31 Advances in Biochemical Engineering/ Biotechnology

Managing Editor: A. Fiechter

# Plant Cell Culture

With Contributions by
L. A. Anderson, J. Berlin, C. A. Lambe,
M. Misawa, J. D. Phillipson, M. F. Roberts,
A. Rosevear, F. Sasse, Y. Yamada

# Plant Cell Culture

With Contributions by
L. A. Anderson, J. Berlin, C. A. Lambe,
M. Misawa, J. D. Phillipson, M. F. Roberts,
A. Rosevear, F. Sasse, Y. Yamada

With 51 Figures and 18 Tables

Springer-Verlag
Berlin Heidelberg New York Tokyo
1985

ISBN 3-540-15489-2 Springer-Verlag Berlin Heidelberg New York Tokyo
ISBN 0-387-15489-2 Springer-Verlag New York Heidelberg Berlin Tokyo


Library of Congress Catalog Card Number 72-152360
Printed in GDR



Typesetting and Offsetprinting: Th. Müntzer, Bad Langensalza
Bookbinding: Lüderitz & Bauer, Berlin

2152/3020-543210

# Table of Contents

# Biosynthesis of Secondary Products by Cell Cultures of Higher Plants

L. A. Anderson, J. D. Phillipson, and M. F. Roberts
Department of Pharmacognosy, The School of Pharmacy, University of London, 29–39 Brunswick Square, London, WC1N 1AX, UK



Biosynthetic studies of alkaloids, phenols and terpenes utilizing plant cell culture techniques have been reviewed from literature dating mainly from 1980. Many of the research papers deal with the investigations into the alkaloids of *Catharanthus roseus* but there are a number of significant publications in other areas of alkaloid biosynthesis, viz. other indole alkaloids, isoquinolines and quinolizidines. Flavonoid biosynthesis continues to be an active area of research in which much use has been made of plant cell cultures. Individual steps in secondary product biosynthesis tend to be the focus of attention with particular emphasis on the isolation and characterization of the enzymes involved. The use of plant cell culture for biosynthetic studies has been greatly enhanced by the development of sensitive analytical techniques.

## 1 Introduction

Although plant cell cultures do not necessarily produce the same secondary metabolites as their parent plants, they are nevertheless valuable for the investigation of biosynthetic problems [1–5]. Cell suspensions are used in preference to callus cultures

in such studies because of the easier administration of precursors and the extraction of products. In some aspects, plant cell cultures possess advantages over intact plants for biosynthetic studies, e. g.,

a) Cultures can be grown under standard conditions for short growth cycles and are not subject to seasonal variation.
b) Cultures are less complex in organization than the entire plant and hence permeability, translocation and segregation of precursors and products do not present the problem of incorporation which are sometimes encountered in whole plants.
c) Purified enzymes and active cell-free systems can be prepared more easily from cell cultures.

The obvious disadvantages of plant cell cultures for biosynthetic studies include the necessity for working under aseptic conditions and the fact that such cultures may not produce the same secondary metabolites as the parent plant. The synthesis of some secondary metabolites is thought to be connected with cell differentiation or with the organization of tissues so that it is to be anticipated in biosynthetic studies that particular secondary metabolites may not be produced in cultures [1, 6].

The major routes of biosynthesis for many secondary metabolites are now largely understood but what is required is the complete elucidation of pathways by determining each intermediate and the characterization of the enzyme(s) involved in its formation.

The combination of cell suspension cultures and highly specific assay procedures such as radioimmunoassay have proved invaluable and this has been well demonstrated in such areas as indole alkaloid biosynthesis [7]. The biosynthesis of polyketides, phenolics, terpenes and alkaloids has been reviewed in 1983 based on literature published during the period 1979 to 1981 and covers some references to plant cell culture techniques [8]. Furthermore, the previous literature devoted solely to the use of plant cell cultures for the study of biosynthetic pathways in higher plants has been reviewed extensively up to 1979 [1–5] and consequently literature references mainly from 1980 have been cited in this text.

## 2 Alkaloids

### 2.1 Indoles

Heterotrophic, photomixotrophic and photoautotrophic cell suspension cultures of *Peganum harmala* have been analyzed for harman-type alkaloids [9]. Only heterotrophic cultures produced these alkaloids and the presence and yield of individual alkaloids was influenced by the plant hormones added to the media. Evidence has been presented for the biosynthetic sequence of tryptophan → tryptamine → serotonin taking place in cell suspension cultures of *P. harmala* [10]. From feeding experiments and enzymatic measurements, it has been concluded that decarboxylase activity is a regulatory control for β-carboline alkaloid biosynthesis. [$^{14}$C] Labelled gramine has been fed to barley *(Hordeum vulgare)* cell suspension cultures and

