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GENERAL

TROPANE ALKALOIDS IN PHARMACEUTICAL AND PHYTOCHEMICAL ANALYSIS[1]

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Abstract: Alkaloids with tropane skeleton, present in many species of *Solanaceae* family, constitute important raw materials for variety of pharmaceutical preparations. Although basic facts concerning chemistry and pharmacology of these compounds date back to XIX century, the wealth of data accumulated recently has challenged many established opinions, particularly in the field of biogenesis. Advances in analytical techniques which made this progress possible are discussed, in reference to contemporary requirements for specification of active pharmaceutical ingredients.

Keywords: tropane alkaloids, medicinal applications, quantitative determination, biogenesis, chromatographic separations.

Tropane alkaloids, which emerged as valued drugs from ancient tribes tradition of witchcraft and folk medicine, constitute one of the oldest chapters of knowledge on secondary plant metabolites. Their presence is common in many members of *Solanaceae* family growing in warm climates, they can be also found in many species of *Atropa, Datura, Hyoscyamus and Scopolia,* native to Europe. When Linneus in 1753 named the plant known as „deadly nightshade": *Atropa belladonna,* after mythological Atropos, whose duty was to cut the thread of life, its poisonous character was already well recognized and documented in written sources such as *Grand Her bier,* issued in Paris in 1504. The first individual compound of the group – atropine, was isolated in 1832 (Mein, Geiger, Hesse). Chemical structure of the principal constituent of the class – tropane [**1**] – was identified by Landenburg as early as 1883. Following studies: partial (Landenburg, 1883) and total synthesis of atropine (Willstatter, 1898), crowned by biomimetic synthesis of tropinone [**2**] (Robinson, 1917) established a solid ground for pharmacological investigations (1). Remarkably, almost a hundred years ago Cushny (2) discovered that laevorotatory hyosyamine [**12**] is considerably more potent antagonist of acetylcholine than the racemic mixture (atropine), starting new but soon neglected field of stereopharmacology (3). Although tropane alkaloids are seemingly amply covered in pharmacology and pharmacognosy textbooks, these presentations often contain confusing nomenclature, particularly relating to stereochemistry, and seldom conform to the standards of clarity required in pharmaceutical registration documents or trade files (e.g. analytical specification of an active pharmaceutical ingredient, drug master file, technology transfer document). It should also be noted that a lot of new data concerning tropane alkaloids biogenesis and taxonomy has been collected recently, by application of newly established sophisticated analytical techniques. Naturally, these new developments are likely to influence current status of analytical requirements formulated within pharmaceutical quality assurance system, and consequently they should be surveyed periodically.

MEDICINAL APPLICATIONS

The solanaceous alkaloids are principally used as anticholinergic drugs, but both: central and peripheral nervous system effects, which are medicinally relevant, are observed. Around 1870 in their classic experiments Schmiedeberg and independently Adamuck, demonstrated that the site of action of acetylcholine antagonism was at or beyond the postganglionic nerve endings (1). Since acetylcholine (ACh) plays pivotal role in neurotransmission

[1] **Dedicated to Professor Osman Achmatowicz on the occasion of his 70th birthday**

* The corresponding author

and different tissue and organs are supplied by cholinergic nerves, it remained in focus of traditional pharmacology, which divides autonomic nervous system into parasympathetic (ACh mediated) and sympathetic (acetylcholine as preganglionic and norepinefrine as postganglionic transmitter) neuron clusters. It is obvious that ganglionic acetylcholine mediated transmission is a very short–time event, since ACh is hydrolysed by ubiquitous enzyme acetylcholinesterase (a serine hydrolase, but also by some less specific enzymes) in the cleft of the synapse at diffusion controlled rates, thus regenerating choline and terminating the chemical signal impulse (4). However, there are also acetylcholine receptors in the region of postsynaptic effector cell membrane, classified as muscarinic (mAChR, metabotropic) and nicotinic (nAChR, ionotropic) named for two natural agonists which can mimick ACh action. Cholinergic receptors belong to a superfamily of guanine–nucleotide–binding regulatory protein–coupled receptors (GCPR) of opsine type, characterized by seven hydrophobic regions spanning through the cell membrane, with extracellular N–terminal and intracellular C–ending (5). Muscarinic receptors (mAChR), for which atropine and scopolamine are prototype antagonists, constitute a subset of at least five distinct protein types (M_1 to M_5), for which specific genes have been identified and cloned. Their products are known to be expressed in different locations of brain as well as in peripheral tissues such as heart, smooth muscle, exocrine glands, vascular endothelium and peripheral ganglia. Selective subtype mAChR agonists are lacking among natural products and are sought after by synthesis, in view of their potential therapeutic applications. More selectivity is found in the group of mAChR antagonists, which offers a better chance to elucidate molecular mechanism of their action. However, classical muscarinic antagonists, such as atropine, do not distinguish between receptor subtypes. Generally, the signal transduction pathway involves secondary messengers. Stimulation of M_1, M_3 and M_5 results in activation of phospholipase C and consequently increases amount of inositol triphosphate and intracellular calcium ions. In case of stimulation M_2 and M_4 subtype receptors, inhibition of adenyl cyclase activity takes place (6). Thus, through action on muscarinic acetylcholine receptors, tropine alkaloids can be implicated in the following physiological roles: cognition, control of motor activity, cardiovascular and respiratory activity, sensory functions (e.g. pain perception), arousal, sleep cycle and stress response (CNS effects) as well as reduction of heart rate and force of cardiac contraction, stimulation of glandular secretion, smooth muscle contraction and vasodilation (peripheral effects). In fact, following effects of atropine on man, in relation to dosage, can be observed:

0.5 mg – slight cardiac slowing, some dryness of mouth, inhibition of sweating;

