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Review

Tropane alkaloids as medicinally useful natural products and their synthetic derivatives as new drugs*

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Abstract:
Secondary metabolites of *Solanaceae* plants, sharing tropane skeleton as a common structural feature, are sharply divided into two classes: tropine and ecgonine derivatives. The first group, represented by well known alkaloids: atropine and scopolamine, which are considered to be model anticholinergic drugs, continues to provide inspiration in the search for more selective muscarinic receptor antagonists. The second class accommodates one of the principal drugs of abuse, cocaine. Synthesis of much needed cocaine antagonists, despite extensive research, has not been particularly successful. Therefore, new concepts of cocaine abuse treatment resort to immunotherapy and biotechnology. Contemporary pharmaceutical industry manufactures over 20 active pharmaceutical substances containing tropane moiety in their structure, which are applied as mydriatics, antiemetics, antispasmodics, anesthetics and bronchodilators. There are two sources of raw materials for this industrial activity: natural products isolated from cultivated transgenic plants (mainly scopolamine and atropine from Australian *Duboisia*) and chemical synthesis based on common intermediate: tropinone, which can be further transformed by synthetic means to the following classes of compounds: tropine and its esters (tropeines), scopine and nortropine derivatives, and tropane quaternary ammonium salts. This survey focuses on new developments in chemistry and pharmacology of tropane derivatives, particularly in view of their prospective industrial applications as therapeutics.

Key words:
tropane alkaloids, tropinone, tropines, tropeines, antiemetic drugs, antispasmodics, mydriatics, cholinergic muscarinic antagonists, tropane quaternary ammonium salts, tropane chemical syntheses, stereochemistry of tropane derivatives

Abbreviations: AcCoA – acetyl coenzyme A, ACh – acetylcholine, AChR – acetylcholine receptor, API – active pharmaceutical ingredient, ATC – anatomotherapeutic classification WHO, ATP – adenosine triphosphate, BChE – butyrylcholinesterase, CNS – central nervous system, CocE – cocaine esterase, DA – dopamine, DAT – dopamine transporter, H6H – 6-hydroxytropine hydroxylase, *im* – intramuscular, *iv* – intravenous, LC-MS/MS – liquid chromatography with tandem mass spectrometry detection, MR – muscarinic receptor, NET – norepinephrine transporter, NMR – nuclear magnetic resonance, PNS – peripheral nervous system, SAR – structure-activity relationship, *sc* – subcutaneous, SERT – serotonin transporter, TR – tropine reductase

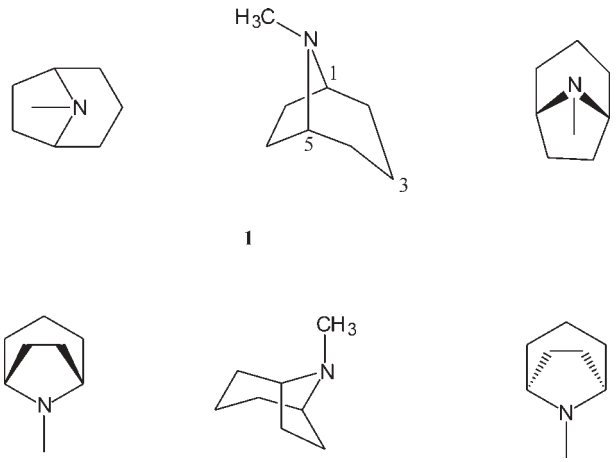
* Dedicated to the memory of Professor Osman Achmatowicz (1899–1988), an eminent organic chemist and the author of seminal papers on alkaloid chemistry.

Introduction

Tropane alkaloids are among the oldest medicines known to man. Poisonous *Solanaceae* family plants, presently classified as genera: *Atropa*, *Brugmansia*, *Datura*, *Duboisia*, *Hyoscyamus* and *Scopolia*, with many alkaloid-containing species [26, 33], were well known already in ancient times, and records of their employment in folk medicine of various ethnic groups are abundant [31, 45, 51, 81]. In 1819 Meissner (who actually coined the term: alkaloid) was the first to realize that the active principles of these poisonous plants are alkaline in character and thus can be isolated by extractive techniques, and consequently individual alkaloid compounds started to be isolated from 1830 onward: atropine from *Atropa belladona* L, and hyoscyamine from *Hyoscyamus niger* L (K. Mein, P.L. Geiger, K. Hesse, 1831–1833), followed by scopolamine [19, 31, 81]. The use of coca plant, which belongs to *Erythroxylaceae* family, as a stimulant can similarly be traced to prehistoric times. Cocaine, the principal alkaloid of *Erythroxylon coca* was first isolated in 1860. Historical aspects of ethnopharmacological tradition and the beginning of medicinal chemistry, in which tropane alkaloid investigations played a prominent role, are well covered in various sources [19, 29, 45, 81]. Contemporary medicine utilizes tons of atropine and scopolamine extracted from genetically modified cultivars, while ever growing demand enhances new, chemical and biotechnological methods of their manufacturing. In parallel, cocaine obtained from two *Erythroxylon* species, which is of limited use in medicine because of very strong addictive properties [74, 90], became a subject of illicit manufacturing and trafficking of "recreational drugs", with socioeconomic and health endangering consequences on global scale. Probably because of this dual assignment and somewhat cloudy economic impact of plant tropane secondary metabolite turnover on local economies, there are a lot of contradictions and misconceptions in generally available publications, concerning these substances, particularly on their origin, manufacturing processes, amounts in circulation, but also on their stereochemistry, properties, purity and even chemical reactivity. The purpose of this review, which extends the scope of our earlier survey [22], is to juxtapose two principal resources and methods of manufacturing utilized for tropane medicinal compounds: harnessing natural products and executing selective chemical synthesis, in the context of advancing pharmacological knowledge and requirements of modern medicine and contemporary pharmaceutical industry.

Tropanes of plant origin and their traditional medicinal applications

Ethnopharmacological tradition lists many plants of moderate climate, whose extracts used throughout ages as poisons and magic potions of various assignments, (e.g. pain killers, hallucinogens, etc.) are today known to contain significant amounts of tropane alkaloids (e.g. up to 2% in ripe seeds of *Datura stramonium*). These include: deadly nightshade (*Atropa belladonna*), mandrake (*Atropa mandragora*), henbane (*Hyoscyamus niger*, *Hyoscyamus albus*), jimsonweed, also called thornapple (*Datura stramonium*) and scopola (*Scopolia carniolica*), among others [26]. Usually all parts of a plant contain alkaloids, which can lead through incidental intake, to poisoning of people or livestock. Acute toxicity data for humans are deducted from forensic material and are said to be a subject to great individual variation. With atropine for example, death can occur after taking 100 mg, but cases of survival after 500 mg dose are also recorded. As the category of medicinal plants that evolved in modern times, these species became a subject of collection (or subsequently, cultivation) and regular trade as a raw material for manufacturing of pharmaceutical preparations. Attempts to isolate the pure active principles, followed by investigation of their biological activity were continued throughout the 19th century [10, 19, 45, 81], while structure elucidation and subsequent syntheses crowning chemical part of the study extended well into the 20th century [18, 21, 34]. The name tropane is given to the bicyclic saturated structure (N-methyl-8-azabicyclo[3.2.1]octane **1**; Scheme 1), characteristic of a class of ca. 200 alkaloids, which are conveniently subdivided according to the number of carbons in the tropane skeleton and stereochemical features. Although tropine and ecgonine derivatives share common biogenetic reaction sequence leading to tropane skeleton, branching only at the tropinone reduction step, their division in scientific literature have another important reason: the ecgonine class is strongly identified only with its principal member –



Scheme 1. Conventional representations of tropane structure (equivalent)

cocaine, a neurostimulant notorious for causing devastating addiction.

Biosynthetic pathway on which the tropane **1** derivatives are formed has been thoroughly elucidated with the help of radioactive labeling experiments [36, 65, 66, 72]. In short, the main precursor of the bicyclic alkamine part is L-ornithine **2**, converted to a diamine, putrescine **3,** by a specific decarboxylase (OrnDC). Putrescine (which can be also obtained biogenetically from arginine) is mono-N-methylated by transferase PMT and subsequently transformed into 4-N-methylaminobutanal by diamineoxidase DAO. Next, spontaneous cyclization-dehydration takes place, with formation of the common intermediate precursor, N-methyl-Δ^1-pyrrolinium cation **4**, from which nicotine, cocaine and tropane alkaloids can be formed. This monocyclic precursor is further transformed into a corresponding 4-carbon side chain β-ketoacid intermediate **5** by the action of two acetyl coenzyme A, (AcCoA) ester molecules. The oxobutanoic acid **5** can cyclize to *exo*-carboxytropinone **6** from which derivatives of tropine **8**, pseudotropine **9** and/or ecgonine **15** are subsequently formed [10, 65] (Scheme 2).

