7 Alkaloids

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1 Introduction

Plants have the ability to produce tens of thousands of highly complex secondary metabolites to assist their survival in the environment, many of which protect the plant from predators. Man has exploited these compounds of self-defence as sources of medicinal agents, poisons, and potions since time immemorial. Throughout the world different communities have discovered plants with pharmacological properties, and many useful drugs have their origins in indigenous ethnopharmacologies. Some notable examples include: the roots of the mandrake plant, known for their sedative properties from the time of HIPPOCRATES (ca. 400 BC) and also used as a deadly poison during Elizabethan times (MANN, 1989); the leaves of the coca plant, which were chewed as an aid to stamina and as part of ceremonies in South America over 5000 years ago (VAN DYKE and BYCK, 1982); and plants whose hallucinogenic properties were used in the preparation of "magic potions" by the Aztec Indians. The compounds responsible for these physiological effects in man were isolated during the 19th and early 20th centuries and were identified as scopolamine, cocaine, and amides of lysergic acid, respectively. SOCRATES' death in 399 BC was the result of consumption of hemlock (*Conium maculatum*) which contains the alkaloid coniine (**1**) (Fig. 1; HENDRICKSON et al., 1970), while CLEOPATRA used extracts from Egyptian henbane (*Hyoscyamus muticus*) during the last century BC to dilate her pupils and increase her beauty. Likewise, medieval European women used extracts of deadly nightshade (*Atropa belladonna*) in their beauty preparations, hence the name *bella donna*, "fair lady".

Other historical uses include extracts from the bark of *Cinchona officinalis* which have been employed as antimalarials. Extracts derived from the opium poppy *Papaver somniferum* comprise another group of important pharmacologically active compounds which possess powerful analgesic properties. It has been reported that extracts of the milky latex material that exudes from the cut unripe seed capsule of the opium poppy were used by the early Egyptians for medicinal purposes; however, results from a recent examination of materials from the tomb of the Royal Architect KHA seem to refute earlier observations (BISSET et al., 1994). It was not until the 19th century that the active compounds, alkaloids, were isolated from the opium. Morphine was also the first alkaloid to be identified and crystallized by the chemist SERTÜRNER in 1805. This was a significant achievement as not only was it the first time that a nitrogenous base had been isolated from a biological source, but it was also the first time that such a substance had been shown to be intrinsically basic. This finding formed the basis of one of the earliest definitions of an alkaloid which was attributed to the pharmacist W. MEISSNER (HESSE, 1981; PELLETIER, 1983). The two authors, HESSE (1981) and PELLETIER (1983), differ on the exact date of the coining of the term (1818 and 1819 being cited, respectively) and the derivation, but the general meaning was taken to be an "alkali like" compound of plant origin, or as BENTLEY (1954) interpreted, a "vegetable alkali". This was extended by WINTERSTEIN and TIET (1910) to include a four part definition stating a "true alkaloid" can be characterized by: (1) the possession of a nitrogen atom as part of a heterocyclic system; (2) a complex molecular structure; (3) significant pharmacological properties; (4) its origin from the plant kingdom (cited in PELLETIER, 1983).

The majority of alkaloids fit this four part definition; however, a number of exceptions exist. The compounds samandarine (**2**) (Fig. 1), samandarone, and cycloneosamandarine, isolated from the skin glands of the European fire salamander (*Salamandra maculosa* Laurenti) all exhibit the usual properties of an alkaloid substance, but do not fit the definition of a "true alkaloid" owing to their animal origin. There are numerous examples of alkaloids furnished by animal, including batrachotoxinin A (**3**), a steroidal alkaloid from the Colombian arrow-poison frog (*Phyllobates aurotaenia*), bufotenine (**4**), a tryptamine-type alkaloid from the common European toad, (−)-deoxynuphradine and (−)-castoramine (**5**) from the Canadian beaver (*Castor fiber* L.), and muscopyridine (**6**) from the scent gland of the musk deer (*Moschus moschiferus*). Alkaloids have also been identified from arthropod, bacterial, and fungal origins. For example, the quinazole

