Isolation, Purification, and Cytotoxicity of 5-Methoxypodophyllotoxin, a Lignan from a Root Culture of Linum flavum

Wim Van Uden, Ben Homan, Herman J. Woerdenbag, Niesko Pras, Theo M. Malingré, Harry J. Wichers, and Marien Harkes

J. Nat. Prod., **1992**, 55 (1), 102-110• DOI: 10.1021/np50079a015 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 55, No. 1, pp. 102-110, January 1992

ISOLATION, PURIFICATION, AND CYTOTOXICITY OF 5-METHOXYPODOPHYLLOTOXIN, A LIGNAN FROM A ROOT CULTURE OF *LINUM FLAVUM*

WIM VAN UDEN,* BEN HOMAN, HERMAN J. WOERDENBAG, NIESKO PRAS, THEO M. MALINGRÉ,

Laboratory for Pharmacognosy, University of Groningen, A. Deusinglaan 2, NL-9713 AW Groningen, The Netherlands

HARRY J. WICHERS,[1] and MARIEN HARKES

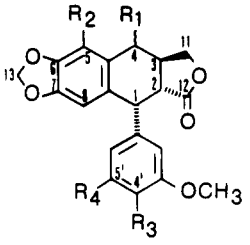
Netherlands Organization for Applied Scientific Research (TNO), Department of Biotechnology, Utrechtseweg 48, NL-3704 HE Zeist, The Netherlands

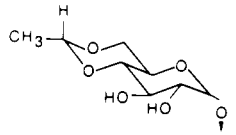
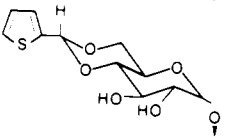
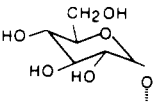
ABSTRACT.—A method has been developed for the large scale isolation of 5-methoxypodophyllotoxin [**1**] from a high-producing root culture derived from *Linum flavum*. A closely related lignan, 5′-demethoxy-5-methoxypodophyllotoxin [**2**], was also present in the root culture and was the cause of the main isolation difficulties. Essential steps in the isolation procedure are CH_2Cl_2 and XAD-4 extraction and XAD-8 cc followed by Si gel chromatography, using two different mobile phases. The isolated 5-methoxypodophyllotoxin [**1**] was very pure (>99%) and possessed the desired stereochemical configuration, namely (−)-5-methoxypodophyllotoxin [**1**]. The in vitro cytotoxicity of 5-methoxypodophyllotoxin [**1**] against EAT and HeLa cells was determined and compared with those of podophyllotoxin [**3**], etoposide (VP-16-213) [**4**], teniposide (VM-26) [**5**], and 5-methoxypodophyllotoxin-4-β-D-glucoside [**6**]. It appeared that 5-methoxypodophyllotoxin [**1**] has about the same cytotoxic potency as podophyllotoxin [**3**].

Etoposide [**4**] and teniposide [**5**] are clinically applied semi-synthetic cytostatics, chemically prepared from the natural lignan, podophyllotoxin [**3**] (1–3). The total chemical synthesis of [**3**] is complicated because of the presence of four chiral centers, a rigid trans-lactone, and an axially locked 1-aryl substituent (4). Therefore, podophyllotoxin [**3**] is still extracted from the rhizomes of *Podophyllum* species (Berberidaceae) (5). However, the supply of *Podophyllum hexandrum* plants, which contain about 4.3% podophyllotoxin [**3**] on a dry wt basis, has become limited, due to intensive collection and lack of organized cultivation (6). As a result, high costs are involved in obtaining **3** from the plant rhizomes. Therefore, the production of **3** and related lignans by means of biotechnological techniques has been considered as an attractive alternative. During the last decade, a series of reports on the biosynthesis of podophyllotoxin [**3**] and related lignans using plant cell cultures have been published (7–20). Several reports have dealt with the production of 5-methoxypodophyllotoxin [**1**] by cell cultures from *Linum flavum* L. (Linaceae). So far, the presence of **1** has only been demonstrated in a few *Linum* species and in *Juniperus sabina* (21,22). Recently, we selected a root-like culture of *L. flavum* and developed a medium for an optimal production of 5-methoxypodophyllotoxin [**1**] (20). In the present study, the isolation and purification procedure for **1** from these roots is described.

