The Chemistry and Biology of Isoquinoline Alkaloids

Edited by
J. D. Phillipson, M. F. Roberts,
and M. H. Zenk

With 178 Figures

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Contents

Plants as a Source of Isoquinoline Alkaloids
N.G. Bisset (With 5 Figures) 1

Chemotaxonomy of the Papaveraceae Alkaloids
V. Preininger (With 8 Figures) 23

Structure Activities and Pharmacological Properties of the Opium Alkaloids
E. Lindner (With 7 Figures) 38

The Occurrence of Simple Isoquinolines in Plants
J. Lundström (With 3 Figures) 47

Erythrina Alkaloids
A.H. Jackson (With 12 Figures) 62

Annonaceae Alkaloids
A. Cavé (With 22 Figures) 79

The Chemistry and Pharmacology of Cularine Alkaloids
L. Castedo (With 28 Figures) 102

Bisbenzylisoquinoline Alkaloids
P.L. Schiff, Jr. (With 5 Figures) 126

Natural Degradative Routes for the Aporphines
M. Shamma (With 5 Figures) 142

Synthesis and Structure-Activity Relationships of Aporphines as Dopamine Receptor Agonists and Antagonists
J.L. Neumeyer (With 8 Figures) 146

The Chemistry and Pharmacology of Morphinan Alkaloids
A. Brossi (With 15 Figures) 171

The Development of a Practical Total Synthesis of Natural and Unnatural Codeine, Morphine and Thebaine
K.C. Rice (With 9 Figures) 191

Biomimetic and Total Synthesis of Monoterpenoid Isoquinoline Alkaloids
R.T. Brown (With 7 Figures) 204

The Biosynthesis of Isoquinoline Alkaloids
R.B. Herbert (With 14 Figures) 213

Biosynthesis of Morphinan Alkaloids
E. Brochmann-Hanssen (With 7 Figures) 229

Enzymology of Benzylisoquinoline Alkaloid Formation
M.H. Zenk (With 12 Figures) 240

Morphinan Alkaloids from Plant Cell Cultures
F. Constabel 257

The Production of Isoquinoline Alkaloids by Plant Cell Cultures
M. Rueffer (With 3 Figures) 265

Cytodifferentiation and *Papaver* Alkaloid Accumulation
T.M. Kutchan, S. Ayabe, and C.J. Coscia (With 8 Figures) 281

Subject Index 295

The Production of Isoquinoline Alkaloids by Plant Cell Cultures

M. RUEFFER[1]

1 Introduction

Under optimal conditions plant cells are able to grow like microorganisms in suspension cultures without limitation or ageing. Moreover they are totipotent, in that one single cell or protoplast has the whole genetic information for the differentiated plant. Therefore one can expect that under appropriate conditions, plant cell suspension cultures have the ability to produce the whole range of natural products which are isolated from the differentiated plants. This could be of extreme importance for the production of substances especially for pharmaceutical use, since a survey indicates that 23% of all prescriptions contain natural compounds (Farnsworth and Morris 1976). Plant cell cultures would have several advantages in comparison to differentiated plants. They are independent for instance of geographical and climatical conditions, of plant diseases or animal destruction and therefore the price of the plant drugs could be stabilized. In addition, they could easily be cultivated under special state control, and thus would be of great importance for example for the production of morphine.

During recent years a variety of natural compounds among them a series of benzylisoquinolines have been isolated from plant cell cultures and the results of these researches are described in this chapter.

2 The Production of Isoquinoline Alkaloids

2.1 Benzylisoquinoline Alkaloids from Plant Cell Cultures

Since Reinhard (1967) described the production of protoberberine alkaloids by callus cultures of *Berberis vulgaris*, many different structure types of benzylisoquinoline alkaloids could be isolated from callus- and suspension cultures (Table 1). The greatest variety in the substitution pattern of the basic structure is found in the group of protoberberine alkaloids. Ikuta and Itokawa (1982b) for example described the isolation of 11 different protoberberine alkaloids from one plant cell culture (*Nandina domestica*, Berberidaceae).

1 Lehrstuhl Pharmazeutische Biologie der Universität München, Karlstraße 29, 8000 München 2, FRG

The Chemistry and Biology of Isoquinoline Alkaloids, ed. by Phillipson et al.