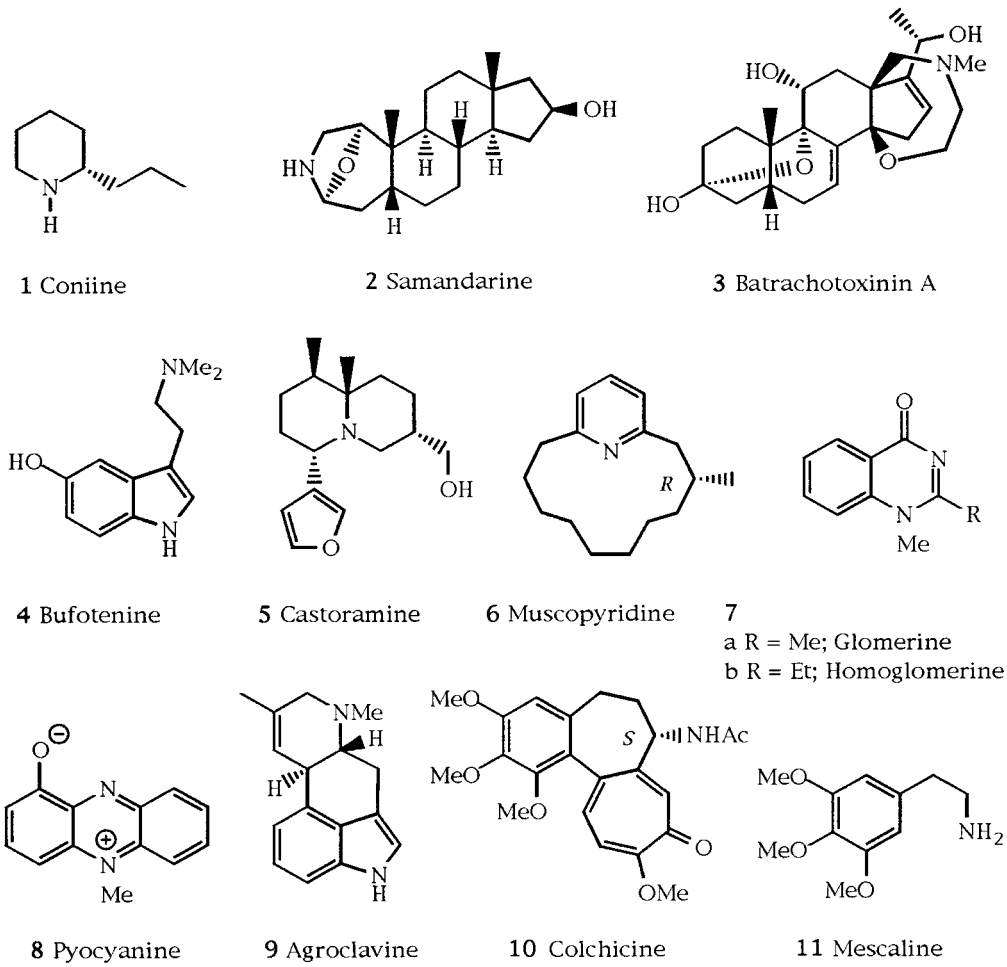


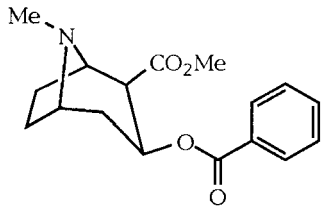
Fig. 1. See text.

alkaloids, glomerine (**7a**) and homoglomerine (**7b**) discharged from the dorsal glands of the European millipede (*Glomeris marginata*), the deep-blue colored alkaloid pyocyanine (**8**), isolated from the bacterium *Pseudomonas aeruginosa*, and agroclavine (**9**), produced by the fungi *Claviceps purpurea* and *Aspergillus fumigatus*. Other examples of alkaloids exist which also do not adhere to the criteria stipulated in the four part definition of an alkaloid. For example, the alkaloids colchicine (**10**) (autumn crocus, *Colchicum autumnale* L.) and mescaline (**11**) (*Lophophora williamsii*) do not possess nitrogen as part of a heterocyclic system. Also colchicine (**10**) is essentially neutral and, therefore, does not conform to the original definition of an alkaloid. PELLETIER (1983), however, provides a reasonable summary of an alkaloid's properties as being an "alicyclic compound containing nitrogen in a negative oxidation state which is of limited distribution among living organisms". Over 10000 compounds fall within this definition (SOUTHON and BUCKINGHAM, 1989) and new alkaloids are continually being reported from various sources. These represent approximately 20% of all known natural products; however, only about 30 of these with biological activity are commercialized (FARNSWORTH, 1990).

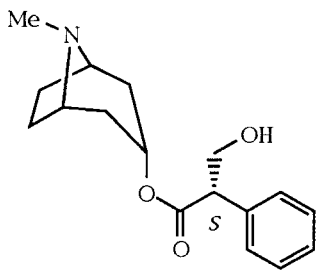
Unlike any other group of compounds the alkaloids exhibit a vast array of skeletal types and are classified accordingly. A typical example is the scheme used by HESSE (1981), who describes 11 classes of heterocyclic alkaloids, differentiating by the nature of the carbon skeleton, e.g., the pyrrolidine and isoquinoline alkaloids. The majority of alkaloids are amino acid-derived, although terpenes, steroids, purines, and nicotinic acid can also act as building blocks of, e.g., aconitine, solanidine, caffeine, and nicotine, respectively. If the anabolic route of an alkaloid is known, this can be used to classify the compound (DALTON, 1979). The tropane, and pyrrolidine alkaloids, for instance, are all derived from ornithine, a derivative of arginine, and thus grouped together under this scheme.

