Bioactive Alkaloids from South American *Psychotria* and Related Species

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Abstract: Many important molecules have been discovered from tropical and sub-tropical plant biodiversity. However, the largest part of the chemical profile of such biodiversity remains unknown. Combining ethnopharmacological and chemotaxonomical investigation can be a good strategy in bioactive compound discovery. South American *Psychotria* species studied by this approach proved to be a rich source of new bioactive alkaloids, some of which bear unique chemical skeletons.

Key Words: Alkaloids, South America, *Psychotria*, Ethnopharmacology, Analgesic.

INTRODUCTION

Plants synthesize an extensive array of secondary metabolites, or natural products, and many of them have pharmacological properties. These bioactive compounds can be found in plants traditionally used for medical purposes, i.e. medicinal plants. Extracts, infusions and other preparations from medicinal plants were the sole alternative to human healthcare needs until the nineteenth century [1]. The isolation of morphine in 1806 is regarded by many as the beginning of phytochemistry [2, 3]. From then on, the development of organic chemistry provided standardized medications through synthethic drugs and active compounds isolated from biological material.

Despite being complex and expensive, biodiversity-screening programs remain productive approaches in drug discovery [4]. Between 1981 and 2006, 36% of all small molecule new chemical entities were natural products or derived from them, usually by semisynthethic modification [5]. Other 24% were "natural product mimics", i.e. competitive inhibitors of natural substrates. High throughput screening techniques [6] as well as advancements in biochemistry and biotechnology of secondary metabolism [7-9] are major contemporary achievements which are accelerating natural products research.

This review focuses on molecules found in Rubiaceae plants that have been evaluated for their bioactivity. To that end, a systematic search for full length articles was carried out in indexed databases (e.g. Science Citation Index Expanded®), highlighting alkaloids discovered within the last decade from South American *Psychotria* species of the Atlantic Forest biome.

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ACTIVE ALKALOIDS FROM RUBIACEAE PLANTS

The Rubiaceae, a large plant family with 10,700 species [10], yielded the most widely used human stimulant, caffeine (reviewed in Ashihara *et al.* [11]). Novel active structures, belonging to diverse biochemical classes such as alkaloids [12-15], anthraquinones [15-17] and peptides [16, 18, 19] are constantly being discovered in Rubiaceae species. However, two South American species deserve special consideration: *Uncaria tomentosa* and *Cinchona officinalis.*

The *Uncaria* genus covers 34 species and is a particularly rich source of medicinal natural products (reviewed in Heitzman *et al.* [20]). Over 150 compounds have been isolated from *Uncaria* plants, alkaloids being the most prominent of them. *Uncaria tomentosa* (Willd.) DC. is endemic to central and south-America and is one of the most important medicinal plants to Peruvian populations [20]. Extracts from *U. tomentosa* have a plethora of traditional uses. Further investigations of extracts revealed activities such as immunomodulator [21, 22], antiviral [22], anti-inflammatory [23], antioxidant [20, 23] and cytostatic [24]. Tetra and pentacyclic alkaloids present in the plant extracts seem to play a major role in some of these effects [21, 24].

From the discovery of the New World until the mid-19th century, malaria was a major cause of death of European people involved in trade and colonization of tropical lands [25]. With the introduction of quinine, an active compound from *Cinchona* bark, the mortality fell dramatically. Quinine is an alkaloid found in *Cinchona officinalis* and some other *Cinchona* species known as "quina quina" by native populations [26]. Other antimalarial alkaloids present in *Cinchona* bark include quinidine, cinchonine and cinchonidine [27]. In present times, quinine-resistant strains of *Plasmodium falciparum* have emerged, and new drug leads to address this problem include medicinal plants [28, 29]. Alkaloids such as cephaeline (Fig. **1a**) and klugine isolated from *Psychotria*