1.0 mg – dryness of mouth, thirst, acceleration of heart, pupillary dilatation;

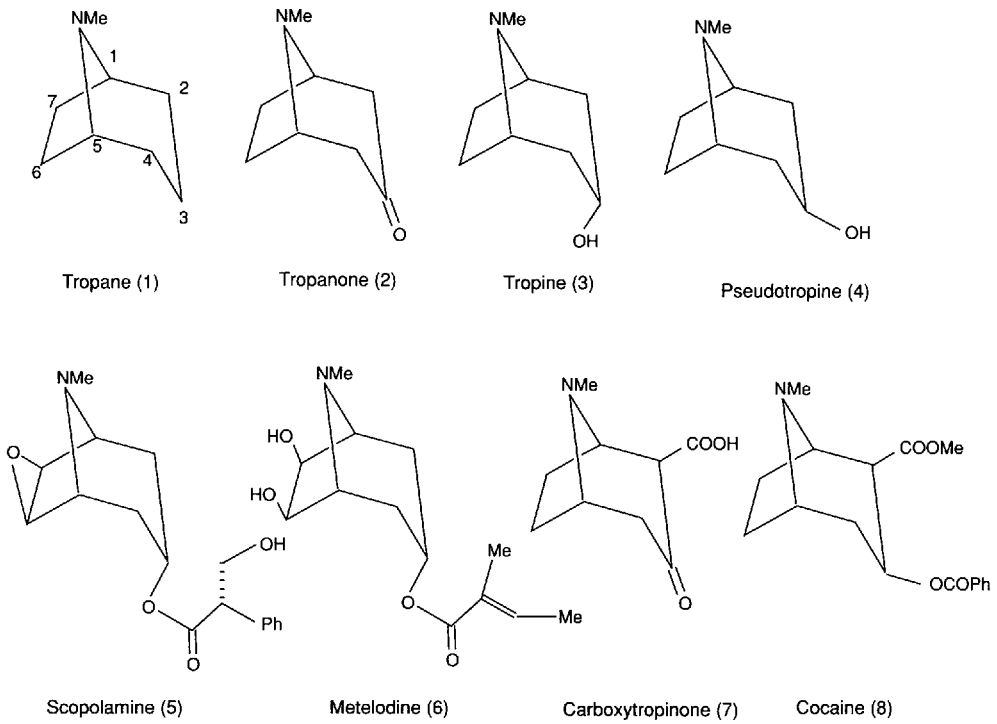
2.0 mg – rapid heart rate, palpitation, dilated pupils, some blurring of near vision;

5.0 mg – above symptoms, disturbed speech, restlessness, headache, dry hot skin;

>10.0 mg – above symptoms, rapid and weak pulse, blurred vision, skin flushed and scarlet, ataxia, hallucinations and delirium, coma.

Present medicinal application of tropane alkaloids include relaxation of smooth muscles of alimentary and biliary tracts, reduction of secretion in the respiratory tract (e.g. in preparation for surgical anesteshia) and local application to the eye to cause mydriasis for ophtalmoscopic examination. As can be seen from the above list of effects, potential CNS disturbances, xerostomia, mydriasis and tachycardia are among side effects, which would limit their wider therapeutic utility.

Atropine is a specific antidote for the central respiratory depression in poisoning with anticholinesterase agents such as organophosphorus insecticides and the nerve gases. Scopolamine hydrobromide in oral doses of 0.5 to 1.0 mg is used as a remedy for motion sickness. Its sedative effects are sometimes employed in obstetrics and more often to subdue manic patients and to control delirium tremens. A number of synthetic derivatives of tropane alkaloids have been introduced into pharmaceutical practice in which either tropic acid residue was replaced (homatropine, apoatropine or Tropisetron® (Scheme 2)) or quaternary salt function was introduced (homatropine methylbromide, methylatropine, methylscopolamine and butylscopolamine, known under trade name Buscopan®), which exhibit similar range of activity as natural products but improved selectivity. In our geographic region, the following plants constitute main source of tropane alkaloids: belladonna (*Atropa belladonna*, leafs and roots), henbane (*Hyoscyamus niger*, leafs), jimsonweed (*Datura stramonium* or *Datura innoxia*, leafs and seeds) and scopolia (*Scopolia carniolica*, roots) and corresponding materials are well represented in nonprescription (OTC) herbal remedies. In addition, many preparations are registered, which are based either on a single active tropane component or contain one of the following substances, atropine sulfate, me-



Scheme 1. Tropane (1) and its derivatives.

thylatropine nitrate, scopolamine hydrobromide, scopolamine borate, scopolamine butylbromide or scopolamine methylbromide in a composite medicine. Formulations range from topical (eye drops or eye oitments) through oral (tablets) to intravenous or intramuscular injections.

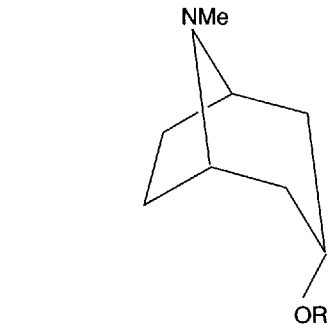
STRUCTURE AND OCCURRENCE

Tropane alkaloids are characterized by the presence of bicyclic ring system: (N–methyl–8–azabicyclo[3.2.1]octane) **[1]** bearing at least one hydroxyl group (in position 3). Such prototypal structure can exist in two configurational (C–3 epimeric) variations, for which common names: tropine **[3]** and pseudotropine **[4]** are used. (Scheme 1) Another bicyclic aminoalcohols (alkamines) encountered in *Solanaceae* plants include: di– and tri– hydroxylated tropines, [e.g. **6** and **28**], 6,7–epoxide [scopine in **5**] and corresponding de–N–methylated compounds (N–nortropane derivatives). Biogenetically related coca alkaloids occuring in several species of *Erythroxylaceae,* which posses additional carboxylic function in position C–2, are considered as drugs of abuse [e.g cocaine **8**] and are outside of the scope of this survey. Tropane alkaloids typically occur as esters of **3** with carboxylic acids (Scheme 2) and one of them: tropic acid **[45]** seems to be chemotaxonomically distinct marker for alkaloid containing plants which belong to species of *Solanaceae* family (7).