Alkaloids have provided a wealth of pharmacologically active compounds; approximately 25% of the drugs used today are of plant origin. These are administered either as pure compounds or as extracts and have often served as model structures for synthetic drugs, e.g., atropine (**13**) for tropicamide, quinine for chloroquinine, and cocaine (**12**) (Fig. 2) for procaine (KUTCHAN, l995). Screening of plant extracts for pharmacologically active compounds still continues and results in new drug discoveries; recent examples include the anticancer drugs taxol from the western yew, *Taxus brevifolia*, and camptothecin from *Camptotheca acuminata*. Alkaloids are generally regarded as speciality chemicals; approximately 300–500 metric tons of quinine and quinidine are produced each year; ajmalicine (**98**) production amounts to about 3600 kg, while compounds like vincristine (**94**) and vinblastine (**95**) (Fig. 21) are produced in the kilogram range. The annual market value of the major alkaloids has been estimated to be in the range of several hundred million dollars (VERPOORTE et al., 1993).

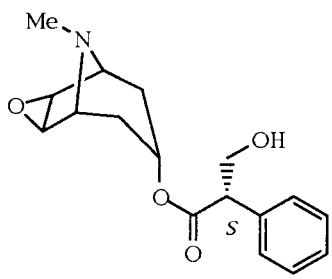
The important pharmacological activity of many alkaloids has spurred chemists to make many derivatives of these natural compounds. The chemical preparation of such semisynthetic alkaloids has resulted in the production of drugs with improved properties, such as the addition of a 14-hydroxy group to the morphine alkaloid structure which has been found to dramatically increase potency (JOHNSON and MILNE, 1981). However, the synthesis of such compounds is often difficult to achieve on a commercial scale due to the chemical complexity of the starting material, cost, and environmental issues, in addition to the precursors being in limited supply. Biotransformations can offer a number of advantages over conventional chemical processes. The specificity of enzyme-catalyzed reactions, e.g., allows the stereospecific transformation of defined functional groups. However, biotransformations of alkaloids, unlike their steroid counterparts, have yet to meet their potential on an industri-



12 Cocaine



13 Hyoscyamine
(Atropine is racemic hyoscyamine)



14 Scopolamine

Fig. 2. Examples of tropane alkaloids.

al scale. This is in part due to the lack of suitable enzymes and partly because no alkaloid-based drug commands a significant share of the therapeutic market, unlike the steroids.

The rate at which new drugs derived from natural products are entering the therapeutic market has declined significantly over recent periods in contrast to synthetic molecules, possibly due to the difficulty of modifying these often complex chemicals for the development of new drugs and the difficulty of producing these natural products in a pure form. The difficulties associated with the development of new drugs are being addressed by the ever increasing interplay of chemistry and biology. Undoubtedly combinatorial approaches (AMATO, 1992) and genetic engineering will play an important role in the development of new drugs.

The use of recombinant DNA technology is beginning to have substantial impact on biotransformation processes, resulting in the development of new approaches using biological systems. Recent advances in the understanding of the genetic and biochemical basis of alkaloid biosynthetic pathways are now beginning to make biotransformations of complex alkaloid molecules more plausible. The expression of plant enzymes, which are often present at very low levels in the plant, in heterologous hosts such as bacteria allows detailed examination of mechanisms of reaction which are often unknown in synthetic organic chemistry. It is possible to add to the genetic repertoire of a plant by incorporating genes from other species allowing the possibility of producing unique compounds with potential biotechnological applications. Microorganisms have been used for the large-scale production of high-value chemicals for many years, and the use of microbial processes to make analogs of naturally occurring alkaloids is achievable. It is now possible to assemble hybrid transformation pathways in microbes using structural genes cloned from different organisms which mediate enzymic processes which are not indigenous to the host organism. These "patchwork" pathways can have the advantage of removing unwanted side reactions, they allow the possibility of increasing the activity of a cell by altering regulatory processes, and avoid the need to supply expensive exogeneous cofactors. Furthermore, it is now theoretically possible to manipulate alkaloid biosynthetic pathways to improve yields and to extend pathways to synthesize new bioactive molecules. Metabolic engineering of plants offers the capability of altering the pattern of alkaloid accumulation in the plant; in addition, the ability to house and express recombinant genes in plants from other organisms offers the potential of both extending pathways and allowing the biological synthesis of semisynthetic derivatives. It is now possible to design strategies to alter the metabolic flux in a variety of organisms, such as the introduction of extra copies of genes encoding enzymes which form bottlenecks in pathways affords a way to attain increases in yields of plant secondary products, or more globally through the expression of one or more regulatory genes (for reviews see BAILEY, 1991; NESSLER, 1994; HUTCHINSON, 1994; KUTCHAN, 1995). Due to their complex structures, alkaloids are still most efficiently produced by the plant and the future success of metabolic engineering of plant secondary products is dependent on having a good understanding of the biochemistry and regulation of the pathways under consideration.

Plant cell culture has been invaluable as a means of providing suitable biomass for the elucidation of pathways for secondary metabolites, particularly for alkaloid synthesis. Cell culture has also been examined for biotransformation purposes (reviewed by VERPOORTE et al., 1993) and extensively investigated as a means of producing plant secondary products on a large scale. Unfortunately, the level and manner of production of alkaloids in plants does not necessarily correlate with production in cell cultures. The use of plant cell cultures for biotransformations of alkaloids will not be considered in detail this chapter. The purpose of this review is to introduce some of the newer technologies which are beginning to make an impact on the area of alkaloid transformations. It also aims to introduce some of the more recent developments in the biochemistry and genetic understanding of biosynthetic and catabolic routes of some of the more pharmacologically important alkaloids in different organisms, without which the rational design of any recombinant alkaloid biotrans-

formation processes would not be feasible. The alkaloids discussed include the tropane alkaloids, the benzylisoquinoline alkaloids, the benzophenanthridine alkaloids, the morphinan alkaloids, and the monoterpenoid indole alkaloids.