Table 1. Benzylisoquinoline alkaloids isolated from plant cell cultures

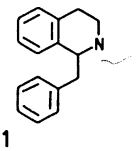
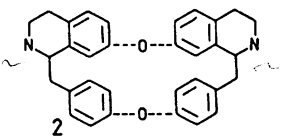
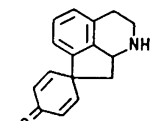
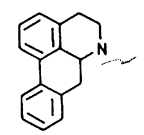
Structure type	Alkaloid name	Source (reference)
Benzylisoquinolines		
 1	Coclaurine	*Fumaria capreolata* (Tanahashi et al. unpublished)
	Reticuline	*Fumaria capreolata* (Tanahashi et al. unpublished)
		Chasmanthera dependens (Rüffer et al. unpublished)
Bisbenzylisoquinolines	Berbamine	*Stephania cepharantha*
 2	Aromoline	(Akasu et al. 1976)
Proaporphines	Stepharine	*Tinospora caffra, Chasmanthera dependens* (Rüffer et al. unpublished)
 3		*Tinospora cordifolia* (Arens unpublished)
Aporphines	Cepharadione A	*Stephania cepharantha* (Akasu et al. 1975)
 4	Cepharadione B	(Akasu et al. 1975)
	Liriodenine	(Akasu et al. 1975)
	Lysicamine	(Akasu et al. 1975)
	Norcepharadione	(Akasu et al. 1975)
	Isoboldine	*Fumaria capreolata* (Tanahashi et al. unpublished)
	Magnoflorine	*Corydalis incisa, Corydalis pallida, Dicentra peregrina, Eschscholtzia californica, Papaver somniferum, P. setigerum, P. bracteatum, P. orientale, P. rhoeas* (Ikuta et al. 1974)
		Thalictrum minus, Coptis japonica, Mahonia japonica, Nandina domestica (Ikuta and Itokawa 1982a,b)
		Tinospora caffra, Chasmanthera dependens, Stephania japonica (Rüffer et al. unpublished)
		Dioscoreophyllum cumminsii (Furuya et al. 1983)

Table 1 (cont.)

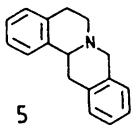
Structure type	Alkaloid name	Source (reference)
	Stephanine	*Stephania glabra* (Gurova et al. 1980)
Protoberberines		
 5	Berberine	*Argemone mexicana* (Khanna et al. 1982)
		Coptis japonica, Nandina domestica, Mahonia japonica, Thalictrum minus (Ikuta and Itokawa 1982a,b)
		Berberis vulgaris (Reinhard 1967)
		Berberis stolonifera (Hinz and Zenk 1981)
		Phellodendron amurense (Kuroda and Itokawa 1973)
	Columbamine	*Berberis stolonifera* (Hinz and Zenk 1981)
		Coptis japonica, Mahonia japonica, Nandina domestica, Thalictrum minus (Ikuta and Itokawa 1982a,b)
		Tinospora caffra Rüffer et al. unpublished)
	Coptisine	*Chelidonium majus* (Böhm et al. 1980)
		Coptis japonica, Mahonia japonica, Nandina domestica, Thalictrum minus (Ikuta and Itokawa 1982a,b)
		Fumaria capreolata (Tanahashi et al. unpublished)
	Dehydrocheilanthifoline	*Fumaria capreolata* (Tanahashi et al. unpublished)
	Dehydrocorydalmine	*Chasmanthera dependens* (Rüffer et al. unpublished)
	Desoxythalidastine	*Thalictrum minus* (Ikuta and Itokawa 1982a)
	Epiberberine	*Coptis japonica, Nandina domestica, Thalictrum minus* (Ikuta and Itokawa 1982a,b)
	Groenlandicine	*Coptis japonica, Nandina domestica* (Ikuta et al. 1974)

Table 1 (cont.)

