PERGAMON

Phytochemistry 54 (2000) 115–120

PHYTOCHEMISTRY

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Molecules of Interest

Podophyllotoxin

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Accepted 9 March 2000

Abstract

Podophyllin, an ethanolic extract of *Podophyllum peltatum* L. or *P. emodi* Wall (syn. *P. hexandnum* Royle), is a good source of the aryltetralin-type lignan, podophyllotoxin. The latter compound, as well as its congeners and derivatives exhibit pronounced biological activity mainly as strong antiviral agents and as antineoplastic drugs. The podophyllotoxin derivatives etoposide, etopophos (etoposide phosphate), and teniposide are thus successfully utilized in the treatment of a variety of malignant conditions. Continued research on the *Podophyllum* lignans is currently focused on structure optimization to generate derivatives with superior pharmacological profiles and broader therapeutic scope, and the development of alternative and renewable sources of podophyllotoxin. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Podophyllotoxin; Aryltetralin lignan; *Podophyllum peltatum*; *P. emodi*; Berberidaceae; Antineoplastic drugs; Etoposide; Etopophos; Teniposide; Tubulin; Topoisomerase

1. Introduction

The aryltetralin lactone (−)-podophyllotoxin **1** occupies a unique position among lignan natural products since its glucopyranoside derivative was recognized as a potent antitumor factor (Jardine, 1980). This discovery entails a particularly fascinating account, involving a multitude of investigations conducted over a period of more than a century (Stähelin and von Wartburg, 1991). The studies culminated in the structure elucidation of podophyllotoxin, the assessment of its biological activity and the discovery of its mode of action. Initial expectations regarding the clinical utility of podophyllotoxin were tempered largely due to its unacceptable gastrointestinal toxicity. This led chemists in the pharmaceutical research department of Sandoz, to investigate the possibility that the *Podophyllum* lignans might occur naturally as glycosides (Stähelin et al., 1991). Using special procedures to inhibit enzymatic degradation, these researchers indeed obtained the podophyllotoxin-β-D-glucopyranoside **5** as the main component and its 4′-demethyl derivative **6** from the Indian *Podophyllum* species. Both of these glucosides and the glucosides **8** and **10** of α- and β-peltatin **7** and **9** (Fig. 1) were also isolated from the American *P. peltatum*. Being less hydrophobic, the glucosides displayed lower toxicity than the aglucones, but their cytostatic activity was reduced to the same degree. The research efforts were then focused on a program to chemically modify both the glucosides and aglucones of a wide range of podophyllotoxin derivatives. Nearly 600 derivatives were prepared and tested over a period of about 20 years (Stähelin et al., 1991). Somehow serendipitously, a radical change in mechanism of action and a quantum step in therapeutic utility were effected by acetalization of the 4- and 6-hydroxy groups of the glucopyranose moiety using aldehydes, eventually leading to the discovery of the clinically important anticancer drugs etoposide **11**, etopophos **12** (Schacter, 1996) and teniposide **13** (Fig. 1).

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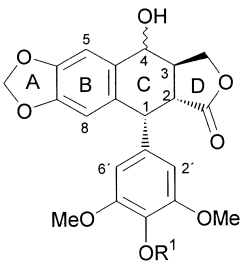
0031-9422/00/$ - see front matter
PII: S0031-9422(00)00094-7

