EXERCISE VI.1

PODOPHYLLOTOXIN: SOURCES, EXTRACTION, AND PREPARATION OF CYTOTOXIC ANALOG COMPOUNDS

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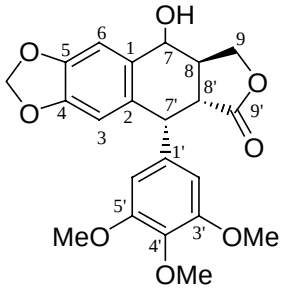
Introduction

Podophyllotoxin is the most abundant lignan isolated from Podophyllin, the resin obtained from species of the genera *Podophyllum* (Berberidaceae) (Ayres, 1990 and refs. cited therein). Lignans are a family of natural products that originated as secondary metabolites through the shikimic acid pathway. They are formed by the union of two phenylpropane units and constitute a complex family of skeletons and characteristic functionalizations, which can be subdivided into four groups: lignans, neolignans, oxyneolignans, and trimers, higher analogs, and mixed lignanoids (Moss, 2000). Among the lignans group, cyclolignans present a carbocycle between both phenylpropane units, created by two single carbon–carbon bonds through the side chains, one of them between the β–β' positions. The aryltetralin structure of podophyllotoxin belongs to cyclolignans.



7-hydroxi-3´,4´,5´-trimethoxi-4,5-methilendioxi-2,7´-cyclolignan-9´,9-lactone

Nomenclature and numering of lignans
(IUPAC recomendations 2000) (Moss, 2000)

Interest in lignans

Plants containing lignans have been used since approximately 1000 years ago as folk remedies in traditional medicine of many diverse cultures. Plants with high lignan content were commonly used in Chinese, Japanese, and the Eastern world folk medicine, for example, *Kadsura coccinea* (Schizandraceae), *Fraxinus sp.* and *Olea europaea* (Oleaceae)1 (Ayres, 1990).

The very extensive use in traditional medicine makes the lignans an important family of starting products for the development of new therapeutic agents based on structural modifications of such compounds. Actually, there are different biological activities[2] in lignans that make them interesting for several investigation groups, like reverse transcriptase inhibition and anti-HIV activity, immunomodulating activity, effects on cardiovascular system, properties against leishmaniosis, effects on high-

density lipoproteins and hypolipemiants properties, 5-lipooxigenase inhibition, antifungic, antirheumatic, antipsoriasis, and antimalarial and antiasmatic properties.

But cytotoxicity and antiviral are the more important activities that maintain the interest in podophyllotoxin and analogs[1,2].

Podophyllotoxin: Pharmacological activity and applications

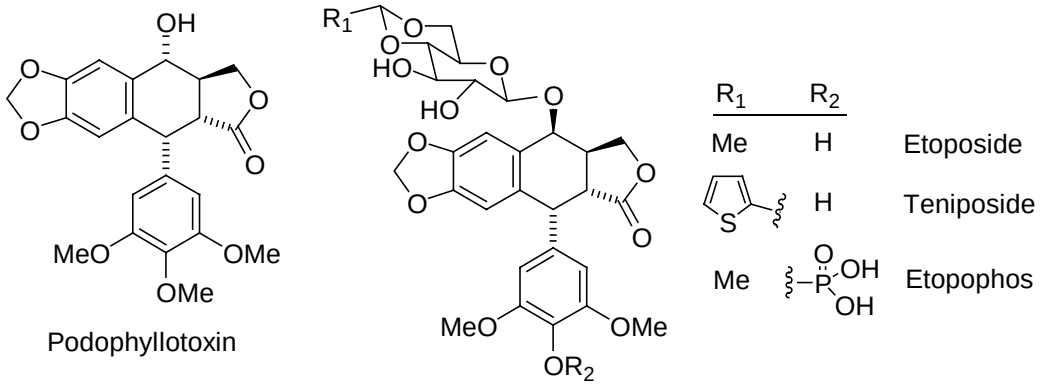
Extracts of *Podophyllum* species have been used for diverse cultures since remote times as antidotes against poisons and toxic, cathartic, purgative, antihelminthic, vesicant, and suicide agents (ref. 1 and refs. cited therein). Podophyllin was included in the U.S. Pharmacopoeia in 1820, and the use of this resin for the treatment of venereal warts was described, attributing this action to podophyllotoxin. The destructive effect of this resin on experimental cancer cells in animals was published too.

