic and other activities (1).

In recent years we have been studying the chemical constituents of *Juniperus* spp. (Cupressaceae) and have identified numerous lignans related to **1** (2–4). The availability of these compounds and the interesting pharmacological properties of some of them have led us to explore the semi-synthesis of podophyllotoxin analogues and their cytotoxic activities.

Derivatives of **1** are known to have two different mechanisms of action as antineoplastic agents: some act by inhibiting the polymerization of tubulin and blocking cell division in the metaphase, while others inhibit the enzyme activity of DNA-topoisomerase II (1). Most of the compounds that act through the latter mechanism are ethers and glycosides of derivatives of the epi- series at position 7. This has led us to consider the possibility of preparing all of the diastereomeric methyl ethers of **1** and its epimers picropodophyllotoxin [**2**], epipodophyllotoxin [**3**], and epipicropodophyllotoxin [**4**], from the corresponding halogenated derivatives at C-7. In this study we also report some modifications of the lactone ring, such as transformation into the corresponding diols and diacetates for subsequent pharmacological assays against several neoplastic cell lines.

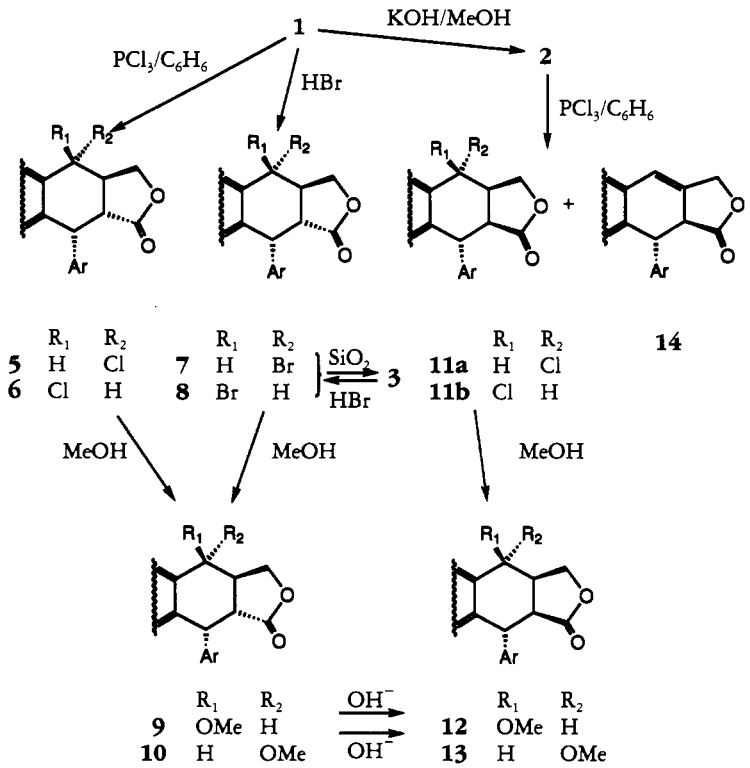
Podophyllotoxin [**1**] was the starting material in all the transformations made in this work and was isolated from commercial Podophyllum resin (*P. peltatum*), by chromatographic procedures.



	R_1	R_2
1	H	OH, H-8′β
2	H	OH, H-8′α
3	OH	H, H-8′β
4	OH	H, H-8′α

RESULTS AND DISCUSSION

The halogenated derivatives were prepared from **1** by reaction with PCl_3 to yield a mixture of the 7α- and 7β-chloro derivatives **5** and **6**, in a 4:6 ratio, respectively (see Scheme 1). The α isomer is described in the literature as a low-yield reaction product of podophyllotoxin and HCl (5). The separation of isomers was not complete, although it was sufficient to be able to characterize them by nmr. The most notable differences observed between them were the chemical shifts and the coupling constants of the H-7 hydrogen atom. In the case of substance **5**, which has an α chlorine atom, H-7 appeared as a doublet at 5.10 ppm (J=10.2 Hz), whereas in the β isomer it resonated at 5.39 ppm (J=3.6 Hz), characteristic for a cis configuration between H-7 and H-8.



SCHEME 1. Preparation of cyclolignan methyl ethers.

