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ALKALOIDS DERIVED FROM HISTIDINE: IMIDAZOLE (PILOCARPINE, PILOSINE)

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Abstract This Chapter will cover the alkaloids containing the imidazole nucleus, the smallest group in terms of structure numbers, which are formed by precursors derived directly from the aminoacid L-histidine. The structures were divided in those using histamine as a precursor and one we called *Pilocarpus* alkaloids that contained a distinct precursor. The state-of-the-art on the biosynthesis of these alkaloids, biotechnological approaches for their production and their biological activity is also discussed.

Keywords Imidazole alkaloids, histidine derivatives, *Pilocarpus* alkaloids, plant sources, biosynthesis, cell culture, biological activities

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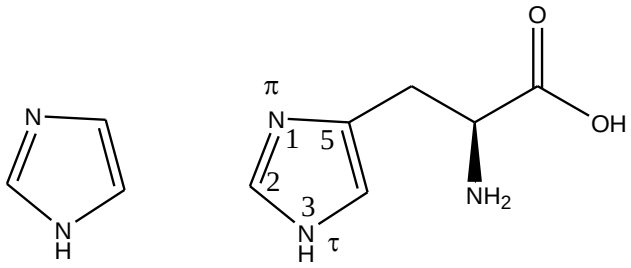
Abbreviations

^{13}C NMR	^{13}C Nuclear Magnetic Resonance
^{1}H NMR	1H Nuclear Magnetic Resonance
2,4-D	2,4-Dichlorophenoxyacetic acid
BAP	6-Benzylaminopurine
CNS	Central Nervous system
ESI-MS	Electron Spray Ionization – Mass Spectrometry
GA_3	Gibberellin A3
HDC	Histidine decarboxylase
HPLC	High Performance Liquid Chromatography
HPLC–ESI–MS/MS	High Performance Liquid Chromatography with Electron-spray Ionization Tandem Mass Spectrometry
HT	Histidine aminotransferase
IUPAC	International Union of Pure and Applied Chemistry
MS medium	Medium Murashige & Skoog
PEG	Polyethylene glycol

27.1 Introduction

Alkaloids form a wide group of secondary metabolites with a structural diversity comparable to that of terpenoids, representing *ca.* 20% of all known natural substances [1]. In spite of this abundance, proportionally few alkaloids contain the imidazole group (1).



1 **2**

In general, the nitrogen atom in alkaloids, normally forming a heterocyclic ring, is derived from amino acids. L-histidine (2) is the only amino acid that contains an imidazole ring (glyoxaline) (1), an aromatic heterocyclic group with one nitrogen atom

hybridized as in pyridine and the other one hybridized as in pyrrole. These two nitrogen atoms confer a relatively high basicity to the imidazole ring because the protonated species can be stabilized by resonance. This amino acid is considered the precursor of all alkaloids containing this ring system because of their close structural relationship [2].

About the nomenclature of histidine and its derivatives there has been some confusion for a considerable time, mainly concerning the numbering of atoms in the imidazole ring, biochemists generally numbered as 1 the nitrogen atom adjacent to the side chain, and organic chemists designating it as 3. In this chapter, we followed the IUPAC recommendation for numbering the nitrogen atoms in the imidazole ring of histidine. The nitrogen nearest the alanine side chain is called *pros* ('near', abbreviated π) and the farthest one *tele* ('far', abbreviated τ). This means that the pros-nitrogen atom (N^{π}) has position 1 and the *tele*-nitrogen (N^{τ}) position 3. Consequently, the carbon atom between the two ring nitrogen atoms is numbered 2 (as in imidazole), and the carbon atom next to the τ nitrogen is numbered 5. The carbon atoms of the aliphatic chain are designated α and β. This numbering is also used for the decarboxylation product histamine and for substituted histidine derivatives [3].

Battersby and Openshaw [4] wrote one of the firsts reviews on imidazole alkaloids. At that time, this group was even smaller and the discussion dealt mainly with pilocarpine and related alkaloids. Since the 1980's, the number of alkaloids containing the imidazole ring has increased mainly from marine organism such as mussels, sea urchins and sponges and in smaller number from plants [5]. These marine alkaloids will be discussed in a specific chapter from this Handbook (Chapter 40).

On the other hand, their occurrence in plants is restricted to some specific genera or even species from different families without a distribution pattern. The only exception is *Pilocarpus* Vahl. (Rutaceae) in which all the species are known to accumulate imidazole alkaloids. No new sources from these alkaloids from higher plant have been reported since 1990.

In this chapter we will only discuss about non-marine alkaloids which are originated directly from histidine and are nor simple peptide alkaloids. The alkaloids here discussed were classified, accordingly to their biosynthetic origin, in simple histamine derivatives and the so called *Pilocarpus* alkaloids which are originated directly from histidine but from different precursors.

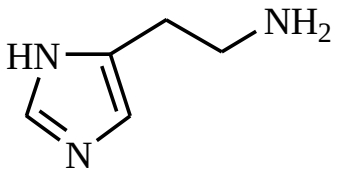
27.2 Hisditine derived alkaloids

Few imidazole alkaloids can undoubtedly be associated to L-histidine as biogenetic precursor. Nevertheless some of these are closely related to this amino acid despite lack of biosynthetic experimental data. In this chapter imidazole alkaloids were divided according to their chemical features as histamine and derivatives, simple histamine amides and *Pilocarpus* alkaloids. Plant species, isolation and detection are highlighted in this topic.

27.2.1 Alkaloids formed from Histamine and derivatives

27.2.1.1 Histamine and simple amines

Histamine is a biogenic amine formed by L-histidine decarboxylation mediated by HDC (histidine decarboxylase) (EC EC 4.1.1.22) and naturally occurs in some fungi, marine and plant species [6, 7]. It was isolated from aerial parts of *Capsella bursa-pastoris* (L.) Medik. (Brassicaceae), *Lolium perenne* L. (Poaceae) [8] and *Spinacea oleracea* L. (Chenopodiaceae) [7, 9]. This amine may act as an intermediate in many imidazole derivatives biosynthesis such as amides.