The search for new cytostatics is still in progress because of the severe side effects of the generally used anti-tumor agents and because of the insensitivity of many neoplastic malignancies for the applied therapeuticals (1, 2, 23–25). Based on the chemical resemblance with podophyllotoxin [**3**], 5-methoxypodophyllotoxin [**1**] may be an interesting starting compound for the preparation of new semi-synthetic derivatives with anti-tumor properties. Therefore, in this study, the cytotoxic activity of the isolated **1**

[1]Present address: Agrotechnological Research Institute, Haagsteeg 6, NL-6708 PM Wageningen, The Netherlands.



	R_1	R_2	R_3	R_4
1	OH	OMe	OMe	OMe
2	OH	OMe	OMe	H
3	OH	H	OMe	OMe
4		H	OH	OMe
5		H	OH	OMe
6		OMe	OMe	OMe

was tested and compared with authentic podophyllotoxin [**3**], etoposide [**4**], teniposide [**5**], and 5-methoxypodophyllotoxin-4-β-D-glucoside [**6**].

RESULTS AND DISCUSSION

The extraction of lyophilized root-like cell material by means of sonification in 80% MeOH for 1 h is the first step in the isolation procedure (Figure 1). This extraction method proved to be very suitable for analytical samples, usually consisting of 100 mg quantities of dried cell material (11–14, 16, 17, 20). The use of larger quantities resulted in a strongly reduced efficiency of the extraction; when more than 100 g of dried cell material was used, the efficiency was lowered by 50–70%. Possibly, the applied energy is insufficient to damage the cells when high biomass concentrations are present. Therefore, the extraction procedure still has to be optimized and experiments using Soxhlet and Ultraturrax (mechanical destruction of cells under blending in MeOH) extractions are under current investigation.

An emulsion occurred sometimes during the extraction of the polar H_2O/MeOH phase with CH_2Cl_2. The addition of one or two drops of concentrated HCl was sufficient to clarify the mixture in order to displace compound **1** to the latter apolar phase. Care was taken not to let the pH of the MeOH/H_2O layer drop below 2. At a pH of 1, hplc analysis revealed partial decomposition of **1** into unknown compounds. The use of base had to be avoided at any case, since this led to dramatic losses of **1**. Strong decomposition of **1** was also observed when a solution in H_2O/MeOH was heated above 40°, although **1** in pure MeOH could be heated or evaporated without decomposition of the lignan. As a consequence, H_2O/MeOH fractions, which were collected from the XAD-8 (Serdolit® adsorbent resin) column, could not be concentrated by rotary evaporation. This problem was solved by adding an extra amount of H_2O to these fractions first, followed by extraction with CH_2Cl_2 and subsequent evaporation of this apolar phase.

Decomposition of **3** in hot 96% EtOH has been described in detail by Buchardt *et al.* (26), while the sensitivity of **3** to acids and bases has long been recognized. For example, even weak bases like NH_3 and NaOAc rapidly induced epimerization leading to picropodophyllin (25,27). As a result of the strong structural resemblance, a similar decomposition pattern for **1** is likely valid.