2 Tropane Alkaloids

The tropane alkaloids occur in the Solanaceae family, but they are also found in the plant families Erythroxylaceae, Convolvulaceae, Proteaceae, and Rhizophoraceae. Their common structural element is the azabicyclo[3.2.1]octane system, and over 150 tropane alkaloids have been isolated. The 3-hydroxy aromatie ester derivatives form the parent alkaloids, examples of which include cocaine (**12**) (*Erythroxylon coca*, coca plant), hyoscyamine (**13**), (*Hyoscyamus niger*, henbane), atropine (**13**) (*Atropa belladonna*, deadly nightshade), and scopolamine (**14**) (*Scopola carniolica*) (Fig. 2). It appears that in most cases atropine (**13**) is formed by racemization of hyoscyamine (**13**) during extraction.

Long before the elucidation of their structures, the pharmacological properties of several tropane alkaloids were exploited. Atropine (**13**), which typifies the action of tropane alkaloids, causes antagonism to muscarine receptors (parasympathetic inhibition) (CORDELL, 1981).

These receptors are responsible for slowing of the heart rate, vasodilation, dilation of the pupil, and stimulation of secretions. The heart rate altering properties of atropine (**13**) have led to its use in the initial treatment of myocardial infraction. Tropane alkaloids have also been used to treat peptic ulcers, prevent motion sickness, and as components of pre-anesthetic drugs. Cocaine (**12**) is perhaps the best known of all the tropane alkaloids mainly because of its use as an illicit drug; it is a powerful central nervous system stimulant and adrenergic blocking agent, and its hydrochloride salt has been used as a local and surface anesthetic in face, eye, nose, and throat surgery (GERALD, 1981). The function of cocaine (**12**) in leaves of the coca plant was unknown until a recent study suggested that the alkaloid has insecticidal properties at naturally occurring concentrations due to potentiation of insect octopaminergic neurotransmission (NATHANSON et al., 1993).

2.1 Tropane Alkaloid Biosynthesis

The biosynthetic pathways for the tropane alkaloids have been studied in considerable detail and are associated with nicotine biosynthesis, since the *N*-methyl-Δ^1-pyrrolinium cation (**15**) is a precursor to both classes of alkaloids. The formation of the tropane nucleus from ornithine and acetoacetate was first investigated in plants using radioactive tracers as long ago as 1954, but it was not until the early 1980s that a biosynthetic scheme was finally elucidated in *Erythroxylon coca* (Fig. 3; LEETE, 1983). The pathway for biosynthesis of hyoscyamine (**13**) and scopolaminc (**14**) is quite complex, since not only is an acetone unit required for the formation of tropinol (**19**), but a second converging pathway is necessary for the conversion of phenylalanine (**20**) to tropic acid (**23**) (Fig. 4). Recent work with root cultures of *Datura stramonium*, suggests that hygrine (**17**) is not an intermediate, but an off shoot from the main pathway (ROBINS et al., 1997). The biosynthesis of cocaine (**12**) is similar to that of hyoscyamine (**13**). The *N*-methylation and cyclization of ornithine-derived putrescine gives the *N*-methyl-Δ^1-pyrrolinium cation (**15**), which condenses with acetoacetyl-CoA (**16**). Methylation of the free carboxylate group followed by ring closure, reduction of the ketone group, and benzoylation results in the formation of cocaine (**12**). The benzoic acid is derived from phenylalanine (**20**).

The molecular biology of tropane alkaloid synthesis is being studied extensively and holds considerable potential for alkaloid biotransformations. An elegant example is the construction of a transgenic species of *Atropa belladonna* that was able to accumulate the important pharmaceutical scopolamine (**14**) instead of hyoscyamine (**13**) (YUN et al., 1992). The final two steps in the pathway for the biosynthesis of scopolamine (**14**) (Fig. 5) are catalyzed by 2-oxoglutarate-dependent hyoscyamine 6β-hydroxylase (hyoscyamine[6β]-di-