2. Biosynthesis

Lignans are biosynthetically derived from the phenylpropanoid pathway. The sequence leading to (−)-matairesinol **19**, the presumed precursor to (−)-podophyllotoxin **1** in *P. hexandrum*, was elucidated using *Forsythia intermedia* as a model system (Davin et al., 1997). In the presence of a one-electron oxidant e.g., laccase, and a 78 kDa dirigent protein, *E*-coniferyl alcohol **14** is converted into (+)-pinoresinol **16** via regio- and stereoselective intermolecular 8, 8′-coupling of the putative enzyme bound intermediate radical **15** (Fig. 2). Sequential stereoselective reduction of (+)-pinoresinol **16** then occurs to consecutively generate (+)-lariciresinol **17** followed by (−)-secoisolariciresinol **18**. The stereoselectivity of this process results in inversion of the configuration at C-2 and C-5 of pinoresinol, a process which is envisaged to occur either by a concerted S_N2 mechanism or via reduction of an intermediate quinomethane. Stereoselective dehydrogenation of (−)-secoisolariciresinol **18** then occurs to give (−)-matairesinol **19**. The efficient incorporation of (−)-[^{14}C] matairesinol into (−)-podophyllotoxin **1**, β-peltatin **9**, 4′-demethylpodophyllotoxin **2** and α-peltatin **7** in *Podophyllum* demonstrates that (−)-matairesinol **19** is probably the common precursor to both groups of the *Podophyllum* lignans (Broomhead et al., 1991). Thus, (−)-matairesinol **19** is presumably converted to yatein or 4′-demethylyatein which are respectively transformed into podophyllotoxin **1**/β-peltatin **9** or 4′-demethylpodophyllotoxin **2**/α-peltatin **7** via the appropriate quinomethane intermediates.

3. Synthesis

Although the natural podophyllin resin was used in folk medicine, it was not until its antitumor activity was confirmed in the 1940's that synthetic studies of the podophyllotoxins were undertaken. Earlier efforts

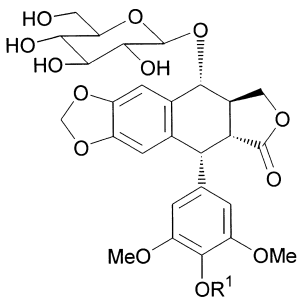


1 R^1 = Me, ⦚ = ⦙

2 R^1 = H, ⦚ = ⦙

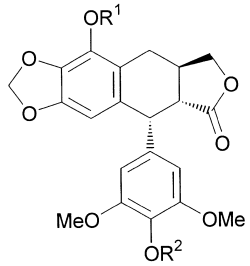
3 R^1 = Me, ⦚ = ▲

4 R^1 = H, ⦚ = ▲



5 R^1 = Me

6 R^1 = H

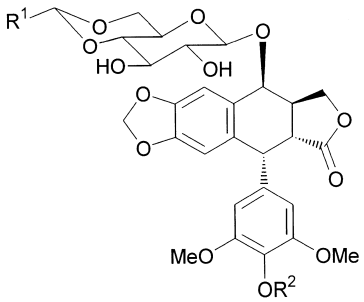


7 $R^1 = R^2$ = H

8 R^1 = β-D-glucopyranosyl, R^2 = H

9 R^1 = H, R^2 = Me

10 R^1 = β-D-glucopyranosyl, R^2 = Me



11 R^1 = Me, R^2 = H

12 R^1 = Me, R^2 = $PO(OH)_2$

13 R^1 =  , R^2 = H

Fig. 1. Structures of (−)-podophyllotoxin **1** and its congeners and derivatives.

on synthetic, structural and mechanistic aspects of podophyllotoxin have provided much of the basis for the synthetic endeavors that followed (Ward, 1992). The challenge of a stereoselective synthesis is embedded in the formation of the four contiguous stereocenters and the presence of a base sensitive *trans*-lactone moiety. The 1,2-*cis*-2,3-*trans* configuration, which is of crucial importance for biological activity (vide infra), constitutes a real thermodynamic trap due to facile epimerization to the less strained but inactive *cis*-lactone, picropodophyllotoxin (C-2 diastereomer of **1**). The stereochemistry at C-4 is of less concern since podophyllotoxin **1** and epipodophyllotoxin **3** are readily interconvertible. Furthermore, in the synthesis of etoposide **11**, etopophos **12** and teniposide **13**, the glucosylation reaction exclusively affords the C-4β glucoside regardless of the C-4 configuration of the starting material **1** or **3** (Stähelin et al., 1991).