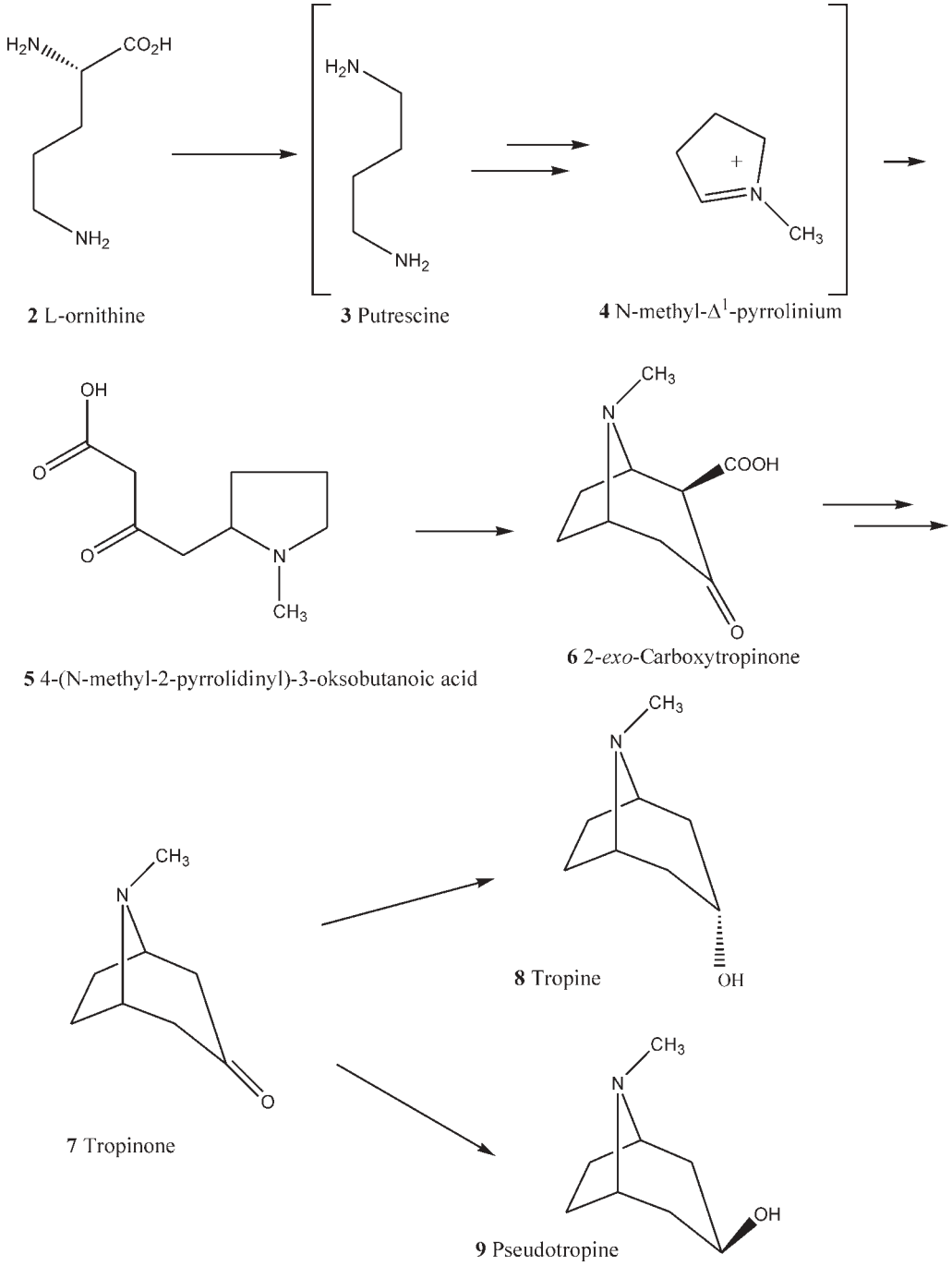
Derivatives of tropine

Decarboxylation of the compound **6** leads to tropinone **7** from which tropines (**8** and **9)** can be obtained by biotransformation or chemical reduction. Two tropinone reductases (TR) with opposite stereospecificities have been identified in plants [57], viz. EC 1.1.1.206 (also known as TR-I) produces tropine (3α-hydroxytropane **8**) whereas EC 1.1.1.236 (TR-II) catalyzes formation of pseudotropine (3β-hydroxytropane **9**). Derivatives of the latter are less common. Similarly to enzymatic pathways, in chemical reduction of tropinone, a considerable degree of stereoselection can be achieved by proper choice of the reaction conditions [53, 80].

Hyoscyamine **12**, the principal alkaloid of the above-mentioned plants is the ester of tropine with levorotatory {l(–)} or (*S*)-tropic acid, which is believed to be biogenetically generated by skeletal rearrangement of phenyllactic acid, which in turn is derived from L-phenylalanine **10**. The metabolic pathway from tropine phenyllactic ester, littorine **11,** to tropic acid ester, hyoscyamine **12,** (Scheme 3), which involves the 1,2-carboxy group shift, generally accepted by modern treatises on alkaloid biosynthesis, was recently contested [66], based on detailed studies with doubly labeled ^{3}H,^{13}C precursors. Although incorporation of an isotopic label is usually low in biogenetic experiments, earlier observation that exogenously added ^{13}C-labeled littorine was metabolized to hyoscyamine by *Datura stramonium* to a significant degree is not denied, but the old standing problem of the molecular mechanism of this transformation still remains unresolved [36, 65, 66].

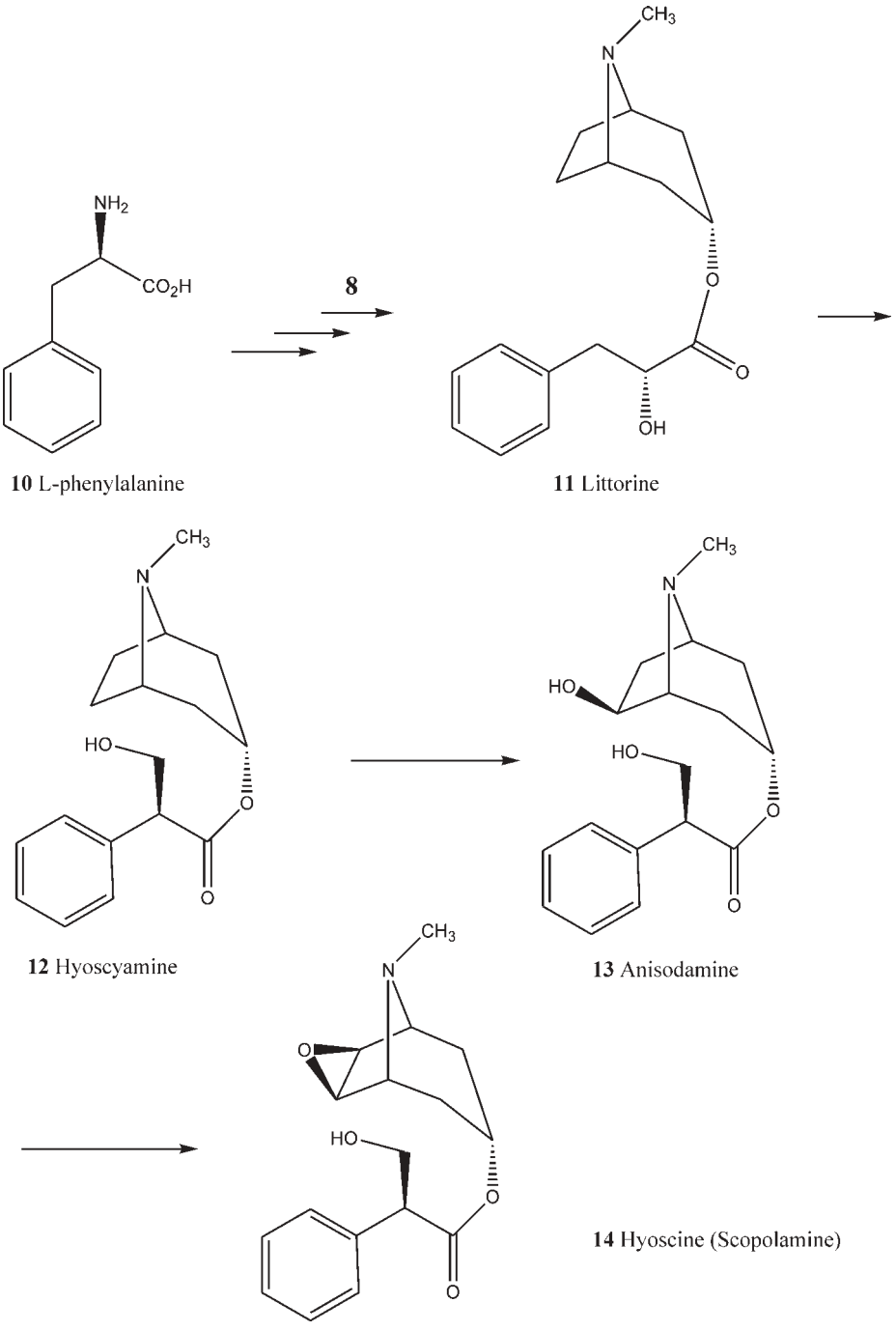
Compound **12** easily undergoes racemization after isolation and the racemic mixture is known under the name atropine. Although l(–)- and d(+)-hyoscyamines differ considerably in their biological activity [10, 28, 46, 51], the racemate, atropine, is a pharmaceutically and medicinally accepted form of the active substance, because it is stable, less susceptible to enzymatic hydrolysis, and can be reliably standardized for potency.

6-β-Hydroxytropine (**13**; this compound is also known as anisodamine), generated by action of the enzyme – hyoscyamine 6β-hydroxylase (also: 6-(*R*)-hydroxylase; H6H), is a precursor to another alkamine, scopine, which contains an additional epoxide ring and constitutes a basic part of hyoscine **14**. Its American name scopolamine reflects the fact that unlike in Europe, in the United States the alkaloid is isolated from *Scopolia carniolica*. It is another important alkaloid with medicinal applications, which is an ester of levorotatory (–) tropic acid. Scopolamine is the most valued tropane alkaloid, which found various



Scheme 2. Crucial steps in biosynthesis of tropines

medical application: from antiemesis to resuscitation, and also serves as a raw material in synthesis of the next generation drugs. The use of compound **14** as an interrogative aid (or "truth serum") is poorly documented, for obvious reasons. Minor tropane alkaloids contain tropine, nortropine or hydroxylated tropines as an alkamine part, and may be esterified in position C-3 with a variety of acids as, for example benzoic, cinnamic, tiglic, truxillic, isovaleric, methylbutyric, and others. They did not became drugs, with a notable exception of anisodamine (3-tropoyl-6-hydroxytropine, **13**) [70].



Scheme 3. Biosynthesis of tropic acid esters

The mind-altering properties of extracts from tropane alkaloid-containing plants (e.g. hallucinogenic) are known since antiquity but the main application of these substances in modern medicine is different [10, 28, 45]. Their antispasmodic action is useful in treatment of bladder spasms, irritable bowel disease, peptic ulcer, colic, cystitis and pancreatitis. All main three alkaloids mentioned above (considering, according to the medical tradition, hyoscyamine and its racemate – atropine as separate entities), are known to cause the pupil dilatation (mydriasis), which is accompanied by impairment of the lens accommodation (cycloplegia).

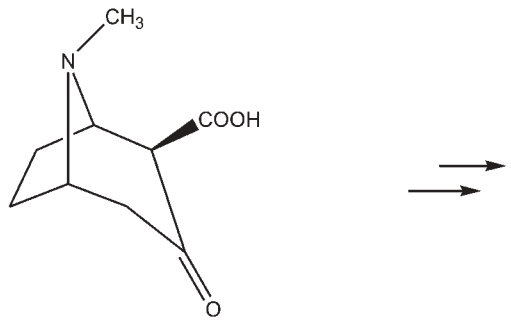
This biological activity, pronounced even after topical application of minute amounts of the substance, makes them useful as agents aiding ophthalmic examinations. The strength of this effect diminishes from hyoscyamine through atropine to scopolamine [10, 46], but atropine became the substance of choice for ophthalmologic applications because it is more stable and easier to standardize than hyoscyamine and because at small doses it is practically devoid of central nervous system (CNS) activity. Its effects on other body systems include: inhibition of the respiratory tract secretory activity and bronchodilation, alteration of the heart rate (bradycardia at low doses and tachycardia at high doses) reduction of gastric secretions, and inhibition of sweating accompanied by rise of body temperature. Atropine is also useful as an antidote against poisoning with organic phosphorous derivatives used as insecticides and also against some nerve gases applied as military weapons. Atropine, which was first isolated in a pure state as early as 1833 (F.F. Runge), crystallizes in needles melting at 114–116°C, is sparingly soluble in water (ca. 2 g per liter) and easily forms much more soluble salts with mineral acids. The most popular preparations of atropine are eye drops, which contain dilute solution (from 0.1 to 2%; typically 1%) of the alkaloid or its sulfate salt [10, 33, 58].

Pharmacological effects of scopolamine are even more diverse and so are the indications for its use. The drug exhibits pronounced effects on the CNS, affecting locomotive activity, neurotransmission and short-time memory. Its clinical applications include alleviation of symptoms of motion sickness, premedication for anesthesia, obstetrical analgesia, sedation in delirium tremens, psychosis or mania, and treatment of parkinsonian tremors. It is also used to induce transient cognitive deficits, to model dysfunctions observed in aging and dementia, which are considered useful for testing new CNS drug candidates [10, 28, 71].