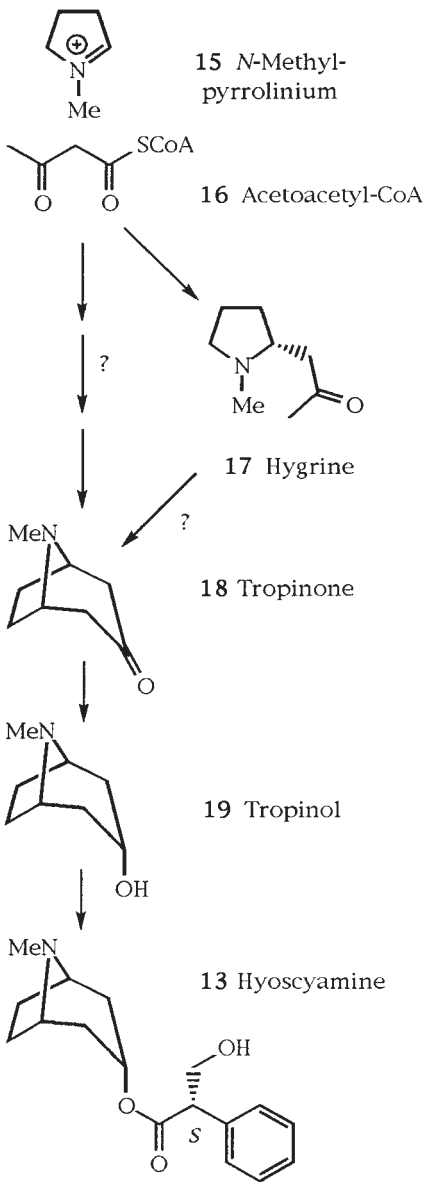


Fig. 3. Biosynthesis of tropane alkaloids (ROBINS et al., 1994).

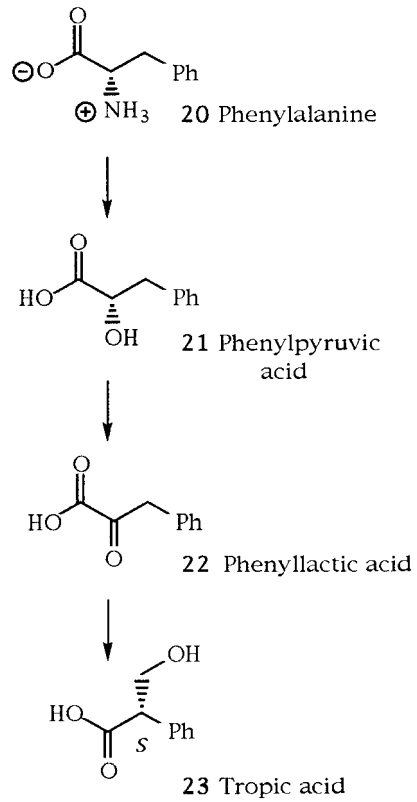


Fig. 4. Biosynthesis of tropic acid (ROBINS et al., 1994).

oxygenase; EC 1.14.11.11). This enzyme first hydroxylates hyoscyamine in the 6β-position of the tropane ring (**24**), which is followed by epoxidation. The use of purified 6β-hydroxylase from root cultures of *Hyoscyamus niger* showed that catalysis of these two steps carried out was by the same enzyme (HASHIMOTO et al., 1987). Analysis of key enzymes of metabolic pathways at the molecular genetic level assists clarification of complex biochemical mechanisms, and hydrolysis and epoxidation of the tropane ring was later confirmed unequivocally by molecular cloning and expression of the structural gene of the 6β-hydroxylase in a heterologous host (MATSUDA et al., 1991; HASHIMOTO et al., 1993b); *A. belladonna* accumulates hyoscyamine (**13**) instead of scopolamine (**14**) because it lacks the 6β-hydroxylase. The cDNA encoding the 6β-hydroxylase from *H. niger* was transferred into *Agrobacterium tumefaciens* and introduced into *A. belladonna*. The regenerated transgenic plants were found to contain elevaled levels of scopolamine (**14**) (YUN et al., 1992). The change in

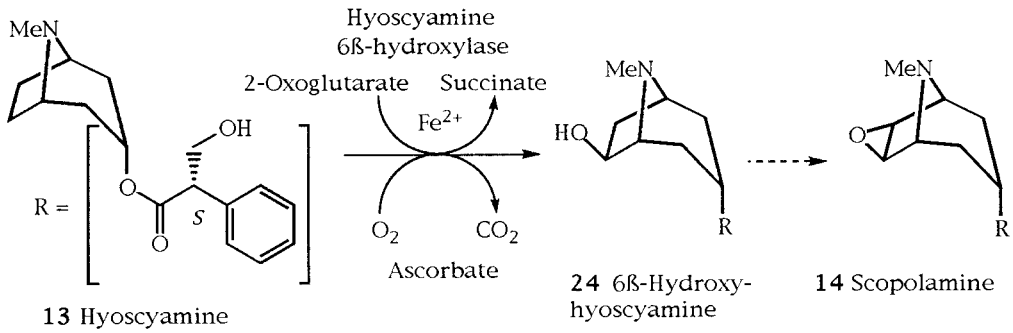


Fig. 5. See text.

alkaloid composition in the transgenic *A. belladonna* was considerable, with scopolamine (**14**) being almost the only alkaloid present in the aerial parts of the plant. It was thus possible to isolate pure scopolamine (**14**) by recrystallization of the total alkaloid fraction, instead of conventional differential extraction and chromatography. Analysis of expression of the 6β-hydroxylase gene by measurements of levels of mRNA and Western blot analysis of protein extracts from various tissues showed that enzyme expression in scopolamine producing species of *Hyoscyamus* was lacking in the stem or leaves, being localized in the roots of these plants, and explains why it has not been possible to produce these alkaloids in significant quantities by cell culture (HASHIMOTO et al., 1991; MATSUDA et al., 1991). HASHIMOTO et al. (1993b) have now engineered transgenic *A. belladonna* hairy root cultures that express the *H. niger* gene encoding hyoscyamine 6β-hydroxylase which exhibited up to 5 times higher activity. These transgenic roots may prove to be useful for enhancing scopolamine productivity *in vitro*. Recombinant strains of *Escherichia coli* expressing the gene encoding hyoscyamine hydroxylase were also capable of transforming hyoscyamine (**13**) to scopolamine (**14**) (HASHIMOTO et al., 1993a; LAY et al., 1994).