It should be remembered that common names of tropane alkaloids were often coined before their structure and configuration was determined and they should be used with proper stereochemical descriptors to avoid confusion. Hyoscyamine **[12]** and atropine **[12+13]**, which differ basically by a degree of enantiomeric purity represent such case. Another example is hyoscine **[5]**, isolated as laevorotatory enantiomer from *Hyoscyamus muticus,* while corresponding racemate obtained from extract of *Scopolia atropoides* was named scopolamine. Presently, the second name prevails and is also used to denote enantiopure compound.

Biogenesis and its stereochemical consequences

Tropane alkaloids were among the first groups of secondary plant metabolites, for which proposed biogenetic pathways were experimentally verified by administration and incorporation of radioactive labelled precursors. Experiments on incorporation of [2–^{14}C] ornithine into hyoscyamine in 1954, which indicated an asymmetric intermediate and its stereospecific transformation, started a long sequ-



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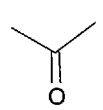
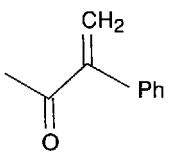
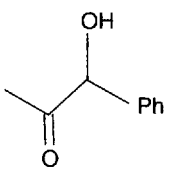


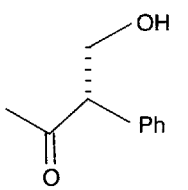
Acetyltropine (9)



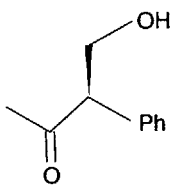
Apoatropine (10)



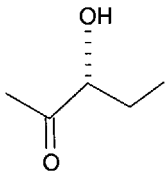
Homatropine (11)



S - (-) - Hyoscyamine (12)



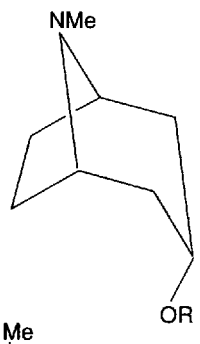
R - (+) - Hyoscyamine (13)

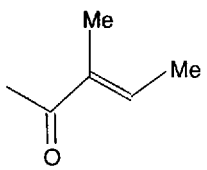


Litorine (14)

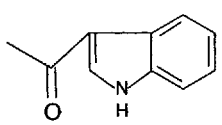
Scheme 2. Tropine esters (part I).

ence of studies from which relatively clear, although not quite complete picture of biogenetic events have emerged (8). Symmetric putrescine (1,4–diaminobutane) has been excluded as a possible intermediate since labelled L–ornithine [**18**] is incorporated into tropane without label scrambling between C–1 and C–5. It is generally accepted that N–methyl–L–ornithine [**19**] undergoes subsequent decarboxylation and oxidative deamination affording N–methylaminobutanal, in equilibrium with N–methylpyrrolinium salt [**20**], the common key intermediate for nicotine, hygrine, tropane and coca alkaloids. The next step of C–alkylation, necessary for bicyclic construction, is a subject of lasting debate. Initially postulated hygrine carboxylic acid was never isolated but intermediacy of a β–ketoester compound (likely equivalent of two acetyl coenzyme A units) seems quite certain. C–Alkylated intermediate is represented in the

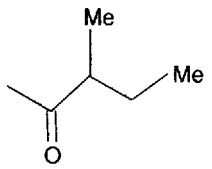




Tigloidine (15)



Tropisetron (16)