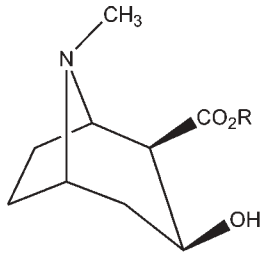
Derivatives of ecgonine

Two out of approximately two hundred *Erythroxylon* plant species (*E. coca* and *E. granatense*, commonly known as coca) indigenous to South America, share an exclusive taxonomic characteristic, namely the ability to produce tropane skeleton alkaloids structurally and stereochemically distinct from tropine derivatives discussed above. Coca's principal alkaloid is cocaine **17**, derivative of ecgonine **15**, in which both tropane O – functional groups are esterified (Scheme 4). Stimulating and energizing properties of coca were known in pre-Columbian populations of Andean ridge for millennia and Inca Indians considered the plant a gift from gods. Apparently, habitual chewing of the coca leaves has never led to the negative consequences associated with the present uses of illicit cocaine preparations [44, 50]. The pure cocaine substance was first obtained from imported coca leaves by A. Niemann (an assistant to F. Wöhler at Göttingen University) in 1860 and after F. Koller's ophthalmologic experiments in 1884, instantly became known in medical circles as a topical anesthetic. Later, under influence of Sigmund Freud, cocaine was recommended as an antidepressant. Around that time, cocaine was supposed to be very closely structurally related to atropine, in part because both exhibited mydriatic and local anesthetic activity. Correct structure of both alkaloids was elucidated in 1898 by R. Willstätter in Munich, who also succeeded in synthesis of cocaine three years later [19, 45, 81].

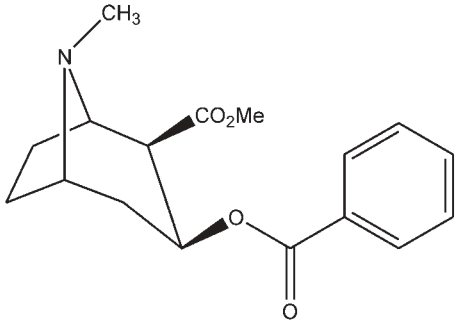
For some time around 1900 cocaine was used in many medicinal products and also in beverages. Following recognition of its addictive properties, the substance became illegal in the USA in 1916 and it is presently regulated by the 1970 Controlled Substances Act. Cocaine has presently very limited medicinal use, particularly in ophthalmologic surgery, where its topical anesthetic and vasoconstrictive properties are particularly advantageous [90]. The drug active substance needed for this purpose is obtained as a by-product of food industry, from processing some beverage raw materials. There is no doubt that at the same time large scale illicit industry operates in South America, extracting hundreds of tons of the alkaloid for illegal distribution on wealthy markets. Well over 30 million Americans have used cocaine and there are over 1.5 million registered cocaine addicts in the USA [44, 64]. Despite lasting effort in search for cocaine antagonists (particularly among the alkaloid structural analogs) [38, 80, 85], there are no effective therapeutics for cocaine addiction available, although a group of synthetic ligands based on 4'-iodococaine exhibited good binding potency for dopamine transporter. Some more recent advances in this field are mentioned in the following section.



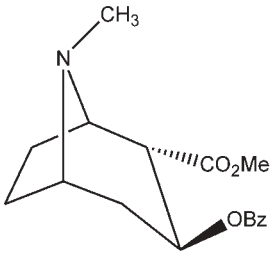
6 2-*exo*-Carboxytropinone



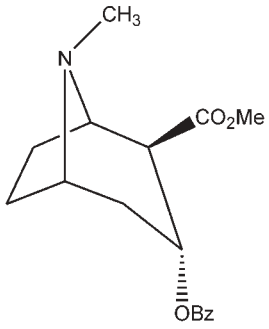
15 R = H Ecgonine
16 R = CH_3 Ecgonine methyl ester



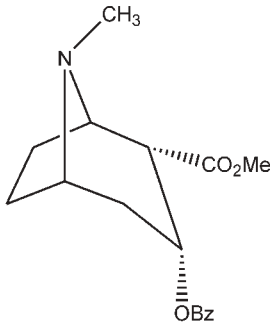
17 *R*-(-)-cocaine



18 *R*-pseudococaine



19 *R*-allococaine



20 *R*-allopseudococaine

Scheme 4. Ecgonine derivatives; stereoisomers of cocaine

Modern pharmacology of tropane derivatives

From the onset of the 20th century chemical approach to medicinal compounds has become firmly established and chemical synthesis was used to seek improvements in properties and efficacies of natural products applied as drugs. As ideas of structure-activity relationship (SAR), pharmacophore, receptor and stereoselectivity of biological action were initiated and developed, rational design of structure-based biological activity flourished, occasionally bringing to life new, effective drugs. Investigation of tropane alkaloids as mydriatics, spasmolytics and local anesthetics were at the forefront of newly born medicinal

chemistry [10, 28, 33, 45, 81]. At first, new chemical analogs of prototypic compounds became available for biological testing, then knowledge about molecular mechanisms of action of biological targes (receptors and signal transduction and transmission) has evolved. In contemporary pharmacology old drugs and new model compounds are studied not only at the molecular or cellular level, but are also presently investigated in organismal context, using specific gene knockout mice as tools to examine physiological and pathological situations [25, 83]. Availability of both kinds of tools, i.e. purposefully designed compounds as molecular probes, and biological models for testing their activity, is equally essential for further progress.

From atropine to tropeines

Atropine was first isolated as an active principle from the roots of belladonna in 1831 by K. Mein, a German apothecary, and as a pure chemical substance by F.F. Runge in 1833, while hyoscyamine was obtained from henbane by P. L. Geiger and K. Hesse in the same year [19, 81]. Their mydriatic effects were also discovered in the same period. Then, it took almost half a century to learn, how the alkaloid substance can be split, by action of simple chemical agents, into two components: tropine base and tropic acid (K. Kraut and W. Lossen, ca. 1880), thus revealing its ester character. Subsequently, A. Ladenburg discovered that gentle heating of these two components in hydrochloric acid results in restoration of the atropine structure [10, 19, 81]. This procedure was soon adopted for esterifying tropine with various organic acids, in search for new mydriatic agents, less irritating to the eye than atropine. Ladenburg succeeded in producing a series of physiologically active compounds, which he called "tropeines". One of them, the mandelic acid ester named homatropine **59**, acted more quickly than atropine and had less detrimental paralytic effects on ciliary muscle of the eye. Although less potent than atropine, it was introduced by E. Merck company in Darmstadt as a new mydriatic in 1883, as one of the very first synthetic drugs (L. Knorr synthesized antipyrine in the same year; aspirin although obtained in crude form in 1853, was not released as a drug until 1899) [81]. Tropeines were later investigated by application of SAR methods but no superior agent was found for this particular application [23, 28, 29, 54].

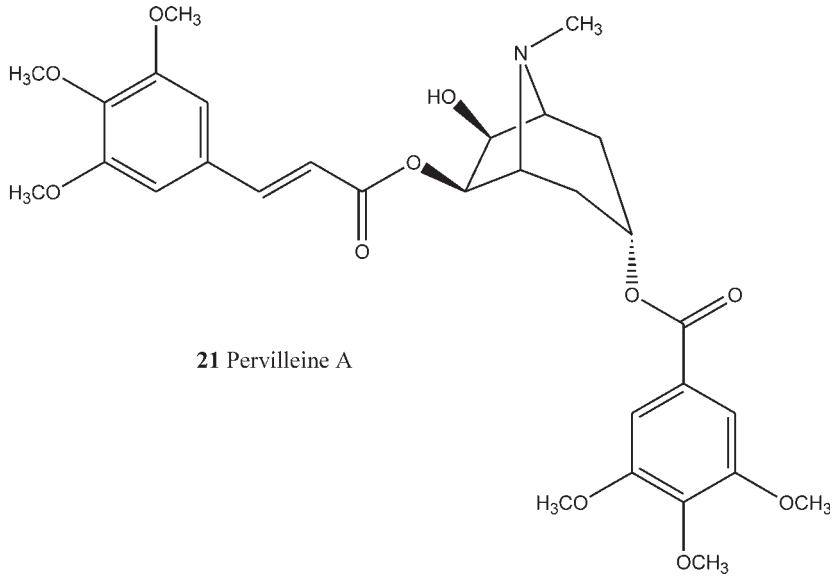
Recognition of anticholinergic character of physiological action of the tropane alkaloids dates back to classical, independent experiments of J. N. Langley and P. Erlich, from which concept of a receptor emerged [33, 45]. The concept of muscarinic pharmacophore is much later and its impact on drug discovery incomparably smaller. Today, cholinergic receptor classification distinguishes five metabotropic muscarinic (M_1–M_5) and several groups of ionotropic nicotinic acetylcholine receptor (nAChR) subtypes. Human muscarinic receptors (MR) are homologous proteins, 460–590 amino acids long, which belong to a superfamily of seven transmembrane spanning receptors that are linked to G-protein, and therefore, their effectors are relatively well recognized [7, 14, 20, 75, 91]. The receptor proteins have been cloned and various ligands' affinity have been studied with the help of radioactive compounds [20]. It is generally accepted that MR action of tropane alkaloids are stereoselective, as a result of a difference between stereoisomers in affinity and binding. Resulting differences in potency, between *S*-(–)- and *R*-(+)- hyoscyamine in various functional tests have been estimated as 30–300 fold, in favor of the former compound [28, 46]. This trend is not necessarily retained in other types of biological activity. For example *R*-(+)-hyoscyamine was found to exert antinociceptive and nootropic action in rodents, while *S*-(–)-hyoscyamine is completely devoid of such activity [23]. Following this lead, new tropeines with analgesic and nootropic potential were obtained. Atropine and related compounds are competitive antagonists of acetylcholine (ACh) actions at muscarinic receptors and they are rather poorly selective towards receptor subtypes [10, 28]. The binding site for acetylcholine (and its competitive antagonists) is acidic in character and it is located in a cleft formed by transmembrane helices of receptor protein. ACh receptors are widespread and present in autonomic effector sites, postganglionic parasympathetic fibers, sympathetic and parasympathetic ganglion cells, skeletal muscles, as well as CNS and peripheral nervous system (PNS) synapses. Their physiological roles in CNS include involvement in cognition processes, control of motor, cardiovascular and respiratory activity, sensory functions (e.g. pain perception) and stress responses. In PNS muscarinic acetylcholine receptors are engaged in heart rate control, contraction of smooth muscles, glandular secretion and vasodilata-

tion. Obviously, MR antagonists can produce a variety of physiological responses, which are of great interest in physiology, and some of them are exploited in clinical practice. Since dysfunction of muscarinic cholinergic system have been implicated in depression, epilepsy, Parkinson's disease and Alzheimer's disease, MR antagonists remain of great interest as potential CNS drugs [3, 15, 27, 33].

Tropane alkaloids are absorbed rapidly from the gastrointestinal tract. Drugs, in the form of injectable solution, are usually administered intramuscularly. Atropine is capable of binding to MR at neuroeffector sites of muscles and gland cells, peripheral ganglia and in the central nervous system. Both, atropine and scopolamine have a characteristic, dose dependent action on the cardiovascular system, which is clinically useful for resuscitation. They inhibit secretion in the respiratory tract and also reduce gastric secretion. Atropine has prolonged inhibitory effect on the gastrointestinal motor activity. The action of tropane alkaloids on the spincter muscle of the iris and the ciliary muscle of the lens, after topical application, leads to pupil dilatation and to temporary paralysis of accommodation. Full recovery may take as long as 7 to 12 days [10, 19, 28]. Atropine is also effective in counterbalancing high systemic concentrations of ACh, which may result from poisoning with organophosphorous insecticides or nerve gases used as chemical weapons, thus it is applied as an antidote. It was also the first effective drug in therapy of Parkinson's disease [27, 28].