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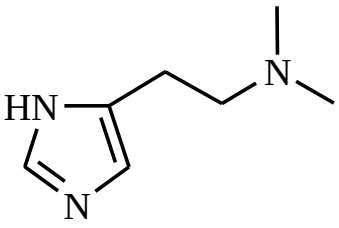
S. oleracea [7], *Echinocereus blanckii* Palm. and *E. triglochidiatus* Engelm. var. *paucispinus Engelm*. ex W.T. Marshall (Cactaceae) [10] provide N^{α},N^{α}-dimethylhistamine (4) in addition to other alkaloids. Extraction and isolation procedures were performed accordingly to classical phytochemical techniques, using partition, column and thin layer chromatography. *E. triglochidiatus* var. *neomexicanus* yielded 0.11% of this alkaloid with the following major mass fragment: 139 (8%) M^{+}, 95 (10%), 58 (100%).



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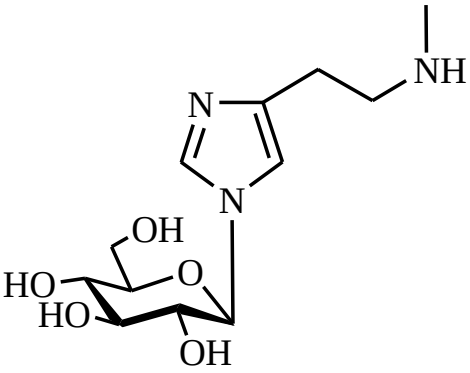
This imidazole alkaloid is also present in *Casimiroa edulis* Llave et Lex (Rutaceae), which also produces an glycosylated derivative of N^{α}-methylhistamine, casimidine (5), and the unusual alkaloid containing a sulphur atom, zapotidine (6) [8, 11-13].



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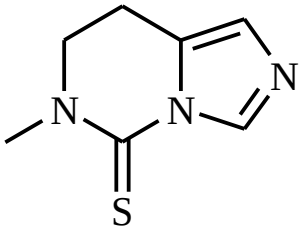

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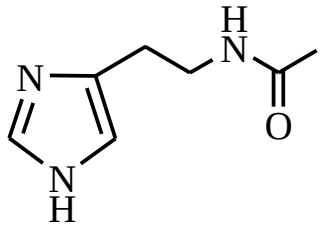
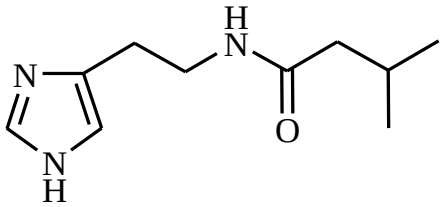
27.2.1.2 Simple histamine amides

The simplest histamine derivative from this group, N^{α}-acetylhistamine (7), was firstly identified by Appel and Werle in *S. oleracea* [7] crude alkaloid extract.



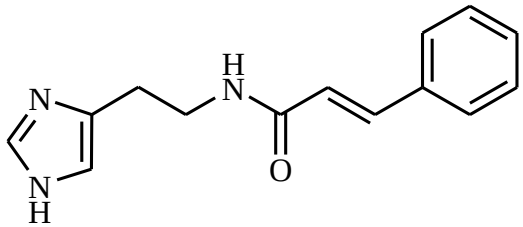
7

Also in this group, dolichotheline (*N*-isovalerylhistamine) (8) was isolated, in 1969, from a small cactus named *Dolichothele sphaerica* (Dietrich) Br. and R. [14, 15]. The alkaloid was obtained from crystallization from non-phenolic fraction with a benzene-acetone mixture. Identification was carried out by thin layer chromatography using chromogenic Pauly's reagent (for histidine and tyrosine metabolites) and spectroscopy methods. Structural identity of dolichotheline was provided by means of mass spectral data with molecular ion at *m*/*z* 195, and an *m*/*z* 85 and *m*/*z* 111 fragments due to the loss the isovaleryl and histamine moieties, respectively [14]. This was the first imidazole alkaloid reported to a Cactaceae plant and out of Jaborandi species.



8

N^{α}-cinnamoylhistamine (9) was isolated from several plant species such as *Argyrodendron peralatum* (Bailey) Edlin ex J.H. Boas (Sterculiaceae), *Acacia* spp. (Fabaceae) and *Glochidion philippicum* (Cav.) C.B.Rob. (Euphorbiaceae) [8, 16]. Using current analytical methods this alkaloid was isolated as colourless crystals (m.p. 178-179°C) and characterized as the *trans* isomer due to high coupling constant for the olefinic protons signs [16].



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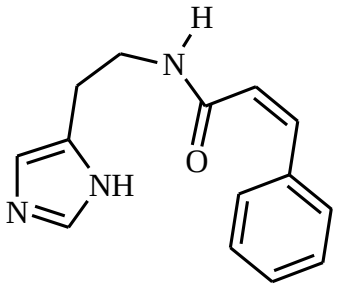
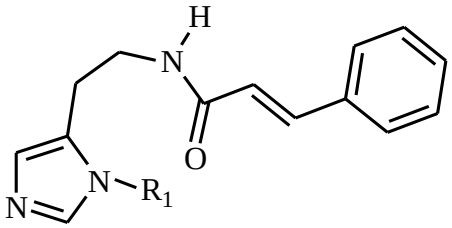
The *cis* isomeric form (9a) was not previously reported in former species but it was isolated from aerial parts of *Lycium cestroides* Schltdl. (Solanaceae) [17, 18]. By means of ^{1}H NMR spectroscopy the *cis-trans* isomers of N^{α}-cinnamoylhistamine were characterized as two quasi-bicyclic forms with a hydrogen bond between the carbonyl

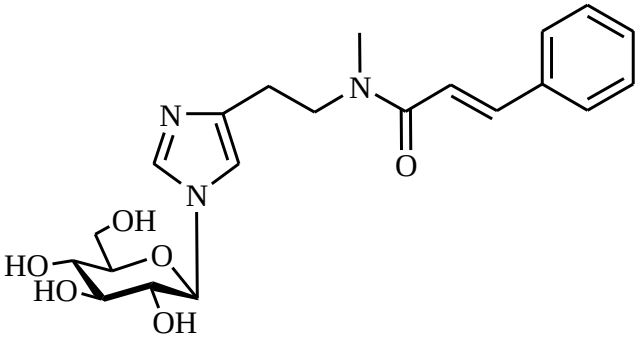
amidic group and the N^{π}-H imidazolic ring [17]. According to the authors, these isomers spontaneously undergo to lineal form especially the *trans* isomer (9b). Methyl derivatives were also isolated from crude ethanolic extract (10a and 10b). The Rutaceae species *C. edulis* affords casimiroedine (11) [11, 12], a glycosylated derivative of N^{α}-cinnamoyl-N_{π}-methylhistamine from *L. cestroides*.





