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Chemistry and biological activity of alkaloids from the genus *Schizanthus*

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Abstract The genus *Schizanthus* (Solanaceae) comprises 12 accepted species primarily endemic to Chile. It is characterized by the presence of numerous tropane alkaloids found as ester derivatives of isomeric acids like angelic, senecioic, or tiglic, as well as mesaconic, itaconic, and citraconic acids, leading to numerous positional and configurational isomers. Up to now 50 alkaloids with these esters have been isolated and most of them are fully characterized. Analytical methods and techniques engaged during the investigation of this class of compounds, as well as their identification capabilities, are discussed. In several cases the absolute configuration is also well established, and some alkaloids are reported as biologically active. This review summarizes updated information on the distribution, botanical characteristics, phytochemical, and pharmacological knowledges of this genus up to mid-2018.

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Abbreviations

AC	Absolute configuration
BA	6-Benzylaminopurine
CE	Capillary electrophoresis
COSY	Correlation spectroscopy
Da	Dalton
DFT	Density functional theory
ECD	Electronic circular dichroism
EI	Electron impact
FID	Flame ionization detector
FTIR	Fourier transform infrared
GC	Gas chromatography
HMBC	Heteronuclear multiple bond correlation
HPLC	High performance liquid chromatography
HPTLC	High performance thin-layer chromatography
HRESIMS	High-resolution electrospray ionization mass spectrometry
HSQC	Heteronuclear single-quantum correlation
IR	Infrared
ITMS	Ion trap mass spectrometry
IUPAC	International union of pure and applied chemistry
LLE	Liquid–liquid extraction

MEKC	Micellar electrokinetic chromatography
MS	Mass spectrometry
MW	Molecular weight
NACE	Non aqueous capillary electrophoresis
NOESY	Nuclear Overhauser effect spectroscopy
NMR	Nuclear magnetic resonance
PGC	Porous graphitic carbon
NAA	1-Naphthaleneacetic acid
NPD	Nitrogen phosphorus detector
SPE	Solid phase extraction
TDDFT	Time dependent density functional theory
TLC	Thin-layer chromatography
TOFMS	Time-of-flight mass spectrometry
UHPLC	Ultra-high performance liquid chromatography
UV	Ultraviolet
VCD	Vibrational circular dichroism

Introduction

According to The Plant List (ThePlantList 2013), the Solanaceae family comprises 115 genera and 2678 species distributed in both warm and temperate regions of the World with the greatest concentration of genera and species in South- and Central-America. This family includes many species of agricultural and economic relevance. Several of them, such as tomato, Cayenne pepper, eggplant or potatoes are widely cultivated since they are among the most commonly edible vegetables in the World. The leaves of dried and fermented tobacco are smoked worldwide. Other plants from this family are toxic and some of them are used recreationally or during shamanic ceremonies (e.g. *Datura* spp., *Brugmansia* spp. (tree-datura), *Brunfelsia* spp.) with poisoning frequently reported. Other species such as *Atropa belladonna* L., *Datura stramonium* L. and to a lesser extent *Hyoscyamus niger* L. are notorious examples of pharmaceutical relevant species. They contain a wide range of alkaloids, some of them with considerable therapeutic activities.

The alkaloid content in the Solanaceae family differs by species and typically accounts between modest 0.01 up to 3% (Oksman-Caldentey and Arroo 2000). The recorded types of alkaloids include tropane, indole, pyridine, pyrrolidine, steroidal and glycoalkaloids (Eich 2008). From them, tropane alkaloids occur in several genera like *Atropa*, *Brugmansia*, *Datura*, *Duboisia*, *Hyoscyamus*, *Latua*, *Mandragora*, *Schizanthus*, and *Scopolia*, but also in other families like Brassicaceae, Convolvulaceae, Euphorbiaceae and Erythroxylaceae (including coca), and more sporadically in Proteaceae and Rhizophoraceae (Christen 2000; Griffin and Lin 2000).