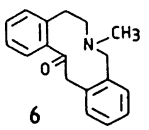
Structure type	Alkaloid name	Source (reference)
	Jatrorrhizine	*Berberis stolonifera* (Hinz and Zenk 1981)
		Dioscoreophyllum cumminsii (Furuya et al. 1982)
		Coptis japonica, Nandina domestica, Mahonia japonica, Thalictrum minus (Ikuta and Itokawa 1982a,b)
		Chasmanthera dependens, Tinospora caffra, Stephania japonica (Rüffer et al. unpublished)
		Tinospora crispa (Sumimoto Chemicals 1982)
	Palmatine	*Coptis japonica, Thalictrum minus, Nandina domestica* (Ikuta and Itokawa 1982a,b)
		Dioscoreophyllum cumminsii Furuya et al. 1982)
		Phellodendron amurense (Kuroda and Itokawa 1973)
		Tinospora crispa (Sumimoto Chemicals 1982)
		Tinospora caffra, Chasmanthera dependens, Stephania japonica, Menispermum canadense (Rüffer unpublished)
	Scoulerine	*Fumaria capreolata* (Tanahashi et al. unpublished)
	Stylopine	*Papaver bracteatum* (Kamimura et al. 1976)
	Thalifendine	*Nandina domestica, Thalictrum minus* (Ikuta and Itokawa 1982a,b)
	Thalidastine	*Coptis japonica, Nandina domestica, Thalictrum minus* Ikuta and Itokawa 1982a,b)
Protopines	Allocryptopine	*Chelidonium majus* (Böhm et al. 1980)
 6		*Macleaya microcarpa* (Koblitz et al. 1975)
	Cryptopine	*Papaver somniferum* (Furuya et al. 1971)

Table 1 (cont.)

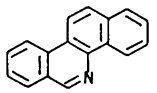
Structure type	Alkaloid name	Source (reference)
	Protopine	*Corydalis ophiocarpa* (Iwasa and Takao 1982)
		Fumaria capreolata (Tanahashi et al. unpublished)
		Macleaya microcarpa (Koblitz et al. 1975)
		Papaver somniferum (Furuya et al. 1971)
		Corydalis incisa, Corydalis pallida, Dicentra peregrina, Chelidonium japonica, Eschscholtzia californica, Papaver setigerum, P. bracteatum, P. orientale, P. rhoeas (Ikuta et al. 1974)
		Chelidonium majus (Böhm et al. 1980)
Benzophenanthridines		
 7	Chelidonine	*Chelidonium majus* (Böhm et al. 1980)
	Chelirubine	*Eschscholtzia californica, Macleaya cordata, Papaver bracteatum* (Ikuta et al. 1974)
	Dihydrochelerythrine	*Eschscholtzia californica* (Berlin et al. 1983)
	Dihydrochelirubine	*Eschscholtzia californica* (Berlin et al. 1983)
	Dihydromacarpine	*Eschscholtzia californica* (Berlin et al. 1983)
	Dihydrosanguinarine	*Chelidonium japonica, Eschscholtzia californica, Macleaya cordata, Papaver somniferum, P. setigerum, P. bracteatum, P. orientale, P. rhoeas* (Ikuta et al. 1974)
	Norsanguinarine	*Chelidonium japonica, Eschscholtzia californica, Macleaya cordata, Papaver somniferum, P. setigerum, P. bracteatum, P. rhoeas, P. orientale, Corydalis incisa, C. pallida, Dicentra peregrina* Ikuta et al. 1974)

Table 1 (cont.)

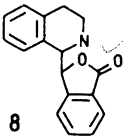
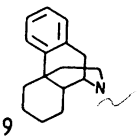
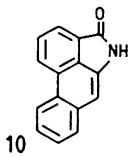
Structure type	Alkaloid name	Source (reference)
	Oxosanguinarine	*Eschscholtzia californica, Chelidonium japonica, Macleaya cordata, Papaver somniferum, P. setigerum, P. bracteatum, P. orientale, P. rhoeas* (Ikuta et al. 1974)
	Sanguinarine	*Chelidonium majus* (Böhm et al. 1980)
		Corydalis ophiocarpa (Iwasa and Takao 1982)
		Macleaya microcarpa (Koblitz et al. 1975)
		Eschscholtzia california, Macleaya cordata, Papaver somniferum, P. setigerum, P. bracteatum, P. orientale, P. rhoeas (Ikuta et al. 1974)
Phthalideisoquinolines		
 8	Narcotine	*Papaver rhoeas* (Khanna and Sharma 1977)
Morphinans	Codeine	*Papaver somniferum* (Tam et al. 1980, Hodges and Rapoport 1982, Furuya et al. 1971)
 9		*Papaver setigerum* (Cdn patent 1980)
		P. rhoeas (Khanna and Sharma 1977)
	Morphine	*Papaver somniferum* (Furuya 1981, Hsu 1981)
		Papaver rhoeas (Khanna and Sharma 1977)
	Thebaine	*Papaver bracteatum* Shafiee et al. 1978, Hsu 1981), Kamimura et al. 1976, Zito and Staba 1982)
		Papaver rhoeas (Khanna and Sharma 1977)
	Pallidine	*Fumaria capreolata* (Tanahashi et al. unpublished)

Table 1 (cont.)

