Historical and Current Perspectives of Neuroactive Compounds Derived from Latin America

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Abstract: Plants and invertebrates in Latin America have contributed to a great extent in the use, discovery and development of novel neuroactive tools. Significantly, these neuroactive drugs have proven to be particularly important for our current understanding of the physiology and pharmacology of the nervous system. In addition, these discoveries have helped to build the modern and successful pharmacological business that we know today. For example, curare helped to introduce the use of muscle relaxing agents into modern surgical techniques. The discovery of cocaine from the leaves of Peruvian coca plants was instrumental in the discovery of local anesthetics. The search and discovery for useful neuroactive compounds derived from Latin America has also been ongoing in other areas and new applications for quinine, capsaicin and epibatidine were recently described. Besides these organic compounds, several peptides produced by spiders and other invertebrates to hunt their prey also induce effects in channels and membrane receptors at very low concentrations, indicating their high potency and selectivity. It is likely that new pharmaceutics will be developed from these molecules.

The interest to renew the search for new compounds is timely, since largely unexplored lands, such as the Amazon and Patagonia, hold an important number of plants and animals that contain exciting new active compounds. With the introduction of new techniques to isolate, identify and characterize the molecular targets and actions of chemical entities, together with the need for more potent and selective compounds to treat neurological conditions, it is necessary to broaden the current exploratory effort in order to find more beneficial uses.

Key Words: Capsaicin, d-Tubocurarine, Cocaine, Epibatine, Batrachotoxin, Sodium channels, Calcium channels, Acetylcholine receptors, Neurotoxins.

INTRODUCTION

Since ancient times plants and animals have supplied a large number of useful neuroactive compounds. Species that are native to Latin America have contributed to a great extent in the use, discovery and development of novel neuroactive tools. For example, indigenous people from the Americas used neurotoxins such as curare, batrachotoxin and histrionicotoxin impregnated on hunting weapons in order to paralyze their prey. Furthermore, these neuroactive drugs have proven to be particularly important for our current understanding of physiology and pharmacology. For example, curare served as a prototype compound to determine the existence of receptive biomolecules (receptors) in cell membranes and introduced the use of muscle relaxing agents into modern surgical techniques. The discovery of cocaine from the leaves of Peruvian coca plants was instrumental in the discovery of local anesthetics, without which our visits to the dentist would be a complete nightmare. The search and discovery for useful neuroactive compounds derived from Latin America does not stop here since new applications for other substances such as quinine, capsaicin and epibatidine have been recently described.

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This review will deal with the more significant data on neuroactive compounds derived from the New World, primarily from Latin America, with special emphasis on how they have contributed to the development of new medicines. We will first analyze classical organic non-protein compounds followed by compounds of peptidergic nature.

THE TARGETS

The nervous system is capable of receiving, integrating and elaborating responses by means of transmitting electrical signals between different brain regions. To integrate all this information in the nervous system, the signal needs to cross from a presynaptic to a postsynaptic neuron by means of a structure known as a *synapse* composed of presynaptic vesicles which contain excitatory or inhibitory neurotransmitters. The neurotransmitter interacts mainly with postsynaptic receptors producing a local response, which facilitates or inhibits the initiation of a self propagating action potential. Since neuroactive compounds can interfere with nerve impulse conduction or neurotransmission, this review will put special emphasis on differentiating compounds that interfere with ion channels or synaptic receptors (Fig. **1**). Finally, we must stress the idea that the interest in characterizing the molecular targets for neuroactive compounds has been renewed since the introduction of modern methodologies such as molecular biology, patch clamp techniques and analytical and combinatorial chemistry.

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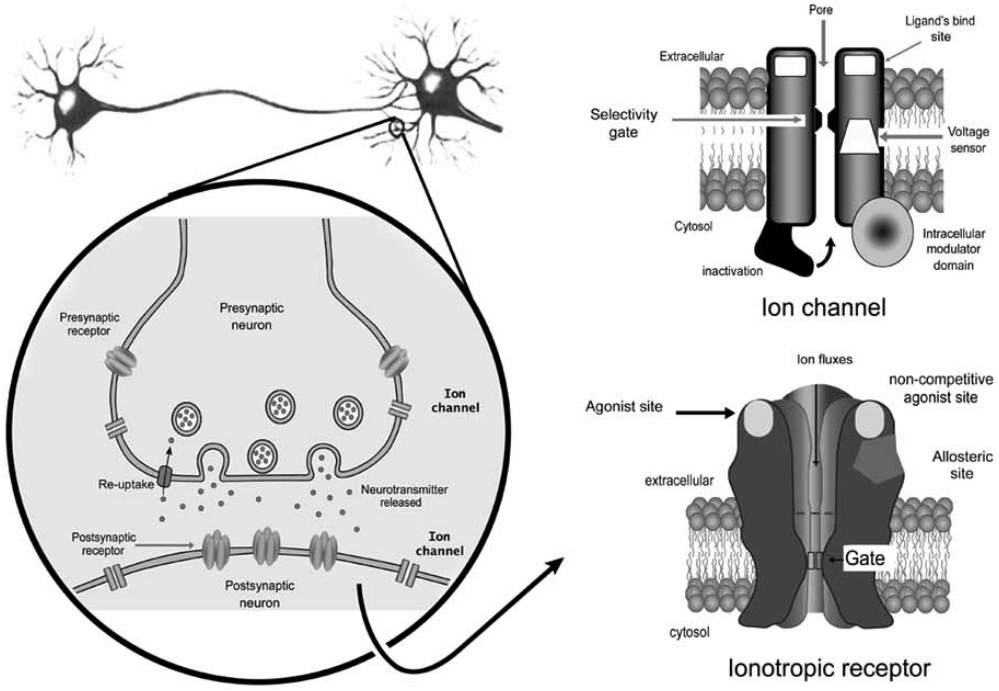

Fig. (1). Left, schematic representation of neuronal synapses and some structures involved in synaptic transmission. Right, voltage-dependent ion channel (upper) and ionotropic receptor (bottom) through their modulatory sites represent important targets for neuroactive compounds.