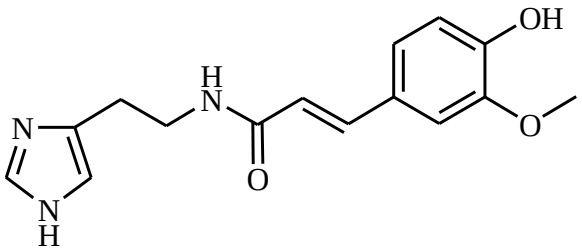
9a R_1 = H
10a R_1 = -CH_3

9b R_1 = H
10b R_1 = -CH_3



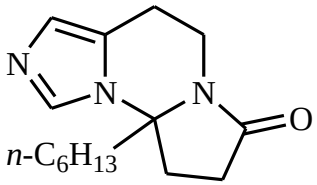
11

Ephedra spp. roots are known as major source of ephedrine and derivatives alkaloids but this plant species also contains an imidazole derivative, feruloylhistamine (12), which was identified in methanolic extract of the drug in 1983 [19, 20]. After column chromatography and crystallization procedures, feruloylhistamine was characterized by ^{1}H and ^{13}C NMR and mass spectroscopy. A molecular ion peak was observed at *m/z* 287 consistent to a $C_{15}H_{17}N_3O_3$ fragment and the ^{13}C NMR spectrum revealed signals for aliphatic and aromatic carbons, and also one carbonyl group. The synthetic derivative was also obtained and afforded the same physical data as the natural compound [19].



12

The isomers glochidine (13) and glochidicine (14) (both with a lactam ring), and *N*-(4-oxodecanoyl)histamine (15) were isolated from *G. philippicum*. Structure elucidation was performed by elementary analisys, ^{1}H and ^{13}C NMR and mass spectroscopy. The *m/z* 176 fragment, which corresponds to *n*-hexyl loss from C-12, was present in both isomeric forms [21].



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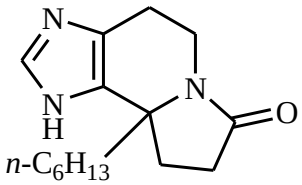

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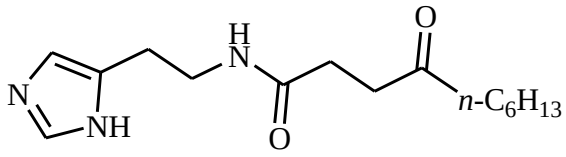
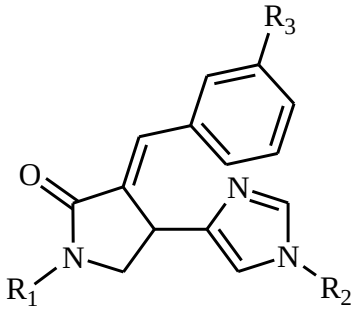
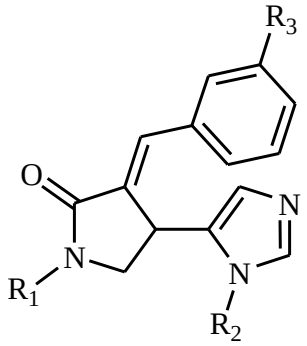


15

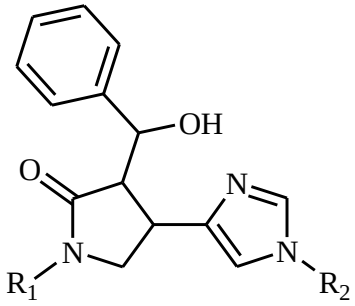
Another example of histamine amides that also contains a lactam portion are the alkaloids from *Cynometra* spp. (Fabaceae). Structurally they are very similar to that one found in *Pilocarpus* species. *C. hankei* Harms, *C. ananta* Hutch. & Dalziel, and *C. lujae* De Wild., are indigenous to west tropical Africa, in which were reported the presence of lactams derivatives in which occur isomerization at imidazole ring level such as anantine (16)/ isoanantine (19) and cynodine (18)/ isocynodine (19) [22]. As these alkaloids are only found in *Cynometra* species possibly they have a chemotaxonomic importance to this genus.



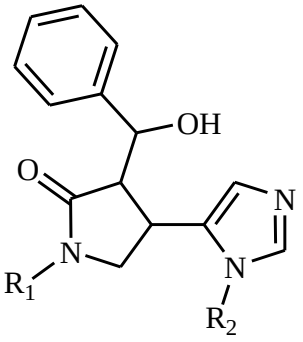
16 R_1 = H R_2 = -CH_3 R_3 = H
17 R_1 = R_2 = R_3 = H
18 R_1 = H R_2 = -CH_3 R_3 = OH



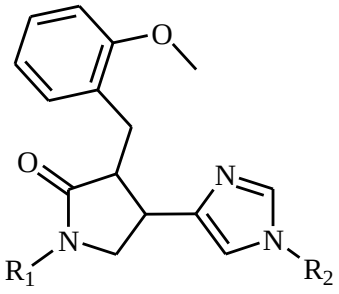
19 R_1 = H R_2 = -CH_3 R_3 = H



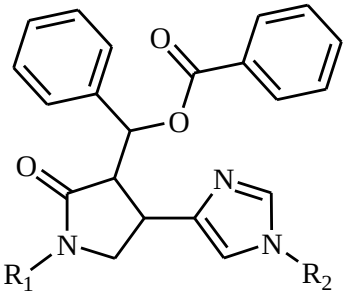
20 R_1 = R_2 = -CH_3
21 R_1 = -CH_3 R_2 = H



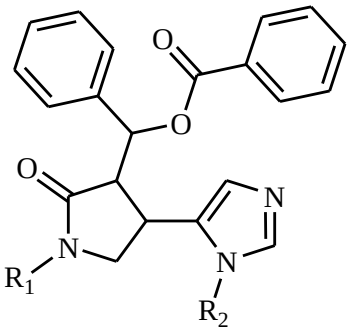
22 R_1 = R_2 = -CH_3



23 R_1 = H R_2 = -CH_3



24 R_1 = R_2 = -CH_3
25 R_1 = -CH_3 R_2 = H



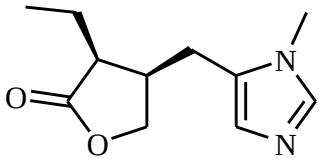
26 $R_1 = R_2 = -CH_3$

27.2.2 *Pilocarpus* alkaloids

This is undoubtedly the most important class of L-histidine derivatives only because pilocarpine (27) therapeutic uses [8, 23, 24]. Since Byasson's first report of Jaborandi alkaloids isolation from the leaves of *Pilocarpus* spp. (Rutaceae) [8], in 1875, several others imidazole alkaloids have been reported in this genus, most of them due to recent advances in hyphenated chromatographic techniques such as HPLC–ESI–MS/MS (high performance liquid chromatography with electrospray ionization tandem mass spectrometry).