Structure type	Alkaloid name	Source (reference)
Aristololactams  10	Cepharanone I Cepharanone II	*Stephania cepharantha* (Akasu et al. 1974)

In contrast only a few compounds from the large group of aporphines have been described so far. However, more complex structures like the benzophenanthridines and morphinans have been isolated not only from callus- but also from cell suspension cultures (see Table 1).

Most remarkable is the callus culture of *Stephania cepharantha* (Menispermaceae). Not only is it able to produce oxoaporphine alkaloids (Akasu et al. 1975) and bisbenzylisoquinolines (Akasu et al. 1976) but also two aristololactams which are only found in traces in the differentiated plants and are discussed as a hint for a possible systematic crosslink from the Menispermaceae to the Aristolochiaceae (Akasu et al. 1974). A further good example of the structure variability that can be produced by one culture is *Fumaria capreolata* (Fumariaceae) cultivated in our laboratory. Many structural types from a simple isoquinoline to a rather complicated structure like

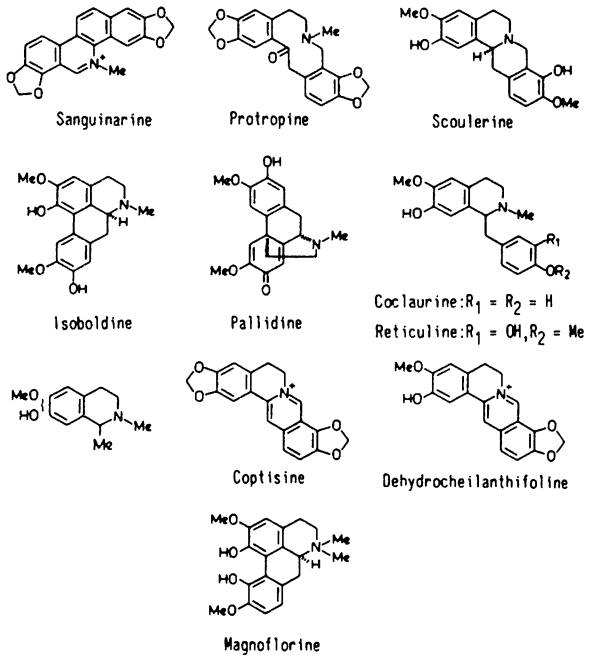


Fig. 1. Alkaloids isolated from a *Fumaria capreolata* (Fumariaceae) cell suspension culture

pallidine are found (Tanahashi unpublished) (Fig. 1). It is interesting to compare the alkaloids which have been produced by differentiated plants with their corresponding cell cultures.

2.2 Comparison Between Differentiated Plants and Cell Cultures

There are only a few detailed comparisons between plant cell cultures and differentiated plants to be found in the literature. Most striking is the lack of production of substantial amounts of morphinan alkaloids in cultures of *Papaver* species. Some examples are described where these alkaloids are detected in minor amounts (see Table 1), but the cell cultures do not reach the productivity of the plants. A possible reason for this may be the lack of specific enzymes in the biosynthetic pathway. On the other hand, one could postulate a correlation between the genetic expression of morphological differentiation and the production of these alkaloids since they are detected in higher amounts in redifferentiating calluses (Schuchmann and Wellmann 1983). The possible lack of specific enzymes is well illustrated by the bisbenzylisoquinoline alkaloids from *Stephania cepharantha* (Menispermaceae). The differentiated plant produces cepharanthine and isotetrandrine as major alkaloids, which are not detected in the cell culture, whereas the culture produces their possible precursors aromoline and berbamine in higher amounts than the plant, and this may be explained by the lack of specific methylating and the methylenedioxybridge forming enzymes (Akasu et al. 1976).

Sometimes the ratio between the amounts of several alkaloids in the plant differs markedly from that of the cell cultures. In the culture of *Berberis wilsoniae* var. *sub-*