a series of products identified. The degradation sequence commences with demethylation to yield methylaminomethylindole and aminomethylindole and is followed by oxidative deamination to yield indole-3-aldehyde which is then either oxidized or reduced to form the corresponding acid or alcohol [11]. The majority of biosynthetic studies of indole alkaloids utilizing plant cell cultures have been concerned with the iridoid alkaloids and have concentrated on *Catharanthus roseus*. The clinically useful antitumor dimeric indole alkaloids vinblastine and vincristine are expensive to produce from whole plants and their potential production by plant cell cultures is an attractive possible alternative source. The first committed step in the biosynthesis of these alkaloids is the coupling of tryptamine (*1*) and the monoterpene secologanin (*2*) to yield the gluco-alkaloid, strictosidine (*3*) (Fig. 1). The enzyme responsible for this process in strictosidine synthase [7] which has been immobilized on CNBr-activated sepharose [12]. It is now possible to synthesize gram quantities of strictosidine for biosynthetic studies whereas previously this compound has proved difficult to prepare and to purify.

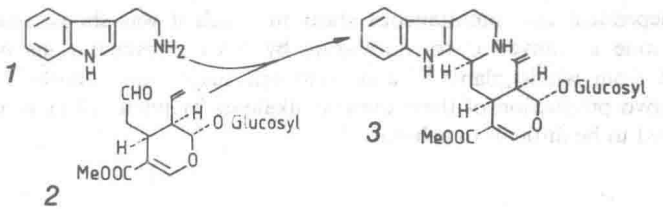



**Fig. 1.** The formation of strictosidine (3), the key intermediate in iridoid-indole alkaloids, from tryptamine (1) and secologanin (2)

One of the problems encountered in studying biosynthetic pathways is the low incorporation of labelled precursors into intact plants. This problem has been overcome for monoterpene incorporation into indole alkaloids by the use of cell suspension cultures of *C. roseus* [13]. Deuterated 10-hydroxygeraniol and 10-hydroxynerol have been incorporated into ajmalicine and strictosidine lactam in approximately 50% and 80% yields, respectively. Based on further experiments, it has been concluded that 9,10-dihydroxygeraniol, 9-oxo-10-hydroxygeraniol and 9,10-dioxogeranial are intermediates in the biosynthesis of ajmalicine. These experiments have led to a proposed biosynthetic pathway between geraniol and loganin [14]. A monoterpene hydroxylase has been isolated from cell suspensions of *C. roseus* and is unlike its counterpart in seedlings because a significant portion of the enzyme appears to be either soluble or only loosely bound [15].

An increasing number of monomeric monoterpenoid alkaloids continue to be isolated from cell suspension cultures of *C. roseus*. In one particular study, 12 alkaloids were isolated from *C. roseus* cell suspensions and 14 alkaloids from *C. ovalis* suspensions [16]. In all, eighteen alkaloids were reported from these two species and they represented the major types of *Corynanthe*, *Strychnos*, *Aspidosperma* and *Iboga* alkaloids. The ability of *C. roseus* cell suspensions to produce such a range of monomeric indole alkaloids has been amply demonstrated by several laboratories [17–23, 26–30]. These studies indicate that the monomeric alkaloids can be produced readily whereas the dimeric alkaloids are not so easily formed. [3-$^{14}$C]-

DL-tryptophan has been incorporated into akuammicine, catharanthine and vindoline by suspension cultures of *C. roseus* [17] and a number of distinct cell lines have been produced [18–20]. One particular strain of *C. roseus* culture has yielded an alkaloidal extract with antimitotic activity but no dimeric alkaloids were detected [22]. In one detailed investigation [23] a particular cell line of *C. roseus* (PRL 200) has been subjected to time-course studies, for alkaloidal yield and constituents, over a 27 day period, utilizing different media conditions. This cell line accumulates catharanthine in particular, together with 9 other indole alkaloids including strictosidine lactam. This latter alkaloid is usually regarded as an artifact produced readily from strictosidine (e.g., in vitro at pH 7.5) however, because of its high yield in this cell line it is possible that it may be formed enzymatically [23].

Cell-free systems from *C. roseus* leaves have been demonstrated to incorporate [2-$^{14}$C] tryptamine (*1*) and secologanin (*2*) into vindoline (*4*) [24]. The same cell-free system was utilized to couple vindoline (*4*) and catharanthine (*5*) to yield the dimeric 3′,4′-anhydrovinblastine (*6*) which in turn was converted to the natural dimeric alkaloids leurosine, catharine and vinblastine (*7*) (Fig. 2). These results corroborate an independent and simultaneous study in which it was shown that 3′,4′-anhydrovinblastine is converted to vinblastine by cell-free preparations of *C. roseus* prepared from whole plants [25] and from cell suspension cultures [26]. The successful de novo production of these dimeric alkaloids by plant cell culture techniques has proved to be difficult to achieve.