Chemically, the majority of *Pilocarpus* alkaloids hold simultaneously an imidazole and a γ-lactone ring. Epimerization at C2 and C3 carbons of the lactone moiety is commonly observed and this change in absolute configuration, gives rise to some compounds that can naturally occur in *Pilocarpus* species or are generated during extraction procedures [23, 25].

Pilocarpine (27) was first isolated, in 1875, by Hardy and by Gerrard, in distinct works, as colourless crystalline salts from *P. jaborandi* Holmes leaves [8, 23]. This alkaloids in also found several other species such as *P. microphyllus* Stapf., *P. pennatifolius* Lemmaire, *P. racemosus* Vahl., *P. trachyllophus* Holmes and *P. carajaensis* Skorupa, where total pilocarpine content range from *ca.* 3.0 to 70% of total alkaloids. Isopilocarpine (28) is the epimerization product of pilocarpine produced by enolization pathway, accordingly to Döpke and d'Heureuse [26].



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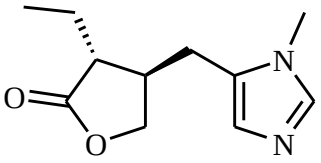

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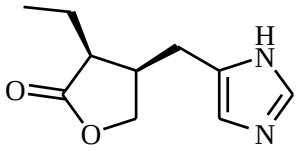
Initially pilocarpine structure was incorrectly attributed as a betaine derivative of a pyridine-lactic acid compound. Further research, based on chemical degradation, synthesis, and X-ray analysis provided pilocarpine structure as (2*S*,3*R*)-2-ethyl-3-[(1-methylimidazol-5-yl)methyl]-4-butanolide, as extensively described in previously reviews [8].

Pilocarpidine (29) and isopilocarpine (30) are the non-N^{π} derivatives of this series of compounds [8, 23, 24]. They were isolated in 1900, by means of classical phytochemical procedures, from *P. jaborandi*, which was confirmed as the only source of these alkaloids by HPLC–ESI–MS/MS [27-29].



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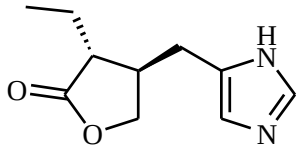
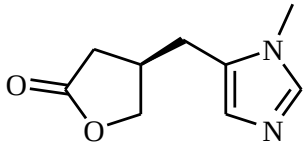


30

Pilosinine (31) is a natural occurring alkaloid in *P. microphyllus*, structurally it resembles pilocarpine (27), since it lacks the ethyl substituent at C2 [8]. This substance plays an important role in pilocarpine synthesis as a key intermediate as indicated by recent works [30, 31]. Tandem mass spectroscopy studies indicated that *P. carajaensis*, *P. spicatus* A. St.-Hil., *P. trachyllophus*, *P. pennatifolius*, *P. jaborandi* and *P. racemosus* accumulated 2,3-dehydropilosinine (32). Confirmatory fragmentation pattern was made by comparison with that described to the synthetic derivative.



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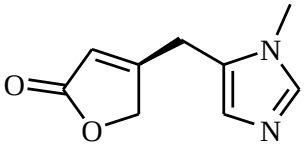
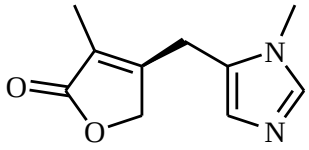


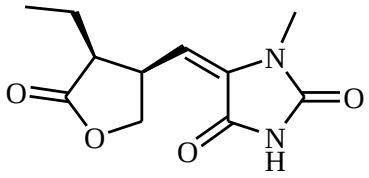
32

The alkaloid 13-*nor*-7(11)-dehydro-pilocarpine (33) was firstly isolated from *P. trachyllophus* [32] as a nitrate salt. Recently afforded, as mentioned before, by chromatographic techniques improvements, this substance was identified en five other species, namely, *P. microphyllus*, *P. carajaensis*, *P. spicatus*, *P. racemosus*, and *P. pennatifolius* [29]. As it was found in this genus, and could possibly participate in different biosynthetic routes.



33

The imidazole alkaloid 4,6-dehydro-1,2,4,5-tetrahydro-2,5-dioxopilocarpine (34) was isolated from *P. grandiflorus* Engl. stems, as an yellowish oil. Its structure was determined by high resolution mass spectroscopy (*m/z* 238) and ^{1}H and ^{13}C NMR that indicated a change in the structure of pilocarpine due to the signals at 177.7, 162.4, 152.4, 132.2 and 112.3 ppm, associated to two additional carbonyl groups and a double bond [33].



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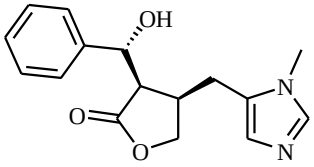
Seven isomers of molecular formula $C_{16}H_{18}N_2O_3$ are reported to *Pilocarpus* species, six out of them have proposed structures: pilosine (35), isopilosine (36), epipilosine (37), epiisopilosine (38) and, the N^{τ}-methyl derivatives piloturine (39) and epiisopiloturine (40) [29, 34, 35]. All they have a α-hydroxylbenzyl group instead of the ethyl substituent at C-2 in the lactone ring. Except for epipilosine (34) all these alkaloids were identified in *P. microphyllus*, *P. jaborandi* and, *P. carajanesis* species [29].