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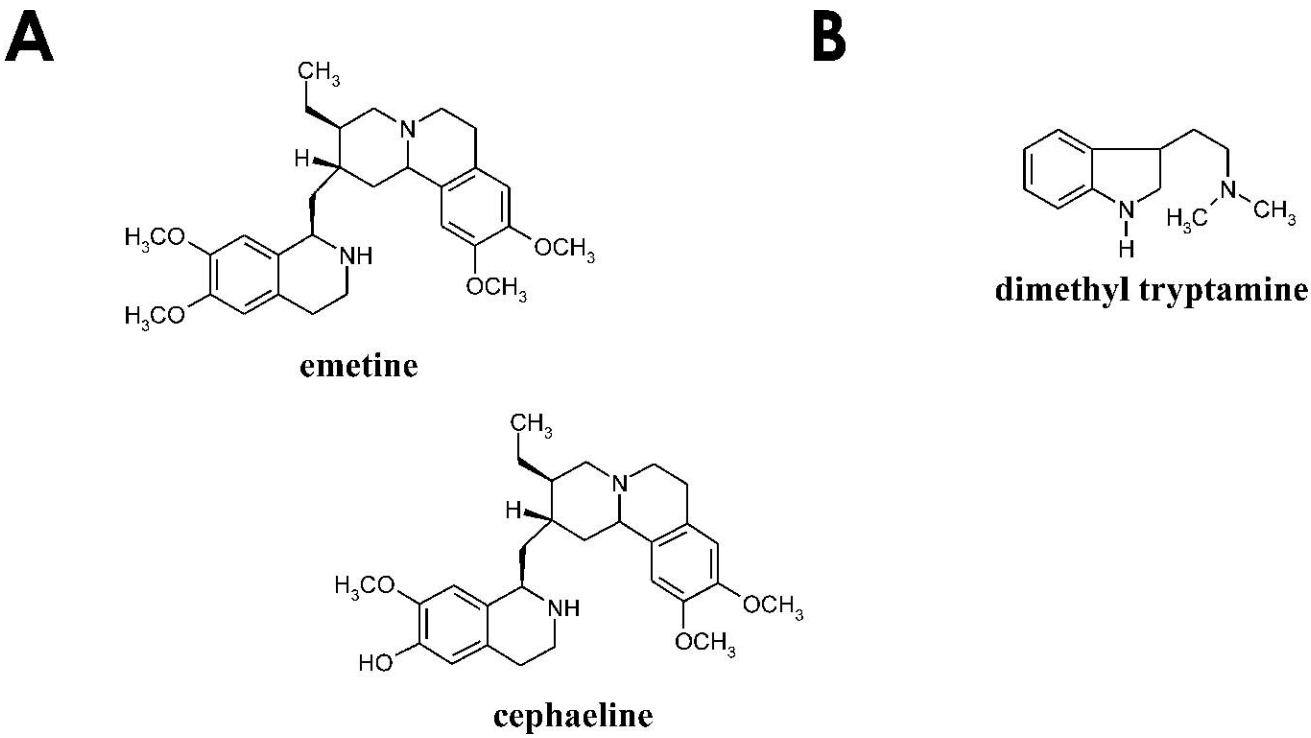

Fig. (1). Important active alkaloids from *Psychotria* species. **A**: The emetic isoquinoline alkaloids emetine and cephaeline from *P. ipecacuanha*, also found in *P. klugii*. **B**: The hallucinogenic dimethyl-tryptamine from *P. viridis*, one of the psychotropic constituents of ayahuasca.

klugii have shown antimalarial and antileishmanial activity [30].

Both *Uncaria tomentosa* and *Cinchona officinalis* are examples of medicinal plants discovered within traditional communities. Plants known by these people, who live in the vicinity of rich biodiversity for centuries, are interesting starting points for phytochemical screening through ethnopharmacological surveys [1, 2, 31]. Important new molecules have been discovered from medicinal plants, including the antitumorals vincristine and vinblastine from *Catharanthus roseus*, the antimalarial artemisin from *Artemisia annua* and the neuroactive reserpine from *Rauwolfia serpentina* [1].

THE *PSYCHOTRIA* GENUS

The genus *Psychotria* is one of the largest genera of flowering plants and the largest within Rubiaceae, with estimated 1000 to 1650 species distributed worldwide [32]. *Psychotria* species often accumulate indole alkaloids, and this trait may be important to chemosystematics, since this genus is taxonomically complex due to a lack of conspicuous morphological differentiating features [32, 33]. A number of *Psychotria* species yielded bioactive extracts. Some examples include antibiotic activity in extracts from *P. microlabastra* [34] and *P. capensis* [35] (Africa), antiviral activity in *P. serpens* [36] (China) and antiviral/antifungal and antiinflammatory activities found in *P. hawaiiensis* [37] and *P. insularum* [38] (Central America), respectively.

Active molecules produced by *Psychotria* species include naphtoquinones [39], peptides [40], benzoquinones [41], pigments [42] and alkaloids [43]. Perhaps the best known compound isolated from *Psychotria* species is the alkaloid emetine. Emetine (Fig. **1a**) is an isoquinoline alkaloid extracted from *P. ipecacuanha* (ipecac) bark, a plant used by traditional communities as stimulant and "antidote to opium" [44] and in the treatment of intoxication due to its emetic effect [45]. Synthethic analogs of emetine, which have less adverse effects, are currently used in the treatment of amoebiasis [46]. Emetine is cytotoxic, inhibiting protein synthesis, and may have applications in drug-induced apoptosis [47].