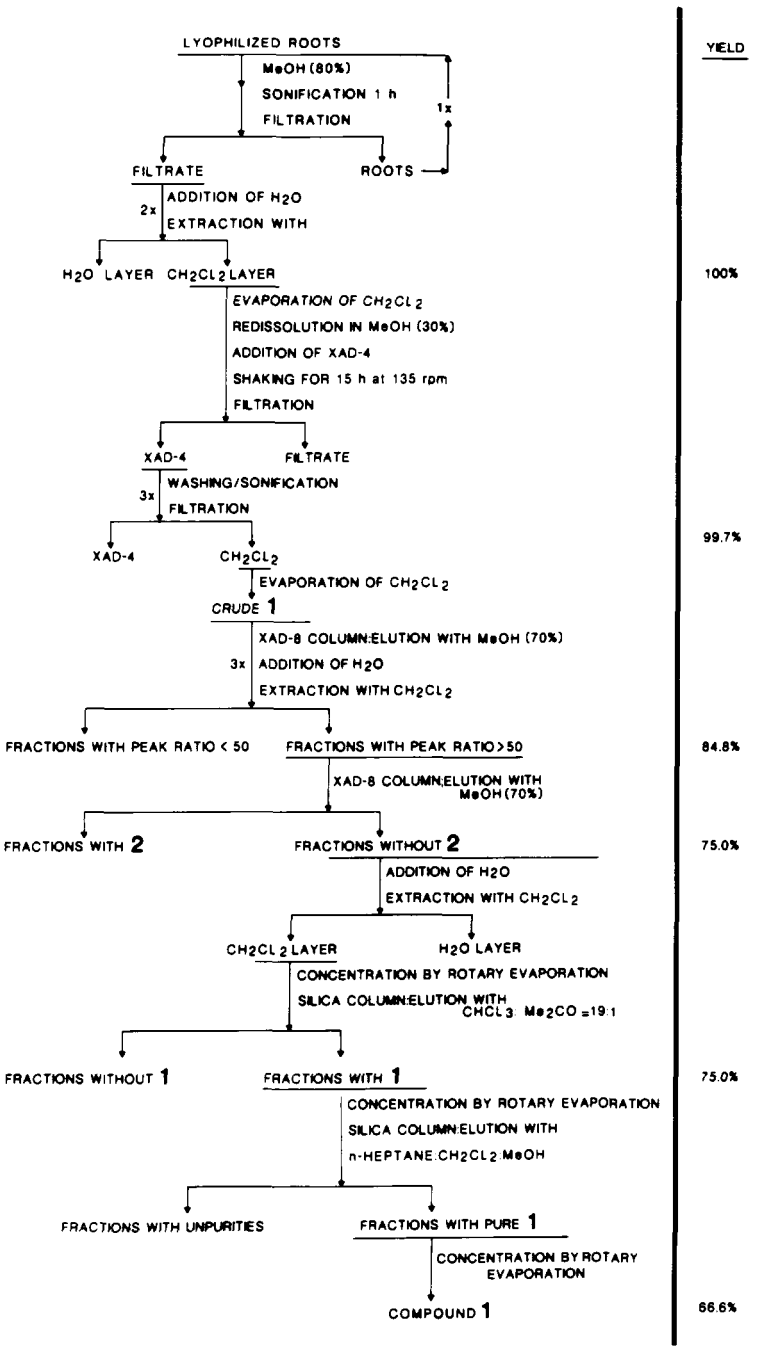


FIGURE 1. Scheme for the isolation of 5-methoxypodophyllotoxin [**1**] from a root-like culture of *Linum flavum*. In the right column the yields are indicated as a percentage of the initial amount.

The use of XAD-4 was introduced to adsorb **1**, while polar inpurities like dyes, proteins, sugars, and salts remained behind in the mobile 30% MeOH phase. Crude **1**, as mentioned in the isolation scheme of Figure 1, was yellowish. The main impurity consisted of an hplc-uv detectable compound with a retention time of 1 min less than the

retention time of **1**, which is approximately 10 min. When the hplc-uv chromatogram peak of the unknown compound was compared with a reference peak of compound **2**, the retention times were identical. The occurrence of this lignan in *L. flavum* plants and suspension cultures has been reported recently by Wichers *et al.* (19). Further analysis of the unknown compound in our material, by means of combined gc-ms and comparison of the ms data with those of the reference, confirmed that the unknown compound was **2**. Based on the very strong chemical resemblance, serious problems in the separation of **2** and **1** were expected. During several steps of the isolation procedure, the two lignans remained unseparable indeed, indicating almost identical physical properties, such as polarity and solubility. To investigate the possibilities of separating these closely related compounds, we chose to examine a number of XAD adsorbent resins.

After eluting the mixture on 10 ml XAD-7 and XAD-8 with 50 ml 80% MeOH, 100% of the applied quantity was recovered. For comparison, using XAD-2 and XAD-4, only 33% and 50% were recovered, respectively. Except for a rapid recovery of the applied sample, the separation ability of the resin is important. From Figure 2 it can be seen that from the four tested XAD-resins, XAD-8 met these requirements the best. Practically, this means that the first fractions collected from an XAD-8 column are en-

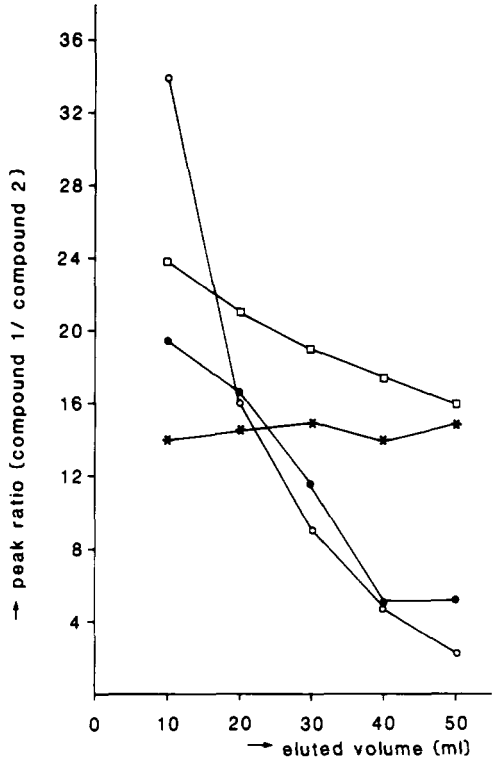


FIGURE 2. Relationship between the peak ratio of 5-methoxypodophyllotoxin [**1**] to 5′-demethoxy-5-methoxypodophyllotoxin [**2**] as detected with hplc-uv at 290 nm and the elution volume, using different XAD-resins and a mobile phase consisting of 80% MeOH; XAD-2 (*), XAD-4 (□), XAD-7 (●), XAD-8 (○).