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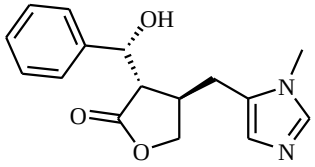

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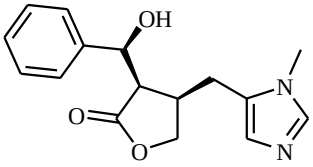

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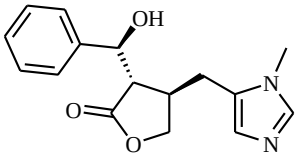

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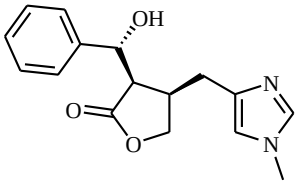

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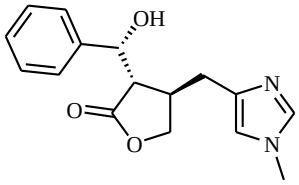

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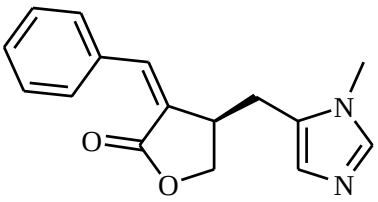
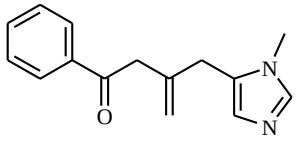
High resolution mass spectrometry of the total alkaloids of *Pilocarpus* species (*P. microphyllus*, *P. spicatus* and *P. carajaensis*) indicated the presence of three isomers of m/z 269. The fragmentation pattern was consistent of that from anhydropilosine (41) [28, 29].



41

As previously mentioned HPLC advances improved *Pilocarpus* alkaloids identification and several new derivatives of this class were characterized. Three protonated molecules (42) (*m/z* 241), (43) (*m/z* 259) and (44) (*m/z* 285) were identified in *P. microphyllus* ESI-MS fingerprints, which structures are shown below. These new compounds display a common and characteristic fragment of the benzoyl ion, $C_6H_5CO^+$ (ion of *m/z* 105). Screening studies in other *Pilocaprus* species showed (42) (and an isomer) were also present in *P. carajaensis*, *P spicatus*, *P. trachyllophus* and *P. racemosus* [27-29].



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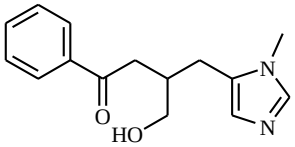

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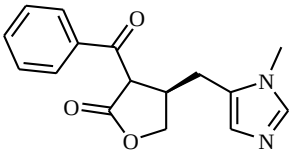

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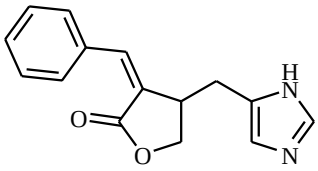
Some of these new *Pilocarpus* derivatives were found specifically in *P. pennatifolius* and *P. microphyllus* samples. Following compounds occur as isomeric forms in which absolute configuration was not elucidated [29].



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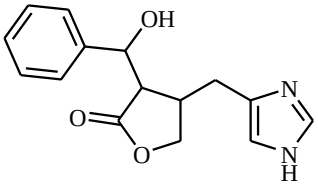

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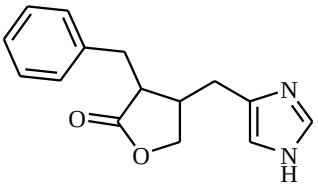

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27.3 Imidazole alkaloids biosynthesis

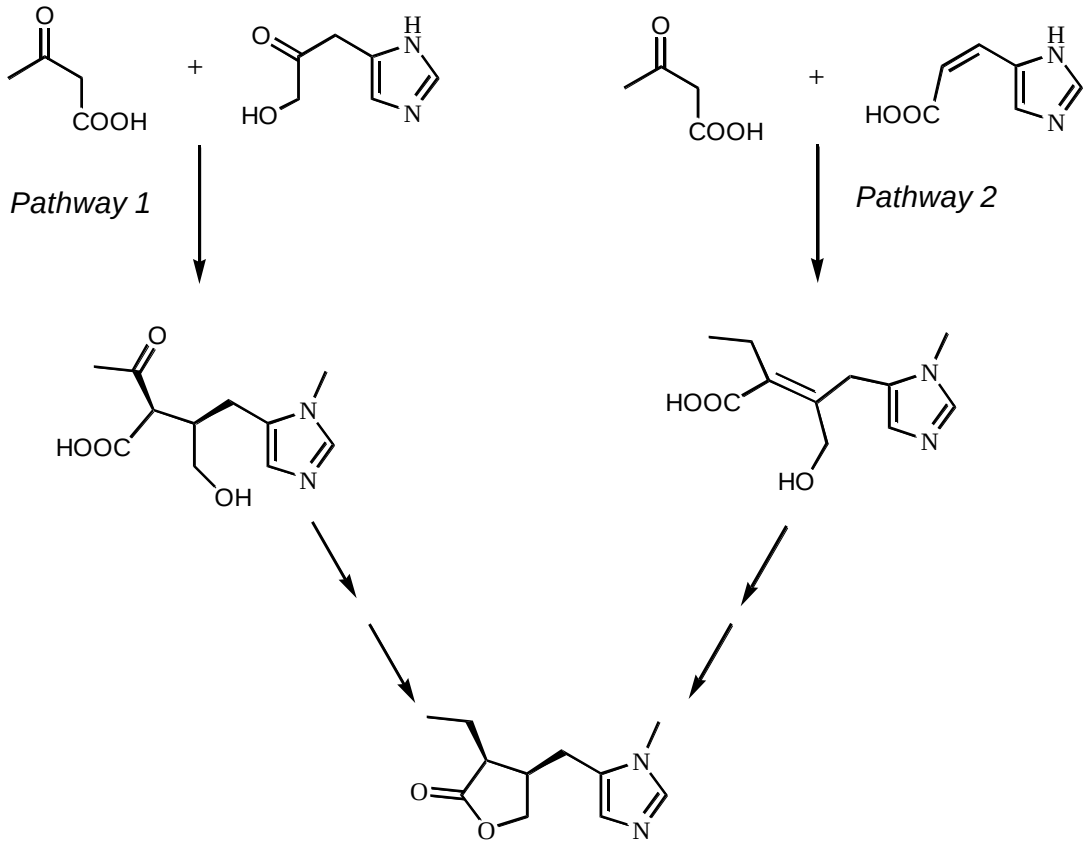
Alkaloids are important secondary metabolites with marked physiological effects and some of them with unmeasured pharmacological interest [23, 36]. It is a general belief that in plants alkaloids may be associated to protection against predators, or as nitrogen storage/transport and even be involved in physiological balance of the plant [23]. The biosynthesis of these nitrogen derivatives depends on the presence amino acid precursors (such as ornithine, nicotinic acid, tyrosine, anthranilic acid, and histidine), carbon units and also key enzymes that conjugate them [23, 36-37].