Another well known *Psychotria* species is the one used in the preparation of the hallucinogenic drink "ayahuasca", *P. viridis*. The decoction is prepared using the plant in combination with the vine *Banisteriopsis caapi*. (Spruce ex Griseb). Morton (Malpighiaceae). *Psychotria viridis* and *B. caapi* are rich sources of the proto-alkaloid dimethyltryptamine (DMT) (Fig. **1b**) and the β-carboline harmine, respectively [48]. Both substances are psychoactive, and the two have a strong synergism when administered together, possibly due to inhibitory effects of harmine on monoamine oxidase, a DMT detoxifying enzyme [49]. The recent popularization of the ayahuasca in the United States and Europe has raised several debates, from mental health issues to conflicts on drug abuse *versus* religious freedom [50, 51].

THE ALKALOIDS FROM *PSYCHOTRIA COLORATA* MÜLL. ARG

Several South American *Psychotria* species are used as medicinal plants by Amazon native populations [52]. An ethnobotanical survey identified species used as painkillers by "caboclos", traditional rural communities from the state of Pará, Brazil, which comprises a large fraction of

the Amazon rainforest. The extracts of *P. colorata* showed analgesic activity, and preliminary tests pointed to alkaloids as major responsibles for the effect [53]. Further chemical investigations demonstrated the presence of several pyrrolidinoindoline and quinoline alkaloids (Fig. **2**) [54], with hodgkinsine, previously isolated from *Hodgkinsonia frutescens* F. Muell. (Rubiaceae) [55], as a major component. Hodgkinsine is a potent analgesic, with results comparable to morphine in murine models using the hot-plate and tail-flick tests [56, 57].

Besides hodgkinsine, three other *P. colorata* pyrrolidinoindoline alkaloids, (+)-chimonanthine and the later isolated *meso*-chimonanthine and psychotridine [58], showed analgesic activity [59, 60]. Interestingly, only pyrrolidinoindoline-type alkaloids, and not quinoline, gave positive results to nociceptive tests, suggesting a structure-activity relationship [58] (see Fig. **2**). From the data with capsaicin-induced pain models, it was suggested that the alkaloids act on opioid and glutamate receptors [57, 59, 60].

BIOACTIVE ALKALOIDS FROM SOUTHERN BRAZILIAN *PSYCHOTRIA* SPECIES

The promising results with *P. colorata* motivated investigations of Southern Brazilian subtropical species. Initial screening of ethanolic extracts from *Psychotria* plants revealed analgesic activity in six species [61] (Fig. **3**): *P. brachyceras, P. carthagenensis, P. leiocarpa, P. myriantha, P. suterella* and *P. umbellata*. All these species belong to the subgenus *Heteropsychotria* [33]. Among these, only *P. carthagenensis* from this region did not show positive reaction for alkaloids [62]. Curiously, *P. carthagenensis* is used in the preparation of ayahuasca brew in substitution to Amazonic *P. viridis* [62]. However, detailed chemical profiles obtained with adequate analytical facilities on *P. carthagenensis* from other regions is lacking and generalization of these results must be done with caution.

Alkaloid extracts from *P. myriantha* shoots, besides analgesic [63], revealed anti-inflammatory activity in chemotaxis assays [64]. Purification and structural elucidation yielded strictosidinic acid and a new structure, myrianthosine (Fig. **3**). *P. suterella* Müll. Arg. leaf extracts, however, did not retain analgesic activity in the alkaloid fractions, which were highly toxic [65]. The alkaloids isolated from this species, lyaloside, strictosamide and naucletine (Fig. **3**), until then had not been reported to the genus. Recently, strictosamide was found as the major component (about 98%) of the alkaloid extract of leaves of *Psychotria nuda* (Cham. et Schltdl) Wawra [66]. Strictosamide was also found in *Psychotria bahiensis* DC. from Trinidad which yielded the bis(monoterpenoid) indole alkaloids bahienosine A and B; these alkaloids incorporate two secologanin units [67].