riched with **1** and contain much lower amounts of **2**. In the case of the actual isolation, the MeOH concentration was adjusted to 70%. The use of this more polar MeOH concentration proved to give a more optimal separation as compared with 80%. When the use of this column was repeated three times, ca. 25% of the initial amount of **1** was lost; however, the problem caused by the presence of **2** was solved.

The Si gel column with $CHCl_3$/Me_2CO as the mobile phase was introduced to eliminate the yellow color, which was present as an impurity in the crude **1**. The choice of this mobile phase was based on the successful isolation of several lignans from *J. sabina* (22), including **1**. Compound **1** was collected in the first fractions, while the colored impurities were retained on the column. For further purification of **1**, another mobile phase was used, in combination with a Si gel column. The eluent was derived from the elution solvent as applied in the hplc system, namely, *n*-heptane–CH_2Cl_2–MeOH (90:10:2). This mobile phase was necessary to remove less polar, unknown, hplc-uv detectable compounds with retention times between 1 and 8 min. Rotary evaporation of the *n*-heptane/CH_2Cl_2/MeOH mixture containing **1** resulted in a white amorphous powder. When **1** was recovered after evaporation from CH_2Cl_2, $CHCl_3$, or MeOH without the presence of *n*-heptane, the result was a transparent residue.

Various amounts of **1**, present in extracts in the range of 100–500 mg, were subjected to the isolation procedure. No additional problems or diminished yields were observed when the initial amount of **1** was increased from 100 to 500 mg, indicating that further upscaling should be feasible. The method developed allowed the reuse of most solvents as well as the chromatographic materials. These advantages are of important economic value for the large scale isolation of **1** and limit environmental pollution.

The isolated **1** was subjected to a series of analytical methods in order to confirm its identity and check its purity. It was necessary to know whether the desired lignan, and in particular which enantiomer, was isolated, in order to allow proper cytotoxicity experiments with the isolated **1** and to use it as a starting compound for the chemical synthesis of chemical derivatives.

The hplc chromatograms recorded at 290 nm showed a purity of >99%. Uv and ir spectra revealed maxima that corresponded fairly well with results reported earlier by San Feliciano *et al.* (22) for **1** isolated from *J. sabina*. The mass spectrum contained fragments that were consistent with the literature ms data on fragmentation patterns as reported previously for **1** (9, 15, 21, 22). The determination of the mp of **1** gave problems, as no unambiguous mp could be detected. A melting traject, between 95 and 100°, was found, while effervescence and foaming were observed at these temperatures. In addition, this melting traject was ca. 100° under the mp as published by San Feliciano *et al.* (22). This phenomenon may be explained by the observation that the crystallization of **3**, itself, has variables. Recently, it has been reported that **3** can be obtained in different crystal forms (28). More importantly, several mp's or trajects were found for **3**. A melting point of 182–183° was found for the orthorhombic form, 162–164° for a trigonal hydrate, and a melting traject from 114 to 118° with foaming for an orthorhombic solvate of H_2O and C_6H_6. It is quite reasonable to assume that **1** is also able to act as a host for certain solvent molecules, because the same hydrophilic and hydrophobic parts are present in both molecules.

Theoretically, of the sixteen stereoisomers of **1** that are possible, only one is of special interest, namely (−)-**1**. ^1H- and ^{13}C-nmr spectral data were compared with previously published data and clearly showed that the isolated lignan was the diastereomer **1** (9, 15, 21, 22). To distinguish between (+) and (−) enantiomers, the optical rotation was measured, and it appeared that the desired lignan with the correct configuration had indeed been isolated. From comparison of the specific optical rotations (at $\lambda = 589.3$ nm, in $CHCl_3$) of **3** and **1**, −132.0° and −129.5° respectively, it can be

concluded that the additional methoxyl moiety at C-5 does not influence the chirality of the molecule.

