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CYTOTOXIC CONSTITUENTS FROM *HYPTIS VERTICILLATA*[1]

MIRIAM NOVELO, JOSÉ G. CRUZ, LOURDES HERNÁNDEZ,[2] ROGELIO PEREDA-MIRANDA,*

Laboratorio de Fitoquímica, Departamento de Farmacia, División de Bioquímica y Farmacia, Facultad de Química, Universidad Nacional Autónoma de México, Apartado Postal 70-265, C.P. 04511, D.F., México

HEEBYUNG CHAI, WOONGCHON MAR, and JOHN M. PEZZUTO

Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612

ABSTRACT.—A new cytotoxic (P-388 ED_{50} 4 μg/ml) arylnaphthalene lignan has been isolated from the Mexican medicinal plant *Hyptis verticillata* (Lamiaceae) and characterized as 5-methoxydehydropodophyllotoxin [**1**]. Eight additional lignans were also obtained by bioactivity-directed fractionation using the brine shrimp lethality test. Of these, the dehydro-β-peltatin methyl ether **2** (P-388 ED_{50} 1.8 μg/ml) is reported for the first time as a natural product isolate. The other bioactive compounds were identified as dehydropodophyllotoxin [**3**], deoxydehydropodophyllotoxin [**4**], (−)-yatein [**5**], 4′-demethyldeoxypodophyllotoxin [**6**], isodeoxypodophyllotoxin [**7**], deoxypicropodophyllin [**8**], and β-apopicropodophyllin [**9**]. Each of these compounds was evaluated against a panel of cell lines comprising a number of human cancer cell types [breast, colon, fibrosarcoma, lung, prostate, KB, and KB-VI (a multi-drug resistant cell line derived from KB)] and murine lymphocytic leukemia (P-388). Lignans **1–4** showed marginal cytotoxic activity against the human cell lines tested. In contrast, compounds **5–9** demonstrated a general nonspecific activity comparable to that of podophyllotoxin [**12**] (ED_{50} $<10^{-2}$ μg/ml). In addition, the antimitotic potential of these compounds was determined in the astrocytoma (ASK) assay. Finally, the plant was also shown to contain the flavonoid sideritoflavone (KB ED_{50} 1.6 μg/ml) and the known pentacyclic triterpenoids ursolic, maslinic, 2α-hydroxyursolic, and oleanolic acids.

Hyptis verticillata Jacq. (Lamiaceae) is a bitter-aromatic herb widely used by the rural population in tropical regions of Mexico (1–4). The leaves of this medicinal plant, popularly known as "hierba martina," are used orally in the treatment of headache, stomachache, and gastrointestinal disorders (3–5). The whole plant is boiled and rubbed for rheumatism (2) and skin infections (4) and used as a bath for undiagnosed ailments (1–5). It is also valued for its anthelmintic and cathartic properties (5). The only previous chemical study of this plant has revealed the presence of two aryltetralin lignans, 4′-demethyldeoxypodophyllotoxin [**6**] and β-peltatin [**10**], which accounted for the antimitotic action of the *H. verticillata* aqueous extracts (6). Recently, organic extracts derived from the aerial parts of this plant were found to demonstrate strong antibacterial activity in qualitative assays (4,7).

As a part of our ongoing investigation on Mexican medicinal plants (8), we now report the isolation and identification of a novel arylnaphthalene lignan, **1**. In addition to the previously reported bioactive compound **6**, seven other lignans were also obtained as the cytotoxic constituents of *H. verticillata*: three arylnaphthalene lignans, including dehydro-β-peltatin methyl ether [**2**], dehydropodophyllotoxin [**3**], and deoxydehydropodophyllotoxin [**4**], a dibenzylbutyrolactone, (−)-yatein [**5**], and three compounds bearing an aryltetralin nucleus, namely isodeoxypodophyllotoxin [**7**], deoxypicropodophyllin [**8**], and β-apopicropodophyllin [**9**]. To the best of our knowl-

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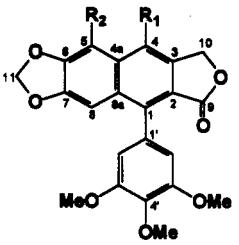
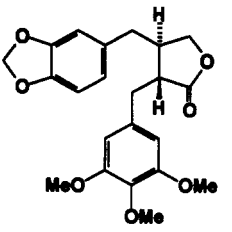
[2]Departamento de Farmacia, Escuela Nacional de Ciencias, Biológicas, Instituto Politécnico Nacional.