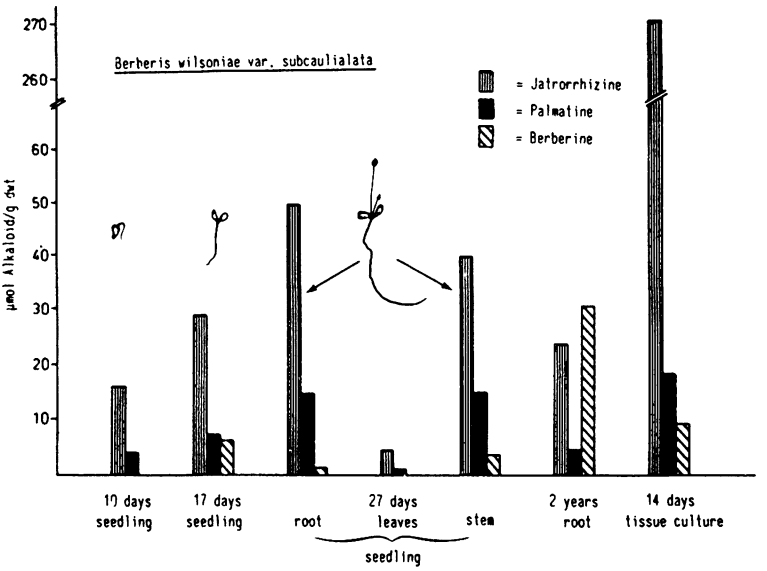


Fig. 2. Comparison between the alkaloid production of the cell culture, the seedling, and the intact root of *Berberis wilsoniae* var. *subcaulialata* (Berberidaceae)

caulialata we could observe that the major alkaloid was jatrorrhizine and the ratio of the three alkaloids jatrorrhizine: palmatine:berberine was 1:0.08:0.04 (Fig. 2). In contrast, a 2-year-old root shows a ratio of 1:0.2:1.4. The ratio of a seedling (1:0.2: 0.03), however, largely resembles that of the cell culture so that one may conclude that the enzyme pattern in the seedling is similar to that of the cell culture. The absolute amount in the cell culture exceeds by far that of the root of the seedling and this is therefore a good example of the capacity of cell cultures for the production of alkaloids in large amounts (Hinz and Zenk 1981).

From these results it can be seen that there may be a promising future for the production of alkaloids by cell cultures. However, it must be realised that there may not be any correlation between the amounts or the type of alkaloids which are produced by the plant or the cell culture.

2.3 Variant Cell Lines

An interesting but insufficiently explained phenomenon is the fact that in cell cultures which seem to be homogenous at the first glance, the amount of alkaloids may differ from cell to cell. Fluoromicroscopy of *Coptis japonica* (Ranunculaceae) cells for instance showed (Sato and Yamada 1984) that they produce varied amounts of protoberberine alkaloids as was shown for the indole alkaloid serpentine in *Catharanthus roseus* (Deus and Zenk 1982). The possibility for selecting cells or small colonies, plated on agar media, with a strong fluorescence to establish cultures with increased productivity was therefore considered practical (Sato and Yamada 1984). Further cultures of *Thalictrum dipterocarpum* (Ranunculaceae) were established by this method. One white cell line produced strongly blue fluorescent compounds in high amounts, whereas a second yellow coloured line gave good production of the protoberberines jatrorrhizine and berberine.

The selection procedure for these cultures producing protoberberine alkaloids is easy due to their yellow colour and their fluorescence in UV light. In the case of other benzylisoquinoline alkaloids, like the morphinans for example, immunological methods, especially the radioimmunoassay (Hodges and Rapoport 1982, Hsu et al. 1983, Wieczorek et al. unpublished) are useful for testing large numbers of small colonies (clones) to find possible cell lines with increased production of these alkaloids.

2.4 Stability

A prerequisite for the industrial use of cell lines selected by the method described above is their stability in producing alkaloids.

Concerning this aspect of cell selection differing results have been obtained so far for the production of benzylisoquinolines. The formation of berberine by cell cultures of *Coptis japonica* for example, produces a very stable culture which even increased its productivity during 3 years of continuous subcultivation and reached a maximal content of 1.67 g l^{-1} medium in one culture period (Fukui et al. 1982). However, Sato and Yamada (1984) could not stabilize the berberine production in their cell lines of *Coptis japonica.* A further example of instability of a culture is that

of *Papaver somniferum* (Papaveraceae) which totally lost its morphinan forming capability after repeated subculturing (Hodges and Rapoport 1982), whereas in our laboratory a *Berberis stolonifera* (Berberidaceae) culture has continuously produced relatively large amounts of the protoberberine jatrorrhizine for the last 9 years (Zenk and Deus 1982, Deus-Neumann and Zenk 1984a). In every case a permanent control of the productivity seems to be necessary and repeated subcloning of the cultures may be necessary for the stabilization of their alkaloid production.

2.5 Localization of Alkaloids in the Cultures

2.5.1 Storage of Alkaloids in the Cells

An important aspect for the commercial production of benzylisoquinoline alkaloids by plant cell cultures is the question whether they are stored in the cells and therefore have to be extracted or excreted into the medium. So far various results have been obtained with different cell cultures.