Four general approaches to the synthesis of podophyllotoxin derivatives have been developed. Several variations and innovations have been introduced within each of the overall schemes. The four routes (Fig. 3) involve either the elaboration of a χ-oxo ester (the oxo ester route, **20** → **21** → **1**) (Kende et al., 1981), the lactonization of a dihydroxy acid (the dihydroxy acid route, **22** → **23**) (Macdonald and Durst, 1988), the cyclization of a conjugate addition product (the tandem conjugate addition route, **24** + **25** + **26** → **27** → **28**) (Ziegler and Schwartz, 1978), or the utilization of a Diels–Alder reaction (**29** + **30** → **31**) (Klemm et al., 1971) to construct the aryltetralin unit. A number of syntheses, particularly some of those in the dihydroxy acid route, also adopt the Diels–Alder protocol to establish the aryltetralin molecular framework.

The majority of useful stereoselective routes to podophyllotoxin and its analogs/derivatives (Andrews et al., 1988; Van Speybroeck et al., 1991; Charlton and Koh, 1992; Bush and Jones, 1995) have also been designed according to the synthetic principles underpinning these approaches. Although a number of synthetic sequences afforded (−)-podopyllotoxin or analogs with excellent enantiopurities, the low overall yields, especially due to the large number of steps (Bush and Jones, 1995), currently still disqualify synthesis as an alternative for naturally produced materials. Finally, extensive modifications at various sites of the podophyllotoxin backbone have been introduced with a view to enhance its therapeutic activity or eliminate undesirable side effects. However, these (and related references) are readily retrievable via the electronic literature network (e.g. Berkowitz et al., 1996).

4. Biological function

Among the plethora of physiological activities and potential medicinal and agricultural applications, the antineoplastic and antiviral properties of podophyllotoxin congeners and their derivatives are arguably the most eminent from a pharmacological perspective. Semisynthetic derivatives of epipodophyllotoxin **3**, e.g. etoposide **11** (Allevi et al., 1993), etopophos **12** and teniposide **13** induce a premitotic block in late S or

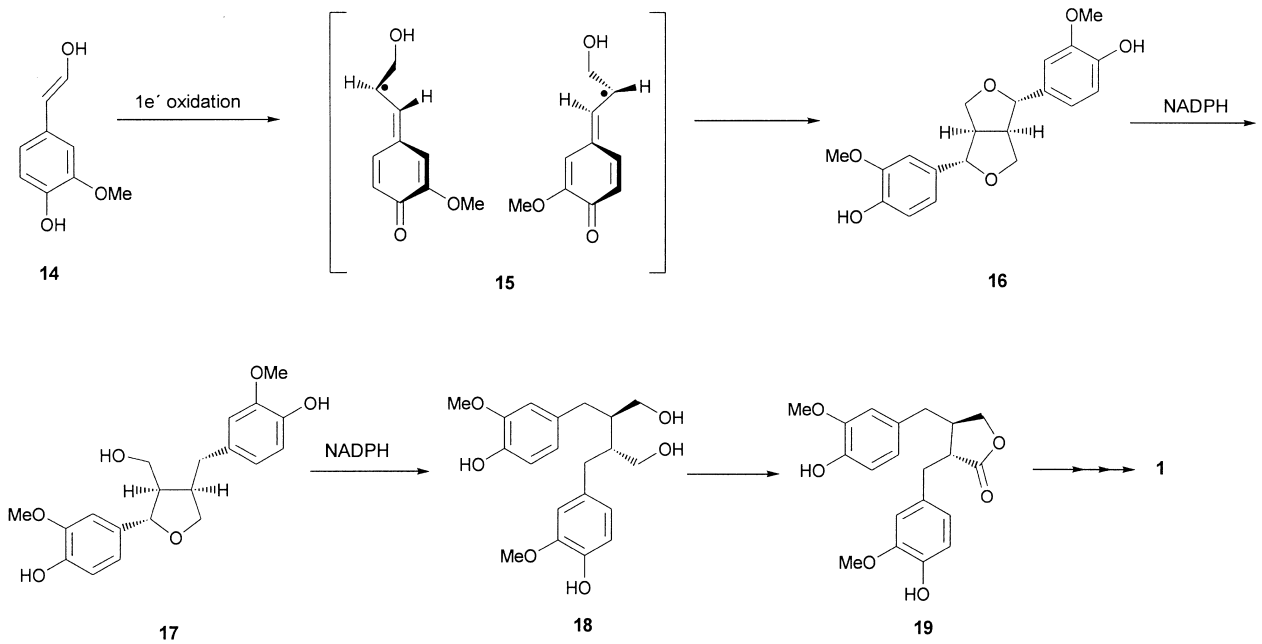


Fig. 2. Proposed biosynthetic pathway to (−)-podophyllotoxin **1**.