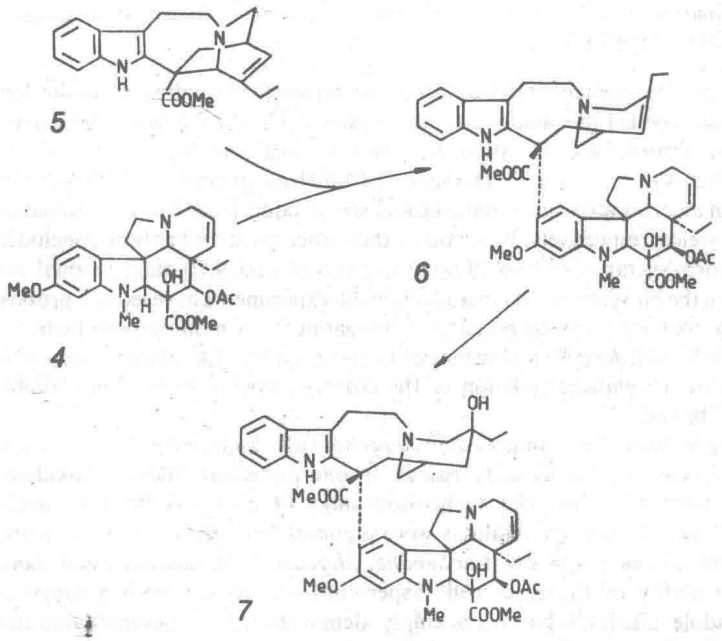
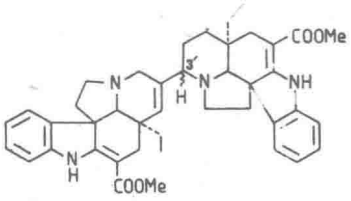



**Fig. 2.** The conversion of vindoline (4) and catharanthine (5) into 3′,4′-anhydrovinblastine (6) and vinblastine (7) by cell-free systems of *Catharanthus roseus* leaves

A cell line of *C. roseus* suspension cultures (PRL 953) which does not produce vindoline, failed to convert vindoline (*4*) and catharanthine (*5*) into dimeric alkaloids [27]. Yet another cell line of *C. roseus* ("916") which lacks the ability to produce the characteristic alkaloids of the plant, has been shown to possess the enzymes which are capable of converting 3′,4′-anhydrovinblastine (*6*) into leurosine and catharine [28–30].

Although it has proved difficult to produce dimeric indole alkaloids by *C. roseus* cultures, two novel dimeric alkaloids, voafrine A (*8*) and voafrine B (*9*), have been isolated from cell suspension cultures of *Voacanga africana* [31] (Fig. 3). It is proposed that these alkaloids are produced enzymatically in the cultures and are not artifacts produced because of the instability of tabersonine, the monomeric component of these alkaloids.




*8* C-3′H α configuration
*9* C-3′H β configuration

Fig. 3. Dimeric indole alkaloids isolated from cell suspensions of *Voacanga africana*

Earlier studies on the alkaloids of *C. roseus* cell suspensions showed that ajmalicine and other heteroyohimbine alkaloids (*Corynanthe*-type) are readily produced. The results of tracer feeding experiments led to the assumption that geissoschizine (*10*) was a central component in the biosynthesis of all of the major types of alkaloids found in *C. roseus*. The use of cell-free systems prepared from tissue cultures has given results which indicate that geissoschizine is only indirectly involved in the biosynthesis of heteroyohimbine alkaloids. Geissoschizine dehydrogenase catalyses the dehydrogenation of geissoschizine (*10*) at the C-21 α position to yield 4,21-dehydrogeissoschizine (*11*) which is a central intermediate in the biosynthesis of heteroyohimbine alkaloids. This enzyme which has now been isolated from a suspension culture of *C. roseus*, has been partially purified and characterized [32]. 4,21-Dehydrogeissoschizine (*11*) is converted to cathenamine (*12*) (19(R),19(S)-isomers and iminium forms) (*13*) which are the direct precursors of ajmalicine (*14*), 19-epi-ajmalicine (*15*) and tetrahydroalstonine (*16*) (Fig. 4). Previously, it had been shown that 17-hydroxy-19-epi-cathenamine, which is known to be a precursor of 19-epi-ajmalicine (*15*) under biomimetic conditions, was not involved in its biosynthesis by cell-free extracts [33]. The enzymatic synthesis of ajmalicine (*14*) and related heteroyohimbine alkaloids by means of cell cultures and cell-free systems has been lucidly reviewed [34]. Subsequently, crude enzyme extracts obtained from *C. roseus* cell suspension cultures have been used to follow the steric course of hydrogen transfer during the formation of the 3α-heteroyohimbine alkaloids [35].