Similar results were observed on obtaining the brominated derivatives at position 7, from cyclolignans **1** and **3** by treatment with dry HBr. In both cases the reaction products contained a mixture of the 7α- and 7β-brominated derivatives [**7** and **8**] in a 4:6 ratio. As in the case of **5** and **6**, this was deduced from the ^{1}H-nmr spectrum of the mixture. Two doublets assignable to H-7 were observed at 5.37 and 5.71 ppm with coupling constants of 10.4 and 3.2 Hz, respectively. As in the case of their chlorinated derivatives, these values correspond to an α configuration of the bromine for **7** (H-7 and H-8 trans) and a β disposition for compound **8** (H-7 and H-8 cis).

Attempts to separate the bromo derivatives by chromatography were unsuccessful and led to their transformation into epipodophyllotoxin [**3**]. Both the chloro and bromo derivatives react readily in the presence of nucleophiles such as H_2O and MeOH. Thus,

the reaction of the chloro derivatives with H_2O is one of the procedures used to obtain epipodophyllotoxin from podophyllotoxin (5,6).

Treatment of mixtures of the halogenated derivatives **5** and **6** or **7** and **8** with MeOH afforded methyl ethers **9** and **10**, in both cases in a 16:1 ratio (see Scheme 1); this would confirm that, under these conditions, the mechanism of nucleophilic substitution occurs through benzylic carbocations (5,7).

Epimerization of **1** in base (8) yielded picropodophyllotoxin [**2**], from which we first obtained chloro derivatives **11a** and **11b** and subsequently methyl ether derivatives **12** and **13**, both hitherto unreported in the literature.

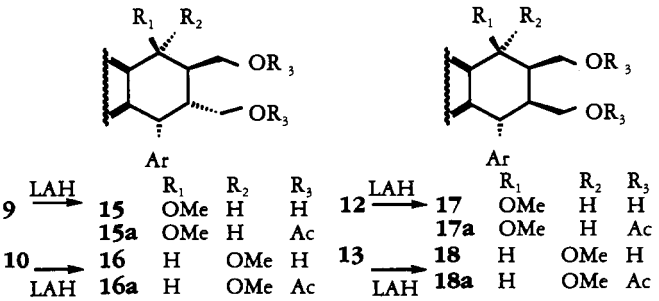
Attempts to obtain the chlorinated derivatives **11a** and **11b** from **2** under the same conditions (benzene reflux with PCl_3) previously applied to **1**, yielded α-apopicropodophyllotoxin **14** (6) and the chloro derivatives **11a,b**. These could not be separated since during cc **11a,b** were hydrolyzed to the hydroxylactones picropodophyllotoxin [**2**] and epipicropodophyllotoxin [**4**]. When the reaction time was reduced to 20 min, only the chloro derivatives were produced. The chloro derivatives were rapidly transformed into **12** and **13**, which were stable and easy to separate by cc.

The stereochemistry of **12** and **13** was determined by study of their ^{1}H-nmr spectra. The signal corresponding to H-7 in **12** appeared as a doublet at 4.25 ppm (J=4.5 Hz), indicating the β configuration of the methoxyl group, whereas in **13** the doublet at 4.04 ppm (J=6.8 Hz) corresponds to the trans configuration for H-7 and H-8. We were able to chemically correlate the methyl ethers derived from the "picro" series with those of the normal series, since epimerization of **9** and **10** in base afforded **12** and **13**, respectively, in quantitative yields.

Once the four methyl ethers had been obtained, reduction to diols with $LiAlH_4$ in Et_2O was carried out in order to maintain the stereochemistry of the different chiral centers (see Scheme 2). This afforded **15–18** and their diacetylated derivatives **15a–18a**, all of them hitherto unreported in the literature; the ^{1}H- and ^{13}C-nmr data (see Tables 2 and 3) were in accordance with the expected stereochemistry for these substances.

Assignment of all signals in the ^{13}C-nmr spectra of these compounds was carried out by comparison with other cyclolignans previously isolated or prepared at our laboratory. Unequivocal assignments were made using two-dimensional heteronuclear ^{1}H-^{13}C correlation experiments (9,10).

