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Biotechnology and synthetic chemistry: Routes to clinically important natural products

J.P. Kutney

Department of Chemistry, University of British Columbia,
2036 Main Mall, Vancouver, B.C., Canada V6T 1Z1

Abstract: The development of plant cell culture methods, in combination with chemistry, affords an attractive and often a powerful route to complex natural products. Several examples of such an interdisciplinary program are cited to illustrate the various types of research strategies which have been pursued. Studies with cell cultures of Catharanthus roseus provide biosynthetic information and subsequently an entry into the efficient synthesis of the clinical anti-cancer drugs vinblastine and vincristine. Experiments with enzymes derived from C. roseus and Podophyllum peltatum cell lines and dibenzylbutanolides as precursors, reveal an attractive route to podophyllotoxin analogues required for synthesis of the anti-cancer drug etoposide. Still other studies with a cell line of Tripterygium wilfordii, an important Chinese herbal plant, allow the production of novel terpenoid systems of pharmacological interest.

Introduction

The plant kingdom has, for many years, provided an important source of natural products many of which have formed the basis for development of medicinally important drugs. Unfortunately, Nature often provides such compounds in low yields and the difficulties associated with their isolation from other less interesting co-occurring constituents can present problems particularly when large quantities of the biologically active compound are required. It is possible to alleviate such difficulties by the use of plant cell culture methodology and when these techniques are coupled with chemistry, a powerful route to such natural products and/or their biologically important analogues, is achieved. This lecture will present results to illustrate how the interplay of plant cell culture methodology, in combination with chemistry, can afford interesting routes to clinically important plant derived medicinal agents. A discussion of the various avenues of research will include: i) studies of biosynthesis and application of biosynthetic information toward development of highly efficient syntheses of clinical drugs; ii) use of plant cell cultures or enzymes derived therefrom , as "reagents" in organic synthesis; iii) use of plant cell cultures to produce higher levels of plant derived natural products and novel compounds for pharmacological screening; iv) an illustration to show how well developed cell lines can afford the opportunity to separate pharmacological activities exhibited by complex mixtures generally employed in herbal medicine practices.

The studies involve the clinical anti-cancer drugs vinblastine, vincristine, etoposide, and natural products of a Chinese herbal plant which possess immunosuppressive activity.

1. Biosynthetic Information Forms a Basis for Efficient Synthesis of the Vinblastine-Vincristine Family.

From a large number of investigations involving enzymes obtained from a stable cell line of Catharanthus roseus and the alkaloids catharanthine (**1**) and vindoline (**3**), we have unravelled the structures of the important late stage intermediates in the biosynthetic pathway of the clinical anti-cancer drugs vinblastine (**7**, R=CH_3) and vincristine (**7**, R=CHO). Fig. 1 summarizes the overall sequence involved. From these data, a highly efficient and commercially important "one-pot" process for the synthesis of the clinical drugs was developed (Fig. 2). The overall process, involving five separate chemical reactions and providing a 40% overall yield of vinblastine, demands that each reaction must proceed with yields in excess of 80%. A recent review (1) summarizes these extensive studies and provides citations to pertinent earlier references.