early G_2 stage (Hainsworth and Greco, 1995). This results from binding of etoposide to topoisomerase II, an enzyme required for the unwinding of DNA during replication. Topoisomerase II forms a transient, covalent DNA–protein link, the cleavable complex, which allows one double strand of DNA to pass through a temporary break in another double strand. Etoposide binds to and stabilizes the cleavable complex preventing repair of the double-strand breaks. Etopophos **13**, launched in 1996 by Bristol–Myers Squib (Schacter, 1996), is a water-soluble phosphate ester prodrug of etoposide. The prodrug can be administered in higher doses than etoposide as a short intravenous injection, whereafter it is rapidly converted to the parent compound by plasma phosphatases, and thus constitutes an improved formulation of etoposide.

The essential modifications which convert (−)-podophyllotoxin from an entity that interacts with tubulin and blocks mitosis to one that arrests the cell cycle at G_2 by interacting with topoisomerase II are: demethylation of the C-4′ methoxy group; epimerization at C-4; glucosylation at C-4; and acetalization of the 4- and 6-hydroxy groups of the glucopyranose units using aldehydes (Imbert, 1998). Some features that affect the

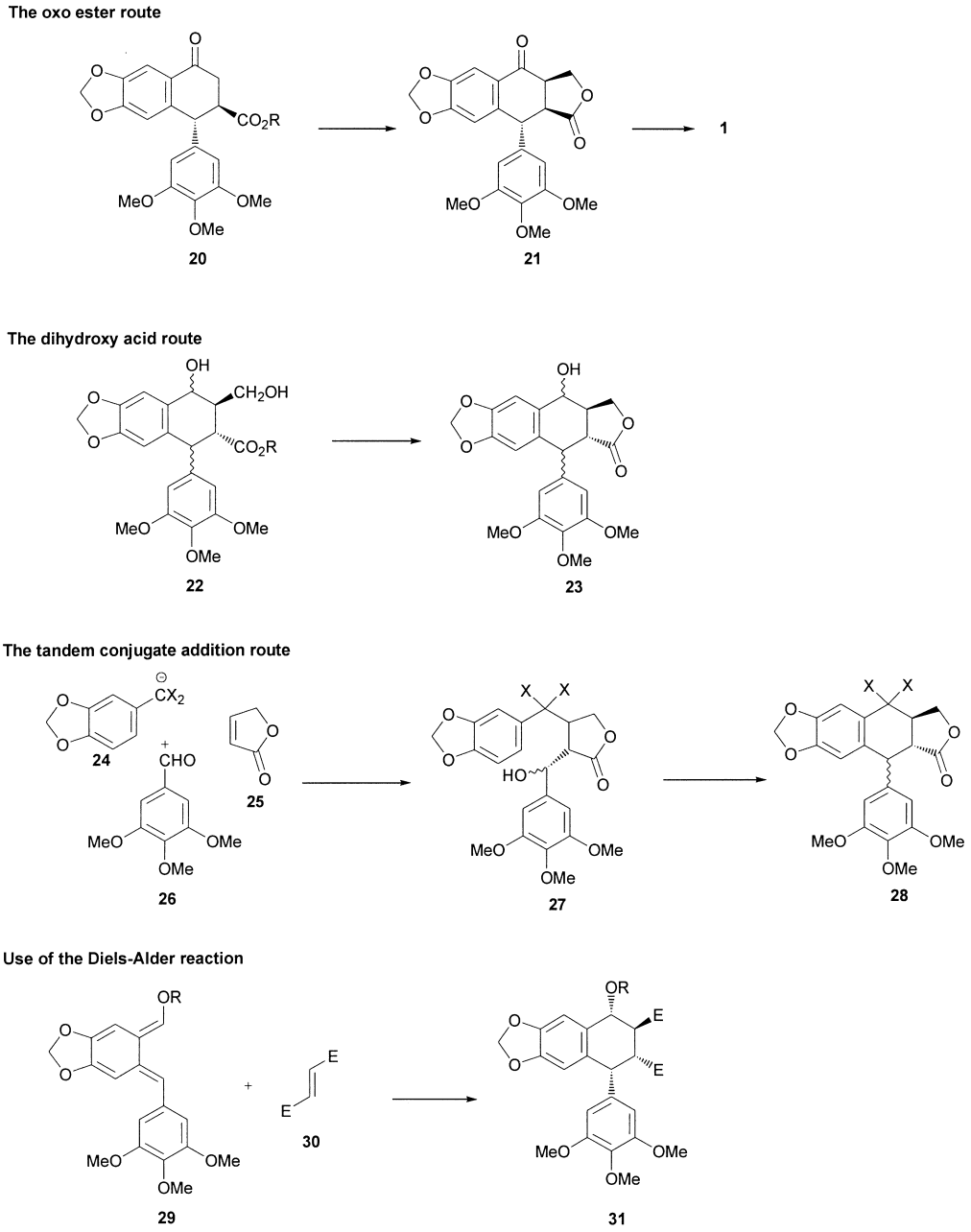


Fig. 3. The four general approaches to the synthesis of podophyllotoxin derivatives.

inhibitory properties of podophyllotoxins analogs have been identified. Compounds with C-4β configuration tend to be cytotoxic and inhibit microtubule polymerization, whereas those with C-4β configuration are less cytotoxic and inhibit DNA-topoisomerase II. All podophyllin-based drugs possess modified 4β-D-glucoside moieties. However, highly active derivatives have been synthesized that have either amino or alkyl residues at C-4 (Huang et al., 1999) with a view to stabilize this stereocenter towards racemization. With few exceptions, the C-2α, C-3β *trans* configuration is of crucial importance for biological activity.

The similarity between the mode of action of aryltetralin lignans as anticancer and antiviral agents is noteworthy. Owing to their ability to bind tubulin, these lignans disrupt the cellular cytoskeleton and thus interfere with viral replication. In addition to tubulin binding, synthetic podophyllotoxin analogs show inhibition of reverse transcriptase which may be exploited to selectively combat RNA viruses such as the human immunodeficiency virus (HIV).