Scopolamine has a very similar mechanism of action to atropine and its profile of medical use is largely overlapping. However, its action on the CNS is much more pronounced, with sedation at low doses and possibility of disorientation and hallucinations observed after higher doses. Since scopolamine is the most effective compound in prevention of motion sickness, transdermal therapeutic system has been designed for this indication, releasing 0.5 mg of the drug over a period of 3 days from 1.5 mg reservoir. Another principal clinical application of scopolamine is premedication before anesthesia by parentheral application (*iv* infusion; *sc* or *im* injection). Although this substance has a very long story of medicinal application, reliable pharmacokinetic data derived from analyses of biological matrices with the help of liquid chromatography with tandem mass spectrometry detection (LC-MS/MS) equipment, were obtained only recently, showing considerable dependence on the dosage form. Maximum drug concentration in plasma occurs after ca 0.5 h and the biological half-life is short. Scopolamine is metabolized mainly by glucuronidation and by ester function hydrolysis [8, 10, 28, 71].

There is a growing body of evidence that tropane alkaloids and their synthetic analogs can interact effectively with receptors other than acetylcholinergic MR. The synthetic tropeine: 1-H-indole-3-carboxylic



21 Pervilleine A

Scheme 5. Pervilleine A

acid ester of tropine, tropisetron **60** was developed as a selective serotonin type 3 receptor antagonist, and it is used clinically for treatment of postoperative and chemotherapy-induced emesis [37, 68, 79]. Further investigation of biological activity of the compound **60** revealed that it can potentiate α1 glycine receptor at femtomolar concentrations, while exerting inhibitory effect in micromolar region. Tropisetron also is an α7 nicotinic AChR selective partial agonist. Another unexpected feature of this drug, is its anti-inflammatory action, probably by targeting the calcineurin pathway, which is likely to find a clinical application in immunomodulation [79].

Another new drug from tropeine category is anisodamine **13** (first extracted from Chinese herb *Scopolia tangutica* Maxim but used in clinical practice as a synthetic substance), well known as an intermediate on biogenetic pathway from hyoscyamine to hyoscine (Scheme 2), which exhibited a remarkable activity as an inhibitor of proinflammatory cytokines, it also inhibits platelet aggregation and has other advantageous cardiovascular actions [17, 61, 96]. Anisodamine, which is thus far not registered beyond Asia, is recommended as a drug for septic shock.

Recently, a group of new tropane alkaloids was isolated from *Erythroxylum pervillei*, with a majority of them featuring 6,7-dihydroxylated tropane moiety, esterified with 3,4,5-trimethoxybenzoic and 3,4,5-trimethoxycinnamic acid residues. Pervilleine A **21** (Scheme 5) shows promising activity as multidrug resistance inhibitor of a potency comparable with verapamil. Interestingly, none of pervilleines produces any significant cholinergic or adrenergic effects [9].

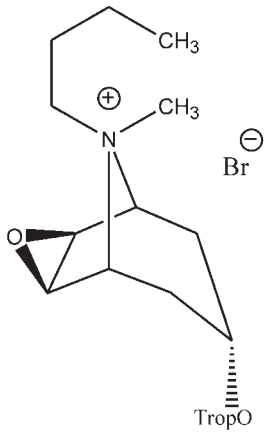
Quaternary ammonium salts

In 1902, the Bayer company introduced first quaternary salt of atropine, methonitrate, under the name of Eumydrin®, as a mydriatic [81]. Originators of the drug acted on an assumption that quaternization significantly increases polarity of the molecule, thus preventing it from readily crossing into the central nervous system. Surprisingly and fortunately, this simple reasoning worked, giving rise to a series of successful drugs of the same chemical character, which are now used as spasmolytics (e.g. hyoscine butylbromide, **23**) or bronchodilators (e.g. ipratropium bromide, **55**). Stereochemical issues involved in the preparation of nonsymmetric quaternary ammonium salts are commented upon on page 457 of this paper. It should be pointed out that various attempts, particularly in SAR systems, have been made to quantify the old idea of lipophilicity as one of principal determinants of the fate of xenobiotic chemicals in the body. Recent simplification in the form of Lipinski's rule [48] is particularly well suited for sorting out molecular property descriptors of tropane derivatives which are tolerant to structural and topological variations. It is generally believed that tertiary tropane amines and their quaternary analogs share many similarities in their anticholinergic action, while their biodistribution differs considerably. Thus, scopolamine butylbromide **22** can be applied safely as an antispasmodic, without risk of such central effects as disorientation, hallucinations and loss of memory, which are characteristic of scopolamine itself [10, 28] (Scheme 6).

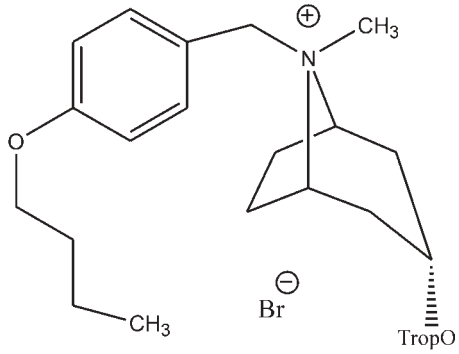
Nortropine derivatives

A great majority of natural tropane alkaloids have N-methyl group at position 8 of the bicyclic system. De-N-methylated derivatives were, until recently, much more often encountered as synthetic intermediates, than secondary metabolites. Polyhydroxylated nortropines, first isolated from *Calystegia sepium*, are now classified as calystegines. It is now supposed that these compounds occur in many other well known plants, and were apparently overlooked because of their very high polarity. Calystegines are nor-pseudotropines with a hydroxyl group at the ring junction positions, which render them a hydroxyaminal properties. It means that they can easily ring-open and the resulting hydroxyaminoheptanones can undergo stereo- and regio-isomerization, as exemplified in Scheme 7 [13].

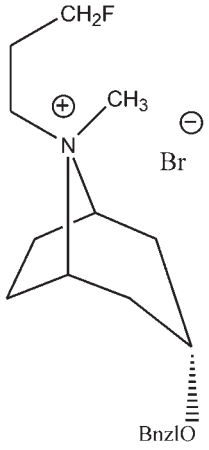
Although nortropinone can be in principle obtained by applying ammonia as a basic component in Robinson's synthesis, chemical de-N-methylation is also applied in a couple of synthetic variants. Thus, tropinone can be transformed to N-formyl nortropinone with ca. 50% yield, through photolytic oxidation [19]. Reaction of tropane derivatives with cyanogen bromide leads to replacement of N-methyl group with N-cyano function, which can be removed by hydrolysis and decarboxylation [31]. N-methyl bond can also



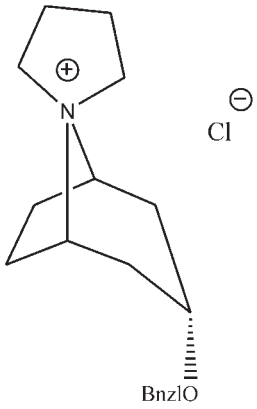
22 Hyoscine butylbromide



23 Butropium bromide



24 Flutropium bromide



25 Trospium chloride

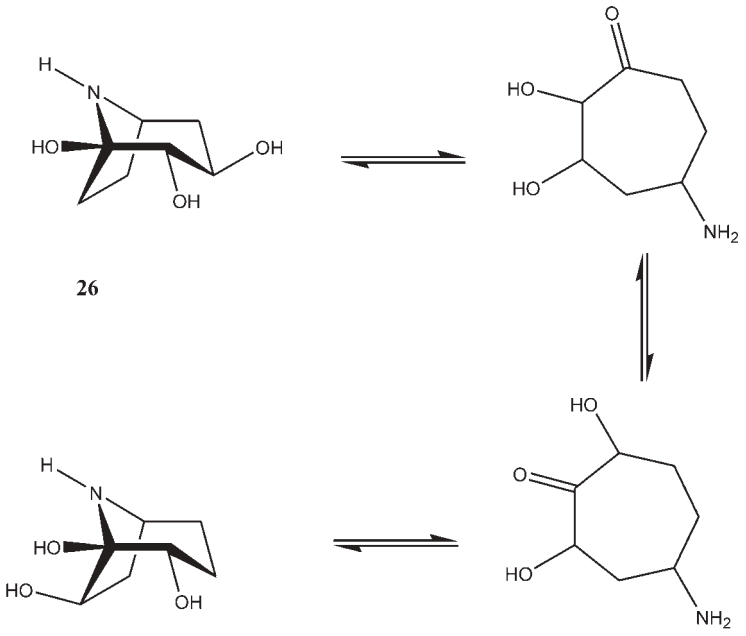
Trop = tropic acid acyl residue Bnzl = benzylic acid residue

Scheme 6. Therapeutic quaternary ammonium salts

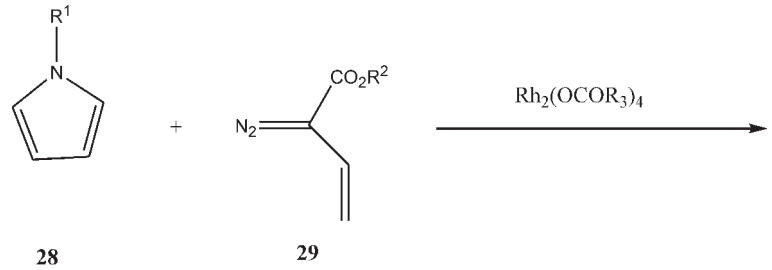
be conveniently split by the action of phosgene or chloroformates, with formation of the corresponding carbamates. Utility of this transformation was first demonstrated on chloroethyl chloroformate, but trichloroethyl ester is more frequently used recently [43]. Suitably protected nortropine derivatives can also be obtained in a variety of reactions, in which N-carboxyalkyl derivatives of pyrrole (e.g. **28**) or pyrrolidine, (e.g. **47**) are applied as C_4 synthon in cyclocondensation reactions. An example of such approach, leading through a protected nortropine **30** to anhydroecgonine derivative **31**, which is a principal intermediate in synthesis of 3-phenyl analogs of cocaine (e.g. **33**), is presented in Scheme 8 [80].