The biosynthetic studies undertaken on cell suspensions and cell-free extracts of *C. roseus* have been facilitated by sensitive analytical techniques. Preparation of

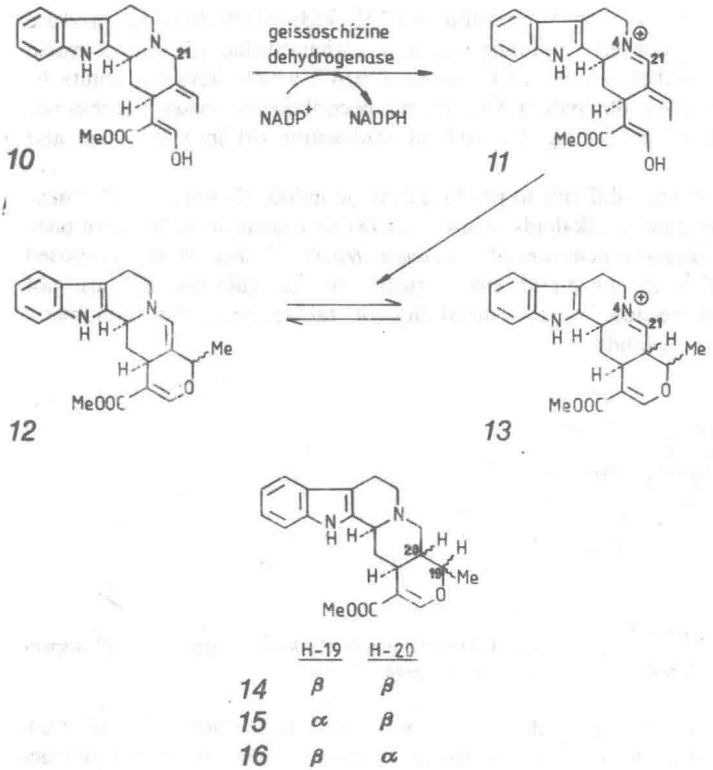



**Fig. 4.** The role of geissoschizine dehydrogenase in the formation of heteroyohimbine alkaloids in *Catharanthus roseus* cell suspension cultures

alkaloidal extracts by Extrelut columns and the identification and quantitation of alkaloids by means of HPLC has recently been described [36]. For the specific identification of a particular compound in plant cell cultures, the method which has proved to be most sensitive is that of radioimmunoassay. The detection of vindoline (*4*) by this technique has been described and it is sensitive within a range of 0.5–100 pmol (0.2–45 ng) [37].

In order to understand the formation and storage of alkaloids in cell suspensions of *C. roseus*, the localization of the alkaloids has been investigated. The alkaloids are stored within the vacuoles of particular cells which possess a vacuolar pH of 3 in contrast to "normal" cells which have a vacuolar pH of 5 [38]. The uptake and retention of tryptamine and alkaloids by cells in suspension have been the subject of several studies [39–41].

The manipulation of plant cell cultures to produce indole alkaloids continues to be an active area of research as exemplified by various studies on *C. roseus* cultures. Factors such as temperature [42] and light [43] have been investigated for their effect on alkaloid production. High sucrose levels stimulated the production of ajmalicine [44] whereas another study revealed that alkaloid synthesis continued with low phosphate levels but not with low sucrose levels [45]. Changes in media

composition have been related to the activities of tryptophan decarboxylase and strictosidine synthase [46]. In an attempt to induce the production of dimeric alkaloids, the effect of several carotenoid-inducers have been investigated. Although ajmalicine and catharanthine production was increased by up to about 20% by concentrations of specific inducers, at levels of 5 ppm, there was no detection of dimers [47]. The omission of 2,4-D from media has resulted in notable increases of ajmalicine and serpentine [48].

Although *C. roseus* currently holds the centre of the stage for biosynthetic studies of indole alkaloids utilising plant cell cultures, there are a limited number of current studies of other genera from the Apocynaceae. Some twelve indole alkaloids have been isolated from cell suspension cultures of *Rauwolfia serpentina* [49]. The major alkaloid proved to be vomilenine (*23*) (ajmaline-type) which was present at much higher concentrations than in differentiated plants. Crude enzyme extracts prepared from the cell suspension cultures converted strictosidine to a series of yohimbine-, heteroyohimbine-, sarpagine- and ajmaline-type alkaloids. Polyneuridine aldehyde esterase (PNA-esterase), isolated and purified from cell suspensions of *R. serpentina*, specifically converts polyneuridine aldehyde (*17*) into 16-epivel-