Tropinone reductase acts at a branch point of biosynthetic pathways leading to a variety of tropane alkaloids. It is an NADPH-dependent enzyme which reduces the 3-keto group of tropinone (**18**) (ROBINS et al., 1994). Two tropinone reductases with different stereospecificities were found in cultured roots of *H. niger* (HASHIMOTO et al., 1992).

These two distinct enzyme activities reduced tropinone (**18**) to 3α-hydroxytropane (**19**) (tropinol, tropine) and 3β-hydroxytropane (pseudotropinol, ψ-tropine, pseudotropine), respectively. Marked differences were observed between the two reductases in their affinities for tropinone (**18**), substrate specificity, and in the effects of amino acid modification reagents. The cDNA clones for the two tropinone reductases have been expressed in *Escherichia coli* and sequenced (NAKAJIMA et al., 1993). Preparation of various chimeric forms of these two enzymes led to the identification of the domain conferring the stereospecificity of the reaction (NAKAJIMA et al., 1994).

These elegant experiments demonstrate a key to future alkaloid biotransformation processes by the manipulation of biosynthetic routes in plants with the use of recombinant DNA technology not just for alkaloids but also other secondary products. It is becoming possible to design strategies to advantageously manipulate the metabolic flux in organisms, or to decrease or increase the production of phytochemicals; however, with regard to biotransformations, it is the possibility of manipulating pathways by altering enzyme function by directed mutagenesis and extending/altering existing pathways by heterologous gene expression to alter the spectrum of plant alkaloids which is particularly challenging and exciting. Although the genetic tools for manipulating biosynthetic pathways in plants lag behind those for prokaryotic organisms, the success of

scopolamine production in transgenic plants will, hopefully, encourage interest and further development.

2.2 Microbial Metabolism of Tropane Alkaloids

Microorganisms possess an incredible variety of metabolic pathways which enables them to degrade a plethora of natural and man-made organic compounds. The elucidation of alkaloid dissimulating pathways has considerable potential for the identification of biotransformation routes for new and existing therapeutic compounds (BRUCE et al., 1995).

The most extensively studied tropane alkaloid, in terms of microbial metabolism is atropine (**13**). Several bacterial species have been shown to possess an esterase that catalyzes the esterolytic hydrolysis of the atropine molecule, to form tropinol (**19**) and tropic acid (**23**) (Fig. 6).

The interest in atropine esterase lies in its similarity to mammalian serine proteases and its use as a possible model of mammalian cholinergic receptors. RÖRSCH et al. (1971) reported the isolation of a number of *Pseudomonas* strains capable of utilizing atropine as a sole source of carbon and nitrogen. The atropine esterase from one of these strains, *Pseudomonas putida* PMBL-1, has been purified and extensively characterized (VAN DER DRIFT, 1983; VAN DER DRIFT et al., 1985a, b, 1987). This esterase showed activity with both enantiomers of hyoscyamine (**13**), but not with cocaine (**12**) (RÖRSCH et al., 1971), despite the close similarity of structure of those compounds. NIEMER et al. (1959) and NIEMER and BUCHERER (1961) reported a breakdown route of atropine (**13**) by *Corynebacterium belladonnae*, which involves the formation of tropinol (**19**) and tropic acid (**23**) by esterase action, followed by dehydrogenation, ring opening, and deamination of the tropane ring (Fig. 6). The first step in their proposed route of tropine catabolism involves a tropine dehydrogenase. Activity, however, was only demonstrated in the reverse direction. The step postulated by NIEMER and BUCHERER (1961) which follows tropinone formation involves ring cleavage and the formation of tropinic acid (**25**), though no enzymes or cofactors were identified. Isolation of a picrate derivative of methylamine from whole cell incubations with tropinone, indicated that nitrogen debridging was taking place. More recent investigations into the microbial metabolism of atropine showed that a strain of *Pseudomonas* sp. (termed AT3) isolated from the rhizosphere of atropine plants was able to utilize tropinol (**19**) as a sole carbon and nitrogen source (LONG et al., 1993). Growth studies revealed a diauxic growth pattern. When this organism was supplied with atropine (**13**) and an exogenous nitrogen source, tropic acid (**23**) was utilized during the first phase of growth and the heterocyclic moiety, tropinol (**19**), was utilized in the second. The enzymes responsible for tropinol (**19**) degradation appeared to be strongly repressed during the first phase of growth. Under nitrogen limitation, however, the nitrogen must be stripped from the tropane ring before growth can occur and under these conditions tropinol (**19**) was utilized in the first growth phase. *Pseudomonas* sp. AT3 initiated the degradation of tropinol (**19**) by attacking the nitrogen atom, yielding a dinitrophenyl hydrazine positive intermediate, identified as 6-hydroxycyclohepta-1,4-dione (**28**), which was oxidized by an NAD^+-dependent dehydrogenase activity to cyclohepta-1,3,5-trione (**29**). The subsequent cleavage of this compound resulted in the formation of 4,6-dioxoheptanoic acid (succinylacetone) (**30**) which was, in turn, the substrate for a second hydrolase yielding succinate (**31**) and acetone(**32**) (BARTHOLOMEW et al., 1993, 1996).