Most of the cyclolignans prepared in this work were then evaluated for their cytotoxic activity. The compounds were subjected to screening against P-388 murine leukemia, A-549 human lung carcinoma, and HT-29 colon carcinoma cells (11,12). In general, although these compounds are less potent than **1**, those derivatives having the lactone ring showed stronger activity than the non-lactone derivatives. Only α-apopicropodophyllin [**14**] exhibited cytotoxic activity similar to that of **1** (Table 1).



SCHEME 2. Reduction of the lactone ring of lignans **9**, **10**, **12**, and **13** with $LiAlH_4$.

TABLE 1. Cytotoxic Activity of Some Podophyllotoxin Derivatives Against Three Cancer Cell Lines.[a]

Compound	Cell Line		
	P-388	A-549	HT-29
1	0.012	0.012	0.0029
2	6.0	6.0	6.0
3	0.06	0.06	0.06
6	0.6	0.6	1.2
9	0.06	0.06	1.2
10	0.06	0.06	0.06
12	0.1	0.13	0.06
13	0.12	0.12	0.23
14	0.013	0.025	0.025
15	11.6	11.6	11.6
15a	9.7	9.7	9.7
16a	9.7	9.7	9.7
17	11.6	11.6	11.6
17a	9.7	9.7	9.7
18	23.2	46.3	23.2
18a	19.4	>38.8	19.4

[a]Data are expressed as IC_{50} values (μm).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined by heating in an external silicone bath and are uncorrected. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter in $CHCl_3$ and uv spectra were obtained on a Hitachi 100-60 spectrophotometer in EtOH solution. Ir spectra were obtained on a Beckman (Acculab VIII) spectrophotometer in $CHCl_3$. Nmr spectra were recorded at 200 MHz for ^1H and 50.3 MHz for ^{13}C in $CDCl_3$ using TMS as internal reference, on a Bruker WP 200 SY instrument. Chemical shift values are expressed in ppm and coupling constants (J) are in Hz. Unless otherwise stated, flash chromatography was performed on Si gel (Merck No. 9385). Tlc and prep. tlc were carried out on Si gel 60 F_{245} (Merck, 0.25-mm thick) and Si gel 60 $F_{254}S$ (Merck, 1-mm thick), respectively. Solvents and reagents were purified by standard procedures as necessary. Elemental analysis was carried out on a Perkin-Elmer 2400 CHN elemental analyzer.

PODOPHYLLOTOXIN [**1**].—*Podophyllum peltatum* resin (50 g) was extracted with hot $CHCl_3$. The soluble fraction was chromatographed on a neutral alumina (activity II) and eluted with $CHCl_3$ to yield **1** (20.4 g, 40.8 %).

REACTION OF PODOPHYLLOTOXIN [**1**] WITH PCl_3.—One gram of **1** was dissolved in 20 ml of dry C_6H_6 and PCl_3 (0.5 ml) was added. The mixture was maintained under reflux for 1 h under an Ar atmosphere. The solution was separated from the yellow residue by filtration through a Buchner funnel. The residue was washed well in hot C_6H_6 and the solvent was evaporated to obtain 1.17 g of reaction product. Flash chromatography of 560 mg of the reaction product afforded 26 mg of a mixture enriched in 7β-chloro-7-deoxypodophyllotoxin [**5**] (^1H-nmr data, see Table 1); 208 mg of a mixture of **5** and **6**; 138 mg of a mixture enriched with 7α-chloro-7-deoxyepipodophyllotoxin [**6**] (^1H-nmr data, see Table 2; ^{13}C-nmr data, see Table 3); and 162 mg of epipodophyllotoxin [**3**].

REACTION OF **1** AND **3** WITH HYDROGEN BROMIDE.—Podophyllotoxin [**1**] (200 mg) was dissolved in 40 ml of dry CH_2Cl_2 and a current of dry HBr was passed through the solution for 30 min followed by a current of N_2 for 10 min. The reaction product (230 mg) represented a mixture of 7-bromo-7-deoxypodophyllotoxin [**7**] (^1H-nmr data, see Table 2; ^{13}C-nmr data, see Table 3) and 7-bromo-7-deoxyepipodophyllotoxin [**8**] (^1H-nmr data, see Table 2; ^{13}C-nmr data, see Table 3), and was used for the next step without purification.