Also, podophyllotoxin is included in many Pharmacopoeias and used as an antiviral agent in the treatment of *Condyloma acuminatum* caused by human papilloma virus –HPV- and other venereal and perianal warts. The application of podophyllotoxin cured almost all the warts completely in less time than other strategies and with fewer side effects. Podophyllotoxin and analog compounds are also active against cytomegalovirus and Sindbis virus. Podophyllotoxin is also effective in the treatment of anogenital warts in children and against *Molluscum contagiosum*, which is generally a self-limiting benign skin disease that affects mostly children, young adults, and HIV patients.

Podophyllotoxin has other uses in dermatology: it is a useful agent in psoriasis vulgaris. Antitumor activity is another outstanding property of podophyllotoxin. It is effective in the treatment of Wilms tumors, different types of genital tumors (e.g., carcinoma verrucosus) and in non-Hodgkin's and other lymphomas

Combination therapies are currently being implemented with other chemotherapeutic agents or with other techniques useful in the fight against viral infections and cancer. In this sense, condyloma acuminata respond best to the cryotherapy-podophyllotoxin combination; multiple myeloma responds best to homeotherapy with podophyllotoxin and intermittent local administration of methotrexate and systemic polychemotherapy. In combination with interferon, podophyllotoxin is active in genital human infections caused by vulvar pruritic papillomatosis and together with *cis*-platin is effective in treating neuroblastomas.

The mechanism of action of podophyllotoxin is based on inhibiting the polymerization of tubulin and arresting the cell cycle in the metaphase[1,2].



Podophyllotoxin, etoposide, teniposide and etopophos

Etoposide, teniposide, etopophos, and new cytotoxic derivatives in clinic assays

Three semisynthetic derivatives of podophyllotoxin—etoposide, teniposide and etopophos—are widely as used anticancer drugs and show good clinical effects against several types of neoplasms, including testicular and small-cell lung cancers, lymphoma, leukemia, Kaposi's sarcoma, etc. However, several limitations, such as myelosuppression, development of drug resistance, and cytotoxicity toward normal cells, still exist.

On the basis of molecular modeling studies, Mac Donald et al. (1992) proposed a composite pharmacophore model with three structurally distinct domains: a DNA intercalating moiety, the minor groove binding site, and the molecular region that can accommodate a number of structurally diverse substituents, which might also bind to the minor groove.