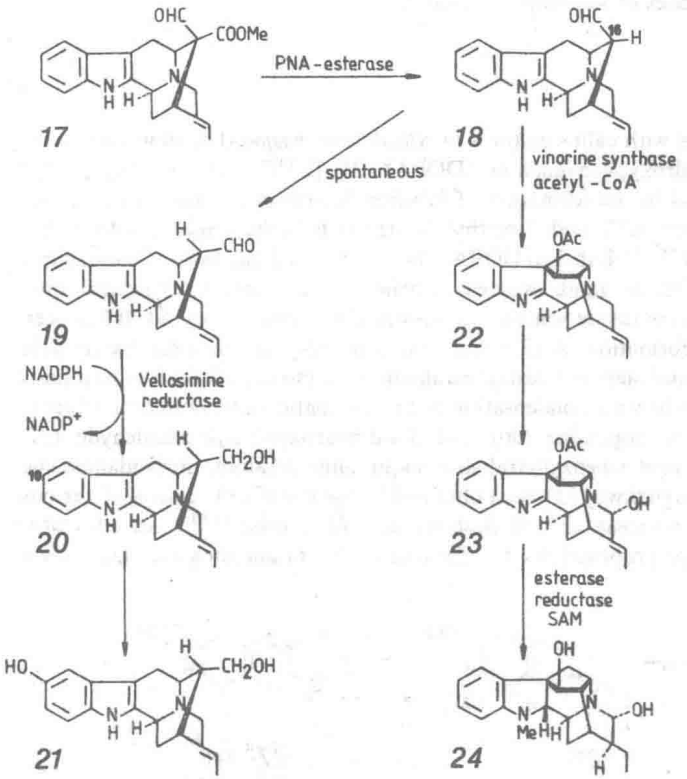
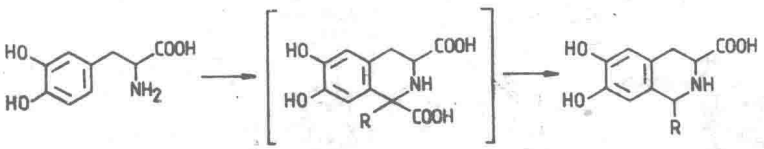



**Fig. 5.** The biosynthesis of sarpagine and ajmaline by means of enzymes isolated from *Rauwolfia serpentina* cell cultures

losimine (*18*) which is at the branch of the biogenetic routes leading to sarpagine- and ajmaline-type alkaloids [50] (Fig. 5). Enzyme extracts of *R. serpentina* cells converted polyneuridine aldehyde (*17*) to vellosimine (*19*) [51]. The same cultured cells were used as a source for the isolation of the next enzyme in the pathway, vellosimine reductase which specifically converts vellosimine (*19*) into 10-deoxy-sarpagine (*20*), the direct precursor of sarpagine (*21*) [52,53]. 16-Epi-vellosimine (*18*) would be anticipated to cyclise to deacetylvinorine and a further enzyme isolated from cell suspensions of *R. serpentina*, named vinorine synthase, is involved in the conversion of deacetylvinorine to vinorine (*22*) [54]. Ajmaline (*24*) is produced via volilenine (*23*) and the specific enzyme involved has been obtained from cell cultures of *R. serpentina* [55] (Fig. 5). Cell suspension cultures of *Tabernaemontana divaricata* and *T. iboga* have yielded a series of monoterpenoid indole alkaloids. The availability of strictosidine by means of immobilized strictosidine synthase [12] means that the role of strictosidine can be investigated by cell-free systems of these *Tabernaemontana* species. In particular, *T. iboga* should prove to be of interest since it produces only two major alkaloids, tubotaiwine (Strychnos-type) and conoflorine (quebrachamine-type) [56]. Further information on the biosynthesis of *Aspidosperma* alkaloids can be expected from studies of plant cell cultures because such alkaloids predominate in cultures of *Voacanga africana* [57].

## 2.2 Isoquinolines

Feeding experiments with callus cultures of *Stizolobium hasjoo* (Leguminosae) using DL-[β-$^{14}$C]-3,4-dihydroxyphenylalanine (DOPA) (*25*) [2-$^{14}$C] pyruvate and [2-$^{14}$C] acetate have resulted in the formation of labelled 3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (*27*) and 1-methyl-3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (*28*) [58,59]. Labelled DOPA was incorporated into both of these simple tetrahydroisoquinoline alkaloids whereas labelled acetate was incorporated only into (*27*) and labelled pyruvate was not incorporated into either alkaloid. It has been proposed that the formation of (*27*) and (*28*) proceeds via the α-carboxylic acid (*26*) (Fig. 6). The inital step in 1-benzyltetrahydroisoquinoline alkaloid biosynthesis has been assumed to be via a condensation of two aromatic units, both derived from tyrosine (*29*) namely dopamine (*30*) and 3,4-dihydroxyphenylacetaldehyde (*31*) to yield the first formed 1-benzyltetrahydroisoquinoline alkaloid, norlaudanosoline (*32*). Support for this pathway has been obtained by the use of cell cultures of various species of the Papaveraceae and of *Berberis* and *Thalictrum* [60,61]. An alternative biosynthetic pathway proposed for the formation of norlaudanosoline (*32*) is via