NON-PEPTIDERGIC NEUROACTIVE COMPOUNDS

Cocaine

Leaves from the *Erythroxylon coca* plant have been used for centuries in mountainous regions of Bolivia, Peru and Argentina to increase resistance to physical exertion. The leaves from this plant were taken to Europe in 1850 where the alkaloid cocaine (1) was isolated (Fig. **2**). The use of this compound as a local anesthetic occurred in 1884 at the hands of Kart Koller during an ophthalmologic intervention. It would later be used in dentistry and neurosurgery. Cocaine has an anesthetic effect due to its blocking action on voltage-dependent Na^+ channels (see Fig. **3**) [1], a characteristic that transformed cocaine into a chemical prototype for the generation of a whole family of local anesthetics [2], thus largely eliminating the clinical use of cocaine. Evidence shows that the affinity of cocaine, and other local anesthetics, for Na^+ channels is dependent on channel state since they can only reach their site of action when the channel is open and display a higher affinity when it is in its inactivated state [3-6]. It has been determined that transmembrane S6 of domain IV (D4S6) of the Na^+ channel plays an important role for the binding of local anesthetics [7]. More recent data has also shown the role of residues from S6 of domains I and III (D1S6 and D3S6) in the binding of local anesthetics [8-10].

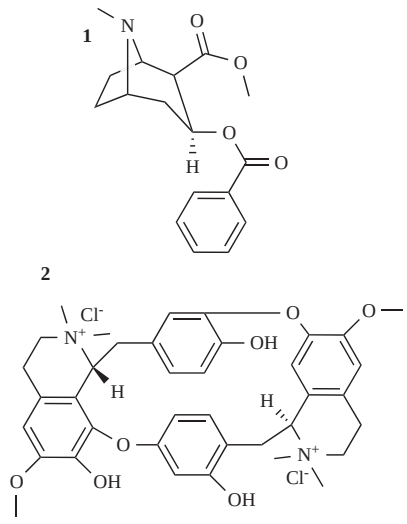

Fig. (2). Chemical structures of the parent natural compounds cocaine **(1)** and d-tubocurarine **(2)** used to develop local anesthetics and muscle relaxants.

At the central nervous system level, on the other hand, cocaine is a potent brain stimulant. Initial studies determined that the mechanism of action of cocaine was the inhibition of dopamine transporters (DAT), which leads to an increase in dopamine concentration in the synaptic space [11,12]. However, studies using DAT knockout mice have questioned whether this explains all the effects of cocaine [13,14]. Thus, it is now accepted that serotonin (5HT-R) and adrenergic receptors also play a role in the effects of cocaine and its derivatives [15,13]. Furthermore, the presence of intracellular regulatory events have generated a series of new questions regarding the mechanism of action of cocaine in the central nervous system [16,17]. Therefore, it is possible that derivatives of cocaine, with less addictive properties, could be used to stimulate the brain in conditions such as aging and attention deficit disorders.

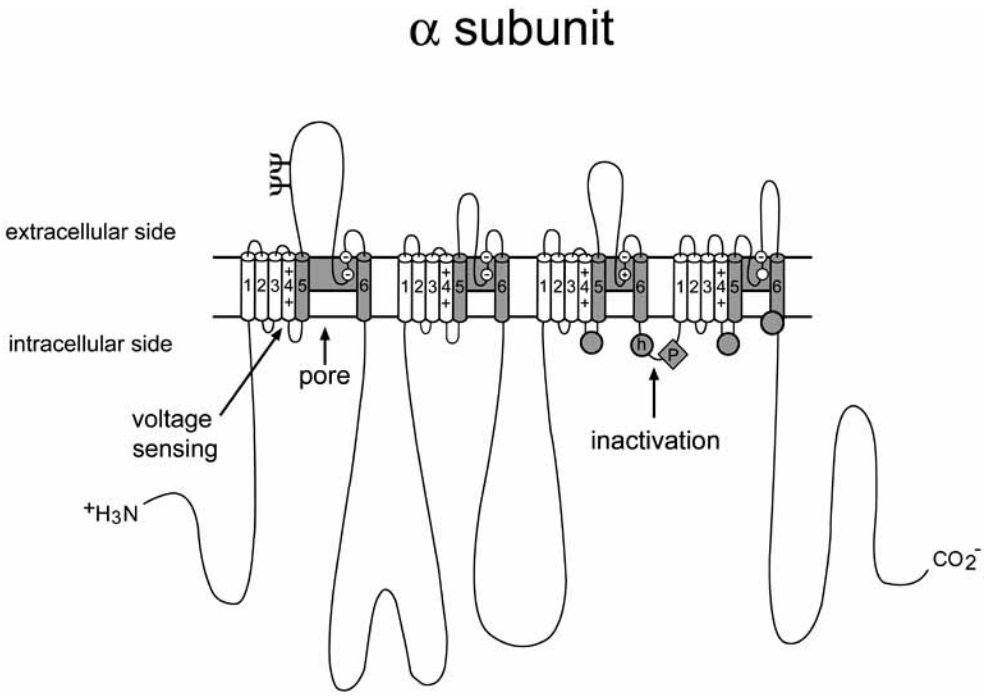

Fig. (3). Topology of sodium channel α-subunits. Each subunit has four transmembrane domains (D) and six transmembrane segments (S) with sites for local anesthetics, toxins and other pharmacologically important ligands. (Modified from Catterall, 2000).

Curare

Curare is a generic term for the poison from a plant known as *Strychnos toxifera* or *S. guianensis* (Fam. Loganiaceae), a chemical utilized for small animal hunting in South America. Classical experiments done by Claude Bernard (1856) led to the postulation of a sort of structural gap between the motor nerve and skeletal muscle. The early clinical uses for curare as a muscle paralyzing agent used in general anesthesia dates back to 1942 [18].

The characterization of the poisonous components of curare revealed the presence of d-tubocurarine (Fig. **2**), a dimeric alkaloid (bisbenzylisoquinoline alkaloid, (**2**)), and its chemical synthesis led to the development of a large number of curare-like agents. These agents produced inhibition of neuromuscular transmission by a competitive action on the acetylcholine (ACh) binding site in the nicotinic receptor [19]. Tubocurarine has two isomers where the d-isomer is several times more potent than the l-isomer [20]. Similarly, other curare-like agents such as cisatracurium have a much higher potency than d-tubocurarine (ED_{95} of 0.48 and 0.05 mg/Kg, respectively) [21].

It is known that the alpha subunit of the nicotinic receptor is fundamental for the binding of both ACh and d-tubocurarine. However, δ and γ subunits contribute to the correct conformational orientation of ligand binding [22,23]. It is suggested that three tyrosine residues (Tyr93, Tyr190, Tyr198) are important for determining receptor affinity [24]. For example, replacement of Tyr198 by Phe decreased tubocurarine potency [24]. Other amino acids, such as Cys192 and Cys193 are also important for the binding of d-tubocurarine to the receptor [25]. The W55L mutation in the gamma subunit results in an eightfold decrease in curare affinity (K_i from 20 nM to 170 nM), suggesting that this subunit modulates binding of tubocurarine to alpha subunits [26]. Depending on the receptor composition (γ or δ subunits), the inhibitory action of d-tubocurarine can vary between 29% and 55% [19].