Compound **3** (100 mg) dissolved in dry CH_2Cl_2 (20 ml) was subjected to a current of dry HBr in a similar manner to yield a mixture of **7** and **8** (118 mg).

7-*O*-METHYLEPIPODOPHYLLOTOXIN [**9**] AND 7-*O*-METHYLPODOPHYLLOTOXIN [**10**].—A mixture of **5**

TABLE 2. ^{1}H-Nmr Data for Compounds **5–10**, **12**, **13**, and **15–18a** [δ values; *J* (Hz) are expressed in parentheses].

Proton	Compound							
	5	**6**	**7**	**8**	**9**	**10**	**12**	**13**
2	7.15, s	6.88, s	7.25, s	6.95, s	6.82, s	7.05, s	6.88, s	6.84, s
5	6.50, s	6.49, s	6.56, s	6.55, s	6.53, s	6.51, s	6.46, s	6.40, s
7	5.10, d (10.2)	5.39, d (3.6)	5.37, d (10.4)	5.71, d (3.2)	4.31, d (3.6)	4.56–4.65, m	4.25, d (4.5)	4.04, d (6.8)
8	3.15, m	3.20, m	2.8–3.0, m	2.8–3.0, m	2.8–3.0, m	2.90, m	3.0–3.2, m	2.91, m
9	4.60, dd (8.9, 6.7)	4.39 d (8.6)	4.65, m	4.65, m	4.32, d (9.0)	4.12, t (9.3)	4.38, d (8.1)	4.43, dd (9.4,6.9)
	4.02, dd (10.5,8.9)		4.45, m	4.45, m		4.56–4.65, m	4.37, d (4.5)	4.24, dd (9.4,3.5)
2′,6′	6.38, s	6.28, s	6.48, s	6.36, s	6.26, s	6.38, s	6.44, s	6.44, s
7′	4.60, d (4.1)	4.65, d (6.2)	4.76, d (5.4)	4.76, d (5.4)	4.59, d (5.3)	4.56–4.65, m	4.31, d (5.0)	4.11, d (4.8)
8′	2.83, dd (14.5,4.1)	3.37, dd (13.7,5.2)	3.45, dd (13.5,5.4)	3.45, dd (13.5,5.4)	3.38, dd (14.0,5.3)	2.80, dd (14.2,4.3)	3.34, dd (10.6,5.0)	3.21, dd (9.7, 4.8)
MeO-3′,5′	3.75, s	3.74, s	3.85, s	3.82, s	3.72, s	3.74, s	3.81, s	3.77, s
MeO-4′	3.81, s	3.79, s	3.89, s	3.87, s	3.78, s	3.81, s	3.86, s	3.81, s
-O-CH_2-O-	6.00, d (1.3)	5.99, d (1.1)	6.06, d (1.9)	6.06, d (1.9)	5.96, d (1.0)	5.97, s	5.93, d (1.0)	5.95, d (1.4)
	5.89, d (1.3)	5.97, d (1.1)	6.05, d (1.9)	6.05, d (1.9)	5.93, d (1.0)	5.96, s	5.95, d (1.0)	5.93, d (1.4)
MeO-7					3.45, s	3.43, s	3.26, s	3.45, s
	15	**15a**	**16**	**16a**	**17**	**17a**	**18**	**18a**
2	6.76, s	6.80, s	6.98, s	6.97, s	6.99, s	6.99, s	6.69, s	6.71, s
5	6.44, s	6.44, s	6.39, s	6.43, s	6.27, s	6.28, s	6.35, s	6.32, s
7	4.42, d (3.1)	4.28, d (3.1)	4.65, d (8.6)	4.59, d (8.4)	4.55, d (4.4)	4.46, d (4.5)	4.11, d (3.0)	4.22, d (3.3)
8	2.20, m	2.50, m	2.6–2.8, m	2.4–2.6, m	2.15, m	2.36, m	2.52, m	2.74, m
9	4.03, dd (11.7,2.7)	4.1–4.3, m	3.5–3.9, m	4.36, dd (11.5,2.8)	3.6–3.9, m	4.33, dd (11.3,5.4)	3.4–3.8, m	3.80, dd (11.5,8.0)
	3.68, m	4.05, dd (11.5,5.5)		4.25, dd (11.5,4.2)		4.25, dd (11.3,7.8)		4.23, dd (11.5,5.4)
2′,6′	6.17, s	6.15, s	6.33, s	6.30, s	6.30, s	6.23, s	6.40, s	6.34, s
7′	4.26, d (6.0)	4.23, d (6.4)	4.09, d (4.0)	4.13, d (6.3)	4.01, d (9.7)	3.84, d (9.2)	3.94, d (10.3)	3.55, d (10.8)
8′	2.82, m	2.78, m	2.6–2.8, m	2.4–2.6, m	2.69, m	2.79, m	2.52, m	2.92, m
9′	3.85, m	4.1–4.3, m	3.5–3.9, m	4.15, dd (11.1,2.6)	3.6–3.9, m	4.06, dd (11.3,7.4)	3.4–3.8, m	4.16, dd (11.3,9.3)
	3.54, dd (11.3,5.9)	3.75, m		4.10, m		3.98, dd (11.3,6.0)		3.98, dd (11.3,5.0)
MeO-3′,5′	3.74, s	3.74, s	3.75, s	3.74, s	3.78, s	3.79, s	3.77, s	3.79, s
MeO-4′	3.80, s′	3.81, s	3.82, s	3.82, s	3.83, s	3.83, s	3.82, s	3.82, s
-O-CH_2-O-	5.93, s	5.93, s	5.93, s	5.93, s	5.87, s	5.90, d (1.2)	5.88, s	5.89, s
		5.91, s	5.92, s			5.88, d (1.2)	5.87, s	5.87, s
MeO-7	3.47, s	3.43, s	3.45, s	3.43, s	3.60, s	3.59, s	3.42, s	3.46, s
OAc		2.07, s		2.07, s		2.02, s		2.02, s
		1.98, s		2.00, s		2.02, s		2.00, s