25 26 27 R=H 28 R=Me

**Fig. 6.** Formation of simple tetrahydroisoquinoline alkaloids by *Stizolobium* callus cultures

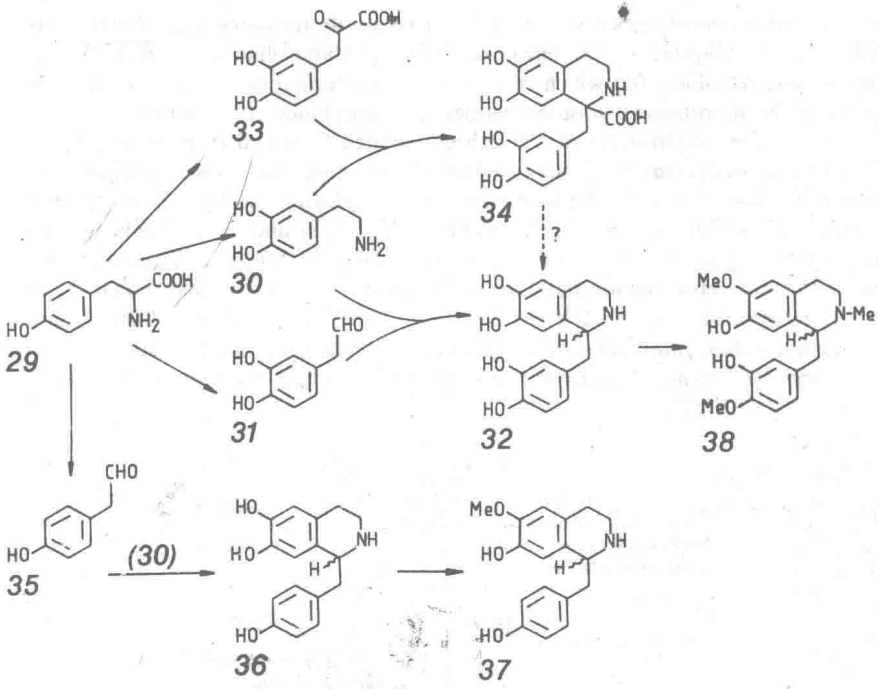



Fig. 7. Putative biosynthetic routes to the 1-benzyltetrahydroisoquinoline alkaloids

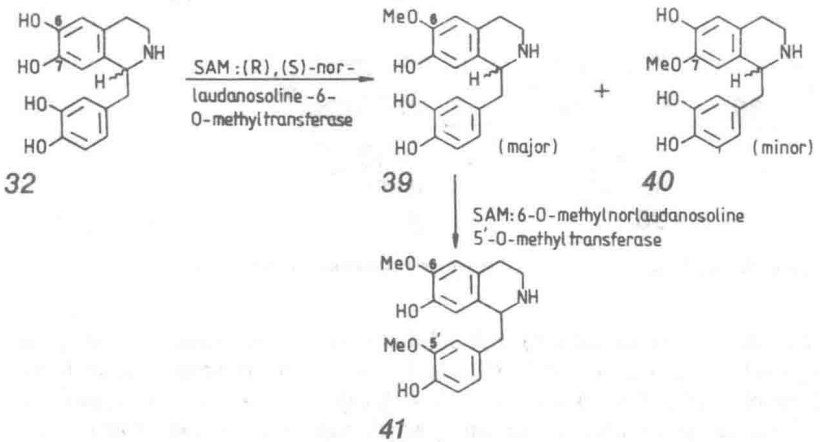
the condensation of dopamine (*30*) with 3,4-dihydroxyphenylpyruvate (*33*) to yield norlaudanosoline-1-carboxylic acid (*34*) which would then decarboxylate to form norlaudanosoline (*32*) (Fig. 7). Experiments with cell-free systems from suspension cultures have failed to produce evidence which supports the existence of this pathway [60,61].

The enzyme (S)-norlaudanosoline synthase has been isolated and characterized from cell cultures and its presence has been demonstrated in those species which produce 1-benzyltetrahydroisoquinoline alkaloids while it appears to be absent from other species which do not contain these alkaloids [60]. Four isoenzymes of (S)-norlaudanosoline synthase have been separated from cell cultures of *Eschscholtzia tenuifolia* and none of these enzymes are capable of catalyzing the condensation of dopamine (*30*) with 3,4-dihydroxyphenylpyruvate (*33*) clearly indicating that norlaudanosoline-1-carboxylic acid (*34*) is not directly involved in the pathway to 1-benzyltetrahydroisoquinoline alkaloids [61] (Fig. 7).