In turn, tropane alkaloids are structurally-related natural products possessing the 8-methyl-8-azabicyclo[3.2.1]octane skeleton, although for simplicity they are generally referred to as substituted tropanes. This important class of natural products has already provided ca. 250 compounds (Christen et al. 2013), which are divided into two main groups, 3α- and 3*β*-hydroxytropanes, according to the *pseudo-axial* or *pseudo-equatorial* orientation, respectively, of the substituent at C-3. The former are the most frequently encountered molecules, whereas 3*β*-hydroxytropanes are mainly found in the *Erythroxylaceae* family (e.g. cocaine). Most representatives are 3-, or 6-hydroxy, 3,6- or 6,7-dihydroxy, or 3,6,7-trihydroxytropanes esterified with various organic acids.

For correct nomenclature purposes it should be noted that unsubstituted tropane has a plane of symmetry that goes through C-3 and the nitrogen atom, and bisects the C-6–C-7 bond, which means for instance that 3-hydroxytropane is a *meso* optically inactive molecule. When a substituent is found at C-6 the molecule becomes optically active and now possesses three stereogenic centers which are defined in Fig. 1 for both enantiomers of 6*β*-hydroxytropane. The atom numbering is now clockwise or counterclockwise according to the *S* or *R* absolute configuration (AC), respectively, of the stereogenic center at C-6, and all three stereogenic descriptors might be given as shown in Fig. 1.

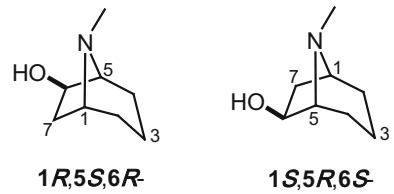


Fig. 1 Both enantiomers of 6*β*-tropanol showing atom numbering and stereochemical descriptors

About 40 organic acids have been identified as esterifying acids so far (Lounasmaa and Tamminen 1993) which include acetic, tropic, angelic, senecioic, tiglic, cinnamic, mesaconic, itaconic, and citraconic acids. The numbering of tropane alkaloids has often been confusing in the literature because the strict application of IUPAC rules is not always followed. Their notation as 3,6/7-disubstituted derivatives has frequently been chosen arbitrarily (Humam et al. 2008; Lounasmaa and Tamminen 1993) although a designation like 3,7-dihydroxytropane is incorrect, since IUPAC recommends the use of the smallest possible numbers. When a 3,6-dihydroxytropane is considered, then four stereogenic descriptors (Muñoz et al. 2016), at C-1, C-3, C-5, and C-6 are required for a complete molecular description, and the careful application of hierarchical digraphs (Zepeda et al. 2011) for the unequivocal assignment of stereochemical descriptors can be of great help.

The development of advanced analytical spectrometric and spectroscopic technologies over the last two decades allowed the isolation and characterization of a large number of new chemical entities. A review reporting 125 tropane alkaloids identified since 1993 together with synthetic approaches has been published very recently (Afewerki et al. 2018). The present review accounts all alkaloids so far isolated from the genus *Schizanthus*, and the biological activities of some extracts and pure alkaloids isolated from them are covered up to mid-2018.

Relationships between the *Schizanthus* species

The species morphological features of this genus are quite variable and their taxonomy is far from being fully resolved. Based on molecular analyses (Pérez et al. 2006), the genus has been split into three main clades: Clade A includes *S. alpestris* Poepp. ex Benth. as a brother clade to an unresolved clade consisting of *S. candidus* Lindl., *S. integrifolius* Phil., and *S. lacteus* Phil. Clade B includes *S. hookeri* Gillies ex Graham and *S. grahamii* Gill. ex Hooker and its *coccinea* variety. From a phylogenetic point of view, *S. grahamii* and *S. hookeri* are very closely related species to form a clade, not only due to genetic or morphological features (Pérez et al. 2006, 2011) but also because their similar alkaloid composition (Peña and Muñoz 2002). Clade C contains *S. laetus* Phil., as well as two brother subclades, one of which includes *S. litoralis* Phil. and *S. porrigens* Graham, while the other one groups together *S. tricolor* Grau & Gronb., *S. pinnatus* Ruiz & Pav., and *S. parvulus* Sudzuki (Scheme 1) (Muñoz-Schick and Moreira-Muñoz 2008).