More distant synthetic relatives of atropine and cocaine

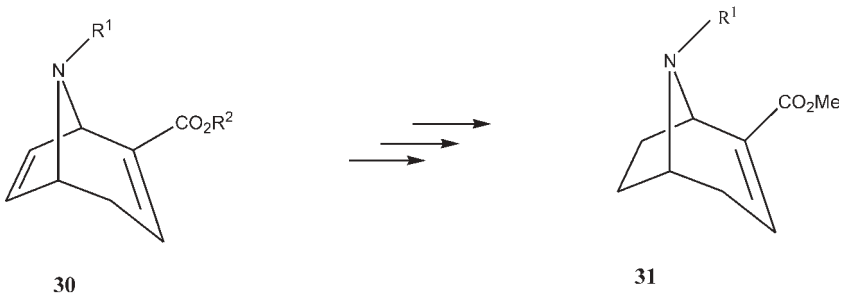
Perhaps the most significant continuation of the early work on semi-synthetic tropane derivatives came from J. von Braun (an eminent and very prolific chemist,



Scheme 7. Isomerization of calystegines



Scheme 8. Synthesis of tropane derivatives from N-protected pyrrole



$R^1 = CO_2Alkyl, CH_3$ R^2, R^3 = alkyl, substituted alkyl

born and educated in Poland, also professor in [then] Breslau 1909–1918 and Rector of Technical University in Warsaw 1915–1917), at the University of Frankfurt (1921–1935), who carried out systematic investigation on the effects of transposing functional groups within a drug molecule. He has managed to place tro-

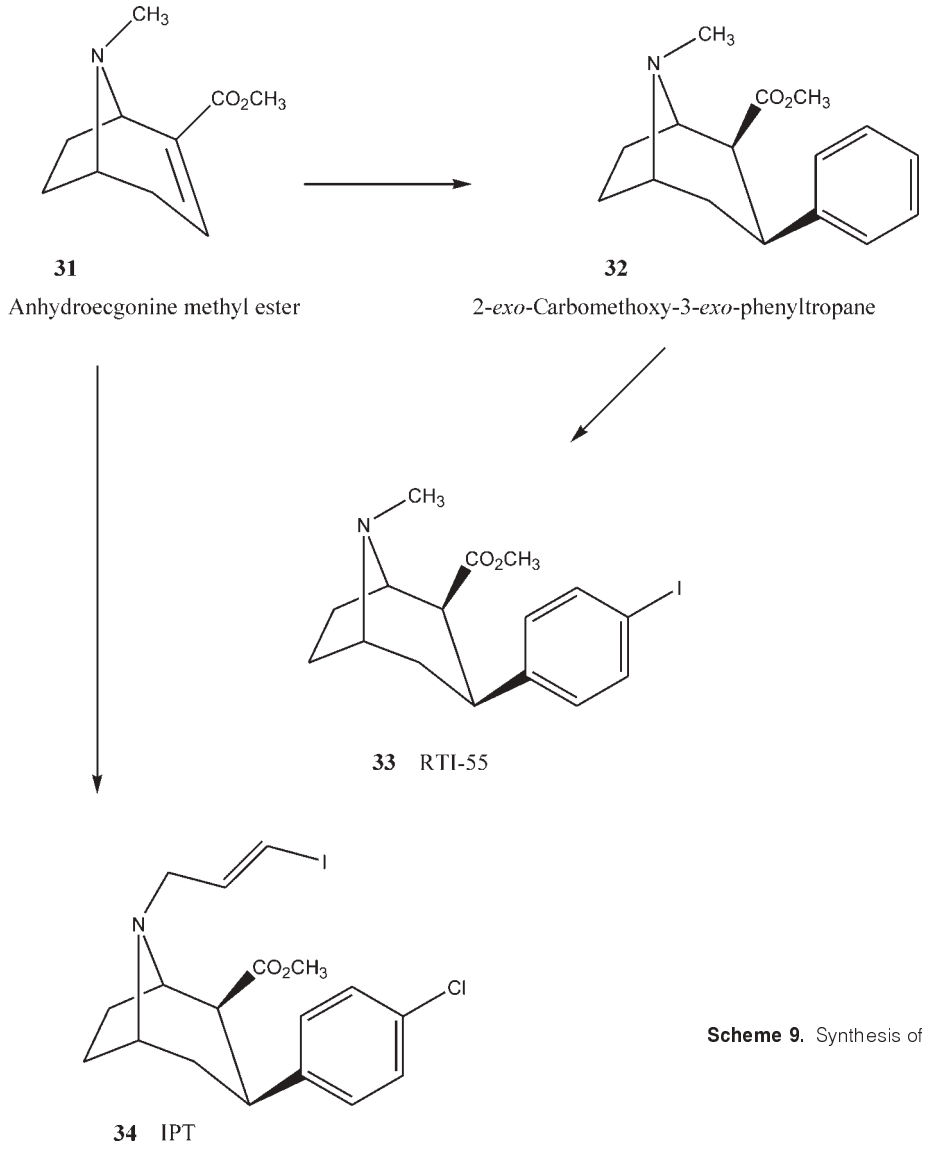
pate ester moiety in different positions around the tropane ring, without losing the mydriatic activity. Further, he demonstrated that intact tropine ring was not essential for activity and could be substituted by a tertiary amine function situated a couple of carbon atoms away from tropate moiety. In parallel investigation concerning minimal pharmacophore of cocaine, conducted by A. Einhorn and G. Merling, it has been found that 2-carbomethoxy substituent of pseudotropine is not essential for anesthetic activity. This discovery started an avalanche of synthetic compounds expected to outperform atropine and cocaine in clinical practice. Eventually, it led to development of a group of synthetic local anesthetics and antispasmodics [81], which bear little resemblance to the alkaloid progenitors, thus validating fundamental concepts of structure-based drug design and pharmacophore optimization. The ethyl ester of p-aminobenzoic acid, known as benzocaine, first introduced in 1903 and still in use today, was the first in a long series, with well known drugs: procaine and amylocaine to follow [45].

Prospects of cocaine abuse treatment

Cocaine abuse has already attained a level of alarming epidemic in the United States and the threat to the rest of the world is obvious. Our understanding of addiction as a physiological and pharmacological phenomenon is certainly not complete, but scientific framework already exists, upon which various lines of research seeking effective means for cocaine abuse treatment, can be based. Among tools, extensively used by pharmacologists, are a variety of behavioral animal models (drug self-administration etc.) and numerous sophisticated neurochemical assays [2, 4, 5, 30, 47, 62]. A considerable evidence has been accumulated indicating that the dopamine transporter (DAT) constitutes the main target, responsible for the reinforcing effect of the drug [44, 86]. On the other hand, dopamine transporter gene deletion studies indicated that this pathway was not the only one. More recently, involvement of another monoamine transporters [serotonin (SERT), norepinephrine (NET)] have been suggested [2, 49, 74]. Besides, cocaine also modulates endogenous opioid system (through μ and κ receptors) and preprodynorphin release.

Search for a cocaine antagonist was initially fuelled by belief that the alkaloid is a competitive inhibitor of dopamine (DA) uptake. A number of DAT blockers (including tropane derivatives: benztropine **61** and a group of 3-phenyl analogs of ecgonine, Scheme 9) have been developed, but they found no clinical use in cocaine addiction treatment. More recent evidence suggests that dopamine and cocaine have separate binding sites on DAT. This finding renders some credibility to continuous effort dedicated to synthesis of cocaine analogs [80]. Other targets, like 5-HT receptors are also actively pursued [49]. The main line of cocaine structural analog production was based on anhydromethylecgonine **31** as the key intermediate capable of stereoselective C-C bond formation at C-3. Addition of phenylmagnesium bromide to **31** gave 2β-carbomethoxy-3β-phenyl tropane **32** with 75% yield. By application of the standard aromatic chemistry, the compound was transformed into a library of derivatives, in which compound **33**, obtained by direct iodination or a sequence of: nitration, reduction and diazotization, proved particularly useful as an experimental cocaine antagonist, molecular probe, and also as a starting material for modern aromatic coupling reactions [80] (Scheme 9). Compound **34** known as IPT, or iptakalim, turned out to be a human nicotinic acetylcholine receptor antagonist and adenosine triphosphate (ATP)-dependent potassium channel opener. IPT inhibits cocaine-induced release of dopamine and glutamate, which suggests its possible application in drug addiction [35].

Much attention has been devoted to development of protein-based therapies of cocaine addiction, like anti-cocaine antibodies [12, 82, 89]. Vaccination of rodents with such construct appeared to reduce self-administration of the drug. Another biotech approach idea is based on enzyme technology. It is known that butyrylcholinesterase (BChE), which splits off benzoic acid from cocaine molecule leaving behind harmless methylecgonine, is a principal cocaine metabolizing enzyme in mammals. The enzyme was applied to experimental animals as such, or in the form of an antibody, with moderate success in cocaine detoxification. Interestingly, another efficient protein catalyst for cocaine hydrolysis has been found in bacteria. *Rhodococcus sp.* strain MB1, which uses cocaine as the sole source of carbon and nitrogen, is equipped with cocaine esterase (CocE), which shows hydrolytic rate constant 1000-fold higher than that of BChE [42]. It seems that both lines of research, synthetic and bio-

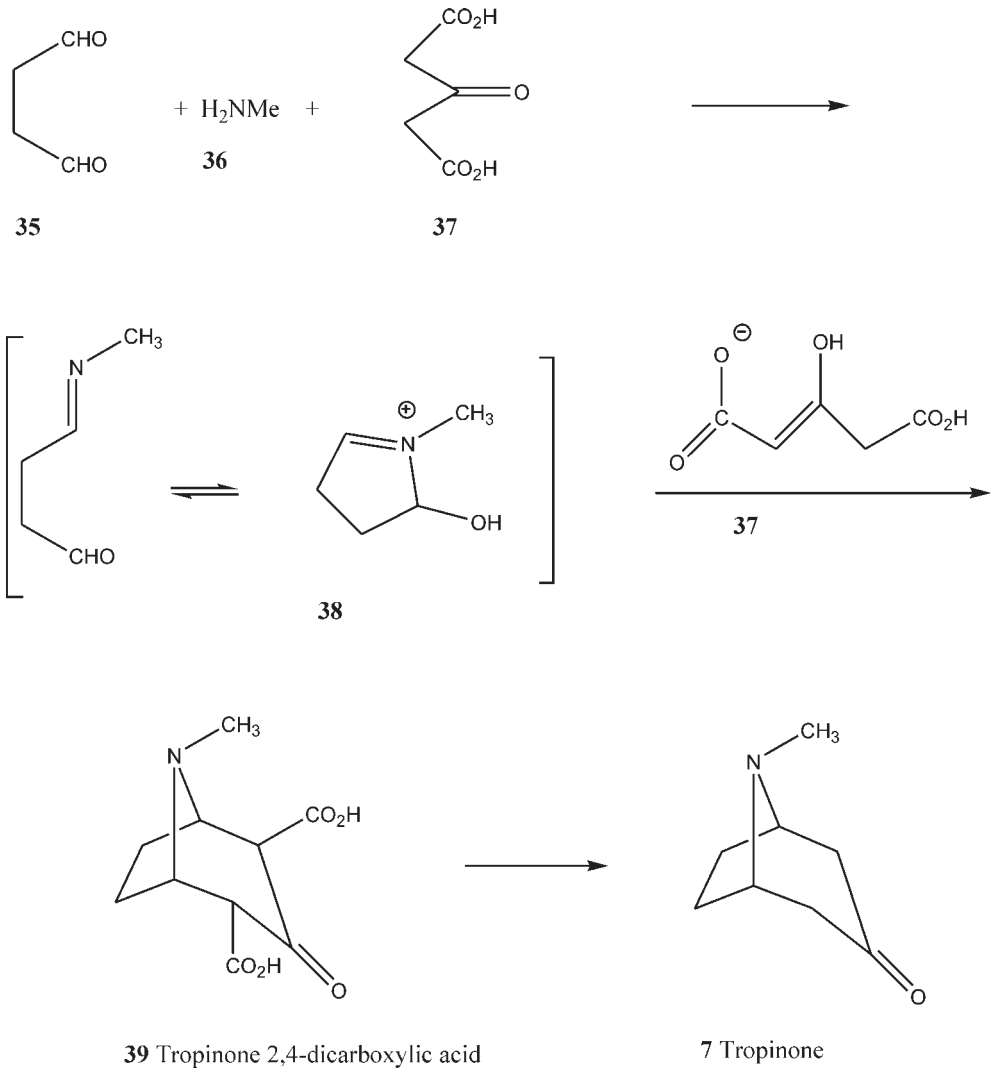


Scheme 9. Synthesis of cocaine analogs

technological, offer reasonable chance for a new molecule (either small ligand or biomacromolecular construct), which will bring a breakthrough in cocaine abuse therapy.