BARTHOLOMEW et al. (1995) identified an $NADP^+$-dependent tropinol dehydrogenase in cell-free extracts of *Pseudomonas* sp. AT3 that was induced by growth on atropine (**13**), tropinol (**19**) or tropinone (**18**). The product of the reaction was tropinone (**18**) and the reaction was shown to be freely reversible. The dehydrogenase showed activity only with tropinol (**19**) and nortropinol (**27**); no activity was detected with a number of closely related compounds including atropine (**13**), scopine, pseudotropinol, ecgonine (**34**) (Fig. 7) and 6-hydroxycyclohepta-1,4-dione (**28**), which suggests thal this inducible enzyme is involved in the metabolism of tropinol (**19**) in *Pseudomo-*

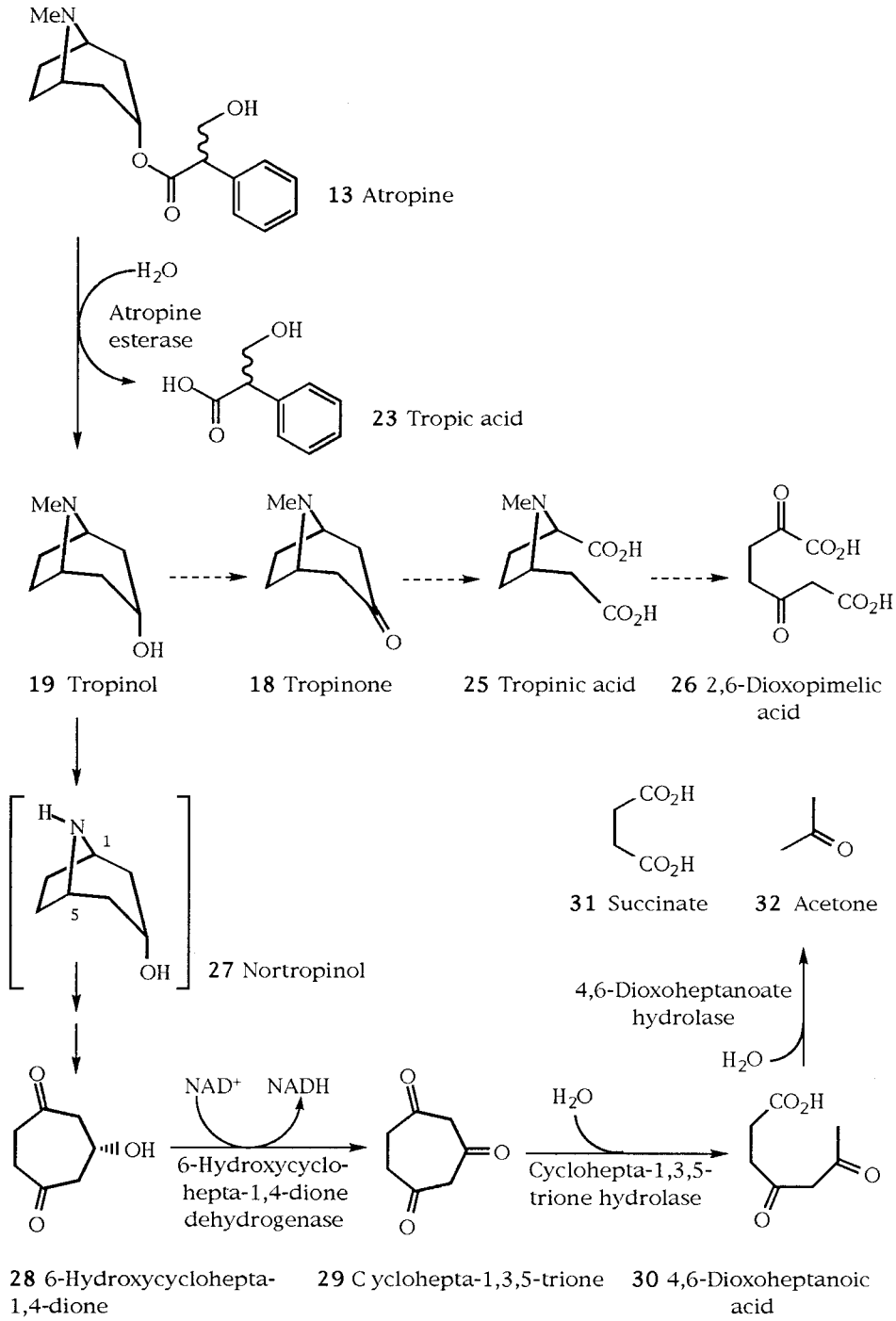


Fig. 6. Proposed pathway for the degradation of atropine (**13**) by *Pseudomonas* sp. AT3 (BARTHOLOMEW et al., 1996). ----► Pathway proposed by NIEMER and BUCHERER (1959) for *C. belladonna*.