The development of synthetic curare analogs have provided many paralyzing compounds, such as chondrocurarine, chondrocurine, chondodine, chondrofoline, curine, isochondrodendrine, L-beberine, L-tubocurarine and tomentocurine. Studies related to structure-activity relationships have demonstrated the effectivity of other curare-like compounds (fazadinium, decamethylene bisatropine, bis-tetrahydroisoquinolinium chloroformates), providing a better understanding for mechanisms of action of nicotinic receptor ligands and receptor structure [21].

Pumiliotoxins

There are more than 180 types of pumiliotoxins (PTX) which are divided into three main groups (A, B and C). The pumiliotoxins have been assigned code names, which consist of a boldfaced number corresponding to the nominal mass and a boldfaced letter for identification of individual alkaloids. The main toxin, pumiliotoxin B (**3**) or **323A** (Fig. **4**), is an alkaloid containing a bicyclic indolizine ring system with two double bonds and a side chain. It is isolated from the skin of frogs from the Dendrobatid family found in the neotropical forests of Panama. Due to its capacity to cause calcium mobilization, **323A** acts as a potent cardiotonic agent [27]. On the other hand, pumiliotoxin A (**4**) or **307A** (Fig. **4**) is much weaker as a positive cardiotonic alkaloid demonstrating the importance of the side chain hydroxyl in **323A** [28]. Besides pumiliotoxins, frog skin also contains allopumiliotoxins (**5**) that encompass a 7-hydroxy constituent.

In sympathetic neurons, **323A** (2 μM) induced repetitive action potential discharge, without changes on resting

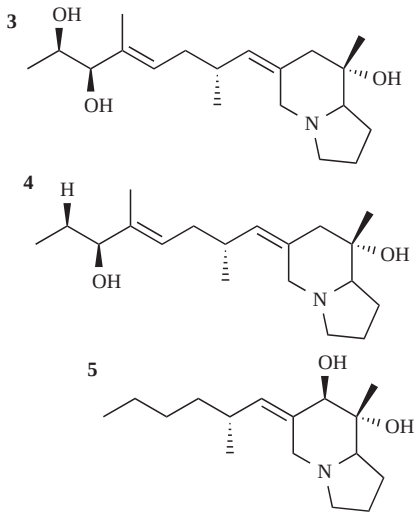

Fig. (4). Chemical structures of principal pumiliotoxins, **(3)** pumiliotoxin **323A** or pumiliotoxin B, **(4)** pumiliotoxin **307A** or pumiliotoxin A, **(5)** allopumiliotoxin.

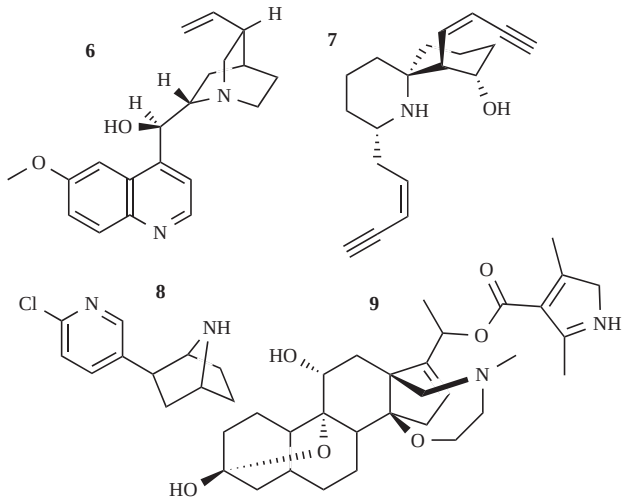

Fig. (5). Structures of **(6)** quinine, a modulator of K^+ and Na^+ channels, two AChR modulators **(7)** histrionicotoxin, **(8)** epibatidine and **(9)** batrachotoxin, a Na^+ channel blocking compound.

membrane potential [29]. The toxin induced a depolarizing after-potential accompanied by a burst of repetitive action potentials. In hippocampal neurons, it caused repetitive discharges at a concentration of 0.1 μM which was associated to a depolarizing shift on the activation curve for Na^+ channels [30]. In addition, **323A** increased the rate of single Na^+ opening and closing. The sodium channel is composed of α and β subunits and has several potential sites of interactions in S4 and the precise site of actions and mechanisms by which PTX modulates these channels is largely unknown. The binding of [^{3}H]-batrachotoxin or [^{3}H]-saxotoxin in the Na^+ channels are not affected by PTX [31].

A high concentration (160 μM) of PTX-C (cis-195A), on the other hand, was reported to decrease neurotransmission by blocking nicotinic ACh receptors in rat muscle [32]. The binding of ^{3}H-perhydro-histrionicotoxin (HTX), but not ^{125}I-α-bungarotoxin, was inhibited by PTX-C. These results indicate that PTX-C and HTX share similar binding sites in the ion channel associated to the receptor. PTX-C, however, is known to be minimally toxic and had been excluded from the pumiliotoxins [33].

Quinine

Quinine (**6**)**,** which is obtained from different plant species of the genus *Cinchona*, is indigenous to the eastern Amazon (Fig. **5**). After discovering that it was effective against malaria fever, it was introduced into the British Pharmacopoeia in 1677. The principal component of the cinchona tree is quinine, which is used in tonic water. Pharmacologically, it is still used in the treatment of malaria, but new applications have involved its use in the treatment of muscle spasms and leg cramps [34,35]. In addition, quinine has several interesting effects on the nervous system. Recombinant α9α10 nicotinic cholinergic receptors are blocked by low μM concentrations of quinine and its optical isomer quinidine [36]. The blockade of ACh-induced currents was a combination of competitive and non-competitive mechanisms. Not only are chemical synapses sensitive to quinine, but gap junction channels formed by connexins 26, 32, 40 and 42 are also blocked at concentrations of 32-73 μM [37]. A study using spiral ganglion neurons showed that quinine inhibits K^+ and Na^+ channels producing an increase in the duration of the action potential together with a reduction on the amplitude [38]. Quinine was found to block three different K^+ currents in sympathetic neurons with the rapidly inactivating K^+ current being the most sensitive to quinine (IC_{50}= 22 μM). In another line of experiments, it was reported that quinine inhibited epileptiform activity induced by picrotoxin, high K^+ or 4-aminopyridine in pyramidal slice preparations [39]. This result is interesting since it suggests an anticonvulsant potential.