Chemical syntheses of tropinone and its derivatives

We owe a great deal of spectacular successes of chemistry as an art of creating new materials, to the tradition established in 19th century, which demanded that elucidation of structure of a new compound isolated from natural sources should be followed by its synthesis, providing the unambiguous proof of structure. Although today structural analysis is based mainly on application of advanced spectral techniques, the art of total synthesis of natural products flourishes, and long, complicated sequences of reactions utilizing most sophisticated molecular transformations, still set academic standards of excellence and serve as teaching aids to education of organic chemists [60]. Although detailed synthetic deliberations are outside of the scope of this review, we will only touch upon some most important topics connected with chemical synthesis of tropane analogs: construction of the bicyclic tropane skeleton, dessymetrization of the *meso*-structure of tropinone (or its



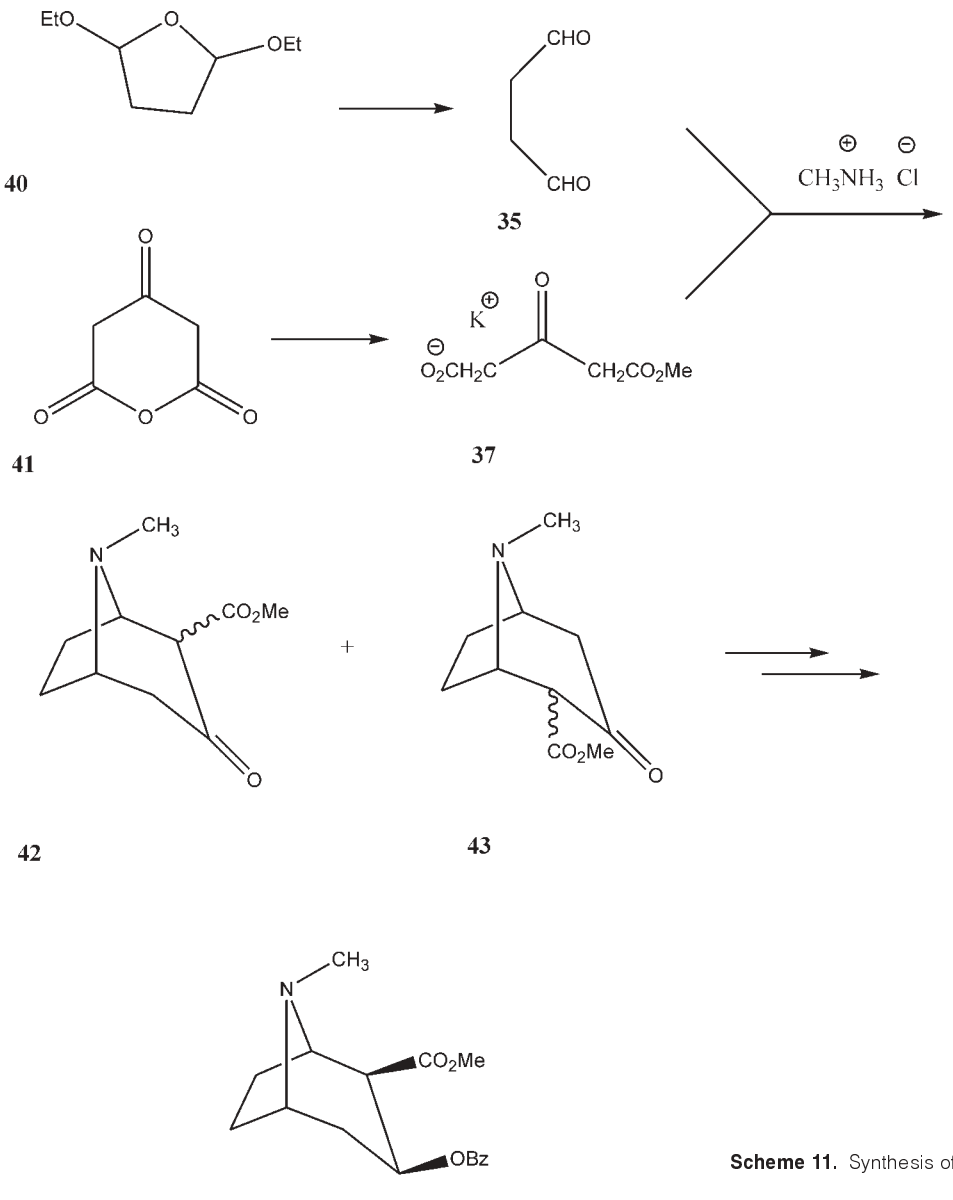
Scheme 10. Robinson's biomimetic synthesis of tropinone

precursors), and stereochemistry of nitrogen atom quaternization, because understanding of their stereochemical features is essential for successful attempts at new drug design in this category. Correct structures of atropine and cocaine were determined by R. Willstätter in 1898 but stereochemical details of tropane derivatives were not completely clarified until 90 years later, and X-ray structures of scopolamine compounds were determined in 2000 [24]. It may, therefore, appear shocking that some syntheses of tropine, atropine and cocaine were successfully completed already in years 1879–1903 (A. Ladenburg; R. Willstätter), when methodology of organic chemistry was still in its infancy. Total syntheses of alkamine part, starting from cycloheptanone, were rather tedious and inefficient, but they served the purpose of proving the structure and additionally provided some pure materials for investigation of biological activity. R. Robinson's synthesis of tropinone, accomplished in 1917, was based on ingenious idea of a three-component biomimetic condensation between succinaldehyde **35**, methylamine **36** and acetonedicarboxylic acid **37**, providing a breakthrough in availability of tropane derivatives [73, 77]. The preparation, depicted in Scheme 10 was modified and improved many times, and eventually made it into the golden collection of the greatest achievements in the total synthesis of natural products [6, 60, 77].

It should be pointed out here that tropanone and tropines are symmetric (chirality of bridgehead car-

bon atoms C-1 (*R*) and C-5 (*R*) is intramolecularly compensated) molecules, which greatly facilitates their preparation. Nevertheless, Robinson-Schöpf procedure for combining methylamine, C_4 (succinaldehyde) and C_3 (acetone) synthons, has some theoretical as well as experimental nuances, connected with substrate and intermediate handling. Succinaldehyde **35**, which is unstable, can be conveniently substituted by its acetals (e.g. 2,5-dimethoxytetrahydrofuran, **40**) or aminals (e.g. 2,5-dimethoxypyrrolidine). Acetonedicarboxylic acid **37**, its salts and esters are frequently used as C_3 synthons, particularly for preparation of ecgonine derivatives. Recently acetone silyl enol ethers have been successfully applied in tropinone synthesis [56]. Discussion of other novel approaches to tropane skeleton synthesis can be found in several references [6, 16, 32, 38, 39, 41, 55, 67, 69]

Since scopolamine is a more valuable product than atropine, the question of chemical epoxidation at C6-C7 arises. In nature, there is an enzyme tropine 6β-hydroxylase (H6H), and its product **13** is converted to the epoxide directly, without dehydration step [65]. A synthetic approach is inevitably multi-step, with intermediacy of 6,7 unsaturated tropene, available in modified Robinson's synthesis by using 2-hydroxysuccinaldehyde (or a more convenient analog: 2,3,5-



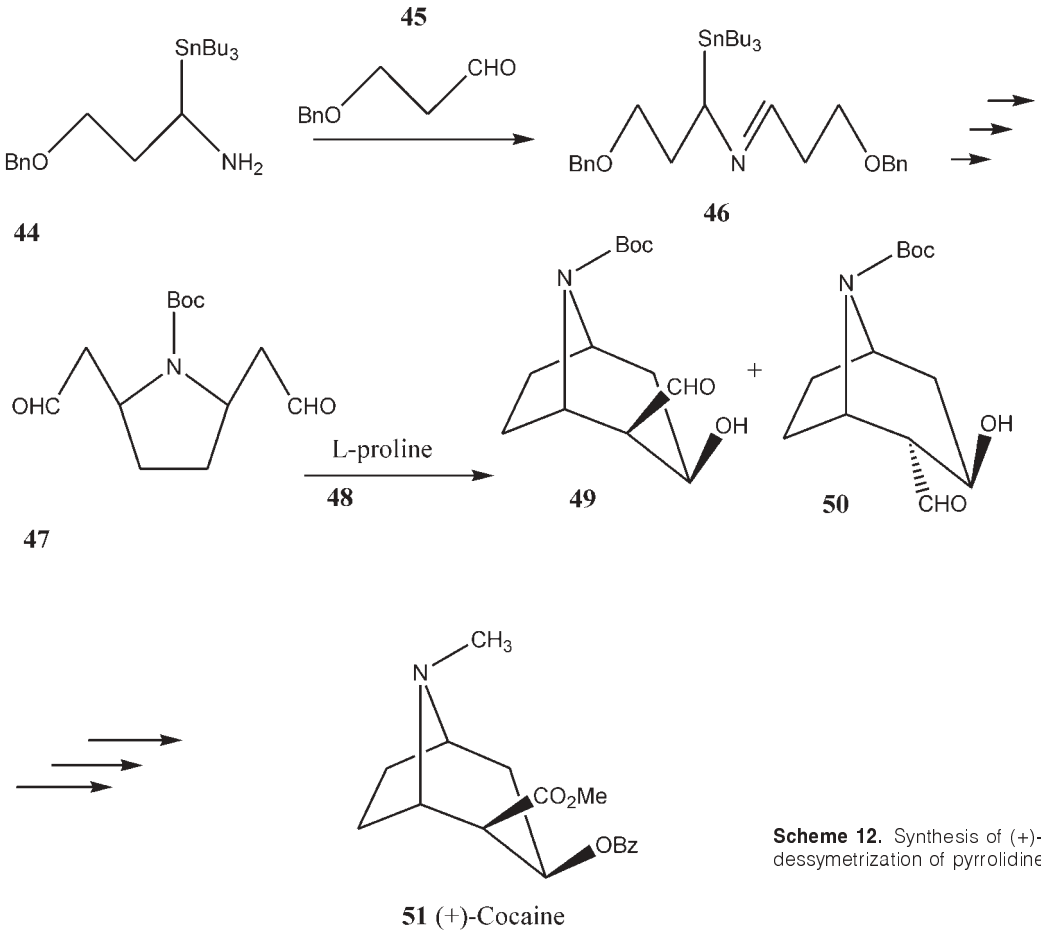
Scheme 11. Synthesis of cocaine

trimethoxytetrahydrofuran) as a C_3 component, and subsequent dehydration of 6-hydroxytropinone. An alternative transformation for chemical functionalization of the tropane C-6 position, which involves intramolecular reaction of N-carbethoxy nortropine 3-α-benzenesulfenate, has also been proposed [67]. This approach, based on generation of O – 3 free radical, followed by 1,5-hydrogen shift with formation of C-6 radical, leads to a 6-phenylthio nortropine derivative, from which the desired 6,7-dehydrotropine can be easily obtained by oxidation/elimination sequence.

