Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Biotechnology

Exploiting plant alkaloids

Sabrina Schläger and Birgit Dräger

Alkaloid-containing plants have been used for medicine since ancient times. Modern pharmaceuticals still rely on alkaloid extraction from plants, some of which grow slowly, are difficult to cultivate and produce low alkaloid yields. Microbial cells as alternative alkaloid production systems are emerging. Before industrial application of genetically engineered bacteria and yeasts, several steps have to be taken. Original alkaloid-forming enzymes have to be elucidated from plants. Their activity in the heterologous host cells, however, may be low. The exchange of individual plant enzymes for alternative catalysts with better performance and optimal fermentation parameters appear promising. The overall aim is enhancement and stabilization of alkaloid yields from microbes in order to replace the tedious extraction of low alkaloid concentrations from intact plants.

Address
Institute of Pharmacy, Martin Luther University Halle-Wittenberg, Hoher Weg 8, D-06120 Halle, Germany

Corresponding author: Dräger, Birgit (birgit.draeger@pharmazie.uni-halle.de)

Current Opinion in Biotechnology 2016, **37**:155–164

This review comes from a themed issue on **Plant biotechnology**

Edited by **John A Morgan** and **Anne Osbourn**

For a complete overview see the Issue and the Editorial

Available online 1st January 2016

http://dx.doi.org/10.1016/j.copbio.2015.12.003

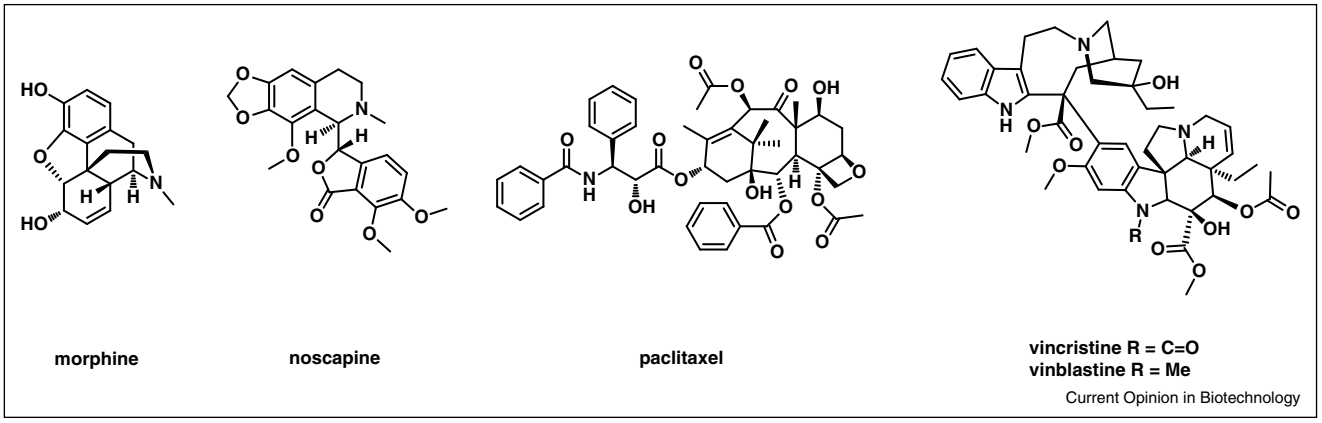
Introduction

Alkaloids are secondary compounds that have been identified predominantly from plants, less frequently from fungi and animals. With more than 12,000 different structures they form a very large group of secondary compounds that is highly diverse. Approaches for a structural definition of alkaloids comprise compounds of low molecular mass with a heterocyclic nitrogen derived from an amino acid as criteria, and alkaline reaction of alkaloids in water of isolated alkaloids was eponymous. However, further plant secondary compounds, for example, ephedrine from *Ephedra sinica* (Ephedraceae) and colchicine from *Colchicum autumnale* (Colchicaceae; meadow saffron), both with non-heterocyclic nitrogen atoms, are also counted as alkaloids. Another alkaloid definition focusses on the strong pharmacological effects that many alkaloids exert. On important example is paclitaxel from *Taxus brevifolia* (Taxaceae; pacific yew), although its core skeleton derives from a diterpene, and the only non-alkaline nitrogen in the molecule is an amide-forming nitrogen from phenylisoserine (Figure 1) [1]. The pronounced effects that alkaloids show in mammals have been exploited since ancient times. The Papyrus Ebers contains a collection of Egyptian medicinal plants and recipes dating from ca. 1580 BC. It lists for example Solanaceae containing atropine and scopolamine, such as mandrake (*Madragora officinarum*) for vitality and longevity, and recommends Egyptian henbane (*Hyoscyamus muticus*) against toothache [2].