TABLE 3. ^{13}C-Nmr Data for Compounds **6–10**, **12**, **13**, and **15–18a**.

Carbon	Compound														
	6	**7**	**8**	**9**	**10**	**12**	**13**	**15**	**15a**	**16**	**16a**	**17**	**17a**	**18**	**18a**
1	131.4	131.7	130.9	132.1	132.0	131.6	131.3	127.4	128.5	129.8	129.3	129.6	129.7	127.4	126.5
2	110.1	108.9	109.4	108.4	107.0	107.5	107.1	108.6	108.6	107.1	107.2	106.2	106.9	109.5	109.9
3	148.9	147.0	148.5	148.2	147.7	147.7	147.7	148.1	148.0	147.4	147.4	147.1	147.4	147.9	148.2
4	147.5	147.8	147.1	146.5	147.7	146.6	146.8	145.8	146.2	147.0	147.2	146.3	146.6	146.0	146.2
5	109.6	109.7	109.6	110.6	109.7	109.6	109.7	110.5	110.3	109.1	109.2	109.5	109.6	110.0	109.7
6	129.6	129.5	130.0	129.0	130.6	129.2	128.9	133.8	132.7	133.3	132.8	131.1	130.6	133.9	133.2
7	59.0	55.9	53.3	75.8	80.9	78.1	79.0	82.7	77.5	78.0	77.9	80.7	78.7	79.5	78.6
8	43.7	42.0	37.2	38.2	37.2	38.4	40.8	38.8	36.9	41.4	38.4	40.7	35.6	41.7	37.4
9	68.9	70.5	70.7	67.3	71.5	66.5	70.0	64.2	64.1	61.3	62.4	59.1	61.7	61.0	62.2
1′	134.8	134.2	134.9	135.4	135.5	137.8	139.1	137.1	137.5	137.7	136.7	140.9	140.4	141.0	140.3
2′	108.2	107.6	108.0	108.3	108.2	106.0	106.1	107.2	107.8	107.6	107.9	106.5	106.3	106.6	106.3
3′	152.6	152.2	152.2	152.4	152.7	153.5	153.4	152.9	153.0	153.0	153.0	153.4	153.4	153.2	153.4
4′	137.3	136.8	137.0	137.2	140.8	137.3	137.2	137.9	137.6	137.7	—	139.2	140.4	136.8	137.2
5′	152.6	152.2	152.2	152.4	152.7	153.5	153.4	152.9	153.0	153.0	153.0	153.4	153.4	153.2	153.4
6′	108.2	107.6	108.0	108.3	108.2	106.0	106.1	107.2	107.8	107.6	107.9	106.5	106.3	106.6	106.3
7′	40.7	43.9	43.4	43.6	45.5	44.3	44.9	48.1	47.1	48.0	47.1	45.8	46.9	44.4	46.0
8′	37.7	46.9	41.2	40.9	44.0	45.1	45.1	37.1	35.9	38.3	34.9	45.4	42.6	42.7	38.0
9′	173.9	173.0	173.4	174.8	174.1	178.7	178.1	63.8	64.5	64.5	64.3	63.1	65.4	63.0	64.7
MeO-3′,5′	56.2	60.2	60.2	56.1	56.2	56.3	56.2	56.2	56.4	56.3	56.2	56.3	56.3	56.2	56.2
MeO-4′	60.6	55.9	55.9	60.5	60.8	60.6	60.7	60.8	60.8	60.8	60.8	60.9	60.8	60.8	60.8
-O-CH_2-O-	101.7	101.5	101.5	101.3	101.5	101.1	101.2	101.1	101.1	101.1	101.1	100.9	101.0	101.1	101.1
MeO-7				57.7	55.4	56.8	57.8	56.9	57.4	54.4	54.7	57.5	57.5	55.9	56.2
OAc									21.0		20.8		20.8		20.8
									20.8						
OAc									170.8		170.6		170.7		170.8
									170.6		170.4				