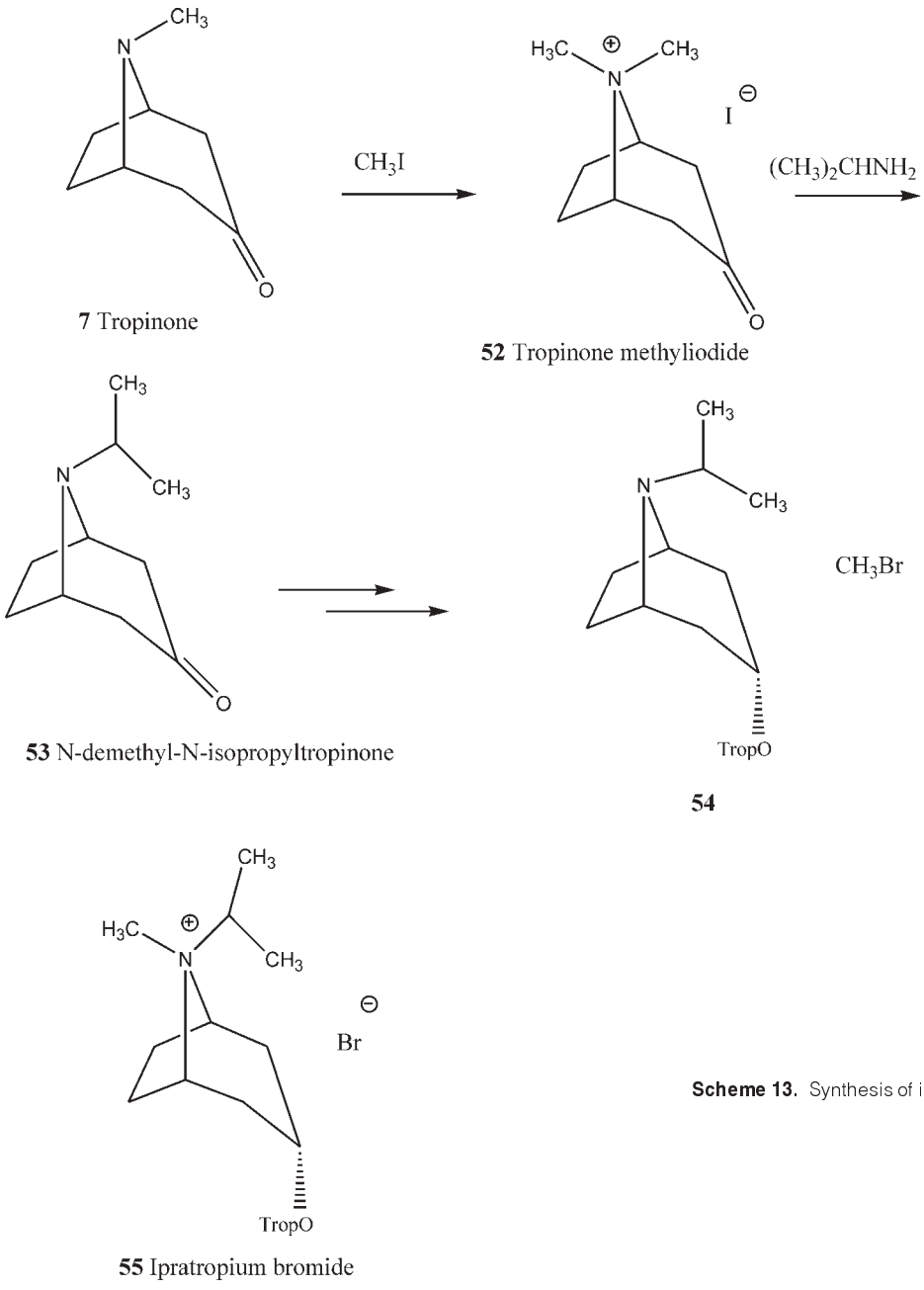
Unlike tropeines, ecgonine derivatives contain a chirality center at C-2 in the alkamine part, which makes their synthesis more demanding, requiring chiral auxiliaries or enantioselective methods. The use of acetonedicarboxylic acid (or its esters) as the C_3 component of the condensation can easily give rise to C-2 carboxylic derivatives, particularly ecgonine **15** and its ester cocaine **17** (Scheme 11). However, control of stereochemistry of the two new centers of chirality in classical version of Robinson's reactions is very difficult and a mixture of all possible diastereoisomers (*R*- and *S*- **17**–**20**) is obtained. Similarly, carboxylation of tropinone with methyl carbonate and metallic sodium gives a mixture of *endo*- and *exo*-products and each of them gives in turn two carbinols upon reduction of the carbonyl group. For ecgonine derivatives, there are four pairs of diasteroisomers (*R*-enantiomers depicted in Scheme 4) and all their members have been isolated from natural sources, but (–)-cocaine, the main alkaloid of *Erythroxylon coca* exhibits quite unusual pharmacological properties, not matched by other isomers [46, 80].

The problem of stereoselectivity of such transformation, as required in ecgonine (and cocaine) synthesis, was addressed by Canadian chemists, who managed to generate enantiomeric anionic intermediates from tropinone by application of lithium derivatives of secondary amines containing chiral substituents [53].

Recently, dissymmetrization of the pyrrolidine intermediate **47** was achieved in a proline catalyzed intramolecular aldol condensation, which gave rise after



Scheme 12. Synthesis of (+)-cocaine by dessymetrization of pyrrolidine derivate



7 Tropinone

52 Tropinone methyliodide

53 N-demethyl-N-isopropyltropinone

54

55 Ipratropium bromide

Scheme 13. Synthesis of ipratropium bromide

additional transformations of aldehyde **49**, to (+)-cocaine **51**, the enatiomer of natural product [55] (Scheme 12). Obviously, at present, chemical synthesis of ecgonine and its esters, which are much sought in illicit markets, is no match for isolation of natural product, because it would have to resort to optical isomers resolution through diastereoisomeric salts [6, 85], which is seldom efficient.

Syntheses of cocaine analogs have spanned over many decades and recent, comprehensive review of this activity totals 100 printed pages, and lists 400 literature positions [80]. The purpose of these efforts can be summarized as the search for better cocaine (that is, devoid of addictive properties) and search for cocaine antagonists, which could block efficiently its interaction with monoamine transporters (particularly DAT). Although these programs have not been particularly successful in terms of pharmacological goal, they served well as an advancement of ecgonine type alkaloid chemistry. For example, phenyltropane chemistry was particularly well developed, based on conjugate additions of aromatic Grignard reagents to anhy-

droecgonine esters, e.g. **31**). A number of new cocaine analogs (e.g. compounds **33** and **34** presented in Scheme 9) have found application as molecular probes for testing interactions with functional biomacromolecules, for example these engaged in dopamine turnover [47, 87].

Tropanes are tertiary amines whose conformational flexibility of the nitrogen atom is somehow restricted by the bicyclic structure. Positional preference of the N-alkyl group has been studied in some detail by spectroscopic methods, ^{1}H and ^{13}C nuclear magnetic resonance (NMR) in particular. It has been concluded that most derivatives of tropine are in equilibrium with preponderance for the axial position of the N-methyl (alkyl) group, which, therefore, is depicted as facing piperidine ring. Both: steric and polar effects can affect the equilibrium and solvent effects can additionally complicate the issue [18, 21, 24, 34]. This situation is of particular importance when bridge nitrogen in a tropane derivative is further alkylated with a substituent other than methyl group and it becomes a center of chirality. Let's consider a case of ipratropium bromide (N-isopropylnortropine methylbromide; 8-*R*; **55**), an important broncholitic medicine, as an example of such situation. Compound **55** can be obtained *via* different routes, but not by direct quaternization of atropine with isopropyl halides! As discovered by Fodor [18, 28], configuration of tropane quaternary ammonium salts is determined by order in which N-alkylations are performed. The last substituent introduced becomes situated equatorially over the pyrrolidine ring [78]. Original synthesis of **55** involved Robinson's $C_4 + C_3$ units condensation in the presence of isopropylamine. The resulting N-isopropylnortropinone was reduced to the corresponding tropine and synthesis was completed by transesterification with formylphenylacetate moiety acting as a precursor of racemic tropic acid [84]. Alternatively, a sequence of reactions involving N-demethylation of atropine, followed by N-isopropylation and N-methylation can be applied. On the other hand, direct quaternization of atropine with isopropyl bromide leads to the isomeric quaternary salt, (in which isopropyl group occupies equatorial position), instead of **55** [78] (Scheme 13).

Recently, some radical progress in synthesis of tropanes with various N-alkyl substituents has been achieved. Although retrosynthetic analysis points out to cycloheptadien-2,6-one as the synthon for one-pot double Michael addition of primary amines, practical realization of this idea proved not entirely satisfactory, because of difficulties in obtaining the amine acceptor. Fortunately, easily available and stable precursor of cycloheptadien-2,6-one: 8,8-dimethyl-3-oxo-8-azoniabicyclo[3.2.1]octane iodide (**52**) was identified, which easily undergoes alkylamine exchange under mild conditions, securing availability of a wide range of N-substituted nortropine derivatives with yields up to 80% [92].