In the present study, the cytotoxicity of the isolated **1** against Ehrlich ascites tumor (EAT) and cervix uteri (HeLa) cells was determined and compared with cytoxicity of **3**–**6**. From Table 1 it can be seen that **1**, **3**, and **6** had about the same ED_{50} values in the experimental setting used. Berlin *et al.* (10), on the contrary, found that the aglycone **1** exhibited a 250–500 times higher cytotoxic activity than **6**, as determined against mouse fibroblast cells L929. The difference may be ascribed to the use of other test systems and to possible impurities of the tested compounds (cell extracts). On the other hand it is known that the glucosides of **3** are less toxic (1).

TABLE 1. Cytotoxicity of the Lignans as Tested on Two Tumor Cell Lines (data are expressed as ED_{50} values in $\mu g \cdot ml^{-1}$).

Compound	Cell Line	
	EAT[a]	HeLa[b]
5-Methoxypodophyllotoxin [**1**]	32.0	22.0
Podophyllotoxin [**3**]	42.8	20.5
Etoposide [**4**]	1.1	7.9
Teniposide [**5**]	0.06	0.3
5-Methoxypodophyllotoxin-4-β-D-glucoside [**6**]	30.0	21.8

[a]Murine Ehrlich ascites tumor cell line.
[b]Human HeLa cervix uteri tumor cell line.

The mechanism of action of **4** and **5**, which is different from that of **3**, resulted in a much stronger cytotoxic action in both tumor cell lines. The EAT cells were less sensitive than HeLa cells for **1**, **3**, and **6**, while the opposite was true for **4** and **5**. Compound **3** is a classical spindle poison, arresting cell division in the metaphase, a process which is connected with the inhibition of microtubule assembly (29). Compounds **4** and **5** do not show any effect on microtubule assembly, although their aglycones (4′-demethylated) behave like **3**, but prevent cells from entering mitosis in the late S or G2 phase of the cell cycle (2, 3, 29, 30). The exact mechanism of action of **4** and **5** is yet unknown, although it is obvious that the induction of DNA strand breaks by **4** can be explained by the ability to inhibit nuclear topoisomerase II (30). Essential for the change from spindle poison to G2 poison are demethylation at position 4′, epimerization at position 4, and presence of a glucose moiety at position 4 which has been condensed with an aldehyde (29,30). Consequently, **1** and **6** are most likely spindle poisons and not G2 poisons.

In this study, it has been shown that large scale isolations of (−)-**1** from root-like cultures of *L. flavum* are possible and that **1** is a potential cytotoxic compound. As already mentioned, **3** and **1** are chemically and physically closely related. Since both compounds exhibit about the same cytotoxic behavior in our test systems, the chances are enhanced for **1** to become a promising new anti-tumor drug. In analogy with **3** from which **4** and **5** are prepared, the synthesis of 4′-demethyl-5-methoxyepipodophyllotoxin-ethylidene-β-D-glucoside, a variant of **4**, is under current investigation.

EXPERIMENTAL

PLANT MATERIAL AND CULTURE CONDITIONS.—A root-like cell line of *L. flavum* was obtained and grown as described recently (20). In this study, the cell material used for the isolation of 5-methoxypodophyllotoxin [**1**] was grown on a production medium consisting of MS salts (Flow Laboratories, Irvine, Scotland) (31), 6% sucrose, no phytohormone, and no vitamins (20). Root-like structures

rapidly developed, and after growing for 2 weeks on this medium the cells were harvested by suction filtration. The 5-methoxypodophyllotoxin [**1**] content generally ranged from 0.35 to 0.68% on a dry wt basis.

GENERAL EXPERIMENTAL PROCEDURES.—All isolation steps were controlled using hplc with uv detection at 290 nm as described previously (12). As a reference solution, 0.1 $mg \cdot ml^{-1}$ of authentic 5-methoxypodophyllotoxin [**1**] in MeOH was used. For the identification of 5′-demethoxy-5-methoxypodophyllotoxin [**2**], an MeOH solution of unknown concentration was used. Both references were obtained from TNO, Zeist, The Netherlands. Gc-ms was performed on a Finnigan 3300 GC-MS system under the following conditions: column fused Si gel CP-sil 5CB, 25 m × 0.33 mm id (Chrompack, Middelburg, The Netherlands); temperature program 200–295°, $6° \cdot min^{-1}$; temperature injection system 250°; carrier gas He 1 $ml \cdot min^{-1}$; injected volume 1 μl of the 5′-demethoxy-5-methoxypodophyllotoxin [**2**] solution in MeOH. Mass identification was performed using ei at an ionization energy of 70 eV; temperature ion source 250°. Cycling time during acquisition of the mass spectrum was 1 sec. The isolated and purified 5-methoxypodophyllotoxin [**1**] was directly introduced into the mass spectrometer by means of the dried insertion probe. ^1H- and ^{13}C-nmr spectra were recorded on a Varian VXR-300 system, with TMS as internal standard. Uv spectra were recorded on a Shimadzu Spectrophotometer UV-160 and ir spectra (KBr-discs) on a Beckman Acculab 2 Infrared Spectrophotometer. Specific optical rotations were determined with a Perkin-Elmer 241 polarimeter equipped with 10 cm cuvettes. The mp's were determined on a Büchi apparatus and are uncorrected.