and **6** (400 mg) was dissolved in MeOH and left to stand at room temperature overnight. The MeOH was evaporated and the reaction product was chromatographed. Compounds **9** and **10** were isolated using CH_2Cl_2-EtOAc (9:1) as eluent.

Compound **9** (320 mg, 81%): mp 206–208° (MeOH); $[\alpha]^{25}D$ −83.5° (c=1, $CHCl_3$); uv (EtOH) λ max (ε) 219 (19200), 290 (3900) nm; ir ν max 1780 (C=O), 1600, 1510, 1490, 1470, 1425, 1340, 1290, 1240, 1130, 1090, 1045, 1010, 940, 860 cm^{-1}; *anal.*, calcd for $C_{23}H_{24}O_8$: C, 64.48; H, 5.60; found: C, 64.13; H, 5.53; ^{1}H-nmr data, see Table 2; ^{13}C-nmr data, see Table 3.

Compound **10** (21 mg, 5.3%): $[\alpha]^{25}D$ −111.6° (c=0.7, $CHCl_3$); uv (EtOH) λ max (ε) 214 (20600), 288 (3000) nm; ir ν max 1785 (C=O), 1600, 1505, 1485, 1470, 1425, 1380, 1335, 1240, 1130, 1100, 1045, 1010, 940, 880 cm^{-1}; ^{1}H-nmr data, see Table 2; ^{13}C-nmr data, see Table 3.

Anhydrous $BaCO_3$ (200 mg) was added to a mixture of **7** and **8** (220 mg) dissolved in absolute MeOH, which was then maintained at room temperature for 5 h. After filtering and extracting with EtOAc, **9**, containing traces of **10**, was isolated (150 mg).

EPIMERIZATION AT C-8′ OF **1**, **9**, AND **10** WITH 5% KOH/MEOH.—All epimerizations were carried out in the following manner: the derivative of the normal series was dissolved in 5% KOH/MeOH and stirred for several h at room temperature. It was then acidified with 2 N HCl, the MeOH evaporated, and the corresponding derivative of the "picro" series extracted with EtOAc: **1**, **9**, and **10** yielded, respectively, **2**, **12** and **13**.