Neumann and Müller (Neumann and Müller 1967) described the ability of *Macleaya microcarpa* (Papaveraceae) callus cultures to differentiate alkaloid accumulating cells. This also could be demonstrated by Kohlenbach (Lang and Kohlenbach 1983) with photographs of differentiation of "alkaloidal cells" from mesophyll protoplasts of *Macleaya*. In some cases these alkaloids even precipitate as crystals in the cells (Böhm and Franke 1982, Lang and Kohlenbach 1983). This phenomenon in *Macleaya* callus cultures suggested some specialized cells with the ability to accumulate alkaloids produced by the surrounding cells. In cell suspension cultures of *Macleaya microcarpa* Franke and Böhm (1982) observed that the smaller a cell aggregate, the lower was its relative amount of "alkaloidal cells". Indeed they could no longer be detected in aggregates consisting of 10 or fewer cells. Instead of being stored the alkaloids, mainly protopine, were excreted into the medium. Similar results were obtained for the cultures of *Chelidonium majus* (Papaveraceae) (Böhm et al. 1980) where the release of the alkaloids chelidonine, protopine, allocryptopine, coptisine, and sanguinarine into the medium could be increased to a factor 1:6.5 (alkaloids in the cell:alkaloids in the medium) by decreasing the cell aggregation and augmenting the auxin content in the medium (2–20 ppm).

These experiments are in contrast to our experiences with *Berberis* and *Thalictrum* cell cultures, where we found no substantial amounts of protoberberine alkaloids in the medium. Even very small cell aggregates in the 2-cell range showed intensive fluorescence, indicating the presence of protoberberine alkaloids within the cells. Furthermore we could isolate fluorescing protoplasts and vacuoles from *Thalictrum glaucum* (Ranunculaceae) suspension cultures, indicating that these alkaloids are stored within the vacuoles. Additionally we tested the uptake of the ^{3}H-labelled protoberberine alkaloids canadine and berberine into the isolated vacuoles of *Thalictrum glaucum* according to methods described by Deus-Neumann and Zenk 1984b) (Fig. 3).

(R)-Canadine was taken up in only small amounts whereas (R,S)-canadine showed a considerably enhanced uptake, indicating that the probably natural form (S)-canadine (Steffens et al. 1984) is selectively accumulated. In contrast the oxidized form of canadine namely berberine stays in the surrounding medium.

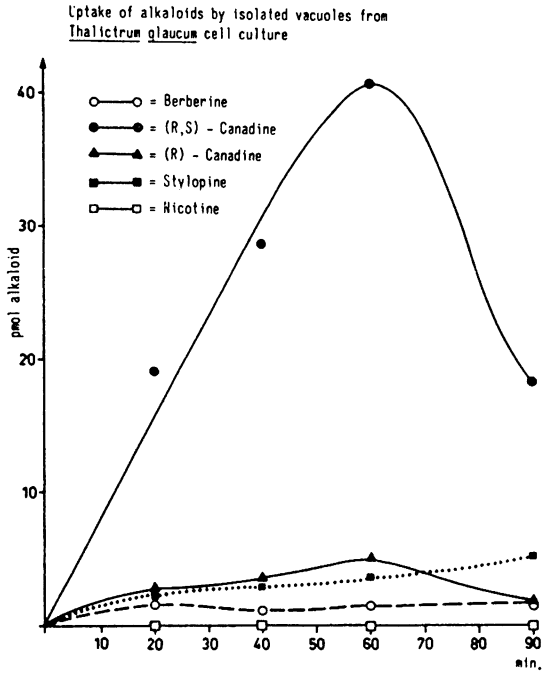


Fig. 3. Uptake measurement of ^{3}H-labelled alkaloids into the isolated vacuoles of *Thalictrum glaucum* (Ranunculaceae)

This phenomenon is in good agreement with data of Deus-Neumann and Zenk 1984b) with isolated vacuoles of *Catharanthus roseus* cultures, where they could observe that ajmalicine and not its oxidized form serpentine is transported into the vacuole.

A hypothetical explanation for this could be, that berberine with its positive charge is too polar to be accepted. Furthermore enzymatic experiments showed (Steffens et al. 1984, Amann et al. 1984) that the last steps in the biosynthesis of berberine do not take place in the cytosol and therefore probably no permeation processes would be necessary for the transfer into the vacuole. Alkaloids like nicotine, morphine or vindoline, which do not occur naturally in *Thalictrum*, are not accumulated in the vacuoles, whereas stylopine, a tetrahydroprotoberberine like canadine, could penetrate the tonoplast, even though only to a small extent, which supports a very specific uptake system located in the vacuole membrane.