The $K_M$ values for 3,4-dihydroxyphenylacetaldehyde (*31*) and for 4-hydroxyphenylacetaldehyde (*35*) proved to be almost identical indicating that the synthase is also responsible for the formation of demethylcoclaurine (*36*). This alkaloid is a precursor for coclaurine (*37*) which acts as a precursor for proaporphines of the crotonosine-type and for the aporphines, roemerine and mecambroline. It is thought that the predominant pathway (Fig. 7) in the biosynthesis of 1-benzyl-tetrahydroisoquinoline alkaloids leads to the formation of the (S)-enantiomers of norlaudanoso-

line (*32*) and of demethylcoclaurine (*36*) which act as intermediates of the morphinans with the (R)-configuration [61]. Methylation of norlaudanosoline (*32*) leads to the key intermediate reticuline (*38*) which is involved in the formation of various alkaloids including the morphinans, protoberberines, proaporphines and cularines.

Two new O-methyltransferases have been isolated from cell suspension cultures of *Argemone platyceras* [62]. S-adenosylmethionine: (R), (S) norlaudanosoline-6-0-methyltransferase catalyses the formation of 6-0-methylnorlaudanosoline (*39*) and to a minor extent 7-0-methylnorlaudanosoline (*40*) from (S)- and (R)-norlaudanosoline (*32*). This enzyme has been shown to be widely distributed in plants which contain 1-benzyltetrahydroisoquinoline alkaloids and is present in plant cell cultures and differentiated plants such as *Papaver somniferum*. S-adenosylmethionine: (6-0-methylnorlaudanosoline)-5'-0-methyltransferase catalyses the formation of nororientaline (*41*) from 6-0-methylnorlaudanosoline (*39*) and appears to be a highly specific enzyme [63] (Fig. 8).




32 39 40 41

**Fig. 8.** Methyltransferases isolated from cell suspension cultures of *Argemone platyceras*

The formation of the protoberberine alkaloid skeleton and the pattern of oxygenation and methylation are intriguing biogenetic sequences. Incubation of (±)-reticuline (*38*) with [$^{14}CH_3$]-S-adenosylmethionine and a crude enzyme preparation from *Berberis aggregata* callus culture has resulted in the formation of labelled palmatine (*42*) and columbamine (*43*). However, similar experiments with the non-phenolic (±)-tetrahydropapaverine (*44*) failed to result in protoberberine production but the corresponding N-methylated alkaloid, laudanosine (*45*) was formed [64] (Fig. 9). The protoberberine alkaloid jatrorrhizine (*48*) is produced in high yields by some cell cultures of *Berberis* species. Doubly labelled 6-0-methyllaudanosoline (*46*) fed to *Berberis stolonifera* callus resulted in the formation of labelled berberine (*47*) and jatrorrhizine (*48*) [65] (Fig. 10). This finding suggests that jatrorrhizine is formed from berberine by reopening of the methylenedioxy group. Since [N-$^{14}CH_3$]-(±)-reticuline (*49*) was shown to be the favoured precursor for jatrorrhizine (*50*) in contrast to other phenolic 1-benzyltetrahydroisoquinoline alkaloids, including [N-$^{14}CH_3$]-protosinomenine (*51*), the results demonstrate that the inversion of A