7-*O*-METHYLEPIPICROPODOPHYLLOTOXIN [**12**] AND 7-*O*-METHYLPICROPODOPHYLLOTOXIN [**13**].—*Method a: Reaction of picropodophyllotoxin* [**2**] *with* PCl_3.—A quantity of **2** (150 mg) was dissolved in dry C_6H_6 (5 ml) and PCl_3 (0.2 ml) was added; the mixture was maintained under reflux for 1 h. After work up as described for **1**, the reaction product (130 mg) was obtained, from which α-apopicropodophyllotoxin [**14**] (76 mg) and a mixture of **3** and **4** (55 mg) were isolated by flash chromatography.

Method b.—A quantity of **2** (100 mg) was dissolved in dry C_6H_6 (5 ml) and PCl_3 (0.2 ml) was added. After 1 h at room temperature and evaporation of the solvent, the reaction product (105 mg) was obtained and shaken with MeOH for 10 min; this was then chromatographed with CH_2Cl_2-EtOAc (95:5), yielding **14** (20 mg); **12** and **13** (68 mg); and **3** and **4** (10 mg).

Method c.—PCl_3 (0.2 ml) was added to a solution of **2** (150 mg) in C_6H_6 (5 ml). After 20 min at room temperature, MeOH was added to the mixture and it was shaken for 10 min. The reaction product was chromatographed with hexane-EtOAc (1:1), yielding a mixture of **12** and **13** (108 mg) and **2** (15 mg, 10%). Using prep. tlc of the previous mixture and eluting with CH_2Cl_2-EtOAc (85:15), compounds **12** and **13** were separated.

Compound **12** (35 mg, 23%): mp 204–206° (MeOH/CH_2Cl_2); $[\alpha]^{25}D$ +24.1° (c=1, $CHCl_3$); uv (EtOH) λ max (ε) 217 (19,800), 290 (3600) nm; ir ν max 1770 (C=O), 1600, 1510, 1490, 1470, 1430, 1390, 1330, 1250, 1130, 1040, 1000, 930, 870, 810, 750 cm^{-1}; *anal.*, calcd for $C_{23}H_{24}O_8$: C, 64.48, H, 5.60; found: C, 63.87, H, 5.63; ^{1}H-nmr data, see Table 2; ^{13}C-nmr data, see Table 3.

Compound **13** (62 mg, 40%): mp 212–214° (MeOH/CH_2Cl_2); $[\alpha]^{25}D$ +36.2° (c=1.01, $CHCl_3$); uv (EtOH) λ max (ε) 222 (13700), 292 (4500) nm; ir ν max 1780 (C=O), 1600, 1500, 1480, 1465, 1420, 1330, 1220, 1130, 1100, 1040, 1010, 940 cm^{-1}; *anal.*, calcd for $C_{23}H_{24}O_8$: C, 64.48, H, 5.60; found: C, 63.91, H, 5.34; ^{1}H-nmr data, see Table 2; ^{13}C-nmr data, see Table 3.

REDUCTION OF LACTONE-METHYL ETHERS WITH $LiAlH_4$.—Compound **9** (35 mg) was suspended in dry ether and $LiAlH_4$ (50 mg) was added. After 12 h at room temperature in an inert atmosphere and after removal of excess hydride, the reaction product (34 mg) was obtained. The product was chromatographed to yield 7-*O*-methylepipodophyllol [**15**] (22 mg, 44%): $[\alpha]^{25}D$ −128.9° (c=0.48, $CHCl_3$); uv (EtOH) λ max (ε) 219 (19300), 292 (4000) nm; ir ν max 3420 (OH), 2930, 1590, 1500, 1485, 1465, 1420, 1390, 1330, 1240, 1135, 1070, 1040, 1020, 945 cm^{-1}; *anal.*, calcd for $C_{23}H_{28}O_8$: C, 63.88, H, 6.53; found: C, 63.53, H, 6.53; ^{1}H-nmr data, see Table 2; ^{13}C-nmr data, see Table 3.

Acetate **15a**.—$[\alpha]^{25}D$ −105.6° (c=1, $CHCl_3$); uv (EtOH) λ max (ε) 218 (26800), 292 (4100) nm; ir ν max 1740 (C=O), 1600, 1510, 1490, 1470, 1425, 1375, 1340, 1240, 1135, 1090, 1045, 945, 915, 875 cm^{-1}; ^{1}H-nmr data, see Table 2; ^{13}C-nmr data, see Table 3.