Many alkaloids still possess prominent places in modern pharmacotherapy. Those that were abandoned have often served as model compounds for the development of synthetic analogues with a milder or more focused effect. For example, local anesthetics used in dentistry and eye surgery mimic structural elements of cocaine such as mepivacaine (Meaverine™). Alkaloids that are still intensively utilized in medication are often applied in life threatening conditions, for example, atropine as antidote against intoxications with nerve agents or insecticides. Paclitaxel and its derivatives are applied against ovary carcinoma and breast cancer. The dimeric alkaloids from Madagascar periwinkle (*Catharanthus roseus*, Apocynaceae), vincristine and vinblastine (Figure 1) and semisynthetic derivatives are frequently used against leukemia and lymphoma. Morphine from opium poppy (*Papaver somniferum*, Papaveraceae) and its derivatives are indispensable for the treatment of severe pain [3]. Recently a phthalideisoquinoline alkaloid from *P. somniferum*, noscapine, has attained high interest because of its antimitotic activity (Figure 1). Intensive testing of noscapine and its semi-synthetic derivatives for application against various cancers is presently performed [4].

The plant species that provide the desired alkaloids show high variation in growth and alkaloid content. Correspondingly, procedures for industrial alkaloid production differ in strategy and efficiency. For example, extraction of atropine and scopolamine from high-yielding Solanaceae such as *Duboisia* hybrids (up to 2.5% scopolamine in leaves [2]) is industrial routine. Equally, quinine and related alkaloids accumulate in Cinchona bark (*Cinchona succirubra*, *C. ledgeriana*, *C. calisaya* and hybrids; Rubiaceae) up to 17% of the dry mass; the trees are cultivated in large plantations [5]. Alkaloids with more simple structures are readily synthesized, such as the purine alkaloids theophylline and caffeine, although most of the caffeine consumed worldwide as mild stimulant originates from natural resources, mostly coffee beans and tea leaves. The

Figure 1



Structures of morphine, paclitaxel, noscapine, vincristine and vinblastine.

production of cytotoxic Catharanthus alkaloids, however, is demanding due to very low concentrations in the plant tissues.

Two groups of alkaloids that are particularly difficult to be exploited from plants are morphinan alkaloids and noscapine (benzylisoquinoline alkaloids, BIA) found in opium poppy and vincristine and vinblastine (monoterpenoid indole alkaloids, MIA) from *Catharanthus roseus*. This review therefore focusses on biotechnology approaches for these groups of alkaloids. Research on alkaloid production strategies usually begins with isolation and description of the plant enzymes that catalyze the often numerous biosynthetic steps to the final alkaloid. For alkaloid production in heterologous hosts, however the application of enzymes different from the original plant enzymes sometimes appears preferable. Alternative enzymes catalyzing alkaloid biosynthetic steps may be more easily expressed in the host cells, or they are necessary because the original plant enzymes in spite of many efforts remain unknown.

BIA biotechnology

BIA from *Papaver somniferum*

Among the large group of BIA that derive from the central metabolite (*S*)-reticuline, the morphinan alkaloids morphine and codeine are of the highest economic importance. Their natural occurrence is limited to *P. somniferum* and to a few other *Papaver* species without economic importance. For traditional alkaloid preparation, opium is used, being the dried latex collected from scraped unripe opium poppy capsules.

The production of opium for medical purpose amounted up to 789 tons in 2011, equivalent to 87 tons of morphine [6]. Morphine and morphinan production for medically purposes relies to a large extent on the extraction of poppy straw [7]. Additionally, this legal production is surmounted by more than tenfold yield from illegal opium poppy cultivation. The total production of opium in 2013 is estimated at up to 6883 tons. The illegal business results in large price differences for the morphinan alkaloids. While a gram of pure heroin is sold in the US black market for around 1000 $, a kilogram of morphine for preparation of pharmaceuticals may attain a similar price.

The main benzylisoquinoline alkaloid compounds in opium are morphine 9.5–12.0%, codeine 2.5% and thebaine 1.0–1.5%. Medicinally, morphine is applied as strong analgesic. It is additionally used as precursor for codeine and other opioids. The consumption of codeine is regularly higher than direct morphine application, resulting from the use of codeine as cough medication and as enhancer for simple pain relief compounds, for example, salicylates or acetaminophen. Thebaine mainly serves as precursor for semisynthetic opioids. Recently another alkaloid from opium attracted attention. The phtalideisoquinoline alkaloid noscapine (= narcotine) was discovered to act as tubulin-binding anti-cancer agent with lower toxicity than established antimitotic drugs. The noscapine content in opium varies widely (2–10%) depending of the cultivar.

Biosynthetic steps: enzymes and genes

All participating enzymes and their encoding genes for the biosynthesis of the morphinan alkaloids and of noscapine have now been assembled. A recent review [8] on BIA biosynthesis in opium poppy lists all but two enzymes that are necessary for the morphinans and for noscapine.

One important step was missing until June 2015, when the conversion of (*S*)-reticuline to (*R*)-reticuline via 1,2-dehydroreticuline was elucidated [9••]. The (*S*)-reticuline to (*R*)-reticuline conversion opens the way from the