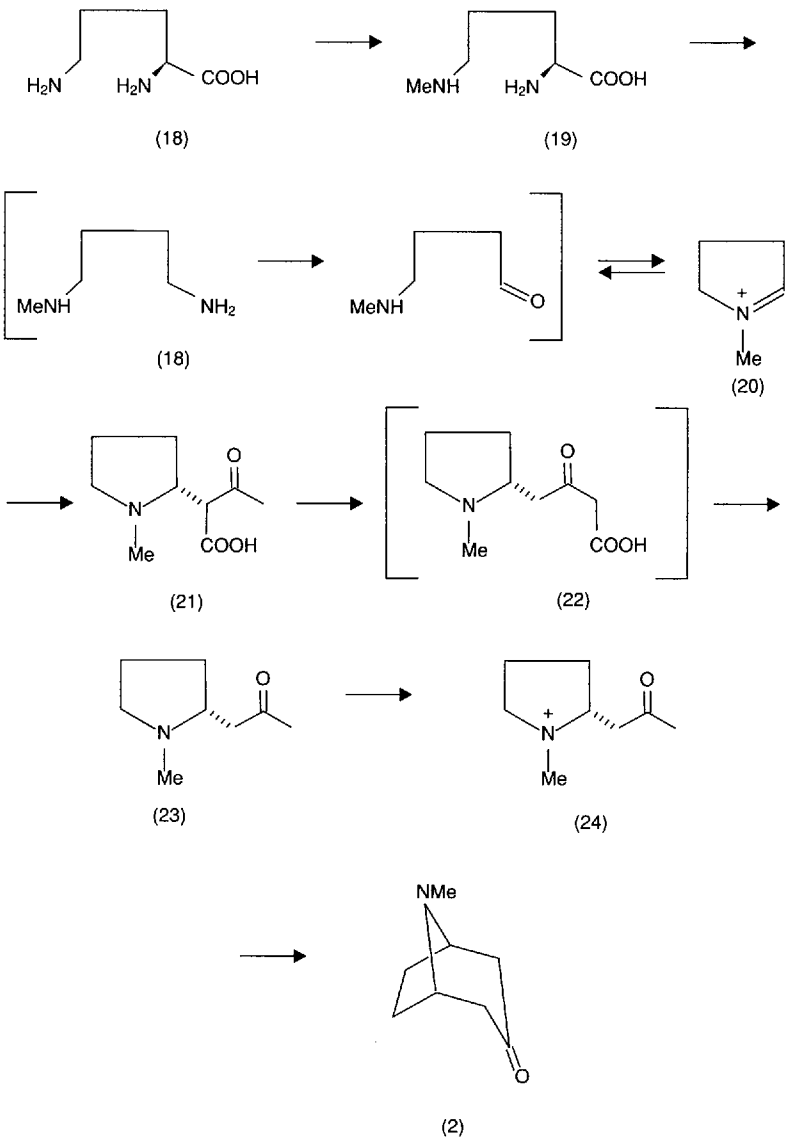


Valtropine (17)

Scheme 2. Tropine esters (part II).

literature by different formulae [**21** and **22**], both leading eventually to tropinone [**2**]. Either of the two possibilities is likely to proceed in an enantioselective way and 2–(*R*)–hygrine [**23**] seems to be the favoured intermediate (9). There are considerable difficulties, however, in sorting out experimental facts concerning this point of biosynthesis. Incorporation of labelled hygrines is considerably more effective for *2–(R)* enantiomer, although susceptibility for racemization even under neutral conditions somewhat blurrs the picture, but labelled hygrine shows a lower incorporation into the tropane alkaloids in *Datura* than labelled ornithine, which is inconsistent with their order in the biosynthetic pathway. Obviously, biosynthesis of tropinone may also involve carboxytropinone intermediate [**7**], *via* decarboxylation, but there is no conclusive evidence at present, to support such notion. In any case, carbomethoxytropinone [**7**] esters seem to be a likely divison between cocaine and hyoscyamine pathways (9), which also differ in configuration of the carbinol center (reduction at the stage of ketoester leads to pseudotropine [**4**] series, while tropinone reduction affords exlusively esters of tropine [**3**]).

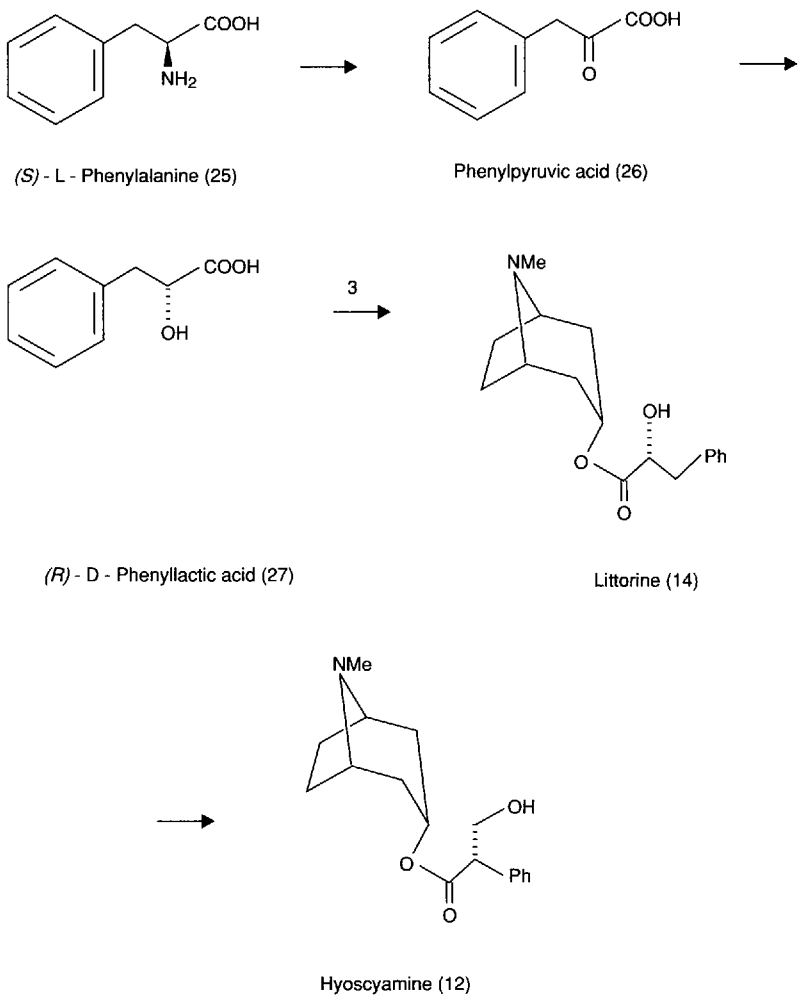
Both isomeric tropines are *meso* compounds and therefore are unresolvable into enantiomers. Stereodifferentiation of tropane alkaloids stems



Scheme 3. Biosynthesis of tropinone (2).