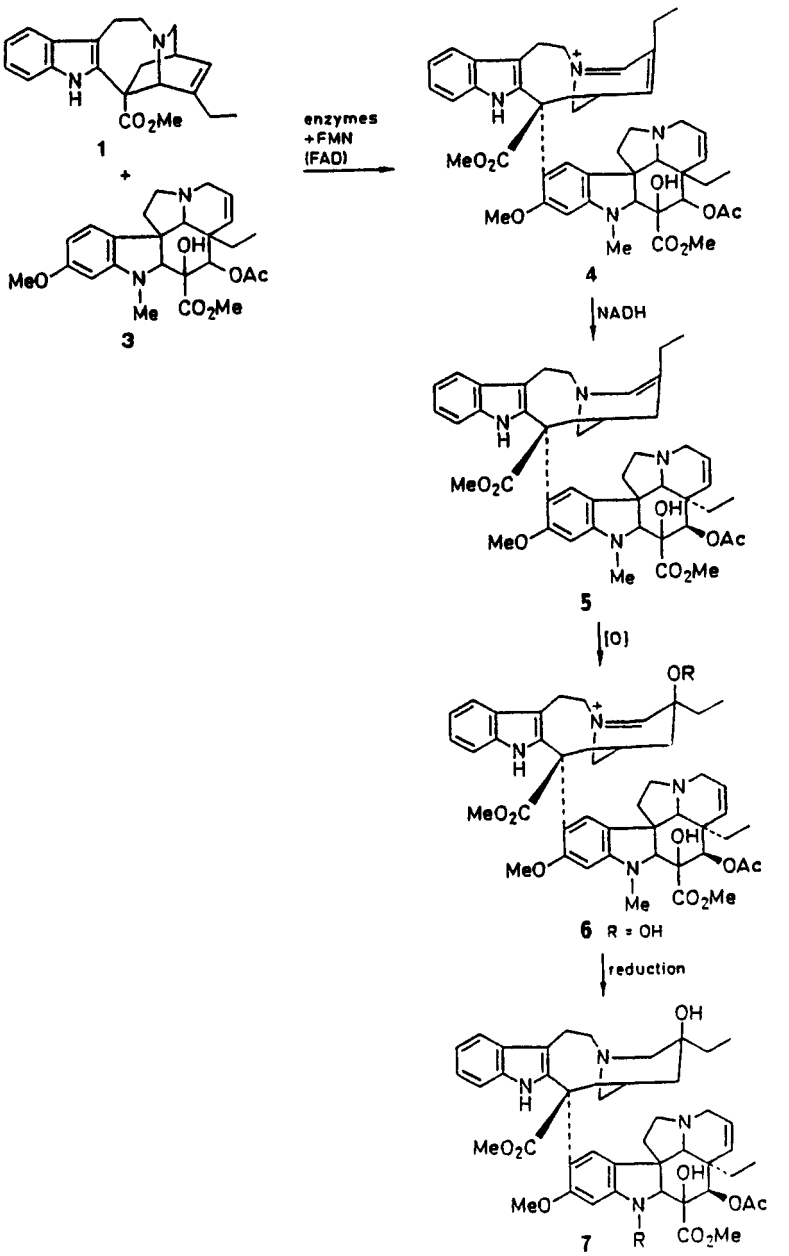


Fig. 1. Overall summary of the biosynthetic pathway of vinblastine (7) from catharanthine (1) and vindoline (3).

2. Plant Cell Cultures as "Reagents" in Studies Related to Etoposide Synthesis.

The podophyllotoxin family (**11**, Fig. 3) of natural products has been extensively studied over the years. One of the important analogues within this family is the clinical anti-cancer drug etoposide (**13**) and studies in our laboratory directed toward the development of an efficient synthesis of **13** are underway. The overall strategy involves the use of appropriate enzymes derived from plant cell cultures as "reagents" in the biotransformation of suitable substrates to end products presently utilized in the commercial production of **13**. Based on biosynthetic information provided from the studies of Dewick and coworkers (2), it appeared that dibenzylbutanolides of general structure **19** (Fig. 4) were appropriate substrates for such studies. A versatile route to **19** and closely related analogues was developed (Fig. 4) and enzyme-catalyzed biotransformations to the