Biosynthetic studies have experienced great development since initial works on imidazole alkaloids biogenesis [36]. The use of isotopic labeled precursors, in the 1950's, is the first boundary in secondary metabolite biosynthesis. Cell cultures were also extensively used as tool to track active enzymes involved in alkaloid production. Advances in molecular biology have led biosynthetic researches to gene level, successfully used in genetic engineering studies guiding alkaloids or other secondary metabolites accumulation in plants [36, 37]. Despite these recent improvements in biosynthetic approaches, biogenesis of imidazole alkaloids remains obscure.

Only the biosynthesis of *Pilocarpus* alkaloids and dolichotheline, from *Dolichothele sphaerica*, were reported in literature as described below.

27.3.1 *Pilocarpus* alkaloids biosynthesis

The amino acid L-histidine is the assumed building block in the biosynthesis of pilocarpine (27) and other imidazolic alkaloids due to the presence of glyoxaline ring [8, 23]. First attempts to clarify this pathway were proposed by Boit and Leete that considered the phosphate derivative of 2-oxo-3-(5-imidazolyl)-propanol, also known as imidazole pyruvic acid, as imidazole ring precursor (Scheme 1- Pathway 1) [8]. This initial biosynthesis proposal was improved detailing the lactone ring formation by aldol condensation. Another biosynthetic approach suggested condensation of 2-oxobutyric acid (lactone moiety) with urocanic acid (imidazole moiety) (Scheme 1- Pathway 2) [8, 39].



Scheme 1

Isotopic studies with labeled potential precursors such as sodium acetate-^{14}C, threonine-^{14}C, histidine-^{14}C, histidinol-^{14}C and L-methionine-(S-methyl-^{14}C) were

carried out with *P. pennatifolius* in attempt to prove the proposed pathways [8, 39]. Only the methylation of pilocarpidine, last step in both mechanisms, was attested by significant incorporation of radioactivity in the methyl group attached to the imidazole nucleus. This data confirmed an important assumption, the one that consider methionine the biological source of the *N*-methyl group of pilocarpine. Also, one should consider that these radiolabeled studies were carried out with stems, no other parts of the plant were analyzed, and thus some other site involved in biosynthesis was not considered in this study.

The presence of the enzyme histidine aminotransferase, (HT) (EC 2.6.1.38) was reported in *P. pennatifolius* roots [40], which could link L-histidine to imidazole alkaloids biosynthesis and reinforce the hypothesis of a specific site for pilocarpine production. The enzyme activity has estimated in 46.09 $nKat.mg^{-1}$ of protein, and optimal reaction pH 8-9. HPLC data from enzymatic reaction indicated the formation of a product with the same retention time of the imidazole pyruvic acid standard obtained by synthesis. Such an enzyme activity could not be detected in *P. pennatifolius* leaves, reinforcing these organs could be involved only in the later steps of the biosynthesis, such as *N*-methylation, or could play a role as accumulation site not production.

A cell suspension study with *P. microphyllus* reported the obtaining of a stable line in pilocarpine and other imidazole alkaloids production, which means, the same alkaloid profile of that found in leaves, after several subcultures [41]. This study also characterized three new alkaloids with imidazole ring in this plant species for the first time, by means of ESI-MS. Posterior studies using the same technique in leaves of several samples of *Pilocarpus* suggested three different pathways may occur in imidazole alkaloids biosynthesis [27, 41].

Identification of the genes involved in imidazole alkaloids biosynthesis could be the next and final boundary in pilocarpine production in plants. Nevertheless, isolation from natural sources is still a feasible way to obtain this alkaloid.

27.3.2 Dolichotheline biosynthesis

Dolichotheline is an amide derivative from histamine of *Dolichothele sphaerica* (Cactaceae) which structure suggests the condensation of the imidazole unit with isovaleric acid [14, 15]. Its biosynthesis was evaluated by means of radiolabeled tracers such as DL-[2-^{14}C]histidine, DL-[2-^{14}C]leucine, DL-[2-^{14}C]mevalonic acid lactone, and

sodium [1-^{14}C]isovalerate [42]. The extension of radioactivity incorporation indicated that histidine was involved in dolichotheline biosynthesis and also that leucine or mevalonic acid could be the carbon unit donor, since both of them can be reputed as isovaleric acid precursor [43].

The enzymatic system that promotes the amide linkage between L-histamine and isovaleric acid in *D. sphaerica* is not specific, so aberrant or unnatural alkaloids (derivatives produced in the absence of natural precursors) can be produce. Different aberrant alkaloids were produced in labeled studies one could infer that this system is suitable to prepare biologically active natural products that are difficult to synthesize [44-46].

27.4 Biotechnological approaches

Plant tissue culture was initially used for growing isolated plant cells, tissues and organs under controlled conditions aiming regeneration or propagation of entire plants. This technique relies on cell totipotency, which means that single cells undergo differentiation, and thus regenerate an entire plant [47].

Secondary metabolites large-scale production by plant cell culture seems to be feasible and attractive to industrial production and it has two major advantages over traditional monoculture methods [48]: (1) controlled production of fine natural chemicals independent of climatic, edaphic, political conditions and (2) higher quality and yield of the final product in well defined systems. In recent years metabolic engineering has opened a new promising perspective for improved secondary metabolites production. This approach can be used to improve production not only in cell culture, but also in plant itself or other plant species even organisms.

Biotechnological approaches in imidazole alkaloids production are not so developed as other classes of secondary metabolites, and the studies deal exclusively with pilocarpine production. Nevertheless, the reported results do not lead to a profitable accumulation of this alkaloid, but are interesting approaches considering that *Pilocarpus* species are threatened plants.