from possibility of biogenetic esterification with a chiral acid by specific acyl transferases. Tropic acid [(*S*)–(–)–**45**] is an unique molecule, present in hyoscyamine and scopolamine and originating from phenylalanine [**25**] by way of an unusual skeletal rearrangement, which evoked a great deal of speculations. This part of biosynthesis, extensively studied over the last few decades, was recently reinvestigated by using doubly labelled (^{3}H, ^{14}C and ^{2}H, ^{13}C) precursors. After excluding intermediacy of cinnamic acid, its epoxide, 3–hydroxy–3–phenyl propanoic and 3–amino–2–phenyl propanoic acids, it has been demonstrated that phenylalanine [**25**] as well as phenylpyruvic [**26**] and 3–phenyllactic acid [**27**] are incorporated into hyoscyamine [**12**] in *Datura* plants and root cultures. There was no doubt that skeletal transformation takes place, in which C–1 and C–3 of the precursor become C–1' and C–2' of the alkaloid tropate moiety, but stereochemical details of the process remained obscure because of some experimental inconsistencies in tracing hydrogen atoms involved in the rearrangement. Finally, O'Hagan and his collaborators have demonstrated that (*R*)–phenyllactate is a closer precursor of (*S*)–tropic acid than (*S*)–phenylalanine. Thus, alkaloid littorine [**14**] was found to be immediate predecesor of hyoscyamine in stereospecific migration of carboxyl group in which 3–*pro–S*– hydrogen remains in place, while *3–pro–R*– is removed (10;11). Overall result is a double inversion but postulated earlier 1,2–vicinal interchange process concept, in which carboxyl group and C–3 hydrogen atom trade places, remained unsupported.

Transformation of hyoscyamine to hyoscine (scopolamine) is apparently catalysed by only one oxidizing enzyme: 6β–hydroxylase (H6H), although it involves three distinct chemical steps

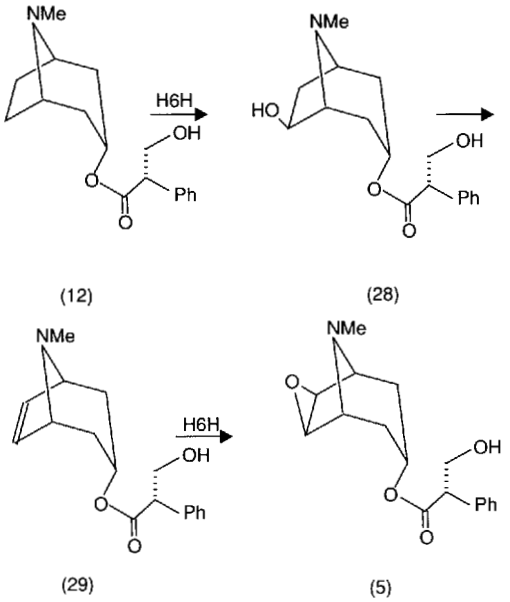


Scheme 4. Biosynthesis of hyoscyamine (12).

– hydroxylation, *cis*–elimination (to account for this rather unusual transformation, additional chemical step, comprising substitution of 6–O–phosphate with a coenzyme A thioloacid type residue, with inversion of configuration, have been postulated) and epoxidation, as shown on Scheme 5 (9).

Tropic acid esters are not particularly stable compounds. Apart from susceptibility towards hydrolysis both, chemical and enzymatic, they are prone to racemization and dehydration reactions. Thus, tropine (*S*)–tropate [hyoscyamine, **12**] is easily converted into racemic mixture (atropine), even upon drying of collected plant material. Apoatropine [**10**] is formed from **12** under variety of conditions, chemical or thermal, promoting dehydration. Fortunately, more valuable phytochemical product – hyoscine [scopolamine, **5**] retains stereochemical integrity of (*S*)–tropic acid moiety much better.