1 R_1=OH, R_2=OMe
2 R_1=H, R_2=OMe
3 R_1=OH, R_2=H
4 R_1=R_2=H



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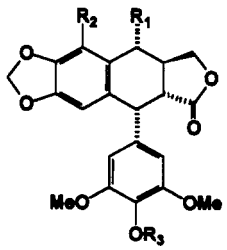
edge, compound **2** is hitherto unreported from any natural source. However, it has been obtained by dehydrogenation of β-peltatin B methyl ether [**11**] (9).

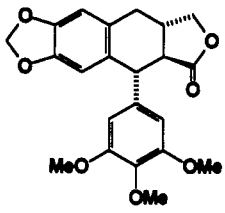
RESULTS AND DISCUSSION

In an initial screening of plant extracts for biological activity, the $CHCl_3$-soluble extract of *H. verticillata* was found to have a noteworthy activity in the brine shrimp lethality test (BST; LC_{50} 13.4 μg/ml). This extract showed significant cytotoxicity when tested with cultured mammalian cells (P-388, ED_{50} 0.3 μg/ml; KB, ED_{50} 0.4 μg/ml). When this $CHCl_3$ extract was subjected to Si gel cc, one of eight combined fractions was found to demonstrate significant cytotoxic activity (BST, LC_{50} 7.9 μg/ml; P-388, ED_{50} 0.1 μg/ml; KB, ED_{50} 0.09 μg/ml). Bioassay-directed fractionation of this active fraction, using lethality to brine shrimp as a monitor, yielded compounds **1–9**. Table 1 summarizes the activities of the total extract, the bioactive fraction (pool IV), and the cytotoxic isolates **1–9** in the preliminary biological screening. Also shown in this table is the cytotoxic activity of these samples with cultured P-388 cells. In general, P-388 cells were more sensitive to the cytotoxic effect mediated by lignans **1-9**, but the activity did show a good correlation with that demonstrated in the BST.

Compound **1** exhibited a molecular formula of $C_{23}H_{20}O_9$, based on its hreims data. The uv absorption maxima at 268, 325, and 357 nm were a clear indication for the presence of a naphthalene nucleus. The ir spectrum showed OH and carbonyl absorption at 3400 and 1763 cm^{-1}, respectively. Consonant with the presence of a phenolic function, the spectrum underwent a bathochromic shift on addition of alkali. The ^{1}H-nmr spectrum of compound **1** was quite comparable to that obtained for dehydro-β-peltatin methyl ether [**2**] (9). The only differences were the absence of the low-field



6 R_1=R_2=R_3=H
9 R_1=R_2=H, R_3=Me, Δ^2
10 R_1=H, R_2=OH, R_3=Me
11 R_1=H, R_2=OMe, R_3=Me
12 R_1=OH, R_2=H, R_3=Me



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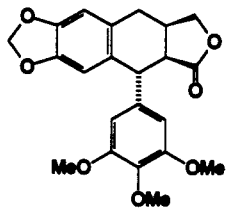

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TABLE 1. Screening for Cytotoxic Activity of Total Extract, Bioactive Fraction IV, and Pure Isolates **1–9** from *Hyptis verticillata*.

Sample	BST[a]	P-388[b]
$CHCl_3$ Extract	13.4	0.3
Pool IV	7.9	0.1
1	>500	4.0
2	434.7	1.8
3	255.0	>5
4	>500	>5
5	2.8	0.4
6	0.2	0.005
7	>500	>20
8	141.5	0.1
9	0.2	0.002
Podophyllotoxin[c] [**12**]	0.2	0.003

[a]Brine shrimp lethality test, LC_{50} μg/ml.
[b]Murine lymphocytic leukemia; ED_{50} μg/ml.
[c]Antitumor agent used as standard for comparison.