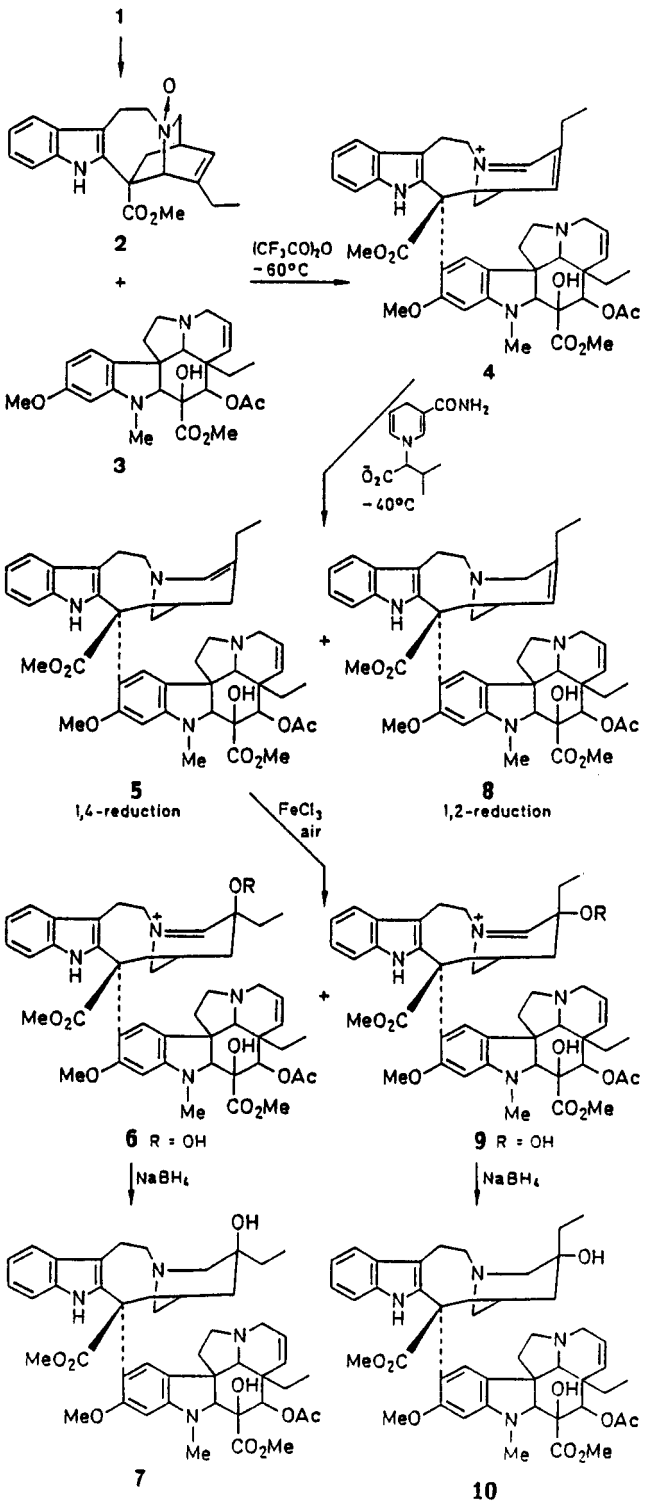
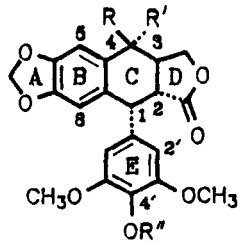


Fig. 2. A highly efficient "one-pot" process for the synthesis of vinblastine (**7**) and leurosidine (**10**) from catharanthine (**1**) and vindoline (**3**).

desired cyclic podophyllotoxin analogues were undertaken. Utilizing enzymes isolated from the above-noted C. roseus cell line and/or whole cell fermentations with a stable cell line of Podophyllum peltatum, the plant from which the podophyllotoxins are normally isolated (Fig.3), highly successful biotransformations were achieved (Fig. 5). With P. peltatum cultures, the biotransformation of **20** (R=OH; R'=H; R"=isopropyl, Fig. 5) to **21**, was achieved via batch and semi-continuous processes. Details of these studies are published (1, 3-5).



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Podophyllotoxin : R=H; R′ =OH; R″ =CH_3

Epipodophyllotoxin : R=OH; R′ =H; R″ =CH_3

Deoxypodophyllotoxin : R=R′ =H; R″ =CH_3

4′ −Demethylpodophyllotoxin : R=H; R′ =OH; R″ =H

4′ −Demethylepipodophyllotoxin : R=OH; R′ =H; R″ =H **(12)**

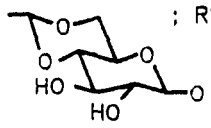
Etoposide **(13)** : R=  ; R′ =H; R″ =H

Fig. 3. The podophyllotoxin family of compounds.