Summarizing the data in this chapter one cannot conclude that generally benzylisoquinoline alkaloids are stored within the cells or excreted into the medium. It may be that specific types like the protopines or the benzophenanthridines are released into the medium whereas others like the protoberberines are deposited in the vacuole.

2.5.2 Stimulation of Releasing the Benzylisoquinolines into the Medium

Addition of Adsorbents. A possible method of enhancing the production of alkaloids by plant cell cultures could be to stimulate the release of alkaloids into the medium. It has been considered that low production of alkaloids in plant cell cultures

may result from minor amounts of alkaloid released into the surrounding medium as a result of the lysis of cells and that this inhibits their further synthesis. To test this hypothesis one could add adsorbent materials like granulated charcoal or perhaps XAD (a non-ionic adsorbent resin based on polystyrol) to the medium to remove the alkaloids and thereby stimulating their further production. Good results were achieved with this method for the formation of natural compounds in *Matricaria chamomilla* cell suspension cultures (Knoop and Beiderbeck 1983). We tried to apply this procedure to the production of protoberberine alkaloids in *Berberis stolonifera* (Berberidaceae). The results were not encouraging, since no enhancement of alkaloid formation was observed, and only 0.1% of the alkaloid amount produced by the cells could be determined as adsorbed on the charcoal. Similar results were obtained by addition of XAD to the medium. However, this method should be reinvestigated with the *Macleaya microcarpa* system, where protopine is released into the medium (Franke and Böhm 1982).

Permeabilization of Cell Membranes. Besides a stimulation of excretion, another approach would be to permeabilize the cell membranes without killing the cells. Brodelius and Nilsson (1983) proposed such a process for the production of natural compounds using DMSO for the intermittant permeabilization of cell membranes of immobilized cells. We tried to apply this procedure to *Berberis stolonifera* cell cultures but only 0.07% of the alkaloids stored within the cells could be released by DMSO treatment. Furthermore microscopy of the treated cells showed that even after a regeneration period of 4 days the cells had not recovered. They were plasmolyzed irreversibly, and therefore it may be concluded that further application of this process is not promising.

2.6 Biotransformation

Besides the possibility of producing the alkaloids in situ the synthetic capacity of the plant cells could be used for biotransformation. So far only few experiments have been described. Furuya et al. (1978) tried to produce morphinan alkaloids by the addition of reticuline, known to be an important intermediate in their biosynthesis. (S)-reticuline was converted to (S)-scoulerine (14.7%) and further on to the protoberberine cheilanthifoline (0.5%). (R)-reticuline, the isomer needed for the morphinan pathway, remained unchanged indicating that probably the enzymes catalyzing the steps to salutaridine and further on to codeine and morphine are missing. The only reaction that could be performed in this pathway was the reduction reaction from codeinone to codeine (67%) but no further metabolization to morphine could be observed. More promising results were obtained in *Corydalis ophiocarpa* (Papaveraceae) (Iwasa and Takao 1982), where alkaloids normally not occurring in the cell culture could be produced by the addition of tetrahydroprotoberberine-α-N-methochloride. 34% of the administered precursor could be transformed to the protopine α-allocryptopine and 1% further on to the benzophenanthridine chelerythrine and therefore one may consider this as a realistic method for the future production of benzylisoquinolines.

2.7 Optimization of Media

Besides the selection of high yielding cell lines and specialized methods for increasing alkaloid formation, the right choice of culture media is indispensable for an optimal production of benzylisoquinolines.

Looking for example at the use of so far described basal media the production of jatrorrhizine in *Berberis stolonifera* cell cultures (Hinz and Zenk 1981) was optimal in LS-medium (Linsmaier and Skoog 1965), the cell suspension cultures of *Coptis japonica* (Sato and Yamada 1984) gave the best results in White medium (White 1963), and *Eschscholtzia californica* (Papaveraceae) (Berlin et al. 1983) was cultivated with the best results in B5 medium (Gamborg et al. 1968).