singlet at δ 8.16, which was ascribed to the proton on C-4 of compound **2**, and the presence of a phenolic-OH proton resonance at δ 9.58, which disappeared on addition of D_2O. Verification of the proposed C-4 position for the OH group on lignan **1** was provided by ^{1}H-nmr nOe experiments. As expected, any enhancement was observed for the methylene protons of the lactone ring (δ 5.34) on irradiation of the signal belonging to the MeO group on the naphthalene nucleus (δ 4.29). This result was in full agreement with the placement of the MeO substituent at C-5. In addition, the H-8 proton (δ 6.87) showed reciprocal nOe's with the singlet at δ 6.48 for the equivalent methine protons on ring C. Assignment of the ^{13}C nmr (Table 2) of compounds **1** and **2** was achieved by selective INEPT experiments (10). The unambiguous chemical shifts values obtained for **2** were used, together with the additive effects induced on the naphthalene nucleus by the introduction of an OH group at C-4 (11), to assign the resonances of compound **1**. Accordingly, these spectral data provided conclusive evidence for the formulation of **1** as 5-methoxydehydropodophyllotoxin.

Known lignans, dehydro-β-peltatin methyl ether [**2**] (9), dehydropodophyllotoxin [**3**] (12), deoxydehydropodophyllotoxin [**4**] (12), (−)-yatein [**5**] (13), 4′-demethyldeoxypodophyllotoxin [**6**] (14), isodeoxypodophyllotoxin [**7**] (15), deoxypicropodophyllin [**8**] (16), and β-apopicropodophyllin [**9**] (17), were also isolated and characterized by comparison of physical and spectroscopic data (uv, ir, and ^{1}H-nmr)

TABLE 2. ^{13}C-nmr Spectra of Lignans **1**, **2**, and **6–9**.[a]

Carbon	Compound					
	1[b]	**2**[b]	**6**[c]	**7**	**8**	**9**
C-1	132.03	139.96	32.40	40.13	32.98	42.71
C-2	120.43	119.16	46.88	48.71	46.36	123.74
C-3	122.98	138.99	43.51	46.70	45.30	157.33
C-4	147.58	113.95	32.34	32.99	32.04	29.18
C-4a	116.00	128.92	131.20	127.72	130.38	129.61
C-5	130.55	135.62	109.36	108.40	108.76	109.52
C-6	136.30	136.02	146.76	146.40	146.79	147.24
C-7	149.10	149.58	146.47	146.63	146.69	147.03
C-8	100.07	98.33	110.26	109.96	109.81	107.74
C-8a	132.75	130.47	131.20	132.23	128.22	128.11
C-9	169.65	169.65	174.44	175.28	178.31	166.10
C-10	66.65	68.26	71.26	70.94	72.72	70.98
C-11	101.81	101.64	100.80	101.09	100.95	101.30
C-1′	130.28	130.37	136.05	138.65	138.13	138.29
C-2,′ -6′	107.18	107.18	108.29	106.49	104.86	105.55
C-3′, -5′	152.85	152.82	147.67	153.08	153.28	153.22
C-4′	136.30	137.59	128.79	136.60	136.67	137.00
3′-, 5′-OMe	56.10	56.11	56.07	56.24	56.17	56.13
4′-OMe	60.90	60.13	—	60.84	60.84	60.75
5-OMe	61.02	60.94	—	—	—	—

[a]Measured at 75.4 MHz in $CDCl_3$ (δ TMS=0).
[b]Assignments confirmed by SINEPT.
[c]Recorded in C_6D_6–DMSO-d_6 (4:1).

with the literature values. Full assignments of their ^{13}C-nmr spectra were carried out for their structural identification (Table 2).

The cytotoxic potential of isolates **1–9** was evaluated with a number of human cultured cell lines. As indicated in Table 3, the dibenzylbutyrolactone **5** and the aryltetralin lignans **6–9** demonstrated general nonspecific cytotoxic activity ($ED_{50}<10^{-2}$ μg/ml) comparable to that of podophyllotoxin [**12**] (18). The intensities of the responses displayed by **6** and **9** were similar to each other and approximately 10- to 100-fold more intense than that demonstrated by the iso- and picroisomers, compounds **7** and **8**, respectively. As expected, the arylnaphtalenes **1–4** were 100- to 1000-fold less active than podophyllotoxin [**12**] because of the planar aromatized naphthalene ring which alters the conformation of the molecule significantly relative to **12**. Compounds **1** and **2** showed only marginal cytotoxicity with the human cancer cell lines, and the most intense activity was observed with the murine lymphocytic leukemia in cell culture (P-388, ED_{50} 4.0 and 1.8 μg/ml, respectively).