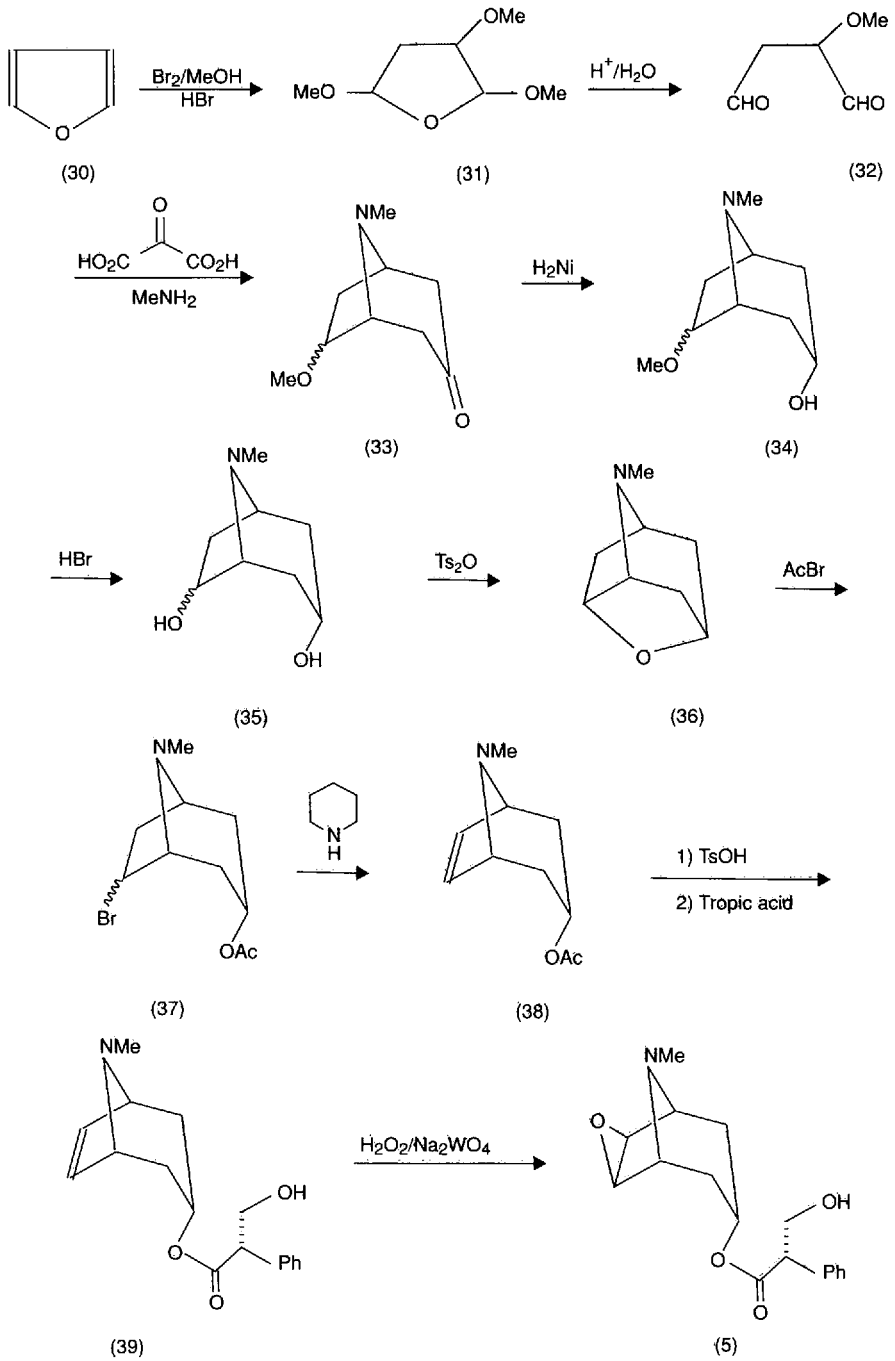
Some aspects of molecular pharmacology of tropane alkaloids may look quite puzzling at first sight. For example, their biological targets – cholinergic muscarinic receptors seem quite sensitive to the ligand stereochemistry (for agonists and antagonists alike), yet natural neurotransmitter, acetylcholine, is an achiral molecule. Muscarinic agonist activity requires positively charged nitrogen atom and it is practically independent of lipophilicity, while antagonists of tropyl tropate type may bear =NH, =NMe or N(Me)(alkyl) group and lipophilic character is important for enhancing their activity. Although considerable wealth of structure–activity relationship data have been accumulated in extensive studies of many choline, tropane and muscarine analogs (12), this approach is still inconclusive, for lack of a complementary biopolymer active site picture. Structural information on muscarinic receptors trans–membrane proteins has emerged only recently, details of its ligand–bound topology are still rather sketchy (5) and even question whether agonists and antagonists have the same binding site is not clarified. As far as stereoselectivity of the ligand action is concerned, it can be taken for granted that any binding site of a functional biopolymer constitute a chiral environment, which should exibit some stereodifferentiation, according to Easson–Stedman rule (13). Formally, achiral ligands are also frequently induced by such an environment to adopt a „preferred conformation" at the binding site, which is not necessarily symmetrical or thermodynamically favoured in solution or solid state.



Scheme 5. Synthesis of scopolamine (5).

Resources of pharmaceutical raw materials

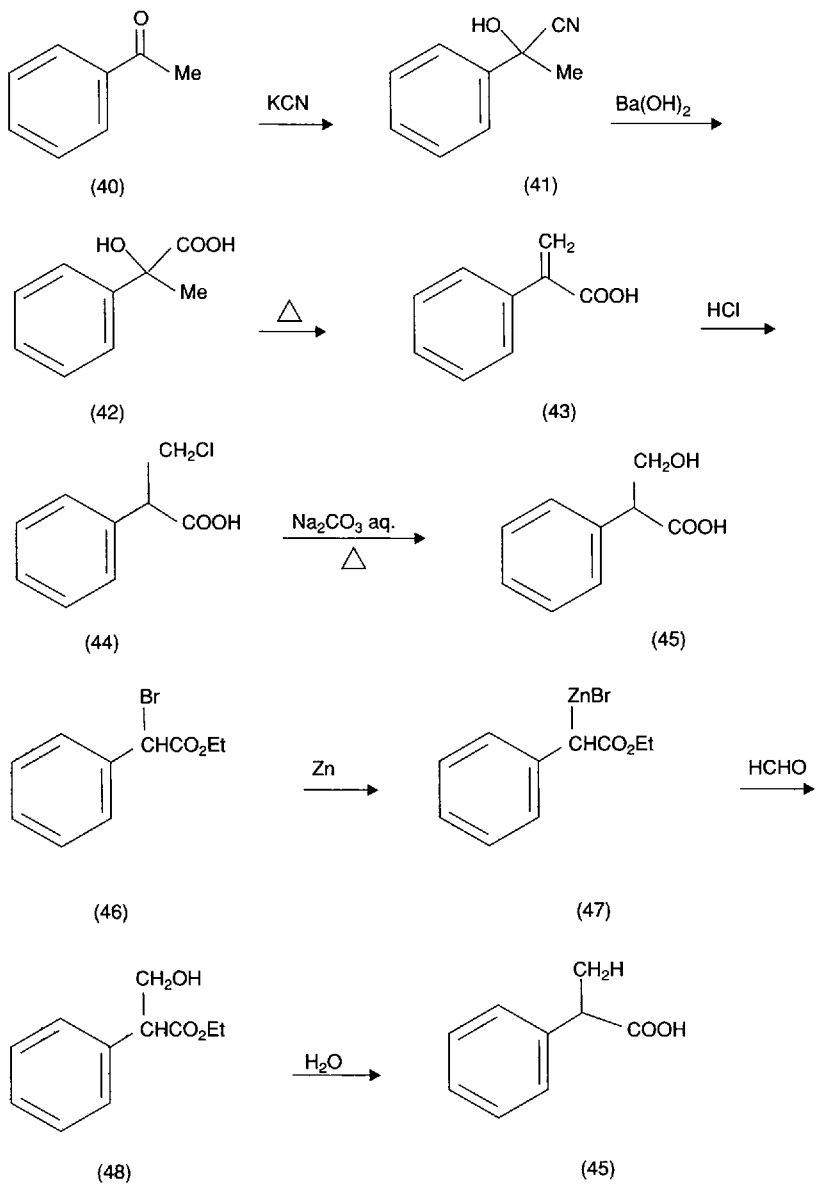
Tropane alkaloids are being isolated from popular plants: *Atropa belladonna, Hyoscyamus niger* and *Datura stramonium* for well over a century and wild plant collection from natural habitats is traditonally employed for this purpose. However, such practice is incompatible with principal requirements of efficiency and quality, which is a driving force of contemporary pharmaceutical industry, and as a result, is quickly becoming obsolete. Among better controlled contract cultivation enterprises, which take over in every climatic zone, *Datura inoxia* seems to hold much promise in our region. It should be pointed out that nowadays, many natural products of considerable structural complexity are manufactured by means of chemical synthesis, total or partial (14). Tropinone (2) and corresponding tropines can be obtained relatively easily and efficiently (ca. 90 % yield) from simple materials: acetal equivalent of succinic anhydride obtainable from furan, methylamine and acetonedicarboxylic acid (15). Although tropine and racemic tropic acid must be prepared in relatively large scale, since they are both commercialized as chemical reagents, to our knowledge technical synthesis of tropane alkaloids has never been launched. The reasons may be following: asymmetric (*S*)–atropic acid [**45**] synthesis, needed as a raw material, may be too expensive to compete with natural hyoscyamine [**12**], which tends to be substituted for many medicinal purposes by cheaper still racemate (atropine). Moreover, current demand for hyoscyamine/atropine is about ten times lower than for