2.7.1 Carbon Source

Nearly all cultures described so far grow best with sucrose as carbon source, but there are exceptions. Yamamoto (1981) for instance observed that his culture of *Coptis japonica* showed the highest growth and production rate on maltose. The optimal concentration of sucrose may differ from culture to culture: 8% sucrose was best for *Eschscholtzia californica* (Berlin et al. 1983), 3% for *Coptis japonica* (Sato and Yamada 1984), 4% for *Tinospora caffra* (Menispermaceae) (Rueffer unpublished), and 3.5% for *Berberis stolonifera* (Hinz and Zenk 1981).

2.7.2 Inorganic Medium Constituents

Variation of the PO_4^{3-}, NO_3^-, and NH_4^+ concentrations of the basal medium did not effect markedly the production of jatrorrhizine in *Berberis stolonifera* (Hinz and Zenk 1981). Various PO_4^{3-} concentrations also did not influence the production of jatrorrhizine in *Tinospora caffra* (Rueffer unpublished), whereas an inhibition of alkaloid production was observed with higher concentrations of phosphate in *Eschscholtzia californica* (Berlin et al. 1983).

2.7.3 Plant Growth Regulators

Plant growth regulators have a remarkable influence on the production of benzylisoquinoline alkaloids in some cultures. Fukui et al. (1982) described an increase of alkaloid yields by addition of kinetin or benzylaminopurine (0.1–1.0 μM). He also could observe a modification in the alkaloid pattern with higher concentrations (10 μM) of these phytohormones: The production of palmatine and jatrorrhizine significantly decreased whereas berberine and coptisine formation remained largely uneffected.

No increase in the alkaloid yields were observed by varying the growth regulators in *Berberis stolonifera* (Hinz and Zenk 1981). In a systematic test of 35 different phenoxyacetic acids and 88 other synthetic growth regulators no better results could be achieved than with the initial LS-medium.

Regarding the growth regulator 2,4-dichlorphenoxyacetic acid (2,4-D) the so far published data show a decrease in alkaloid production as the concentration of this growth regulator is increased in the basal medium. In *Thalictrum minus* (Ranunculaceae) cultures (Ikuta and Itokawa 1982a) 7 different protoberberine alkaloids were produced in the medium containing 0.1 ppm 2,4-D (berberine, palmatine, jatrorrhizine, columbamine, thalifendine, thalidastine, and desoxythalidastine). A rise to 1.0 ppm 2,4-D resulted in a loss of desoxythalidastine formation and with 5.0 ppm 2,4-D in the medium no columbamine, thalifendine, and thalidastine were synthesized. A similar decrease of productivity was observed in *Eschscholtzia californica* (Berlin et al. 1983) and *Chasmanthera dependens* (Menispermaceae) (Rüffer et al. unpublished).

2.7.4 Biosynthetic Precursors

Another essential factor that might induce the production of benzylisoquinolines could be the addition of biosynthetic precursors to the medium. Kamimura et al. (1976), for instance, described a stimulation of thebaine formation with the addition of tyrosine and dopa to the medium of *Papaver bracteatum* cultures. An increase from 0.05% of dry weight to 0.07% in the production of berberine in *Argemone mexicana* (Menispermaceae) suspension cultures (Khanna et al. 1982) also was due to the addition of tyrosine, whereas phenylalanine in the medium only stimulated the growth index and the berberine yield remained constant.

In summary, one has to conclude that every culture has to be optimized individually for alkaloid production and no general composition of one optimal medium for all cultures can be given.

3 Conclusions

On surveying the accomplishments with plant cell cultures and alkaloid production, one may be optimistic for the future of producing benzylisoquinoline alkaloids by this technique. Disappointing is the fact that so far no good production of morphinans has been achieved, but perhaps this problem can be overcome by employing the proposed selection methods and intensifying the search for an optimal medium. Promising is the production of berberine, a useful antibacterial and stomachic, in *Coptis japonica*. The root of the intact plant synthesizes 200 mg (10% of dry weight) within 5 years whereas the cell suspension culture produces 1.67 g l^{-1} medium (11.4% of dry weight) within 3 weeks (Fukui et al. 1982). The best results so far have been the formation of the protoberberine alkaloid jatrorrhizine in *Berberis stolonifera* cell cultures (Hinz and Zenk 1981, Zenk and Deus 1982), where the second highest ever reported production of a natural compound could be observed with 2.7 g l^{-1} medium. Similar results were reported, also for the synthesis of jatrorrhizine, in *Berberis wilsoniae* where the first experiments for the cultivation in a fermentor were carried out (Rothenberger 1982).

These last examples suggest the future development of plant cell suspension cultures for a biotechnological use is a real possibility.

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