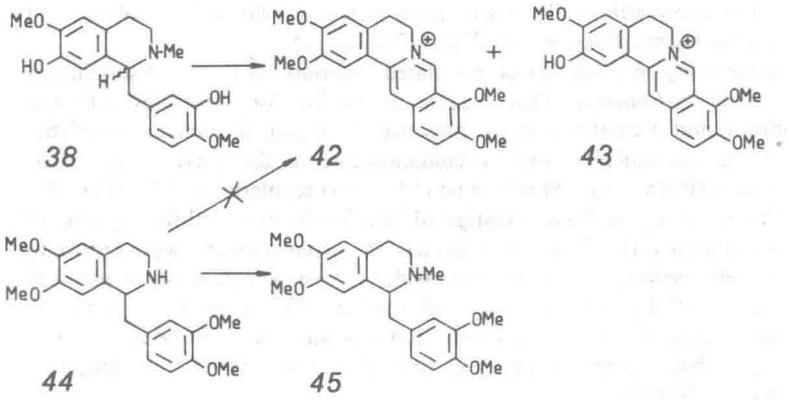



**Fig. 9.** Formation of protoberberine alkaloids from (±)-reticuline by callus cultures of *Berberis aggregata*

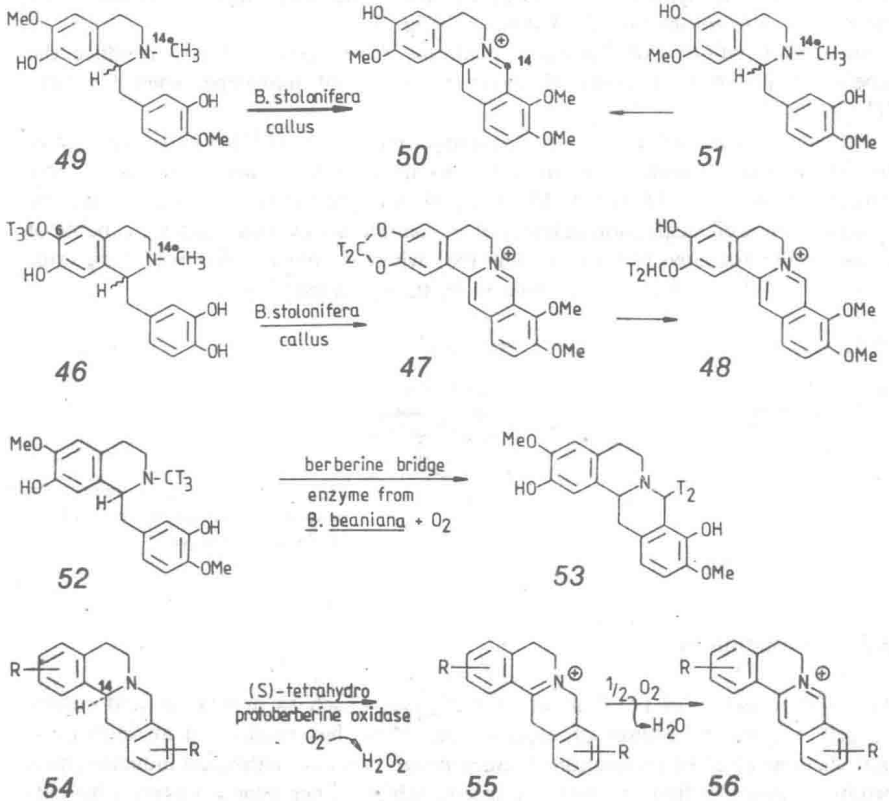



**Fig. 10.** Formation of protoberberine alkaloids in *Berberis* cell cultures and the "inversion" of methoxyl/hydroxyl substitution in ring A

ring methoxyl/hydroxyl substitution pattern, i.e. reticuline (*46*) → jatrorrhizine (*48*) proceeds via a methylene dioxy intermediate (*47*) (Fig. 10).

The berberine bridge enzyme has been isolated and purified from cell suspension cultures of *Berberis beaniana*. This enzyme is specific for substrates with the (S)-configuration and it catalyzes, in the presence of oxygen, the conversion of (S)-reticuline, (S)-protosinomenine and (S)-laudanosoline to the corresponding (S)-tetrahydroprotoberberines e.g. (S)-reticuline (*52*) → (S)-scoulerine (*53*) [66] (Fig. 10). Although this enzyme has been investigated previously in a cell-free system of *Macleaya microcarpa* cell cultures, the mechanism of conversions with regard to the proposed intermediacy of reticuline-N-oxide, the participation of oxygen and the steric course of the reaction remained unclear. The experiments with the enzyme isolated from *B. beaniana* indicate that reticuline *N*-oxide does not serve as a substrate that oxygen is involved and that only the (S) configuration precursors act as substrates [66].

A new flavin enzyme has been isolated from cell cultures of several *Berberis* species. In the presence of oxygen, this enzyme catalyses the oxidation of (S)-tetrahydroprotoberberines (*54*) to protoberberines (*56*) via the 7,14-dehydroberberine intermediate (*55*). This (S)-tetrahydroprotoberberine oxidase is the final enzyme involved in the biosynthesis of protoberberine alkaloids [67] (Fig. 10).

Suspension cultures of *Papaver somniferum* have been shown to produce the labelled morphinan alkaloids, thebaine, codeine and morphine when fed with $^{3}$H-tyrosine [68].

Protein synthesis inhibitors, cycloheximide, puromycin (PUM) and actinomycin (ATM) in low concentrations promoted alkaloid synthesis and in particular low concentrations of PUM and ATM increased the accumulation of codeine but not of morphine. Cell suspension cultures of *P. somniferum* cv Marianne are capable of transforming thebaine (*57*) to neopine (*58*) whereas codeine, neopine, papaverine and laudanosoline were not metabolized by these cultures [69] (Fig. 11).


57 58

**Fig. 11.** Conversion of thebaine to neopine by cell suspension cultures of *Papaver somniferum*

## 2.3 Quinolizidines

The administration of non-quinolizidine alkaloids such as papaverine and coniine to cell suspension cultures of *Lupinus polyphyllus* has resulted in an increase in quinolizidine alkaloid production [70]. Surprisingly, similar methods of induction have produced quinolizidine alkaloids in species which either produce other alkaloids or no alkaloids, viz. *Conium maculatum*, *Daucus carota*, *Atropa belladonna*, *Chenopodium rubrum*, *Spinacia oleracea* and *Symphytum officinale*. These results