nas sp. AT3. However, the occurrence of 6-hydroxycyclohepta-1,4-dione (**28**) during the metabolism of tropinol seemed to dispute this (BARTHOLOMEW et al., 1993). An elegant set of experiments with tropinol (**19**) and pseudotropinol labeled with deuterium in the C-3 position provided an answer (BARTHOLOMEW et al., 1995). The labeled alcohol group at C-3 was shown to remain intact past the point of removal which indicated that tropinone (**18**) is not an intermediate in the pathway of tropinol (**19**) metabolism. What then is the role of tropinol dehydrogenase in the metabolism of tropinol? Tropinone (**18**) serves as a growth substrate for *Pseudomonas* sp. AT3 and it is likely to be encountered in nature, along with atropine (**13**) and tropinol (**19**), as it is an intermediate in the biosynthesis of the tropane alkaloids in plants (LANDGREBE and LEETE, 1990). A mutant strain of *Pseudomonas* sp. AT3 blocked in 6-hydroxycyclohepta-1,4-dione dehydrogenase activity was grown on tropinone; this resulted in the accumulation of 6-hydroxycyclohepta-1,4-dione (**28**), an indication that tropinone (**18**) is metabolized via the same route as tropinol (**19**) and that its keto group is reduced in the process. Thus, the tropinol dehydrogenase may function primarily as a reductase in order to channel tropinone (**18**) and nortropinone into the pathway of tropinol (**19**) metabolism in *Pseudomonas* sp. AT3.

The elucidation of the pathway for microbial metabolism of the related alkaloid cocaine (**12**) has proven to be slightly more elusive. A strain of *Pseudomonas maltophilia* (termed MB11L) was isolated from samples taken in and around a pharmaceutical company that processes cocaine. *P. maltophilia* MB11L was capable of utilizing cocaine (**12**) as its sole source of nitrogen and carbon for growth. The bacterium possessed an inducible cocaine esterase which converted cocaine (**12**) to ecgonine methyl ester (**33**) (Fig. 7), and benzoic acid. Both degradation products supported growth of *P. maltophilia* MB11L, although only cocaine induced high activities of the cocaine esterase (BRITT et al., 1992). Benzoic acid was further metabolized via catechol and the 3-oxoadipate pathway; however, the pathway for the metabolism of ecgonine methyl ester (**33**) was not further elucidated.

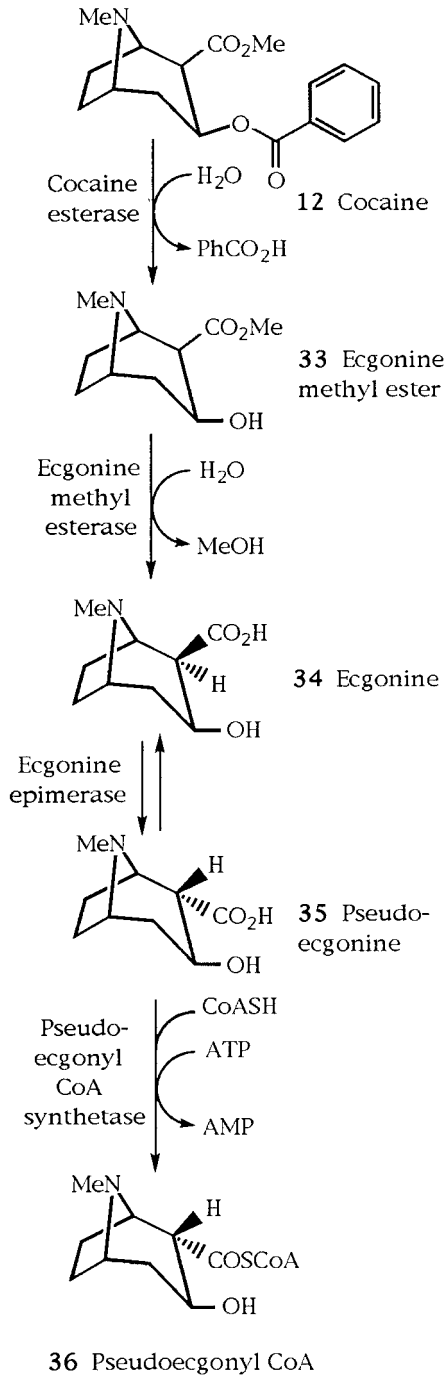


Fig. 7. Proposed pathway for the metabolism of cocaine (**12**) by *P. fluorescens* MBER and *C. acidovorans* MBLF (LISTER et al., 1995).