7-*O*-Methylpodophyllol [**16**] (16 mg, 83%) was obtained from **10** (19 mg) when treated in the same manner as **9**: $[\alpha]^{25}D$ −96.1° (c=1, $CHCl_3$); uv (EtOH) λ max (ε) 214 (25700), 292 (3200) nm; ir ν max 3520 (OH), 3420, 1590, 1500, 1470, 1465, 1420, 1330, 1240, 1130, 1040, 1015, 940 cm^{-1}; *anal.*, calcd for $C_{23}H_{28}O_8$: C, 63.88, H, 6.53; found: C, 63.21, H, 6.32; ^{1}H-nmr data, see Table 2; ^{13}C-nmr data, see Table 3.

Acetate **16a**.—$[\alpha]^{25}D$ −132.5° (c=0.4, $CHCl_3$); uv (EtOH) λ max (ε) 207 (28700), 292 (1800) nm; ir ν max 1740 (C=O), 1600, 1510, 1485, 1470, 1425, 1400, 1375, 1330, 1240, 1130, 1100, 1040, 1010, 945 cm^{-1}; ^{1}H-nmr data, see Table 2; ^{13}C-nmr data, see Table 3.

7-*O*-Methylepipicropodophyllol [**17**] (25 mg, 71%) was obtained from **12** (35 mg) when treated in the same manner as **9**: $[\alpha]^{25}D$ −28.8° (*c*=0.63, $CHCl_3$); uv (EtOH) λ max (ε) 215 (21400), 290 (3200) nm; ir ν max 3400 (OH), 1600, 1500, 1480, 1465, 1420, 1330, 1200, 1130, 1040, 1005, 935, 875 cm^{-1}; *anal.*, calcd for $C_{23}H_{28}O_8$: C, 63.88, H, 6.53; found: C, 63.60, H, 6.03; ^{1}H-nmr data, see Table 2; ^{13}C-nmr data, see Table 3.

Acetate **17a**.—Mp 152–158° (hexane/EtOAc); $[\alpha]^{25}D$ −43.3° (*c*=1, $CHCl_3$); uv (EtOH) λ max (ε) 214 (41100), 292 (5000) nm; ir ν max 1740 (C=O), 1600, 1510, 1490, 1470, 1425, 1375, 1335, 1220, 1135, 1050, 940, 880 cm^{-1}; ^{1}H-nmr data, see Table 2; ^{13}C-nmr data, see Table 3.

7-*O*-Methylpicropodophyllol [**18**] (25 mg, 83%) was obtained from **13** (30 mg) when treated in the same manner as **9**: $[\alpha]^{25}D$ −58.0° (*c*=0.5, $CHCl_3$); uv (EtOH) λ max (ε) 211 (35000), 292 (3500); ir ν max 3630, 3400 (OH), 1600, 1510, 1490, 1470, 1430, 1400, 1340, 1210, 1140, 1100, 1050, 985, 950 cm^{-1}; *anal.*, calcd for $C_{23}H_{28}O_8$: C, 63.88, H, 6.53; found: C, 63.45, H, 6.30; ^{1}H-nmr data, see Table 2; ^{13}C-nmr data, see Table 3.

Acetate **18a**.—$[\alpha]^{25}D$ −69.7° (*c*=1.14, $CHCl_3$); uv (EtOH) λ max (ε) 212 (64100), 292 (5400); ir ν max 1740 (C=O), 1600, 1510, 1490, 1470, 1425, 1375, 1340, 1210, 1130, 1100, 1040, 940, 875 cm^{-1}; ^{1}H-nmr data, see Table 2; ^{13}C-nmr data, see Table 3.

Cytotoxicity assays.—Cytotoxic activity was screened against the cell lines P-388, A-549, and HT-29, as described previously (11,12).

ACKNOWLEDGMENTS

Financial support for this work came from DGICYT, Spain (PB 93/616) and Junta de Castilla y León (Consejería de Cultura y Turismo, SA-64/12/92). We thank Dr. B. Macias, Universidad de Salamanca, for the elemental analyses.

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Received 30 December 1994