In addition, demonstrable antimitotic activity was observed for all the isolates as judged by the astrocytoma (ASK) assay (19). As summarized in Table 4, the dose required (0.032 μg/ml) for compounds **6** and **9** to effect 100% reversal conversion of cultured ASK cells was comparable to that of podophyllotoxin [**12**]. Compound **5** was less active, with an antimitotic activity of 0.16 μg/ml. The ASK system activity of lignans **1–4**, **7**, and **8** was in the concentration range of 0.8–100 μg/ml (Table 4).

Finally, the aryltetralin lignans **6** and **9**, as well as (−)-yatein [**5**], were found to show strong inhibitory activity against *Candida albicans* at the concentrations of 0.2–1.0 μg/ml when examined by the standard dilution technique (8). This activity, which could

TABLE 3. Evaluation of the Cytotoxic Potential of Lignans **1–9** from *Hyptis verticillata* and of Podophyllotoxin [**12**].

Compound	Human cell line[a] ED_{50} (μg/ml)									
	A431	BC-1	Col-2	HT	KB	KB-V1	LNCaP	Lu-1	U373	ZR-75-1
1	6.2	7.6	12.8	15.6	6.0	8.7	11.6	11.7	16.3	>20
2	>20	2.9	3.2	3.4	2.2	4.2	3.2	4.3	5.9	>20
3	>20	15.2	8.9	9.7	5.0	13.0	11.7	10.6	>20	>20
4	6.2	>20	16.7	>20	11.4	>20	11.6	18.8	>20	>20
5	>20	0.05	0.08	0.07	0.08	0.06	0.16	0.1	0.3	0.5
6	0.08	0.01	0.03	0.01	0.01	0.02	0.02	0.03	0.1	2.1
7	6.2	17.5	8.9	10.7	6.7	11.5	12.0	15.9	2.9	13.2
8	>20	2.1	0.3	0.2	0.1	0.7	0.2	0.09	0.1	0.6
9	4.3	0.001	0.01	0.003	0.05	0.06	0.01	0.002	0.001	2.0
12[b]	0.03	0.03	0.005	0.003	0.08	0.06	0.04	0.008	0.004	0.4

[a]Abbreviations: A431, epidermoid carcinoma; BC-1, breast cancer; Col-2, colon cancer; HT, fibrosarcoma; KB, nasopharyngeal carcinoma; KB-V1, vinblastine resistant KB; LNCaP, hormone-dependent prostate cancer; Lu-1, lung cancer: U373, glioblastoma; ZR-75-1, hormone-dependent breast cancer.

[b]Data for podophyllotoxin [**12**] were reported previously (18) and are listed here for comparison.

TABLE 4. Evaluation of the Antimitotic Potential (ASK Assay) of Pure Isolates **1–9** from *Hyptis verticillata*.

Compound	Concentration[a] (μg/ml)							
	100	20.0	4.0	0.8	0.16	0.032	0.0064	0.00128
Colchicine[b]	100%	100%	100%	70%	0%	0%	0%	0%
Vincristine[b]	100%	100%	100%	100%	100%	0%	0%	0%
Vinblastine[b]	100%	100%	100%	100%	100%	100%	0%	0%
Maytansine[b]	100%	100%	100%	100%	100%	100%	100%	0%
1	100%	100%	0%	0%	0%	0%	0%	NT[c]
2	100%	100%	100%	0%	0%	0%	0%	NT
3	100%	100%	0%	0%	0%	0%	0%	NT
4	100%	0%	0%	0%	NT	NT	NT	NT
5	100%	100%	100%	100%	100%	0%	0%	NT
6	100%	100%	100%	100%	100%	100%	0%	NT
7	100%	100%	0%	0%	0%	0%	0%	NT
8	100%	100%	100%	100%	0%	0%	0%	NT
9	100%	100%	100%	100%	100%	100%	0%	NT
12[d]	100%	100%	100%	100%	100%	100%	0%	NT

[a]Percentage of reversal astrocyte formation.
[b]Antimitotic drug used as standard for comparison.
[c]Not tested.
[d]Podophyllotoxin used as an antimitotic lignan standard.

account for the antiseptic properties of the infusions prepared from *H. verticillata*, is presumably due to the established antimitotic activity of the podophyllotoxin lignan series (20), and is consistent with the ASK activity observed for the cytotoxic isolates **1–9** in this study.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's, optical rotations, ir, ms, and nmr spectra were measured as described previously (8). Selective INEPT experiments were performed on a Nicolet NMC-360 (90.8 MHz) spectrometer. ^{1}H-nmr nOe experiments were performed on a Varian VXR-300S instrument.