EXTRACTION AND ISOLATION.—The root-like tissue was dried by lyophilization and subsequently powdered in a mortar. The isolation of 5-methoxypodophyllotoxin [**1**] was performed according to Figure 1. For sonification of the cells in MeOH, 100 g powdered cells were incubated with ca. 0.8 liter of 80% MeOH in a sonification bath (Branson, Danbury, USA). XAD cc for crude 5-methoxypodophyllotoxin [**1**] was an important step in the isolation procedure. For optimization, several Serdolit® XAD-adsorbent resins were applied. The XAD-2, -4, -7 and -8 adsorbance resins (300–1000 μm; research grade) were all purchased from Serva (Heidelberg, Germany) and regenerated by shaking with H_2O, MeOH, and CH_2Cl_2. Small scale optimization experiments with the XAD resins were performed using 10-ml syringes packed with 10 ml XAD resin. Samples of 5-methoxypodophyllotoxin [**1**] (ca. 30 mg) contaminated with **2** (absorbance ratio at uv 290 nm is ca. 14), were eluted with 80% MeOH. The XAD-8 column as used in the larger scale isolation procedure consisted of a glass tube (60 cm × 16 mm i.d.) and was packed with 102 ml XAD-8 resin. The flow of the eluent was 2 $ml \cdot min^{-1}$, and the first four 100-ml fractions were collected. Subsequently, two columns packed with Si gel (Baker I.T., Deventer, The Netherlands, 70242; average particle size = 40 μm) were prepared. The column, which was eluted with $CHCl_3/Me_2CO$ (50 cm × 16 mm i.d.), was packed with 78 g silica. Fourteen fractions of 10 ml were collected. From the column eluted with *n*-heptane/CH_2Cl_2/MeOH (60 cm × 32 mm i.d.) containing 376 g Si gel, 102 fractions of 50 ml were collected. In the isolation scheme of Figure 1, the addition of H_2O has been mentioned in several purification steps. Generally, two times the volume of the MeOH fraction was added for dilution. When extraction with CH_2Cl_2 has been indicated, this solvent was added in a volume equal to the MeOH fraction.

STRUCTURAL IDENTIFICATION.—*5-Methoxypodophyllotoxin* [**1**].—Mp 95–100°; effervescence; uv λ max (MeOH) (log ε) 219 (4.54), 279 (3.38) nm; ir ν max (KBr) 3560, 2960, 2900, 2840, 1785, 1620, 1595, 1505, 1480, 1250, 1125, 1095, 1000, 940, 845 cm^{-1}; $[\alpha]^{20}$ (λ) −129.5° (589.3), −136.5° (578), −156.3° (546), −284.4° (436), −494.9° (365) ($c = 0.99$, $CHCl_3$); eims *m/z* (rel. int. %) $[M]^+$ 444 (100), 258 (14), 219 (16), 181 (21), 168 (79), 153 (21); ^1H nmr (300 MHz, $CDCl_3$) δ 6.44 (2H, s, H-2′, H-6′), 6.30 (1H, s, H-8), 5.95 (2H, s, H_a-13, H_b-13), 5.03 (1H, d, H-4, $J_{4\text{-}3} = 8.1$), 4.64 (1H, dd, H_a-11, $J_{11a/3} = 7.1$, $J_{11a\text{-}11b} = 8.8$), 4.54 (1H, d, H-1, $J_{1\text{-}2} = 4.4$), 4.16 (3H, s, 5-OMe), 4.07 (1H, dd, H_b-11, $J_{11b\text{-}3} = 10.6$), 3.81 (3H, s, 4′-OMe), 3.77 (6H, s, 3′-OMe, 5′-OMe), 2.75 (1H, dd, H-2, $J_{2\text{-}3} = 14.8$, $J_{2\text{-}1} = 4.4$), 2.87 (1H, m, H-3); ^{13}C nmr (75.4 MHz, $CDCl_3$) δ 174.24 (C-12), 152.41 (C-2′ + C-5′), 149.27 (C-7), 141.40 (C-5), 136.90 (C-6), 134.76 (C-1′), 134.54 (C-4′), 132.64 (C-9), 124.76 (C-10), 107.93 (C-2′ + C-6′), 104.18 (C-8), 101.22 (C-13), 71.77 (C-11), 70.35 (C-4), 60.81 (4′-OMe), 59.79 (5-OMe), 56.03 (3′-OMe + 5′-OMe), 44.95 (C-2), 44.42 (C-1), 38.88 (C-3).