Distribution of the genus

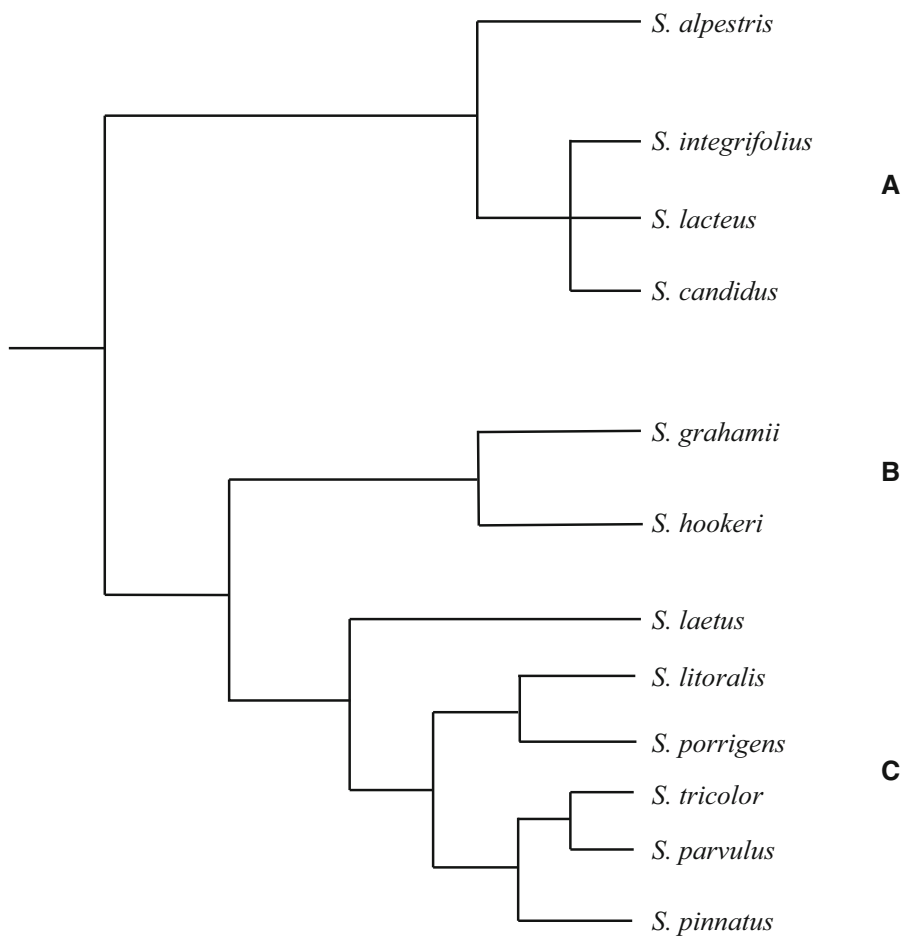
This genus is primarily native to Chile and the species grow in a large diversity of habitats, from the desert to the coast in the Tarapacá region, as well as from the high Andes to areas cleared of forest in the southern Chilean region of Los Lagos (Fig. 2). The distribution of *S. grahamii* and *S. hookeri* reaches central Chile areas bordering Argentina.

The most widely distributed species is *S. pinnatus*, inhabiting the Coquimbo and Los Lagos regions, while the species with the smallest distributions are *S. lacteus* on coast areas of the Antofagasta region, and *S. parvulus* on the coast and inland in the Choapa province of the Coquimbo region (Muñoz-Schick and Moreira-Muñoz 2008).

The 12 species comprising the genus *Schizanthus* are commonly known as little bird, little bird flower, little butterfly, or butterfly of the coast. *S. pinnatus* was the first species described by the Spanish explorers and botanists Ruiz and Pavón as early as 1794 (Ruiz and Pavón 1794), while the last valid species, *S. parvulus*, was described by Sudzuki in 1945 (Sudzuki 1969). Some species have been imported to Europe for cultivation. Several species were described in England like *S. porrigens*, and *S. grahamii* in 1824, while later *S. retusus* Hook. (currently *S. grahamii*) and *S. hookeri* were described in 1831. *S.* × *wisetonensis*, which is successfully cultivated in gardens in both Europe and the United States of America (USA), is a hybrid between *S. pinnatus* and *S. grahamii* commonly known as poor man's orchid. There are several cultivars of this hybrid, some of which have been patented in the USA (Muñoz-Schick and Moreira-Muñoz 2008).