5. Current demand/alternative sources

While the traditional source of podophyllotoxin, the endangered Indian *P. emodi* species, becomes scarcer, the demand for the compound continues to increase. U.S. sales of etoposide tripled in 1995 and have since risen at an annual rate of more than 10%. Etoposide is used in combination therapy in refractory testicular, lymphoid and myeloid leukemia, stomach, ovarian, brain, breast, pancreatic, and small and large cell lung cancers. Approximately 60 clinical trials are under way to test etoposide for new indications, and a further 140 trials use the drug as positive control. In addition, numerous new podophyllotoxin derivatives are currently under development and evaluation as topoisomerase inhibitors and potential anticancer drugs.

Such a growing demand for podophyllotoxin thus exerts severe pressure on the natural source, *P. emodi* and has already drastically reduced the size of natural populations. This explains the urgency regarding the development of shorter synthetic routes, the utilization of biotechnological and enzymatic approaches, manipulation of the biosynthetic pathway, and above all, the aggressive search for alternative and renewable natural sources like the American *P. peltatum* (mayapple) (Moraes-Cerdeira et al., 1998).

6. Conclusions

The introduction of etoposide **11**, etopophos **12**, teniposide **13** and various other analogs and derivatives of (−)-podophyllotoxin **1** into the armory of antitumor drugs, is an excellent example of the manner in which useful pharmaceuticals may be developed from folk remedies. Thus, more than fifty years after the first medicinal application of the antimitotic activity of (−)-podophyllotoxin was proposed, this aryltetralin lignan continues to be the subject of extensive research. Efforts to rationally design a better antineoplastic drug based on the aryltetralin framework have hitherto failed. This fact, together with the large number of potential structural variations, makes the fortuitous discovery of etoposide all the more remarkable.

Acknowledgements

This work was partly funded by USDA grants 97-35501-4886 and 58-6408-7-012.

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