P. leiocarpa Cham. et Schlecht is found in Argentina, Paraguay and the Southern Brazilian state of Rio Grande do Sul. The species is dominant in the understorey of subtropical semi-deciduous forests, possibly due to production of allelopathic compounds [68]. Ethanolic leaf extracts from *P. leiocarpa* yielded *N*, β-D-glucopyranosyl vincosamide (GPV) (Fig. **3**), an unusual indole alkaloid bearing two glucose residues, as major compound [69]. The indole features a glucose residue attached to the nitrogen, an

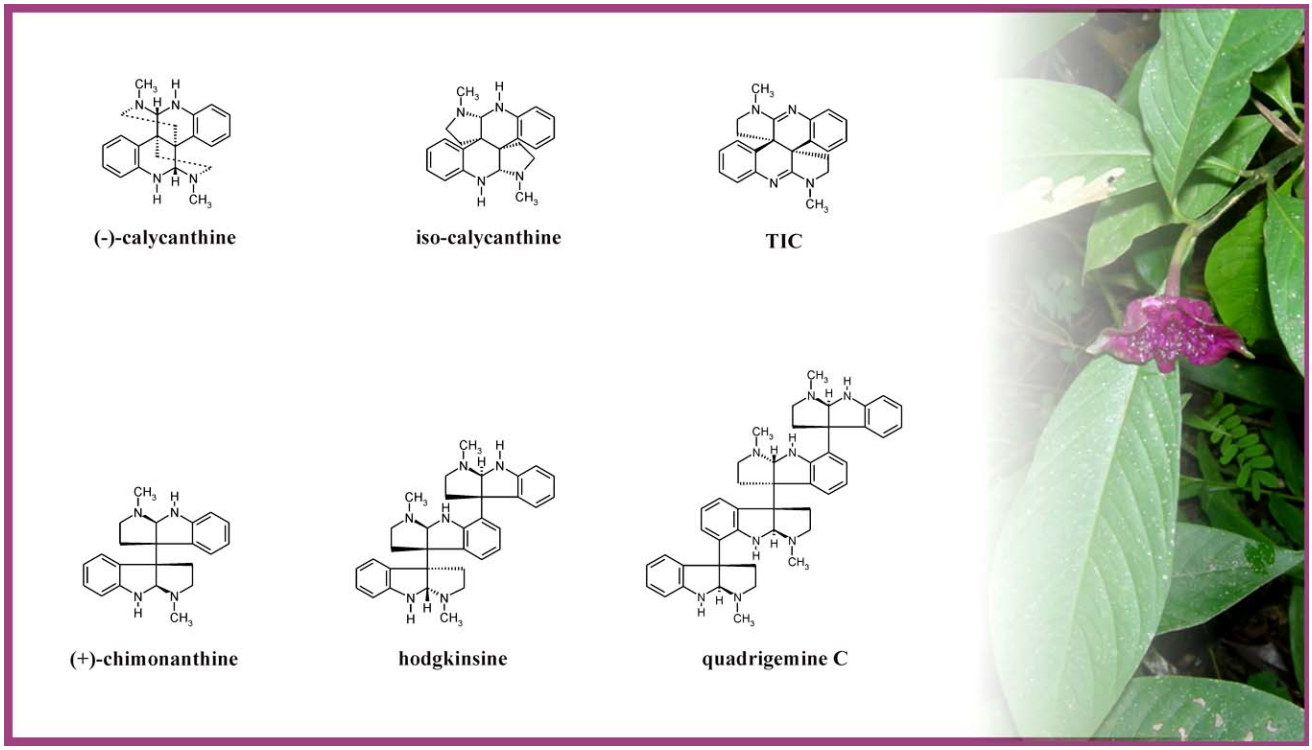

Fig. (2). Alkaloids isolated from *Psychotria colorata*. The upper and lower structures are quinoline and pyrrolidinoindoline alkaloids, respectively. TIC: (8-8a),(8´-8´a)-tetradehydroisocalycanthine 3a(*R*), 3´a(*R*). Photo modified from [87].

A very similar MIA was isolated from *Psychotria brachyceras* Müll. Arg. [76]. The compound, named brachycerine (Fig. **3e**) is a singlet oxygen [77] and superoxide (Porto *et al.*, unpublished) quencher, and gave positive results for anti-inflammatory activity in chemotaxis assays (Henriques, A. T., unpublished). Brachycerine, as well as psychollatine, was capable of protecting *S. cerevisae* strains from oxidative stress, with higher protection against superoxide anion [78].

Both brachycerine and psychollatine are unusual MIAs. Most alkaloids of this class are derived from strictosidine, which is the product of the condensation of tryptamine and the iridoid secologanin, a reaction catalysed by strictosidine synthase [79]. However, brachycerine and psychollatine seem to be products of tryptamine condensation with epiloganin and geniposide derivatives, respectively [71, 76]. The putative pathway for brachycerine and psychollatine biosynthesis is novel, and may establish a new group of MIAs, together with croceaine A [73].