Histrionicotoxin

Histrionicotoxin (**7**) (HTX, Fig. **5**) is the parent member of a family containing 11 compounds. This spiropiperodine cyclic alkaloid is isolated from the skin of a neotropical frog belonging to the Dendrobatidae family. The spirocyclic core of this alkaloid is unique. Two compounds, HTX and its perhydro derivative, show strong anti-cholinergic effects in the neuromuscular junction [40]. Their non-competitive mechanisms of action on the ACh receptor are not related to interactions with the binding sites of acetylcholine in the extracellular domain of the receptor. On the other hand, it is believed that HTX interacts with the ion channel associated to the receptor [41]. In addition, micromolar concentrations of HTX reduced the amplitude and decay of the action potential suggesting blockade of Na^+ and K^+ channels [42]. The effect of HTX is not restricted to peripheral nicotinic receptors, since it was shown that H_{12}-HTX inhibited the nicotine-activated neurotransmitter release from striatal and hippocampal nerve terminals [43]. Furthermore, HTX can also interact with nicotinic receptors in the chick visual system [44].

Interestingly, it was reported that a low concentration of HTX (1 μM) blocked the current induced by acetylcholine in motoneurones from the cockroach Periplaneta americana [41].

This finding suggests that this type of alkaloid, or a derivative, could be used as an insecticide.

Epibatidine

The alkaloid epibatidine ((**8),** Fig. **5**) (a 7-azabicyclo-[2.2.1]-heptane derivative) is a potent nicotinic-like compound isolated from the skin of *Epipedobates tricolor* (dendrobatideaceae), a native South American frog found in the southwest Ecuadorian Andes region and extending down to the mountainous areas of northern Peru [45]. It was later established that its mechanism of action involved activation of nicotinic receptors (R_N), which explained its reported analgesic activity [46,47].

Epibatidine is chlorinated in the 2´ of the heterocyclic ring (Fig. **5**) and contains three chiral centers (1R, 2R, 4S) and two enantiomers [48]. Both are more potent than nicotine (1.00 and 0.93 μg/kg for (+) and (-), respectively compared to 0.4 mg/kg for nicotine [46]), and they do not show differences in their affinities for the receptor [49,50]. Structure-activity relationship studies have shown that substitutions in N-H for methyl, ethyl or allyl groups did not significantly alter the affinity for the receptor. On the other hand, changing the 2´-chloropyrinidyl ring from an exo to an endo orientation or the addition of electron-donating groups in the 2´ position of 2´-chloropyridine ring decreased its affinity, indicating that the 2´-chloropyridine ring may play an important role in the biological activity of the compound [51].

Epibatidine and its analogs display a higher affinity for heteromeric AChR subtypes having the β subunit (see [51] for review). Its increased affinity for $\alpha_4\beta_2$ receptors, found at a high density in the mammalian CNS, is interesting because it would allow for a selective non-opioid analgesic agent [47], however its clinical use is not acceptable due to its toxicity [52]. Nevertheless, the ongoing search for less toxic synthetic derivatives would be advantageous especially with regards to reducing the consumption of live frogs [53]. For instance, a method to synthesize epibatidine derivatives has been described [54]. Although the clinical application of epibatidine as an analgesic agent is still under discussion, a new use for this compound as an imaging tool has been reported [55].

Batrachotoxin

Batrachotoxin (**9**) (20-α-2,4-dimethylpyrrole-3-carboxilate, Fig **5**) is the principal component found in the venom of *Phyllobates aurotaenia*, a native frog from the Columbian jungle [56], and is one of the most toxic alkaloids isolated up to date [57]. It is characterized as being a potent and irreversible activator of muscle and nerve Na^+ channels (Fig. **5**) having a lethal dosis (LD_{50}) of 2 μg/Kg as assayed in mice [56]. The efficiency of batrachotoxin has been shown to be dependent on both pH and temperature; being more effective in alkaline conditions and at a temperature close to 37ºC [58]. Batrachotoxin acts by shifting the activation curve towards more hyperpolarizing potentials which allows for the opening of Na^+ channels at the cell resting potential [59]. This modulation appears to be more effective when the channel is in the open state, thus stabilizing this conformation [60]. More recent studies have demonstrated that batrachotoxin generates a concentration-dependent increase in the Na^+ current (TTX resistant) associated to $Na_v1.8/\beta_1$ subunits [61]. Interestingly, the $Na_v1.8$ channel isoform is related to nociceptive transmission [62], and it was suggested that batrachotoxin binding sites overlapped with sites for local anesthetics in the DIS6 and DIVS6 regions of the α subunit [63].

Finally, it was recently reported that mutations in the S6 region of the subunit channel, which forms the permeation pathway, modified the sensitivity of the channel for batrachotoxin. For example, mutations in phe1710 reduced or eliminated the sensitivity of the channel for batrachotoxin [64]. On the other hand, replacement with cysteine (Phe 1710C) resulted in a rapidly reversible response [64].

Brevetoxin

Brevetoxin (Fig. **6**) belongs to a family of lipophilic toxins isolated from the hairs of *Karenia brevis* microalga found in the Gulf of Mexico. It causes massive death in fish and in humans causes *neurotoxic shellfish poisoning* characterized by tingling in the face, nose and fingers, as well as fever, chills, nauseas and muscle aches. Respiratory symptoms in humans can occur with pM concentrations, which demonstrates its high potency [65,66].

Studies have revealed the existence of 10 different toxins generically named Brevetoxins (PbTx1-10) and subsequently other related structures known as Brevenals (**12**), which display antagonistic properties. Brevetoxin B (**10**) (PbTx-2) and brevetoxin A (**11**) (PbTx-1) were characterized as the main precursors [67]. They are composed of 10 or 11 polyether rings which confer their high lipophilic properties. Four regions in the structure of these toxins have been identified and defined as: a "rigid region" essential for the binding of the toxin to a site (DIS6 and IVS5) in the Na^+ channel [66], a "spatial" region which separates the rigid region from the "head" ring which has lactone functions that modulate the inactivation and opening time of the channel [68,69] and finally the "tail" whose function is not well established.

It has been proposed that the general mechanism of action of this "toxin complex" is related to the proportions of the subtypes (PbTx1-10) and Brevenals (**12**), with the latter having a heterocyclic sequence of 6-7-6-7-7 atoms, contrary to the brevetoxins which have a 6-6-6-7-7-6-6-7-6-6-6 sequence. Its mode of action in the Na^+ channel is by binding at the S5 site of the α subunit producing a shift in the channel activation curve (similar to batrachotoxin) and by slowing inactivation kinetics or by increasing the channel open time [66].