5′Demethoxy-5-methoxypodophyllotoxin [**2**].—Eims *m/z* (rel. int. %) $[M]^+$ 414 (100), 258 (30), 231 (23), 151 (56), 139 (98).

CYTOTOXICITY ASSAYS.—The cytotoxicity of the lignans was tested against the murine Ehrlich ascites tumor (EAT) and the human HeLa (cervix uteri) cell lines. These cell lines were cultured routinely at 37° in a humidified incubator with 5% CO_2 at the Department of Radiobiology, University of Groningen, The Netherlands. EAT cells were grown in suspension culture in RPMI 1640 medium (Gibco, Paisley, Scotland) supplemented with 10% fetal calf serum (Gibco) plus 50 $\mu g \cdot ml^{-1}$ streptomycin and 50 $IU \cdot ml^{-1}$ penicillin G. HeLa cells were cultured in Joklik's modification of MEM (Gibco) with 10% fetal

calf serum, 50 $\mu g \cdot ml^{-1}$ streptomycin, and 50 $IU \cdot ml^{-1}$ penicillin G. The doubling time of the EAT cells was 12–14 h and of the HeLa cells ca. 24 h. The experiments were initiated with exponentially growing cells. The viability of the cells used in the experiments exceeded 95%, as determined with trypan blue. The lignans, 5-methoxypodophyllotoxin [**1**], podophyllotoxin [**3**] (Sigma), etoposide [**4**] (Bristol-Myers), teniposide [**5**] (Bristol-Myers, via Dept. of Radiobiology, Groningen, The Netherlands), and 5-methoxypodophyllotoxin-4-β-glucoside [**6**] (TNO, Zeist, The Netherlands), were dissolved in 96% EtOH just before use at a concentration of 0.5 or 5 mM, depending on the solubility of the compounds. In addition, a dilution containing 75% of the former solutions was prepared. In the experiments, microtiter test plates (Cel-Cult, Sterlin Ltd., Feltham, UK) with eight rows of 12 concave-bottomed wells each were used. In these wells, 24 twofold dilutions of the lignans were made in 100 μl culture medium. Subsequently, 2000 viable cells in 100 μl medium were added. The test plates were incubated at 37° in humidified incubator with 5% CO_2 for 4 days (32,33). The cytotoxic effect of the lignans was then visually determined by comparing the diameter of the sedimented cell pellets. The ED_{50} values, defined as the drug concentration causing 50% growth inhibition of the tumor cells, was calculated. As a regular control in the experiments, the growth of the tumor cells without addition of the cytotoxic agent was checked. The medium concentration of EtOH causing growth inhibition was beyond the cytotoxic concentration of the lignans.

ACKNOWLEDGMENTS

We thank Prof. Dr. A.W.T. Konings from the Department of Radiobiology, University of Groningen, for kindly providing facilities for the cytotoxic testing. We also thank Dr. A.J.R.L. Hulst for taking care of ^1H- and ^{13}C-nmr spectra and Dr. J.F.G.A. Jansen for the determination of optical rotation, both from the Department of Organic Chemistry, University of Groningen, The Netherlands. Bristol-Myers Squibb B.V., Woerden, The Netherlands is thanked for the generous gift of etoposide and teniposide. Finally, we are grateful to R. Bos for providing the gc-ms and ms data.