REGULATION OF ALKALOID PRODUCTION IN *PSYCHOTRIA* SPECIES

Despite the significant advances achieved in biochemistry, molecular biology and biotechnology of natural products, the production of active compounds in controlled conditions (i.e. cell and tissue culture) often results in economically impracticable yields [9]. Chemical synthesis or semi-synthesis of structurally complex active molecules discovered from plants is not always possible. In those cases, industry relies on the plant itself to isolate the active compound in large scale, either from plantations or naturally-occurring specimens. In both cases, isolation from adult plant tissues is often expensive and resulting in low yields [80], and in the second case, the consequences to the plant natural population and its habitat can be disastrous [81].

Molecular biology techniques, along with elicitor-induced accumulation of plant secondary metabolites, may improve bioactive molecule production in plant systems [8, 9, 82, 83]. With this in mind, a series of studies were carried out in order to investigate the regulation of *Psychotria* alkaloids production.

The effect of white light and presence of sucrose in the culture medium on GPV content was assessed in *P. leiocarpa* seedlings. GPV production was restricted to shoots, strongly promoted by light and inhibited by exogenous sucrose, indicating the importance of photoautotrophic metabolism and photomorphogenic development for alkaloid accumulation. Also, the higher content in older seedlings (150 days) in comparison to younger ones (100 days) showed developmental control of GPV accumulation [69].

Brachycerine accumulation, in *P. brachyceras*, was restricted to shoots. The highest concentration occurred in inflorescences (0.3%), followed by fully expanded leaves (0.18%), stems (0.13%), young leaves (0.12%) and fruit pulp (0.04%) [84]. The leaf alkaloid content in field-grown trees showed seasonal variation in some years, with lower concentrations in the summer. *In vitro* cultured seedlings 14 days after radicle emission accumulated brachycerine contents comparable to those of adult plants [84].

Leaf brachycerine content in cuttings was responsive to several stimuli. Mechanical damage or jasmonic acid, an herbivory-related hormone [85], induced brachycerine accumulation (Gregianini *et al*., 2004) [84], suggesting a deterrent role for the alkaloid. However, no toxic effect was detected in generalist herbivore bioassays (Porto *et al.*, unpublished data). Ultraviolet (UV) radiation, both in UV-C (254 nm, germicidal lamp) and UV-B (280-315 nm) ranges, induced up to 10-fold brachycerine accumulation in leaves of cuttings [77]. The antioxidant properties of brachycerine and its strong UV-regulation indicate a protective role for the alkaloid *in planta*.

Differently from brachycerine, psychollatine accumulation in *P. umbellata* cuttings is rather insensitive to the treatments described. However, the plant naturally accumulates a relatively high amount of psychollatine (1-4% of dry weight) [9]. An *in vitro* somatic embryogenesis protocol was established from cultured rhizogenic callus, and, after three months of acclimatization, psychollatine-accumulating plants were obtained with alkaloid contents similar to those found in adult plants; in addition, the capacity to produce the alkaloid was closely associated with the differentiation of somatic embryo shoots [86].

The alkaloids of *Psychotria* species from Southern Brazil represent relatively primitive structures compared to the bisindolic alkaloids of the best studied species in monoterpene indole alkaloid metabolism, *Catharanthus roseus*. However, these simpler *Psychotria* alkaloids also display interesting pharmacological properties. The presence of new structures, such as the *N*-glycosylated GPV and the non-secologanin derived terpene moieties of brachycerine and psychollatine highlight the prominent chemical diversity in this genus. Other features that are common to most of the described alkaloids of *Psychotria* include the retention of glucose residues (which may favor solubility in aqueous environments), antioxidant capacity, the requirement of differentiated shoots for active alkaloid production, and the accumulation in reproductive structures.

CONCLUDING REMARKS

The alkaloids from *Psychotria colorata* (Fig. **2**) [87] were a direct result of ethnopharmacological surveys in the Amazonian region. The simple expansion of chemical investigations to include related species from a different biome (chemotaxonomical survey of *Psychotria* species of Southern Brazil, [88]), involving plants that even lack popular names, revealed several other bioactive alkaloids. Further research on the biological activity of these new compounds, elucidation of their biosynthetic origins and regulation of production, along with studies on plant propagation, may convert relatively unknown species in viable sources of new drugs.

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