Mescaline and N,N-Dimethyl Tryptamine

These two compounds (**13, 14**) are interesting because they have strong hallucinogenic properties. Mescaline (3,4,5-trimethoxyphenethylamine, Fig. **7**) is a phenethylamine [70] present in several cactus species, principally San Pedro (*Trichocereus/Echinopsis pachanoi,* Familia Cactaceae) found in the mountainous regions of South America. Mescaline is also present in Peyote (*Lophophora williamsii*), a species used by indians from Mexico and Texas for at least 5700 years [71]. On the other hand, N,N-dimethyltryptamine

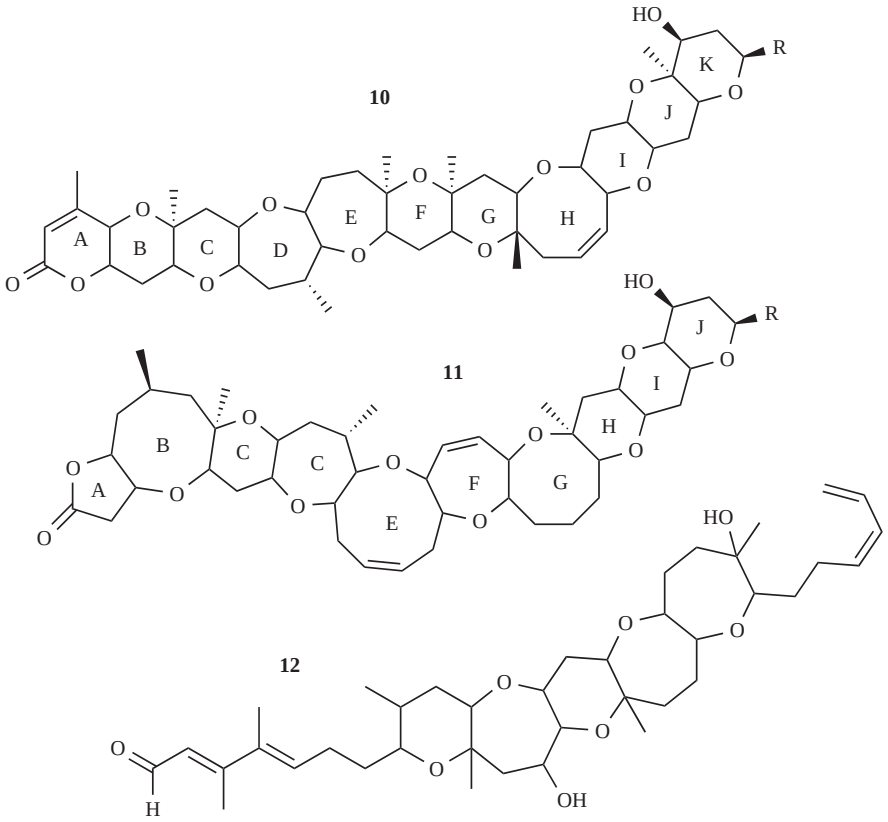

Fig. (6). Chemical structures of toxins derived from *Karenia brevis*. **(10)** brevetoxin B is characterized for a heterocyclic ring sequence of 6-6-6-7-7-6-6-7-6-6-6 atoms. **(11)** Brevetoxin A has a heterocyclic ring sequence of 5-8-6-7-8-7-8-6-6-6 atoms. **(12)** Brevenal, with the antagonist bone-structure of brevetoxins.

((**14**), DMT, Fig. **7**) is the active compound in *ayahuasca*, a traditional drink consumed by several Amazonian tribes, whose use has been established since the prehispanic period. The drink is made with *Psychotria viridis* whose long lasting effect is only induced when monoamine oxidase (MOA) is inhibited by the simultaneous use of another plant named *Banisteriopsis caapi*. Studies have revealed that the principal active compounds in this drink are DMT and several MOA inhibitors (MOA-I) such as harmaline, harmine and 1,2,3,4-tetrahydroharmine [72,73].

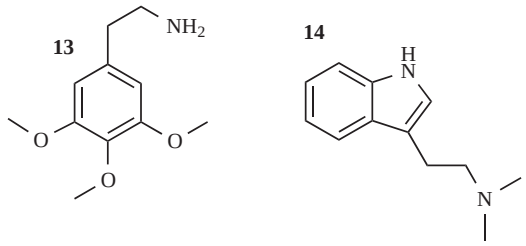

Fig. (7). Chemical structures of molecules with important hallucinogenic actions; **(13)** mescaline, **(14)** dimethyl-tryptamine.

Mescaline was the first psychedelic isolated by Arthur Heffter in 1896. The accidental finding regarding the hallucinogenic effects of a synthesized substance, D-Lysergic acid diethylamide (LSD), by a chemist named Albert Hoffman, and the later discovery (1948) of serotonin in bovine blood serum [11], helped to elucidate that these hallucinogens stimulate serotoninergic receptors (5-HT) in frontal cortical neurons [74]. Studies with mescaline are few in number, but it is known that its effects are accompanied by a "hyperfrontal pattern of increased blood flow", which correlates to mescaline-induced psychological effects [75,76]. A common effect of hallucinogenic compounds is the sensitization of serotonin and norepinephrine receptors. Thus, it is suggested that a mechanism of receptor sensitization might account for the altered perception produced by hallucinogens [74].

It has been determined that the molecular targets for mescaline and DMT are serotonin receptor types 2A and 2C, which are coupled to Gq proteins, whose activation increases the concentration of IP_3 and DAG (inositol 3-phosphate and diacylglycerol, respectively) [77]. Although it was postulated that DMT had antagonistic action in some types of serotonin receptors [78], a comprehensive pharmacological study using phosphoinositide hydrolysis assays showed that DMT acts as an agonist for $5HT_{2A}$ and $5HT_{2C}$ receptors with EC_{50}´s of 983 nM and 49 nM (Smith *et al*. 1998), respectively.

Mescaline served as the prototypical compound for structure-activity relationship studies linking molecular structure to hallucinogenic-like or psychedelic activity [79]. Thus, other mescaline-like drugs have been synthesized containing piperazine or homopipera-zine rings. It was found that the elimination or substitution of the amine groups in mescaline for these heterocyclic groups significantly modified

their activity, with both analogs (piperazine and homopiperazine) showing sedative activity [80]. The synthesis of a new class of psychomimetic compounds was based on structural modifications of the mescaline molecule where sulphur atoms were incorporated above the aromatic ring, and 4-n-propoxy, 4-n-butoxy and corresponding 4-thio analogs were incorporated in the lateral chain (ethylamine).