Scheme 6. Chemical synthesis of scopolamine (5).

hyoscine/scopolamine [5], so there is not much economical incentive to develop a suitable industrial process. Several chemical syntheses of scopolamine have also been elaborated but they do not seem to have much potential for commercialization. The problem is exemplified on Scheme 6 presenting one of the more efficient variants of the preparation.

The synthesis again starts with furan, leading through trimethoxytetrahydrofuran [31] to 6–methoxytropinone [33]. Further transformations, involving anhydro– and dehydro– tropine derivatives



Scheme 7. Synthesis of racemic tropic acid.

[**36** and **38**] are rather straightforward. The point is, that in order to achieve proper stereoselection at the epoxidation step [transforming **39** into **5**], 6,7–dehydrotropine has to be esterified with (*S*)–tropic acid, which is unavailable apart from natural sources or racemate resolution. Several syntheses of racemic tropic acid [**45**] have been described (16; 17). Two of them, combining simplicity and efficiency, are depicted on the Scheme 7.

On the global scale, natural resources for industrial manufacturing of tropane alkaloids tend to concentrate in tropical climate countries, where continuous selection for better productivity of scopolamine is taking place. For example, *Brugmansia*

sanguinea plantations in Equador supply over 400 tonns of dry leaves annualy, with average content 0.8% of scopolamine [**5**]. Commercial *Duboisia* hybrid leafs can contain as much as 1.54% of scopolamine and only 0.1% of hyoscyamine [**12**]. Australian *Brugmansia candida* contains ca. 0.34% of scopolamine, yielding about 15 kg of the alkaloid from hectare per annum. New *Brugmansia* hybrids were estimated to contain 1.5 – 2.5% scopolamine and to give between 14 and 20 kg of the alkaloid per hectare/year (18). Presently, two different experimental approaches supplement hybridization efforts in strive for higher plant efficiency. It is well known that some chemical substances (elicitors) can significantly enhance intensity of secondary metabolism. Elicitation of tropane alkaloids biosynthesis in transformed roots of *Atropa belladonna* and *Datura stramonium* using as different materials as: metal ions, gluthatione, chitin, fungal cell–wall elicitors, oligogalacturonide, yeast extract and jasmonic acid esters, was attempted and some increase in accumulation of the target metabolites was observed (19;20). More straightforward approach relies on genetic transformation of whole plants or selected tissue cultures. An obvious idea is to obtain *Solanaceae* plants in which the gene responsible for scopolamine synthesis is overexpressed. Japanese researchers have succeded in introducing hydroxylase H6H (EC 1.14.11.11) gene with aid of *Agrobacterium rhizogenes* vector into *Atropa belladonna* and observed enhanced transformation of hyoscyamine to scopolamine in the transgenic hairy root culture (21). It seems likely that pharmaceutical industry will soon benefit from this line of research.

ADVANCES IN QUANTITATIVE ANALYSIS

As can be seen from preceeding paragraphs, efficiency and accuracy of analytical methods often determines progress of research in many areas crucial for advancement of natural products chemistry towards medicinal applications and pharmaceutical development. Although alkaloids are among the compounds for which necessity of quantification was always evident, because of their acute toxicity, precise analytical methods for their determination are still in development. Traditional methods, which rely on chemically induced color development in a dissolved sample followed by spectroscopic (colorimetric) evaluation of its intensity, are nonspecific and inaccurate and although they are still listed in analytical monographs (16; 17) as well as many formularies and pharmacopeias, they will not be discussed here. Instead, recent applications of various chromatographic techniques will be surveyed, in an attempt to exemplify their usefulness for such important tasks as pharmaceutical quality control. General problems involved in chromatographic separations of inherently basic analytes on weakly acidic sorbents, including most popular silica gel, are well known and have been largely reduced by introduction of new generations of solid supports and stationary phases (22). In particular case of tropane alkaloids, we are facing need for a method which can simultaneously and accurately determine following materials: tropine and nortropine alcohols and polyalcohols; tropine N–oxides and quaternary ammonium salts; scopine derivatives and esters of all above listed categories with aromatic as well as aliphatic carboxylic acids, including chiral ones. Even making allowance for the fact that certain sub–categories should be missing in specific samples (e.g. there is more need for simultaneous determination of quaternary salts in pharmaceutical than phytochemical materials) difficulty of the task is overwhelming. The main problem is not so much in wide range of polarity of individual analytes as in the lack of simple and universal detection system. Only a few principal tropane alkaloids contain aromatic moiety, which renders them detectable by UV light absorption – still the most popular way to record an analysed or separated sample signal. Therefore, more sophisticated detecting systems, like electrochemical (coulometric) or mass spectrometry (MS) based, have been frequently employed for their quantitative determination.