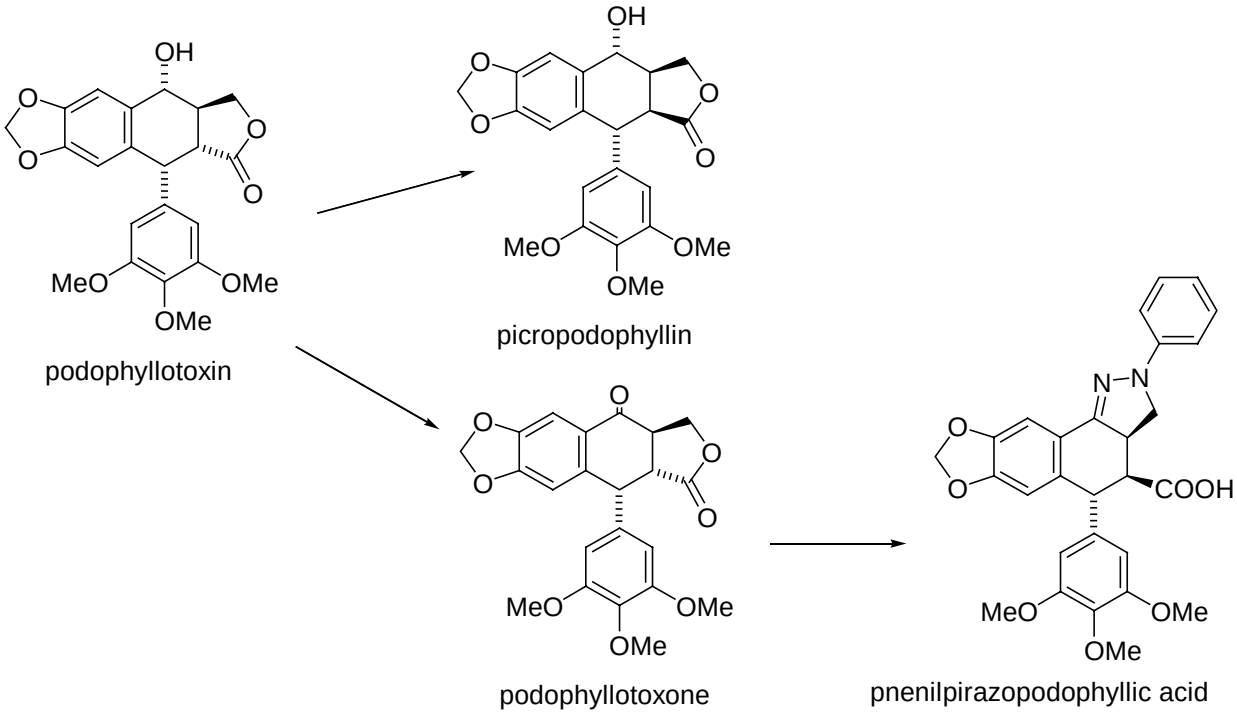
Materials and method

a) Podophyllotoxin: Sources and extraction

Podophyllotoxin has traditionally been isolated from podophyllin, resin of *Podophyllum* rhizome. *Podophyllum emodi* (Indian podophyllum) is preferred to *Podophyllum peltatum* (American podophyllum). The new extraction protocol was applied to other genera: *Linum*, *Juniperus*, *Hyptis*, *Teucrium*, *Nepeta*, *Dysosma*, *Jeffersonia*, *Thymus*, *Thuja* (Bedir, 2002), resulting in another alternative source of podophyllotoxin: needles from *Juniperus virginiana* L. showed 4.7 % of dry weight of podophyllotoxin.

Procedure of isolation: The resin (50 g) of *P. peltatum* was extracted with hot $CHCl_3$ over a day. The soluble fraction was chromatographed on neutral alumina (activity II), and eluted with $CHCl_3$ to yield 20.4 g (40.8 %) of podophyllotoxin. The main lignan compounds of the rest of extract are: deoxypodophyllotoxin (1 %), β-peltatin (9 %), α-peltatin (7 %)[3].

b) Chemistry



Preparation of picropodophyllin: 150 mg of podophyllotoxin in 10 mL of 1 % KOH in MeOH was stirred for 30 min at r.t. After neutralization and extraction with AcOEt, 140 mg (93 %) of picropodophyllin was obtained[3].

Preparation of podophyllotoxone: Pyridinium dichromate (PDC) (1.3 g, 3.45 mmol) was added to a solution of podophyllotoxin **1** (1 g, 2.4 mmol) in dry dichloromethane (25 mL) and stirred at r.t. for 4 h. The excess of PDC was removed by filtration followed by CC of the residue on silica gel to give 750 mg (76 %) of podophyllotoxone and 200 mg (20 %) of unreacted podophyllotoxin[4].

Synthesis of phenylpyrazopodophyllic acid: Phenylhydrazine (0.4 mL, 4.06 mmol) was added to a solution of podophyllotoxone (1 g, 2.4 mmol) in 5 mL of glacial acetic acid and stirred at room temperature for 24 h. After addition of water, the unreacted ketone (90 mg) precipitated and was filtered off. The filtrate was treated with sat. aq. $NaHCO_3$ and extracted with ethyl acetate. After removing the solvent, 900 mg (74 %) of phenylpyrazopodophyllic acid were obtained. M.p. 142–145 °C (CH_2Cl_2)[4].

Note: The spectroscopic data for identification of each compound can be obtained from the cited references.

References

1. Ayres, D.C., Loike J.D., 1990. "Lignans. Chemical, biological and clinical properties", Cambridge University Press, Cambridge.
2. a) Gordaliza, M.; Castro, M.A.; Miguel del Corral, J.M., San Feliciano, A., 2000. "Antitumor properties of podophyllotoxin and related compounds". *Curr. Pharm. Design* 6, 1811-1839.

b) Gordaliza, M., García, P.A.; Miguel del Corral, J.M. Castro, M.A., Gómez-Zurita, M.A. 2004 "Podophyllotoxin: distribution, sources, applications and new cytotoxic derivatives". *Toxicon* 44, 441-459

3. Miguel del Corral, J.M. Gordaliza, M.; Castro, M.A.; Morales, L.J.; López, J.L.; San Feliciano, A. 1995 "Methyl ethers of podophyllotoxin-related cyclolignans" *J.Nat. Prod. 58*, 870-877
4. Gordaliza, M., Miguel del Corral, J.M., Castro, M.A., López-Vázquez, M.L., San Feliciano, A., García-Grávalos, M.D., Carpy, A., 1995. "Synthesis and evaluation of pyrazolignans"!. A new class of cytotoxic agents. *Bioorg. Med. Chem.* 3, 1203-1210.

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