Floral morphology and pollination

The Greek word *Schizanthus* literally means split or cracked flower in reference to the divisions of the petals (Fig. 3). The flowers vary greatly in form and color, they are bilabiated and zygomorphic, a feature



Scheme 1 Phylogenetic relationships in the genus *Schizanthus* (Muñoz-Schick and Moreira-Muñoz 2008)

that is uncommon in the Solanaceae family and appears similar to the Fabaceae flowers. The species are annual or biennial, glandulous-pubescent herbaceous plants measuring 25 to 75 cm high, usually sticky to the touch due to their glandular trichomes. The leaves are dissected, alternate, pinnatid or bipinnatisect, and the flowers appeared on terminating summits (inflorescences). The two species *S. pinnatus* and *S. grahamii*, as well as the hybrid *S.* × *wisetonensis* are cultivated as ornamentals in gardens (Hunziker 2001). Most of the species are melittophylous, excepting *S. integrifolius*, *S. candidus*, and *S. lacteus*, which are pollinated by nocturnal butterflies (sphingophily) (Cocucci 1989).

Alkaloids from the genus *Schizanthus*

Over recent years the development of analytical and spectrographic techniques has provided tremendous improvements for the isolation of a considerable number of new constituents from Nature, which currently can be detected when using GC–MS or HPLC–MS methodologies, as detailed later, although the presence of a new isomer cannot totally be ruled out by this initial approach for the study of a plant extract. Therefore in the present review the alkaloids isolated from *Schizanthus* species are tabulated according to their molecular weight, from the smallest constituent, tropinone (**1**), MW 139, to macrocycle **49**, MW 871, and its isomer open macrocycle **50**, MW 889 (Table 1), both containing three tropane units.

The genus is characterized by numerous tropane alkaloids produced in small amounts in complex stereochemical mixtures with a large structural variety. It accumulates various types of alkaloids such as pyrrolidine derivatives, mono- and dihydroxytropane esters, ditropane diesters, as well as cyclobutanetricarboxylic acid derived triesters. Most of them are