Several gas liquid chromatography (GLC) systems using flame ionization detection have been described for identification and quantification of tropane alkaloids. Typically, analyses were performed on a glass columns packed with SE–30 supported on Chromosorb, operated with temperature gradient with helium carrier gas, while later versions make use of capillary columns and mass spectrometry detection (23,24). Planar chromatography (TLC) enjoys great popularity as an operationally facile, low cost analysis, suitable for phytochemical studies and identification of pharmaceutical components and preparations (25). Some obvious advantages of this approach include availability of wide range of basic and modified stationary phases, easy solvent selection and simple options for derivatization and multiple detection systems. Most of the methods, described for TLC analysis of tropane alkaloids, seem suitable for quantification by use of densitometry, but relatively few examples of such approach are found in recent literature (26;27). The most popular method for quantitative deter-

mination of organic compounds, liquid chromatography (HPLC), have been repeatedly applied to tropane alkaloids, but not without some technical problems. While earlier papers concentrated on selection of proper conditions for reverse phases (RP) HPLC to avoid peak tailing (28;29), more recent studies tend to focus on sensitivity issue. Noteworthy, satisfactory separation on underivatized silicagel eluted with a buffer – methanol mixtures were also reported (30). As already mentioned, discussed compounds have intrinsically low UV absorption, which calls for expensive detection systems (e. g. electrochemical (31) or MS (32)) or chemical derivatization (33). Alternatively, dual detection system (e.g. UV/RI) can be employed to cover all constituents of examined mixture (34). Although enantioselectivity is an important concern in determination of tropane alkaloids, little attention has been paid to their chiral HPLC thus far. Japanese designers of cellulose based chiral stationary phases (35) have demonstrated facile resolution of atropine and homatropine on cellulose tris(3,5–dimethylphenylcarbamate) column in hexane–2–propanol–diethylamine (80:20:0.1), but similar achievements would be needed in chemically stable RP mode in order to make such analysis more useful. Nevertheless, enantioselective HPLC has already provided valuable insight into tropane alkaloids producing plant physiology. Thus, it has been unequivocally established that *R*–(+)–enantiomer of hyoscyamine is present in leaves of *Datura metel L.* and its content increases during the plant growth (36), while generally accepted opinion holds that (*S*)–hyoscyamine racemization is an abiotic process connected with drying and processing of the plant material.

Since alkaloid molecules are, as a rule, easily protonated, ion pairing reagents which have been extensively used in chromatographic techniques have also enhanced separation studies exploiting electrokinetic phenomena (37). Capillary zone electrophoresis (CE; CZE) method, suitable for simultaneous determination of atropine, homatropine and scopolamine in ophtalmic preparations, have been designed and validated (38). Since this technique is easily adaptable to using a variety of chiral selectors admixtures to the electrolyte solution, it seems to hold better potential for combining requirements of being chemoselective as well as enantioselective, than already discussed HPLC methods. Indeed, successful application of enantiomeric separation of tropane alkaloids in pharmaceutical and plant analysis by CE has been described with using neutral (39) as well as acidic (40) derivatives of cyclodextrines. Recently, micellar electrokinetic capillary chromatography (MEKC) has been applied for analysis of tropane alkaloid mixtures, present in transformed and genetically modified plant material from various *Solanaceae* species (41). The column was interfaced with electrospray ionization mass spectrometer with additional use of collision induced dissociation to distinguish fragmentation patterns of nonseparable compounds.

It should be borne in mind that requirements of clinical analysis may differ considerably from these discussed above in this respect, that accurate determination of one particular compund may be consider sufficient, disregarding other components. Determination of scopolamine in urine by a simple and quick radioligand binding assay, which does not require prior extraction of the sample (42), may serve to illustrate this point.

Finally, immunological methods of tropane alkaloids determination should be mentioned, which have been applied to analysis of crude plant samples (43).

Considering, already mentioned, lack of uniform structural and spectral characteristics of tropane derivatives, only most recently developed analytical techniques, combining chiral CE selectivity of separation with MS sensitivity and accuracy of detection, seem to offer capability for simultaneous determination of all components present in complex mixtures of tropane alkaloids occurring in natural sources or pharmaceutical preparations.

CONCLUSIONS

Tropane alkaloids remain valuable as pharmaceutical raw materials and active substances, with no synthetic processes in sight, which could effectively compete with natural sources. Almost every method practiced in analytical chemistry of organic compounds have been applied to tropane alkaloids, but simple, universal system for their quick and affordable individual detection and quantification is still lacking. Considering steady interest in biogenesis and phytochemistry and growing demand for detailed specifications of biological and medicinal materials containing these alkaloids, it is obvious that every step marking progress in selectivity and sensitivity of their quantitative determination will be welcome by academic and industrial natural product specialists. At the present level of technological sophistication in preparation of solid phases and elaboration of detection systems, it is likely that the next step in separation enhancement, by chromatography as well as electrophoresis, will have to rely on a computer assisted multiparameter optimization of conditions for any given analytical task (44).

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