PLANT MATERIAL.—The aerial parts of *H. verticillata* were collected in San Juan Guichicovi, Oaxaca, Mexico, in March 1986. A voucher specimen (MEXU-431167) is deposited at the National Herbarium, Instituto de Biologia, Universidad Nacional Autonoma de Mexico.

EXTRACTION AND ISOLATION.—The air-dried, milled plant material (1.5 kg) was defatted exhaustively by maceration with hexane. The residual material was extracted with $CHCl_3$ three times overnight. After filtration, the solvent was removed under high vacuum to yield 133.3 g of a dark-green resinous extract (KB, ED_{50} 0.6 μg/ml; P-388, ED_{50} 0.1 μg/ml). The whole isolation procedure was directed by the brine shrimp lethality test (BST) (21). The crude extract (BST, LC_{50} 13.4 μg/ml) was fractionated by chromatography on a Si gel (1 kg) column, using a gradient of $CHCl_3$/EtOAc in hexane and collecting 310 fractions of 500 ml each. The eluates were combined, based on tlc similarities, into eight pools (I–VIII) and assayed for toxicity. Fractions 145–172 (pool IV) were most toxic to brine shrimp, with LC_{50} values of 7.9 μg/ml. Fractions 242–250 (pool VII) and 124–144 (pool III) had LC_{50} values of 260 and 340 μg/ml, respectively, while fractions 1–37 (pool I), 38–99 (pool II), 178–189 (pool V), 210–241 (pool VI), and 251–310 (pool VIII) had LC_{50} values >500 μg/ml. A white precipitate was removed from bioactive pool IV (10 g; KB_{50} 0.09 μg/ml) and subjected to preparative tlc with C_6H_6-$CHCl_3$-MeOH (1:1:0.1). Three clearly defined zones were located by uv and separately eluted.

Zone 1 (R_f 0.14) contained 54 mg of dehydropodophyllotoxin [**3**]. Zone 2 (R_f 0.42) afforded 36 mg of 4′-demethyldeoxypodophyllotoxin [**6**]. Zone 3 (R_f 0.57) was further purified by preparative tlc [hexane-$CHCl_3$-Me_2CO (3.3:6.0:0.7)] to give 33.2 mg of β-apopicropodophyllin [**9**] (R_f 0.37), 58.3 mg of isodeoxypodophyllotoxin [**7**] (R_f 0.43), 56.6 mg of deoxypicropodophyllin [**8**] (R_f 0.56), 15 mg of dehydro-β-peltatin methyl ether [**2**] (R_f 0.64), and 16.3 mg of a mixture of compounds **1** and **4** (R_f 0.61). The mother

liquors of bioactive pool IV (1 g; BST, LC_{50} 0.1 μg/ml) were subjected to cc to afford 19.7 mg of additional compound **1** and 20.5 mg of (−)-yatein [**5**] (R_f 0.68).

In order to accomplish the resolution of the mixture of lignans **1** and **4**, 14 mg of this mixture was dissolved in $CHCl_3$ (1 ml) containing 0.1 M NaOH (0.5 ml) and stirred for 2 h. Then H_2O (2 ml) was added, and the mixture was extracted three times with $CHCl_3$ (1.5 ml each). The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated to afford 6.4 mg of pure compound **4**. The aqueous alkaline solution was adjusted to pH 6 with 1 N HCl and extracted with $CHCl_3$ (2×1.5 ml). The resulting organic phase was dried to give an oily residue which was purified by tlc to yield 5.8 mg of compound **1**.

Pool III contained 16.1 g of a mixture of oleanolic and ursolic acids. Pool V afforded 150 mg of sideritoflavone, 5,3′,4′-trihydroxy-6,7,8-trimethoxyflavone (KB, ED_{50} 1.6 μg/ml). Pool VII left a residue which was recrystallized from MeOH to yield 1.32 g of a mixture of maslinic and 2α-hydroxyursolic acids. All triterpene acids were identified by comparison of the physical and spectral data with those of authentic material in our files (22).