Capsaicin

Capsaicin (**15**) (8-methyl-N-vanillyl-6-nonenamide, Fig. **8**) is the pungent ingredient of hot *Capsicum peppers* (family *Solanacea*) and it was initially used by the Aztecs [81]. Capsaicin selectively activates a subset of small-diameter mammalian peripheral neurons in the cranial and dorsal root ganglia (DRG) [82,83]. Interestingly, capsaicin and its more potent congener resiniferatoxin (**20**) (Fig. **8**), itself derived from a *Moroccan cactus* [84], allowed first the identification and then cloning of membrane proteins that are activated by nociceptive stimuli [85]. Thus, today it is known that capsaicin elicits its main effects through the transient receptor potential vanilloid receptor (TRPV1), also known as vanilloid receptor 1 [85]. TRPV1 responds to multiple noxious stimuli including protons and heat [86,87]. These receptors are not only found in peripheral sensory neurons, but other tissues, such as spinal cord, hypothalamus, hippocampus, substantia nigra, kidney and urinary bladder also express TRPV1 receptors [88,89]. Activation of these receptors results in a rapid increase in intracellular Ca^{2+} with an EC_{50} close to 200 nM [91,92]. Interestingly, TRPV1 receptors in the brain might be a target for the endocannabinoid anandamide suggesting some kind of interaction with cannnabinoid receptors [93]. Remarkably, several biotechnological companies are trying to develop high affinity TRPV1 antagonists to treat chronic painful conditions [94]. In addition, capsaicin appears to be an effective model for Huntington´s disease, a disorder associated to the degeneration of dopaminergic neurons [94]. Overall, these exciting new data delineate potentially productive lines of research, all starting from a single hot pepper.

It is interesting that the application of capsaicin into substantia gelatinosa neurons produced an increase in spontaneous synaptic currents together with an inward current suggesting the presence of pre and postsynaptic actions [95]. Similarly, neurons in the medial preoptic nucleus of the hypothalamus were shown to respond to capsaicin with increases in glutamatergic and GABAergic transmissions. The effect was resistant to tetrodotoxin, but dependent on extracellular calcium and capsazepine (**16**), the receptor antagonist [96]**,** suggesting that TRPV1 receptors are present in these central neurons [97]. Similar conclusions supporting the presence of central TRPV1 receptors were reported in a study on rat locus coeruleus neurons [98].

Structure-activity studies examining increases of Ca^{2+} uptake in dorsal root ganglion revealed three regions important for the action of capsaicin on its receptor: aromatic and aliphatic regions at both extremes and a connecting ester or amide linker (Fig. **8**). A homovanillyl (3-methoxy 4-hydroxybenzyl) group in the aromatic region conferred maximal potency on VR1 receptors. The optimization of the

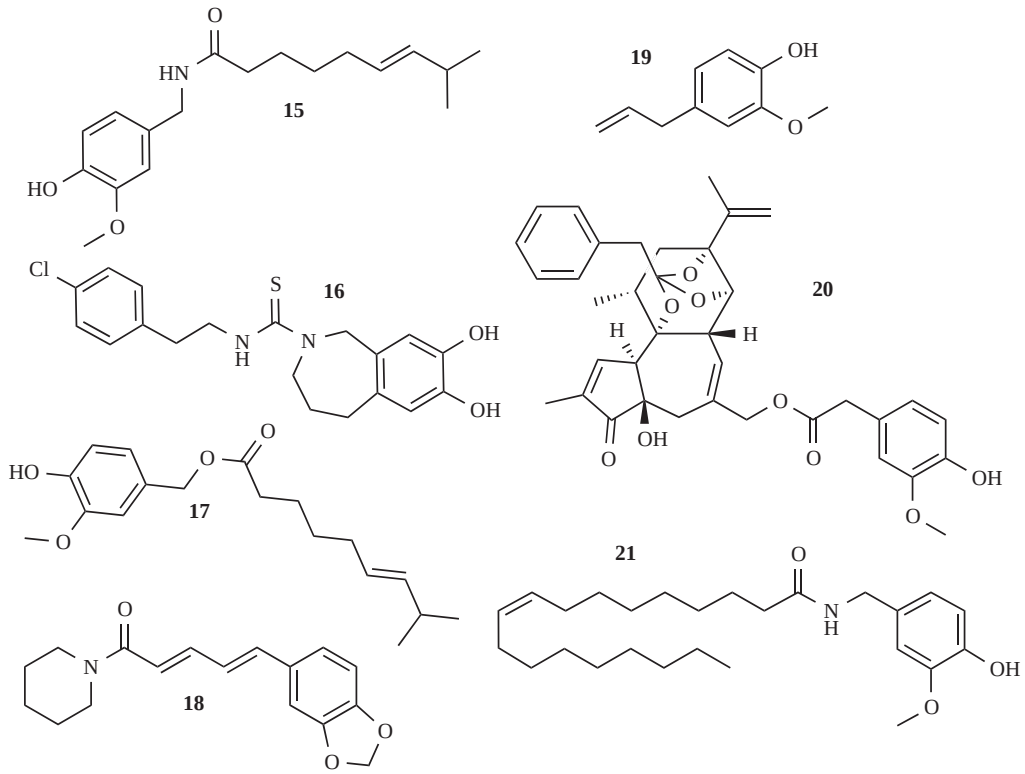

Fig. (8). Chemical structures of capsaicin-like agonists and its VR1 antagonist related to the transient receptor potential channel, vanilloid receptor (TRPV1). Capsaicin **(15)**, capsazepine **(16)**, capsiate **(17)**, piperine **(18)**, eugenol **(19)**, resiniferatoxin **(20)** and olvanil (**21)**.

hydrophobic region was with octanyl or *p*-chlorophenethyl moieties suggesting that the analog interacted with a hydrophobic pocket, which maximally accommodates a 10 carbon chain [99-102]. The binding site for capsaicin is in the intracellular loop that connects S2 and S3 in the receptor, and it has a single amino acid Tyr511 that is critical for this interaction since mutations at the site abolished the action of capsaicin and resiniferatoxin (**20**) [103]. It is unknown where capsazepine (**16**) binds in the receptor.