LITERATURE CITED

1. H. Stähelin and A. Von Wartburg, *Prog. Drug. Res.*, **33**, 169 (1989).
2. P.J. Clark and M.L. Slevin, *Clin. Pharmacokinet.*, **12**, 223 (1987).
3. J.J.M. Holthuis, *Pharm. Weekbl. Sci. Ed.*, **10**, 101 (1988).
4. S.P. Forsey, D. Rajapaksa, N.J. Taylor, and R. Rodrigo, *J. Org. Chem.*, **54**, 4280 (1989).
5. B.F. Issel, A.R. Rudolph, and A.C. Louie, in: "Etoposide (VP-16-213)—Current Status and New Developments." Ed. by B.F. Issel, F.M. Muggia, and S.K. Carter, Academic Press, Orlando, 1984, p. 1.
6. R. Gupta and K.L. Sethi, in: "Conservation of Tropical Plant Resources. Botanical Survey of India." Ed. by S.K. Jain and K.L. Mehra, Howrah, New Delhi, 1983, p. 101.
7. P.G. Kadkade, *Naturwissenschaften*, **68**, 481 (1981).
8. P.G. Kadkade, *Plant Sci. Lett.*, **25**, 107 (1982).
9. J. Berlin, V. Wray, C. Mollenschott, and F. Sasse, *J. Nat. Prod.*, **49**, 435 (1986).
10. J. Berlin, N. Bedorf, C. Mollenschott, V. Wray, F. Sasse, and G. Höfle, *Planta Med.*, **54**, 204 (1988).
11. W. Van Uden, N. Pras, J.F. Visser, and Th.M. Malingré, *Plant Cell Rep.*, **8**, 165 (1989).
12. W. Van Uden, N. Pras, E.M. Vossebeld, J.N.M. Mol, and Th.M. Malingré, *Plant Cell Tissue Organ Cult.*, **20**, 81 (1990).
13. H.J. Woerdenbag, W. Van Uden, H.W. Frijlink, C.F. Lerk, N. Pras, and Th.M. Malingré, *Plant Cell Rep.*, **9**, 97 (1990).
14. W. Van Uden, N. Pras, and Th.M. Malingré, *Plant Cell Rep.*, **9**, 257 (1990).
15. H.J. Wichers, M.P. Harkes, and R.J. Arroo, *Plant Cell Tissue Organ Cult.*, **23**, 93 (1990).
16. W. Van Uden, N. Pras, and Th.M. Malingré, *Plant Cell Tissue Organ Cult.*, **23**, 217 (1990).
17. W. Van Uden, N. Pras, S. Batterman, J.F. Visser, and Th.M. Malingré, *Planta*, **183**, 25 (1991).
18. A.G. Hyenga, J.A. Lucas, and P.M. Dewick, *Plant Cell Rep.*, **9**, 382 (1990).
19. H.J. Wichers, G.G. Versluis-De Haan, J.W. Marsman, and M.P. Harkes, *Phytochemistry*, **30**, 3601 (1991).
20. W. Van Uden, N. Pras, B. Homan, and Th.M. Malingré, *Plant Cell Tissue Organ Cult.*, **27**, 115 (1991).
21. A.J. Broomhead and P.M. Dewick, *Phytochemistry*, **29**, 3839 (1990).
22. A. San Feliciano, J.M. Miguel del Corral, M. Gordaliza, and A. Castro, *Phytochemistry*, **29**, 1335 (1990).
23. J.A. Green, *Eur. J. Cancer Clin. Oncol.*, **25**, 913 (1989).
24. D.A. Stringfellow and J.E. Schurig, *Cancer Treat. Rev.*, **14**, 291 (1987).

25. D.C. Ayres and J.D. Loike, "Chemistry and Pharmacology of Natural Products. Lignans. Chemical, Biological and Clinical Properties." University Press, Cambridge, 1990, p. 156.
26. O. Buchardt, R.B. Jensen, H.F. Hansen, P.E. Nielsen, D. Andersen, and I. Chinoin, *J. Pharm. Sci.*, **75**, 1076 (1986).
27. J.L. Hartwell and A.W. Schrecker, in: "Progress in the Chemistry of Organic Natural Products. Vol. 15." Ed. by L. Zechmeister, Springer Verlag, Wien, 1958, p. 83.
28. K.V. Andersen, O. Buchardt, H. Frydenlund Hansen, R. Boe Jense, and S. Larsen, *J. Chem. Soc., Perkin Trans 2*, **11**, 1871 (1990).
29. H.F. Stähelin and A. Von Wartburg, *Cancer Res.*, **51**, 5 (1991).
30. J.M.S. Van Maanen, J. Retèl, J. De Vries and H.M. Pinedo, *J. Natl. Cancer Inst.*, **80**, 1526 (1988).
31. T. Murashige and F. Skoog, *Physiol. Plant.*, **15**, 473 (1962).
32. H.B. Lamberts, A. Van der Meer-Kalverkamp, J.C. Van de Grampel, A.A. Van der Huizen, A.P. Jekel, and N.H. Mulder, *Oncology*, **40**, 301 (1983).
33. H.J. Woerdenbag, H. Hendriks, Th.M. Malingré, R. Van Stralen, K.J. Van den Berg, and A.W.T. Konings, *Phytother. Res.*, **2**, 109 (1988).

Received 10 June 1991