5-Methoxydehydropodophyllotoxin [**1**].—White needles, mp >225° (dec); uv λ max (MeOH) (log ε) 223 (4.52), 268 (4.36), 325 (3.68), 357 nm (3.38); ir ν max (KBr) 3400, 1763, 1614, 1582, 1465, 1448, 1251, 1199, 1125, 1089, 1055, 1035, 1022, 1004, 939 cm^{-1}; ^1H nmr ($CDCl_3$) δ 9.58 (1H, s, OH), 6.86 (1H, s, H-8), 6.48 (2H, s, H-2′, H-6′), 6.07 (2H, s, -OCH_2O-), 5.34 (2H, s, lactone-CH_2), 4.29 (3H, s, 5-OMe), 3.95 (3H, s, 4′-OMe), 3.82 (6H, s, 3′-, 5′-OMe); ^{13}C nmr ($CDCl_3$) see Table 2; eims (70 eV) *m/z* $[M+2]^+$ 442 (5), $[M+1]^+$ 441 (26), $[M]^+$ 440 (100), 425 (28); hreims 440.1119 (calcd for $C_{23}H_{20}O_9$, 440.1107).

Dehydro-β-peltatin methyl ether [**2**].—Mp 265–267° [lit. (9) mp 266–268°]; spectroscopic data (uv, ir, ^1H nmr) comparable to literature values (9); ^{13}C nmr see Table 2; eims (70 eV) *m/z* $[M+2]^+$ 426 (5), $[M+1]^+$ 425 (24), $[M]^+$ 424 (100), 409 (29), 394 (28), 379 (12), 351 (5).

Dehydropodophyllotoxin [**3**].—White amorphous powder, mp 280°; spectroscopic data (uv, ir, ms and nmr) identical to those reported (12).

Deoxydehydropodophyllotoxin [**4**].—Colorless needles: mp 269–270°; spectroscopic data (uv, ir, ms, nmr) comparable to literature values (12).

Yatein [**5**].—Pale yellow glassy solid: $[\alpha]_D$ −28.9° (*c*=0.4, $CHCl_3$). It was identified by comparison of the physical and spectral data (uv, ir, ms, nmr) with those reported for yatein (13).

4′-Demethyldeoxypodophyllotoxin [**6**].—White small cubic crystals: mp 246–248°; $[\alpha]_D$ −130° (*c*=0.2, $CHCl_3$); spectroscopic data (uv, ir, ms) identical to those previously reported (14); ^1H nmr (C_6D_6) δ 8.09 (1H, s, OH), 6.61 (2H, s, H-2′, H-6′), 6.56 (1H, s, H-5), 6.55 (1H, s H-8), 5.52 (2H, dd, -OCH_2O-), 4.50 (1H, d, *J*=4.8 Hz, H-1), 3.98 (1H, dd, *J*=7.1, 10.6 Hz, H-10α), 3.59 (6H, s, 3′-, 5′-OMe), 3.42 (1H, dd, *J*=8.3, 10.6 Hz, H-10β), 2.56 (1H, m, H-3), 2.48 (1H, dd, *J*=5.3, 15.9 Hz, H-4α), 2.32 (1H, dd, *J*=4.8, 13.7 Hz, H-2), 2.21 (1H, dd, *J*=4.6, 15.9 Hz, H-4β); ^{13}C nmr see Table 2. When compound **6** (10 mg) was methylated with CH_2N_2, 9.5 mg of a product identical (mp, ms, nmr) to deoxypodophyllotoxin was obtained (23).

Isodeoxypodophyllotoxin [**7**].—White needles: mp 246–248° [lit. (15) 256–258°], $[\alpha]_D$ −38.5° (*c*=0.4, $CHCl_3$); ^1H nmr (C_6D_6) δ 6.62 (2H, s, H-2′, H-6′), 6.57 (2H, s, H-5, H-8), 5.57 and 5.52 (2H, s each, -OCH_2O-), 4.15 (1H, dd, *J*=6.7, 8.0 Hz, H-10α), 4.01 (1H, d, *J*=11.4, H-1), 3.79 (3H, s, 4′-OMe), 3.60 (1H, dd, *J*=8.0, 11.3 Hz, H-10β), 3.56 (6H, s, 3′-, 5-OMe), 2.75 (1H, dd, *J*=11.4, 13.4, H-2), 2.63 (1H, dd, *J*=13.0, 13.4 Hz, H-4α), 2.49 (1H, dd, *J*=4.5, 13.0 Hz, H-4β), 2.23 (1H, m, H-3); ^{13}C nmr see Table 2; eims (70 eV) *m/z* $[M+2]^+$ 400 (4.3), $[M+1]^+$ 399 (24.8), $[M]^+$ 398 (100) 383 (10).