Capsiate (**17**), piperine (**18**) and eugenol (**19**) (Fig. **8**) are naturally occurring capsaicin-like compounds and are helpful to understand important features of receptor activation. The first, capsiate (4-hidroxy-3-methoxybenzyl (E)-8-methyl-6-nonenoate) has several interesting properties. For example, whereas the parent compound is pungent, the latter is not and this is probably related to the replacement of the amide bond in capsaicin with an ester bond in capsiate. Since capsiate induced increases in Ca^{2+} currents and pain when injected subcutaneously, similar to capsaicin, it is believed that its lack of pungency is due to its instability and high lipid solubility, which impedes its transport to the nerve terminals [104,81]. Thus, capsiate shows similar properties to olvanil (**21**), a synthetic analog [105] (i.e increases Ca^{2+}), but with-out pungency. The second, piperine is an alkaloid from *Piper nigrum* (Piperaceae) that can activate TRPV1 with a notable degree of fast tachyphylaxis [106,107]. Finally, eugenol, a phenolic compound found mainly in *Eugenia carophyllata* (Myrtaceae) and *Ocimum gratissimun* (Lamiaceae), can induce increases in membrane conductance and intracellular Ca^{2+} sensitive to capsazepine and ruthenium red suggesting that it also activates TRPV1 [108]. Interestingly, Eugenol is used for treatment of dental pain [81].

PEPTIDERGIC NEUROACTIVE COMPOUNDS

Grammotoxin and Hanatoxin

In spite of the confusion as to the definition of tarantulas, only those pertaining to the Theraphosidae family are recognized as belonging to this class of spiders. Bad reputation aside, their venom does not produce serious consequences in humans and in severe cases only causes muscle spasms and cramps that last several hours. While these effects may be caused by some components that affect voltage dependent ionic channels, the painful sensation may be related to its low pH (pH 5) and to the presence of biogenic amines such as serotonin and histamine [109].

Concerning the pharmacology of the compounds found in tarantula venom, one of the first species extensively studied was the Chilean tarantula, *Grammostola spatulata*, due to its popularity as a pet and the facility to produce large quantities of venom. Eight different toxins have been obtained from this specie: GSAFI, GSAFII, grammostola mechanotoxin 2 (GsMTx2), grammostola mechanotoxin 4 (GsMTx4), hanatoxin 1 (HaTx1), hanatoxin 2 (HaTx2), voltage sensor toxin 1 (VSTx1) and ω-grammotoxin SIA (GsTx). Out of these toxins, ω-grammotoxin SIA and hanatoxin have been the most extensively studied [109].

ω-Grammotoxin-SIA (GsTx) is a 36 aminoacid peptide (4.190 Da) present in the venom of *Grammostola spatulata* which displays a reversible inhibitory effect on voltage-sensitive calcium channels [110,111]. Pharmacological studies showed that the toxin had inhibitory activity over K^+ evoked Ca^{2+} influx in chick and rat synaptosomes having IC_{50}s of 270 and 180 nM, respectively. For Ca^{2+} channels, the targets are N and P channel subtypes [110,112]. Other studies elucidated that the mechanism of action of GsTx involved a shift in voltage-dependent gating to more depolarizing potentials [112-114]. Despite that saturating concentrations of GsTx completely block the current activated by moderate depolarization, the channels can be activated by sufficiently large depolarizations [115]. GsTx is closely related to hanatoxin, a 36 aminoacid peptide with selectivity for voltage-dependent K^+ channels responsible for the delayed rectifier currents (K_v2.1) [116-118]. Three dimensional structural studies with these two toxins have shown structural similarity that is directly related to their common effect (Fig. **9**), which is the modification of voltage-sensitive channel gating properties [119,120] since they most likely interfere with the translocation of the voltage-sensitive transmembrane segment (S4) preventing activation of the channel [116,121]. However, the nature of target selectivity for both toxins in Ca^{2+} and K^+ channels is still unknown. For example, although GsTx at low concentrations can interfere with Ca^{2+} channels, at higher concentrations it affects K^+ channels, which leads to the idea that part of its mechanism of action is conserved [122]. It has been determined by using mutated voltage-dependent K^+ channels that these two toxins have their site of action located between the transmembrane S3 and S4 segments [123].

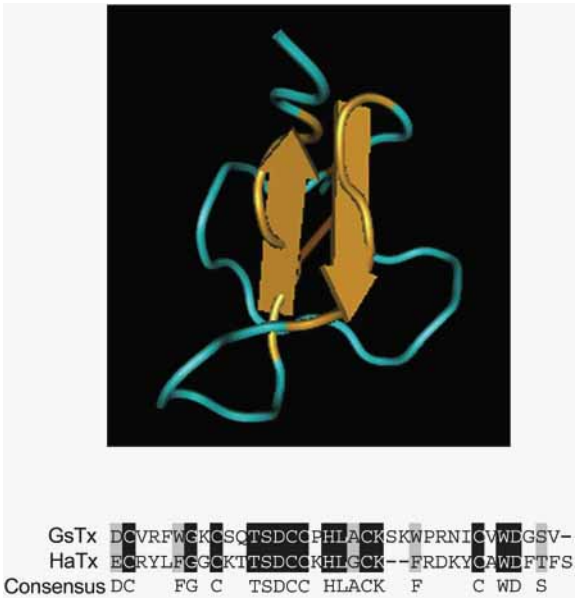

Fig. (9). Upper, three-dimensional structure of ω-Grammotoxin-SIA. Lower, aminoacidic comparison between GsTx and HaTx.

Margatoxin

This toxin is found in the venom of *Centruroides margaritatus*, a scorpion that inhabits an extensive area in Northern South America, Central America and Mexico. This 39 aminoacid peptide displays inhibitory action on voltage-dependent K^+ channels with IC_{50}s of 30 pM for K_v1.3 (KCNA3), 50 nM for K_v1.6 (KCNA6) and 150 nM for Shaker-H4 (K_v1.2, KCNA2) channels. Margatoxin (MgTx)

has a close sequence homology to other toxins such as charybdotoxin (44%), iberiotoxin (41%) and caliotoxin (54%) [124], which also block K^+ channels.

It has also been described that saturating concentrations of MgTx (10 nM) induces the release of dopamine in striatal slices, with an EC_{50} of 2.1 nM. This represents close to 11% of the total dopamine that can be released by depolarization with 60 mM K^+ [125]. Most likely, the slowing down of repolarization should be sufficient to produce an increase in the release of dopamine from nerve terminals. A similar effect was seen with the release of acetylcholine from "striatal slices" exposed to MgTx. This reversible effect was dependent on the concentration of MgTX and sensitive to TTX [126].