Tropane-derived drugs in contemporary pharmaceutical industry

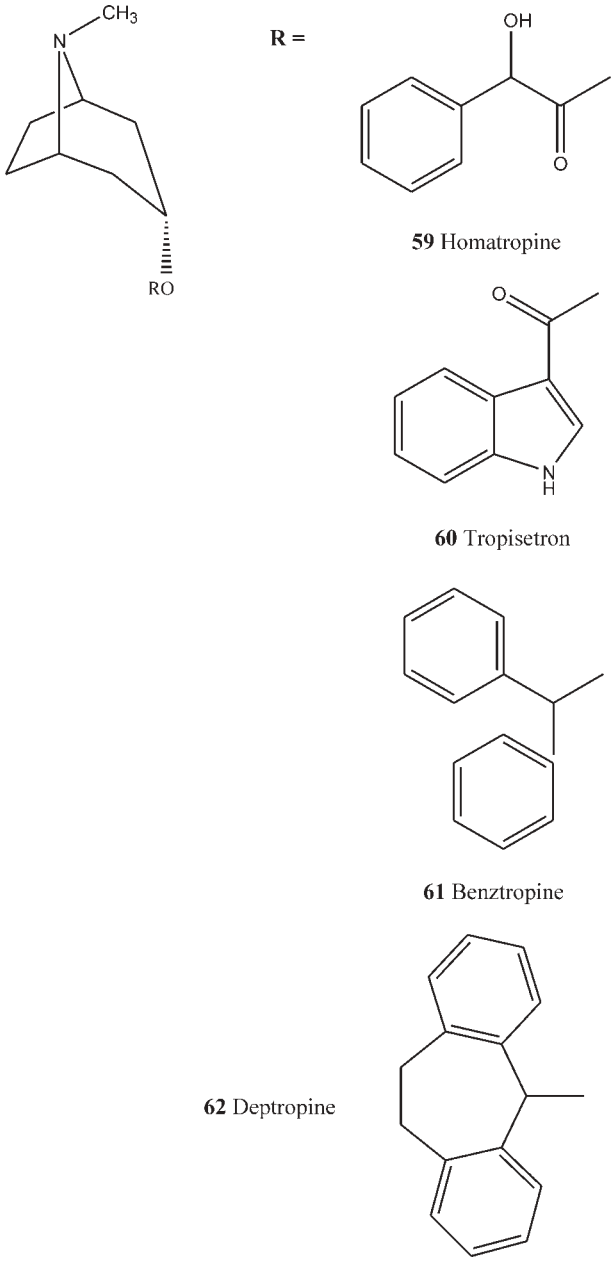
Among active pharmaceutical ingredients (API), which contain tropane moiety in their structure, the most significant in terms of production volume and value are natural products: atropine, hyoscyamine and scopolamine, and a group of their semisythetic derivatives obtained by a single chemical step – N-alkylation, which results in formation of quaternary ammonium salts [10, 28, 51]. Pharmaceutical products based on tropane alkaloids developed at the beginning of the 20th century in Europe, evolved from ethnopharmaceutical tradition and relied on supply of raw materials from wild plant collection. As soon as medicinal application of pure chemical entities started to depart from herbal preparations, the need for efficient, well standardized sources of tropane alkaloids became obvious and cultivation replaced collection of the plants from wild habitates. European plants of *Atropa* and *Datura* species contain on average 0.2–0.8% of total alkaloids and proportion of the most valuable constituent: scopolamine (hyoscine) is fairly low, which hardly justifies industrial isolation based on solvent extraction. In the beginning of the 19th century, phytochemical investigations revealed that Australian plants, classified as *Duboisia*, are a much richer source of tropine derivatives [88]. Presently, *Duboisia myoporoides* obtained by hybridization of local variations, is cultivated on large scale in Queensland, Australia, providing 10–15 tons of fresh leaves per hectare upon harvest, which can be made 3 times a year. The plant is known to contain 2–4% of total alkaloids with ca. 60% hyoscine and constitutes the basis for supply of the global pharmaceutical industry [11, 88]. Experimental plantations of *Duboisia* have already been started in India and other Asian coun-

tries. Biotechnological research towards efficient scopolamine production is also in progress but known technical obstacles with hairy roots growth and processing hamper the progress [52, 94, 97].

The biggest product in terms of volume, in tropane API class, is scopolamine butylbromide **22** (original preparation: Buscopan®, 10 mg coated tablets from Boehringer Ingelheim) with indications for intestinal tract problems (anatomotherapeutic classification WHO – ATC – category A 03 B). It is manufactured on the scale of ca. 21 tons of API and the *ex factory* price value of corresponding preparations is 250 mln $. The methylated analog is manufactured on the one order of magnitude smaller scale. Several other registered products, like trospium chloride **25** with indication for treatment of overreactive bladder [76, 98] or structurally related butropium bromide **23** and flutropium bromide **24**, do not even belong to the list of the top 1000 drugs. For comparison: global production of scopolamine amounts to 600 kg (sales over 77 mln $), atropine to 1000 kg ($ 131 mln) and hyoscyamine to 140 kg ($ 67 mln), according to 2005 data.

Interestingly, 6-(*S*)-hydroxyhyoscyamine **13**, known as a biogenetic precursor of scopolamine, has a long record of pharmacological investigation in China, as a natural compound named anisodamine, isolated from the Tibetan regional plant *Anisodus tanguticus* (or *Scopolia tanguticus* Maxim). It is less toxic than atropine and it has been proposed for a number of therapeutic uses, including treatment of septic shock (possibly by inhibition of cytokine production and improvement of blood flow in the microcirculation), various circulatory and gastric disorders, etc. [70]. Since availability of the natural compound is very scarce, a synthetic compound is also being investigated. It has to be pointed out that such substance consists of four individual entities, grouped in two pairs of enantiomers. Elaboration of stereoselective capillary electrophoresis system of analysis allowed to determine that pharmacokinetics of all isomers was practically identical [95].

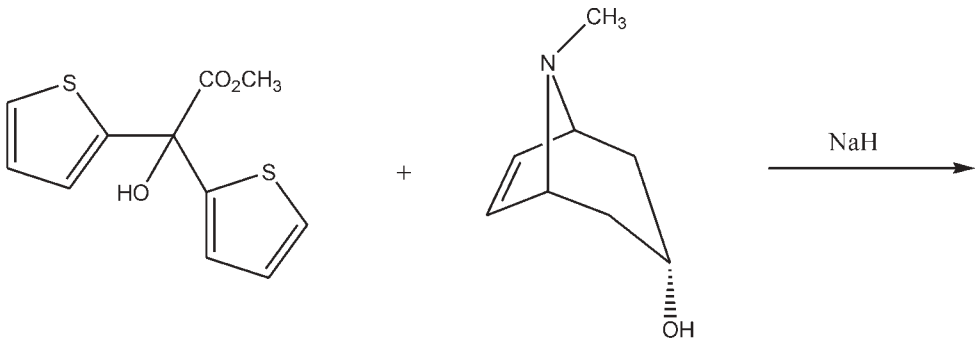
Tropisetron **60**, launched in 1992, is an important synthetic antiemetic (A 04 A4 03) applied as an auxiliary in cancer chemotherapy and radiotherapy. It is obtained by chemical esterification of tropine with indoyl chloride, in the presence of butyllithium [79]. Benztropine (**61**, antiparkinsonic agent used as mesylate) and deptropine (**62**, antihistaminic, applied as citrate) are tropine benzylic ethers, obtainable from **8** by one-step alkylation (Scheme 14).



Scheme 14. Some synthetic tropine therapeutics (homatropine, tropisetron, benztropine, deptropine)

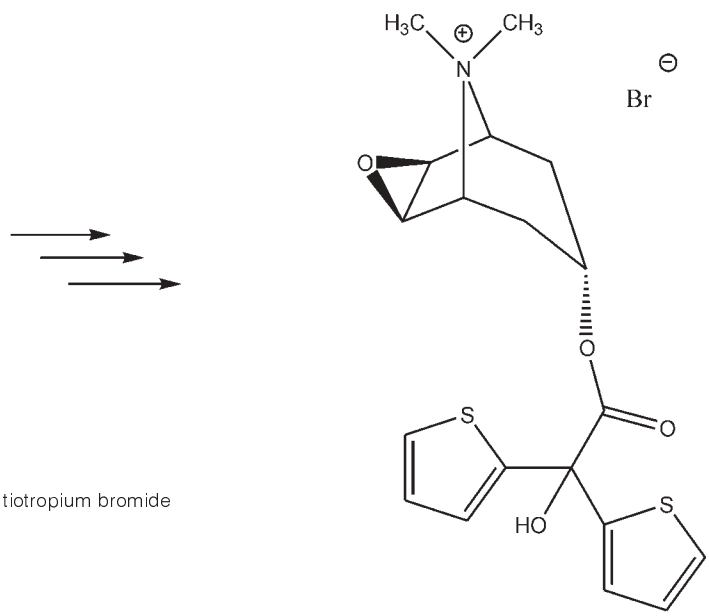
Ipratropium bromide, a synthetic bronchodilator **55** (syntheses discussed in the previous paragraph) (R 03 BB), is a growing product with ca. 2,000 kg of API manufactured annually and corresponding value of preparations exceeding $ 1.7 bln!

Even more dynamic and promising is market performance of tiotropium bromide, another synthetic bronchodilator **58**, launched in 2002, which is used for maintenance treatment in patients with chronic ob-



56 Methyl bis(2-thienyl)-glycolate

57 6,7-Dehydrotropine



Scheme 15. Synthesis of tiotropium bromide

58 Tiotropium bromide

structive pulmonary disease. The drug is relatively long-acting, which allows for patient friendly therapy, based on once daily application of 18 μg dose from a dry powder inhaler. This therapeutic regimen compares very favorably with ipratropium, which is applied four times daily. Market data indicate that tiotropium makes globally 1.2 bln $ out of 12 kg of the active substance produced annually [1, 93]. Synthesis of **58**, using a thiophene analog of benzylic acid as the tropine esterifying moiety [39, 63], is presented in Scheme 15.

As quality management system became an integral part of pharmaceutical management, analytical methods for tropane alkaloids, both as single constituents and also in mixtures of alike compounds, attained high level of sophistication. Various chromatographic separation techniques, coupled with multiple mass spectroscopic detection can assure quick sample composition determination, needed for police investigation in suspected drug trafficking, trace analysis for forensic purposes, metabolite identification and quantification for pharmacokinetics, or impurity profiling for pharmaceutical studies [8, 11, 40, 58, 59].

Although the shade of illicit cocaine industry constitutes a serious problem for global healthcare and economy, there is no reason why closely structurally related derivatives of tropine and ecgonine should not be discussed jointly, in terms of pharmacological and

pharmacogenetic research and development. The research tools and methods used for fighting cocaine addiction are basically the same as used by medicinal chemistry focused on new drug discovery. Development of new drugs for respiratory alignments in recent years proves a good potential of tropines outside of fields of their traditional use. Commercial availability of intermediates: tropanone, tropine and nortropine, facilitates the use of these synthons instead of agricultural raw materials. Additionally, new, useful chemical transformations presented above, offer good prospect for designing new tropane-containing entities, for targeting more specific physiological phenomena than cholinergic transmission in general. Thus, progress in stereoselective chemical synthesis continues to provide principal driving force for development of this traditionally important line of medicinal products.

Conclusions

Plant-derived tropane metabolites made rich contribution to history of medicine – from antique homicidal poisons, through cornerstone test substances for physiological experiments, to a collection of modern pharmaceuticals. Principal individual tropane alkaloids, known for nearly 180 years, exert pleiotropic physiological effects in humans and experimental animals. They are now relatively well recognized and classified in terms of molecular pharmacology. Several of these compounds are among the prime textbook examples of important medicines derived from natural sources. Although their biological actions are nonselective, they are still applied today for many therapeutic indications. Intensive research on tropane alkaloids chemistry and pharmacology laid in the past foundations for important chapters of medicinal chemistry and has consequently led to new generations of structurally distinct synthetic drugs (e.g. local anesthetics and spasmolytics). Now, examples are gathered for their novel medicinal uses, illustrating new capacity of tropane derivatives, crossing beyond traditional cholinergic MR targeting classification. Facts from different scientific disciplines and fields of research were combined in this survey, in an attempt to illustrate how thorough chemical research, supported by modern analytical technology, facilitate progress in pharmacology, providing not only new drugs but also much needed molecular probes for testing novel mechanisms of biological action.

Today, industrial manufacturing operations providing tropane-containing API are based largely on agricultural technology, close to traditional field plant cultivation. Both, advanced biotechnological and chemical methods of tropane derivative synthesis have in principle demonstrated their technical potential as an alternative to the plant material extraction. However, chemical synthetic procedures for tropane API manufacturing are already in place, with all needed data for scaling up, while biotechnological alternatives (e.g. hairy roots cultures with continuous secondary metabolite recovery) are still at the equipment and process design stage.

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