27.4.1 Biotechnology in Pilocarpine production

Despite pilocarpine pharmaceutical importance and structural simplicity, synthetic approaches available for large-scale production are time consuming and do not achieve high yields. Even under ideal storage conditions *Pilocarpus* spp. leaves can lose at least half of their alkaloid content after one year via regular degradation routes [25, 49]. Alternative methodologies aiming increased production of this secondary metabolite are thus a major objective to fine chemicals industries.

First attempts to obtain pilocarpine in cell cultures were performed during 1990's. Seedlings and cell suspension cultures obtained from *P. microphyllus* species accumulate pilocarpine in a content of one-fifth to one-twentieth of the amount of naturally growing plant [50]. Hairy roots, obtained from genetic transformation by *Agrobacterium rhizogenes*, exhibit about the same or greater biosynthetic capacity for secondary metabolite production compared to their mother plants. Thus a hairy root culture of *P. microphyllus* was established in a Murashige and Skoog medium supplemented with 2,4-D and kinetin as growth regulators. Pilocarpine was accumulated in cells and also excreted to the medium in which the amount of alkaloid varied from 300 to 500 μg/g and were equivalent of the original plant [51].

Micropropagation of *P. microphyllus* seedlings were carried out in the lack of GA_3 caused a higher percentage of germination and a lower rate of contamination [52]. The apical segment was the most efficient for shoot emission with best average length under different concentrations of BAP and different combinations of zeatin and kinetin.

P. microphyllus seedlings were subjugated to different stressing factors and elicitors, such as hypoxia, salt stress, wounding, nutrient (nitrogen/ potassium) omission, salicylic acid and methyl jasmonate [53]. A time of exposure/ concentration relationship of salicylic acid and methyl jasmonate treatments increased pilocarpine accumulation over control in a 4-fold factor. Further conditions in other hand reduced pilocarpine production.

In *P. microphyllus* model callus were also subjected to conditions and elicitors in a MS liquid medium supplemented with 2,4-D as growth regulator [54]. Different nutrient concentration, pH values, type/ concentration of elicitors (histidine, threonine and methyl jasmonic acid) and osmotic and salt stress (PEG and NaCl) were evaluated. Light incidence influence was also analyzed. Pilocarpine was released in the liquid medium from calluses kept in the dark. Detection was carried out using HPLC-MS/MS techniques and pilocarpine quantification was performed by HPLC. Histidine and threonine elicitors induced highest accumulation of the alkaloid in a time/ concentration

relationship. Light incidence and methyl jasmonic acid inhibited pilocarpine release to the medium.

This model was also used in the evaluation of stable cell lineages with the same alkaloid profile than that of *Pilocarpus* leaves [55]. After 12 subcultures cell culture imizadole alkaloids composition was similar to that of the leaves thus, one can infer that imidazole alkaloid metabolism in *P. microphyllus* cells is similar to that found in leaves. After 24 subcultures the overall alkaloid distribution remained indicating that cell cultures can be cultivated for long periods and, identification of three new imidazole derivatives suggested that this is a suitable model to biosynthesis studies. The effect of pH variations (from 4.8 to 9.8) on stable cell lineages changed pilocarpine/ pilosine distribution between the cell and the medium, and highest pilocarpine accumulation was reached at pH 8.8-9.8 [56].

Callus and cell suspension culture of *P. pennatifolius* was established using young leaves on a MS medium (pH 5.7) supplemented with 2,4-D and kinetine as growth regulators [49]. Light-green to yellowish coloured friable callus was obtained after six subcultures in solid media, and cell suspension culture was obtained (same growth regulators composition) afforded 35 g/L of biomass after 20 days of maintenance. The callus cultures and suspended cells accumulated, respectively, 1.01 and 0.045 µg/g dry weight of pilocarpine.

27.5 Biological Activity

Some authors do not consider histamine, (2-[5-imidazolyl] ethylamine), as an alkaloid because it is a universal compound, mostly it is regarded as a biogenic amine. However, histamine is the simplest derivative from histidine presenting a remarkable biological activity. Histamine is formed in living cells by the 1 rate-limiting step exothermic decarboxylation of L-histidine, catalyzed by HDC [6, 7, 57]. This compound was discovered in 1910 by Dale and Laidlaw, as an isolate from the mold ergot capable of not only stimulating smooth muscle contraction but also inducing a shock-like syndrome when injected into mammals [58].

Nowadays, histamine is classified as a natural body constituent and a mediator on anaphylactic reactions and it is implicated in several physiological functions, being one of the most extensively studied chemical compounds playing a central role in

inflammation processes, gastric acid secretion, and neurotransmission. The research with histamine receptors allowed the development of drug therapies specifically targeted for allergies, gastric ulcers, asthma, and anaphylaxis. The control of histamine receptors has also some applications in sepsis control, hemorrhagic shock, anaesthesia, surgery, cardiovascular disease, cancer, CNS disorders, and immune-mediated diseases [59-61].

Some histamine amides have been isolated from some cactus species also showed pharmacological properties. In general, cactus alkaloids belong to β-phenethylamines or to the related tetrahydroisoquinolines that are potentially psychoactive compounds. The studies with *Dolichothele sphaerica* started due to its ethnobotanical relationship with the hallucinogenic *Lophophora williamsii* (Lem.) Coult., commonly known as peyote [62]. The chemical investigation of this cactus resulted in the isolation of the unusual imidazole alkaloid dolichotheline (*N*-isovalerylhistamine) [14], but no pharmacological studies were carried out with the isolated alkaloid.

Feruloylhistamine has also been isolated from the underground part of *Ephedra* plants, which was considered the hypotensive principle of the crude drug "maō-kon" [19]. Based on this finding, some feruloylhistamine analogues were prepared and examined their hypotensive and some other potential pharmacological properties. The feruloyl group in the amide was changed by a caffeoyl, cinnamoyl, *p*-coumaroyl and synapoyl substituent maintaining some hypotensive activity but much lower than that of histamine itself. Among the analogues, *p*-coumaroylhistamine presented a remarkable histidine decarboxylase inhibitory activity which was comparable with that of α-fluoromethylhistidine, a reference inhibitor for this enzyme. Consequently, it was expected that this analogue might decrease the gastric acid release, leading to new antiulcer drugs. However, *p*-coumaroylhistamine exerted no antiulcer activity either in the anti-stress ulcer formation assay or in the anti-acid secretion test. All the feruloylhistamine analogues also exhibited some antihepatotoxic activity. Caffeoylhistamine was the analogue that presented the most significant antihepatotoxic activity in the two model assays [20].