Crotoxin

Crotoxin is one of the main peptidergic toxins present in the venom of *Crotalus durissus terrificus* found in desert and semi-desert areas in Brazil and Argentina. This snake toxin is a heterodimer formed by two subunits: one acidic (A) and the other basic (B) [127,128]. The A subunit is approximately 9.5 kDa and is formed by three polypeptide chains joined by 7 disulfide bridges [127]. The B subunit is approximately 14.5 kDa and is made up of a simple chain of 122 aminoacids with a similar number of disulfide bridges [129]. Contrary to the B subunit which displays a potent enzymatic activity similar to phospholipase A_2 (PLA_2), the A subunit lacks activity [130]. Crotoxin has neuro and myotoxic actions [130-132] that can be explained, in part, by findings that indicate that the A subunit acts as a chaperone for the B subunit whose target is the nicotinic cholinergic receptor [129,131,133]. Although its precise site of action is not clear, studies with compounds having PLA_2 activity suggest that the initial cause of cell damage may be due to an increase in Na^+ influx [134]. Alternatively, it has been found that crotoxin caused membrane depolarization as an early indicator of cell damage quickly followed by disruption of the plasma membrane and an increase in creatinine kinase release resulting in cell necrosis [135]. Phase 1 clinical and pharmacokinetic studies have been carried out using crotoxin as a therapy in some solid tumors resistant to classical therapy [136].

Crotamine

Crotamine was also identified in the venom of *C.d. terrificus* having a molecular weight of 4.89 kDa with 42 aminoacids containing 3 disulfide bridges [137]. It has been found that its activity is less potent than crotoxin (LD_{50}= 3,400 μg/kg as compared to 110 μg/kg) [138]. Experiments with whole venom showed that antinociceptive effects are mediated by the action of crotamine on δ subtype opioid receptors and by the release of neuronal NO [139]. In analgesic experiments, pure crotamine was 30 times more potent than morphine and was antagonized by naloxone [140]. More recently, crotamine has been shown to induce release of ACh, dopamine and insulin [141,142]. The effects of crotamine can be blocked by TTX and potentiated by veratridine suggesting the participation of Na^+ channels [143].

Phoneutriatoxin

The venom of the spider *Phoneutria nigriventer* contains a large number of neurotoxins. This species inhabits the central and south part of Brazil, Argentina, Uruguay and Paraguay. There is evidence that peptide toxins present in this venom can act on several ion channels, and for that reason it is important in public health and pharmacological studies. The bite from *Phoneutria* is reported to cause severe symptoms like cramps, tremors, tonic convulsions, spastic paralysis, priapism, sialorrhea, arrythmias and visual disturbance [144].

The main neurotoxic effect of this venom appears to be based on its action on voltage gated Na^+ channels. There is evidence indicating that some components of the venom activate the voltage-dependent sodium channel in muscle and nerve cell membranes in a TTX-dependent way [145] and inhibit voltage gated Ca^{2+} channels [146]. However, it must be taken into consideration that this venom is capable of modulating other pharmacological processes as well [147].

The venom from *Phoneutria* is composed by approximately 17 peptides (with molecular weights ranging from 3.500 to 9.000 Da) that have been studied by classical separation methods [148] and with the use of molecular biology [149,150]. The first two toxins characterized were PhTx1 and PhTx2 both having an effect on voltage gated Na^+ channels [151,152]. Three other toxins (Tx3-2, Tx3-3, Tx3-4) purified from the venom were shown to block voltage gated Ca^{2+} channels. Tx3-3 and Tx3-4 are potent blockers of Ca^{2+} channels activated by Tityustoxin (TsTx, a toxin purified from the Brazilian scorpion *Tityus serrulants*) with an IC_{50} of 0.32 and 7.4 nM, respectively, as shown in rat brain cortical synaptosomes [146, 153]. It is interesting to note that while Tx3-4 is active against N and P/Q type Ca^{2+} channels [154], Tx3-2 can partially block L-type Ca^{2+} channels [149]. It has been demonstrated that Tx3-1, on the other hand, is a potent blocker of A-type K^+ channels, having no effect however on outward K^+ rectifier channels, BK or L-type Ca^{2+} channels [147]. It is interesting to note the striking similarity in the primary structures between several of these toxins such as PhTx2-1, 2-5 and 2-6, PhTx1 and 3-4, and PhTx3-1 and 3-2 (Fig. **10**). With respect to this last subgroup, it also displays a similar homology to ω-agatoxin IVA, a toxin present in the spider Agenelopsis aperta wich blocks type P/Q calcium channels. Therefore, *Phoneutria nigriventer* is a real factory of neuroactive compounds. It is interesting to consider the extraordinary similarity and coincidence in the mechanism of action of venoms from different species. All these venoms are a molecular adaptation for defense against predators, and many of them have a highly similar primary structure or share their effector target.

CURRENT AND FUTURE OPPORTUNITIES FOR THE DISCOVERY AND DEVELOPMENT OF NOVEL NEUROACTIVE COMPOUNDS

It is evident that neuroactive compounds derived from Latin American flora and fauna have been very important for the development of key therapeutics used in modern medicine. Interestingly, these lands still hold an impressive number of unexplored plants and animals that most likely

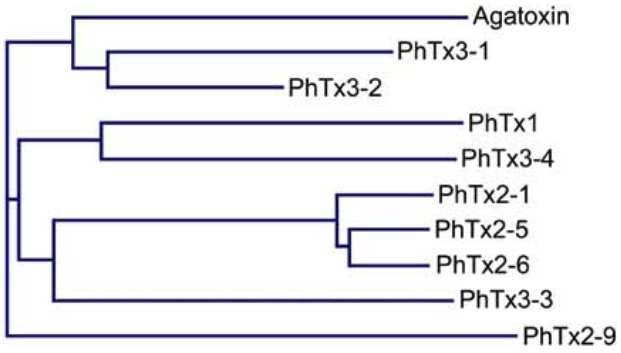

Fig. (10). Philogenetic relationship between aminoacidic sequence of toxins present in *Phoneutria nigriventer* and Agatoxin present in venom of *Agelenopsis aperta*.

contain exciting new active compounds. With the introduction of new techniques to isolate, identify and characterize the molecular targets and actions of new chemical entities, together with the need for more potent and selective compounds to treat neurological conditions, it is essential to advance our research in order to capitalize on the enormous potential still yet to be discovered within our natural resources. Nevertheless, it is important to mention that, unlike previous drug development, economical and intellectual compensation to the local providers should be guaranteed. Furthermore, in this present stage of development, we need to consider legal and ethical viewpoints that would prevent the depletion of exotic species and infringe on the sovereignty and economical potentials of developing nations. The term "Biopiracy" describes the concept of "take and run" and because of these "exploratory" activites, successful medicines were developed and the native providers were never benefited. In the actual era of discovery, we need a change of mentality geered towards three main principles: conservation, development and benefit sharing. For the local community, the benefits must include opportunities for further research and conservation, improvement in the technologies and grants for innovation, as well as royalties for the national economy. We should even consider the possibility of canceling patents on natural product inventions if obtained with "tainted research" that does not meet the above requirements.

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