Deoxypicropodophyllin [**8**].—White amorphous powder: mp 163–165°; ^1H nmr identical to those reported (16); ^{13}C nmr see Table 2; eims (70 eV) *m/z* $[M+2]^+$ 400 (4.3), $[M+1]^+$ 399 (24.8), $[M]^+$ 398 (100) 383 (18).

β-*Apopicropodophyllin* [**9**].—White amorphous powder: mp 218° [lit. (17) 220–222°]; $[\alpha]_D$ +96.8° (*c*=0.5, $CHCl_3$) uv λ max (MeOH) (log ε) 250 (3.8), 301 (3.6), 317 (2.7); ir ν max (KBr) 3016, 2993, 2840, 1754, 1619, 1589, 1506, 1485, 1463, 1385, 1231, 1181, 1099, 1036, 940, 902, 869 cm^{-1}; ^1H nmr ($CDCl_3$) δ 6.72 (1H, s, H-5), 6.63 (1H, s, H-8), 6.37 (2H, s, H-2′, H-6′), 5.95 (2H, brs, -OCH_2O-), 4.87–4.79 (3H, m, H-1, H-10α and H-10β), 3.84 (1H, dd, *J*=2.3, 27.8 Hz, H-4α), 3.79 (3H, s, 4′-OMe), 3.78 (6H, s, 3′-, 5′-OMe), 3.74 (1H, dd, *J*=3.7, 27.8 Hz, H-4β); ^{13}C nmr see Table 2; eims (70 eV) *m/z* $[M+2]^+$ 398 (3), $[M+1]^+$ 397 (24), $[M]^+$ 396 (100), 395 (5), 394 (8), 381 (5), 379 (8), 351 (10), 337 (10).

Sideritoflavone.—Yellow crystals: mp 216–218° [lit. (24) 196–198°]; identified by comparison of the spectral data (uv, ^1H nmr, ms) with those reported for 5,3′,4′-trihydroxy-6,7,8-trimethoxyflavone previously isolated from *Sideritis leucantha* (24); ^{13}C nmr (DMSO-d_6) δ 182.98, 164.99, 153.02, 150.63, 148.97, 146.32, 145.80, 136.32, 133.15, 121.93, 119.84, 116.79, 113.74, 106.67, 103.13, 62.65, 62.23, 61.34.

CYTOTOXICITY ASSAYS.—Extract, fractions, and compounds were evaluated for cytotoxic potential as described previously (8,25). The culture cell lines P-388, human fibrosarcoma (HT-1080), human oral epidermoid carcinoma (KB), human epidermoid carcinoma (A431), human hormone-dependent breast cancer (ZR-75-1), prostate cancer (LNCaP), and the human glioblastoma (U373) were purchased from the American Type Culture Collection. The multidrug-resistant cell line KB-V1 was developed from KB cells by treatment with sublethal doses of vinblastine over an extended period of time. Other human cancer cell types, which include breast (BC-1), colon (Col-2), and lung (LU-1) cancers, were established from primary human tumors in the Specialized Cancer Center, University of Illinois College of Medicine at Chicago.

ASTROCYTE FORMATION ASSAY (ASK).—Pure cytotoxic lignans were evaluated for antimitotic potential using cultured rat glioma (ASK) cells essentially by the procedures reported by Swanson *et al.* (19).

ANTIMICROBIAL ASSAY.—Pure compounds were evaluated for quantitative antimicrobial activity against *C. albicans* (ATCC 10231) by the dilution technique (8). A solution of the test compound (0.5 ml, 1 mg/ml) dissolved in MeOH-H_2O (1:1) was added to 4.5 ml of glucose (2%)-Sabouraud broth. Doubling serial dilutions were aseptically prepared from this broth with concentrations ranging from 100 to 0.2 μg/ml. Each dilution was inoculated with 10 μl of a suspension of *C. albicans* to a final concentration of 10^6/ml. After overnight incubation at 28°, the minimum inhibitory concentration was determined.

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