The most important pharmaceutical alkaloids in this group are the so called *Pilocarpus* alkaloids, mainly pilocarpine, which is used as an ophthalmic cholinergic drug. Pilocarpine and other related alkaloids were isolated from several *Pilocarpus* species which are popularly known by the name of "jaborandi" which was derived from

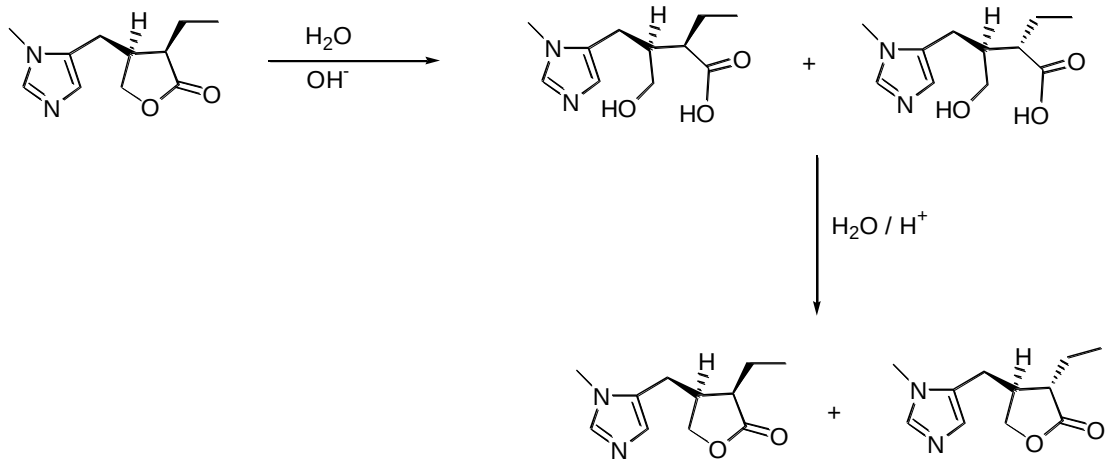
the Tupi-Guarani language, *ya-mbor-endi* (the one who causes slobbering). The first record in Europe on the use of these species was made in 1570 by Gabriel Soares de Souza, a Portuguese explorer who travelled through Bahia, describing that the indigenous population used these plants to treat mouth ulcers. Adolf Weber was the responsible for introducing pilocarpine as a medical treatment for glaucoma in 1877, soon after its isolation by Hardy and Gerrard in 1875 [63].

Pilocarpine is a parasympathomimetic agent that binds no selectively to muscarinic receptors and exerts a broad spectrum of pharmacological effects, which include stimulation of bladder, tear ducts, sudoriferous and salivary glands. These effects make this alkaloid the drug of choice in glaucoma treatment, because it increases the intraocular aqueous outflow through the trabecular meshwork by contracting the ciliary muscle and a mechanical pull on the trabecular meshwork [64]. Pilocarpine is also used as a cholinergic sialogogue to treat xerostomia, a subjective feeling of a dry mouth, which is relatively common in patients on chronic hemodialysis and after radiotherapy treatment of head-and-neck cancers. The beneficial effect of pilocarpine on xerostomia probably can be attributed to the stimulation of the salivary glands function [65, 66].

Sjögren's syndrome is a systematic autoimmune disease characterised by dysfunction of the lacrimal and salivary glands, its therapy remains largely empirical and symptomatic targeting, mainly the alleviation of the dryness symptoms. The stimulation of muscarinic M3 receptors to increase aqueous secretions by pilocarpine is the cornerstone of the current therapy [67, 68].

Systemic or intracerebral administration of pilocarpine hydrochloride in high-doses induces seizures in rodents. These seizures are characterized by a sequential development of behavioural patterns and electrographic activity [69]. The pilocarpine seizure induces *status epilepticus* as in humans mimicking the pathogenesis and progression of mesial temporal lobe epilepsy [70]. It can be useful to exploit these model properties to design new therapeutic approaches for treating refractory epilepsies

Isopilocarpine coexists with pilocarpine in nature, in aqueous solutions pilocarpine can hydrolyze to both pilocarpinic and isopilocarpinic acids. The latter, in acidic pH, can recyclize to isopilocarpine (Scheme 2). The contrary does not happen, because the isopilocarpine hydrolysis yields only isopilocarpinic acid [25]. This change in the spatial structure decreases the binding affinity of isopilocarpine to approximately one-tenth that of pilocarpine in bovine muscarinic cholinergic receptors [71].



Scheme 2

Epiisopiloturine (40) was found in the leaves of *P. microphyllus* presented an effect against *Schistosoma mansoni*, causing death on schistosomes from several ages at doses from 150-300 μg/mL and was also able to inhibit in 100 % worm egg laying at 100 μg/mL with no cytotoxicity to mammalian cells [72].

Although more than a dozen alkaloids have been isolated from *Pilocarpus* spp., the majority has not been screened for biological activities. Besides their structural resemblance, several are epimers, only pilocarpine posses a relevant CNS activity.

27.5 Concluding Remarks

Imidazole alkaloids are one of the smallest groups within alkaloids. This group does not have a defined distribution pattern among the plant species as observed for other groups, the only genus where all species accumulate imidazole alkaloids is *Pilocarpus*. Although the medicinal value of pilocarpine is widely recognized and the fact that the control of histamine receptors might have an important role in several therapies, the majority of the imidazole alkaloids have not yet received a thorough investigation.

The biosynthetic route leading to imidazole alkaloids is a puzzle to be solved. These alkaloids are most likely formed from L-histidine, either by its decarboxylation or deamination. The biosynthesis of those alkaloids derived from histamine, decarboxylation route, is already well established. On the other hand, the biosynthesis of *Pilocarpus* alkaloids not yet completely elucidated. These alkaloids are thought to be

formed by the deamination route having either imidazole pyruvic acid or urocanic acid as precursors. The hypothesis that the former acid is in fact the initial precursor in pilocarpine biosynthesis was supported when only HAT activity was detected in *P. pennatifolius* roots. However, the identification of new alkaloids in *P. microphyllus* cultures suggested that other biosynthetic pathways might be involved, making this an interesting field to be explored.

Acknowledgment

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