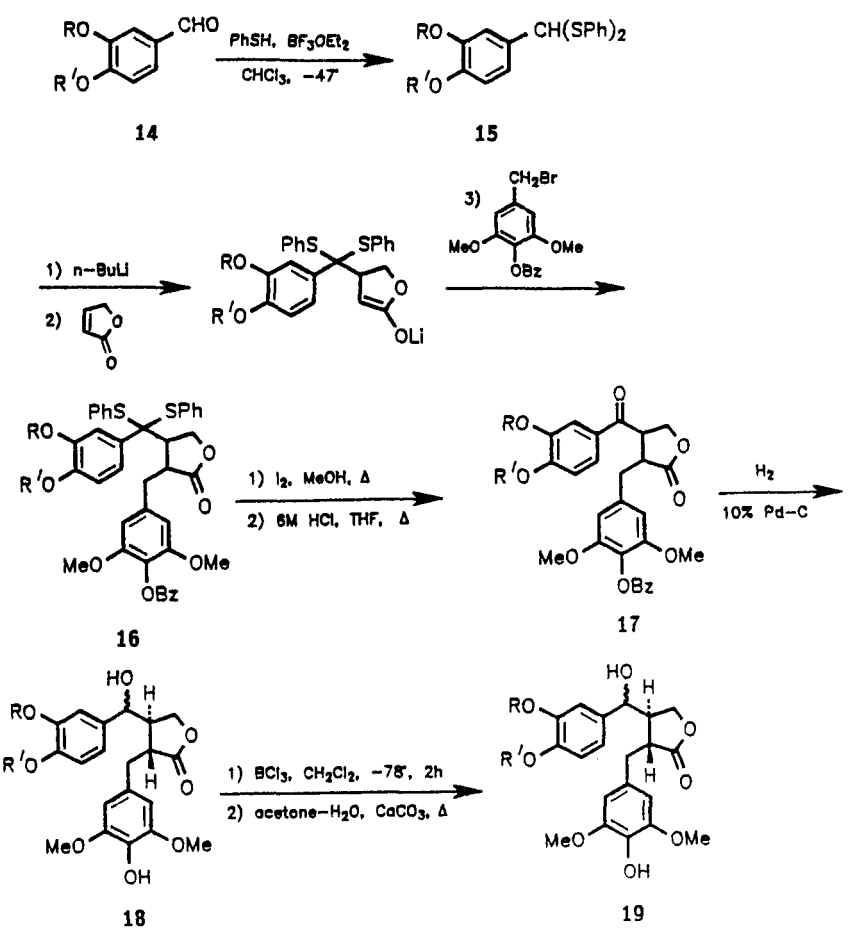


Fig. 4. Synthesis of a 4'-dimethylepipodophyllotoxin precursor.

Fig. 5. Biotransformation of dibenzylbutanolide **20** with cell free extracts of C. roseus cell cultures and whole cells of P. peltatum.

3. Use of Plant Cell Cultures to Produce Higher Levels of Pharmaceutically Interesting Compounds and Separation of Pharmacological Activities in Complex Herbal Medicine Extracts.

Plant cell culture methodology in combination with chemistry, can provide significantly higher levels of plant derived medicinal agents, and their isolation from the culture is much less complicated than from a typical plant extract. For example, in our studies with a well developed cell line of the important Chinese herbal plant Tripterygium wilfordii (1, 6), we have obtained the highly interesting diterpene triepoxide tripdiolide (**22**, Fig. 6) in yields 36 times greater than in the living plant. Furthermore, by the isolation of various metabolites from the cultures with the di- and triterpene structural types summarized in Fig. 6, and subsequent biological screening, we have been able to separate their respective pharmacological properties. The diterpenes **22** and **23** are highly active as immunosuppressive agents and likely related to their possible use for rheumatoid arthritis treatment while the triterpenes (**24**, **25**) exhibit anti-inflammatory activity with application for dermatological disorders.

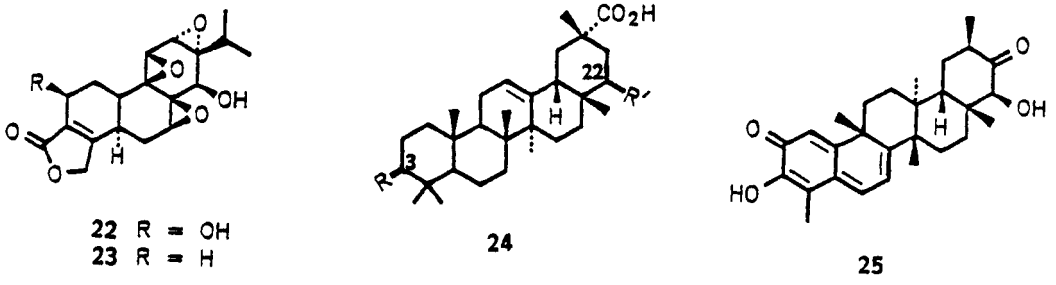


Fig. 6. A summary of structural types of secondary metabolites isolated from T. wilfordii cell cultures.

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