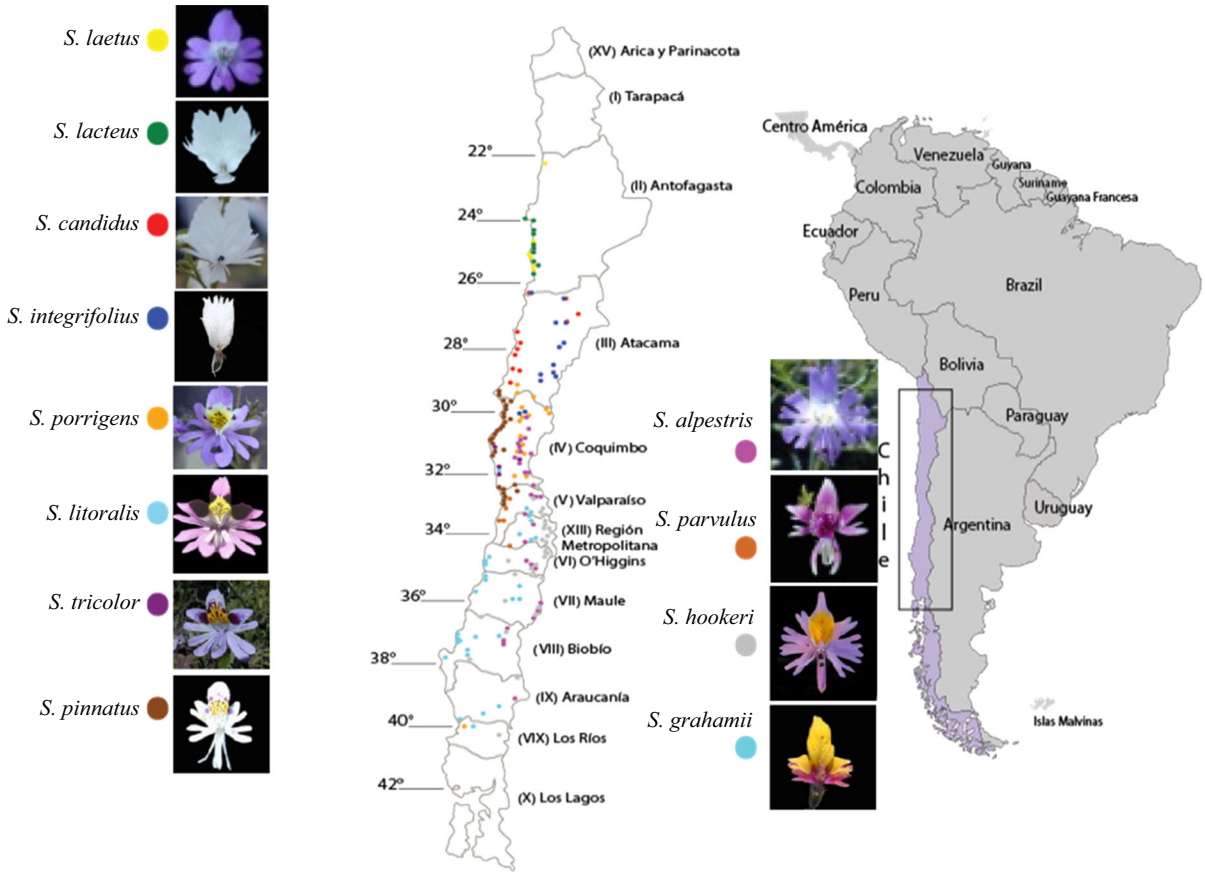


Fig. 2 Distribution of the genus *Schizanthus* in Chile (Pérez et al. 2006)

ester derivatives from angelic, senecioic, tiglic, itaconic, mesaconic, citraconic, and cinnamic acids, which generate numerous positional and configurational isomers. To date over 50 alkaloids have been isolated and characterized from this genus. The chemical formulas of *meso*-tropanes are given in Fig. 4, those of alkaloids derived from 3,6-dihydroxytropane are shown in Fig. 5, the alkaloids containing two tropane scaffolds are summarized in Fig. 6, six alkaloids containing a cyclobutane ring are shown in Fig. 7, and six pyrrolidine derivatives are presented in Fig. 8. It should be noted that no AC representations are attempted in Figs. 4, 5, 6, 7 and 8, as this subject is discussed later in this review. From all known *Schizanthus* species the following eight have already been investigated phytochemically: *S. alpestris*, *S. grahamii*, *S. hookeri*, *S. integrifolius*, *S. litoralis*, *S. pinnatus*, *S. porrigens*, and *S. tricolor*.

Schizanthus hookeri Gillies ex Graham

Schizanthus hookeri, popularly known as the poor-man's orchid, grows on the western slopes of the Andes in fairly dry areas between 1500 and 3000 m in the regions of Coquimbo and La Araucania in central Chile, as well as in the provinces of Mendoza and Neuquen in Argentina. It is an herb of 30 to 80 cm high, carrying zygomorphic lilac flowers with a yellow trimmed, deep purple center (Fig. 3A).

A study of the roots (San Martin et al. 1980) allowed the isolation and characterization of the five alkaloids 3α-hydroxytropane (**3**), the diastereoisomeric hygrolines A and B (**4**), 3α-hydroxy-6β-angeloyloxytropane (**9**), and 3α-senecioyloxy-6β-hydroxytropane (**12**). In addition, 3α,6β-dihydroxytropane (**5**), 3α-senecioyloxytropane (**7**), and 3α-hydroxy-6β-tigloyloxytropane (**11**) have been isolated from the stems and leaves of this species (Gambaro et al. 1982, 1983). From the aerial parts,

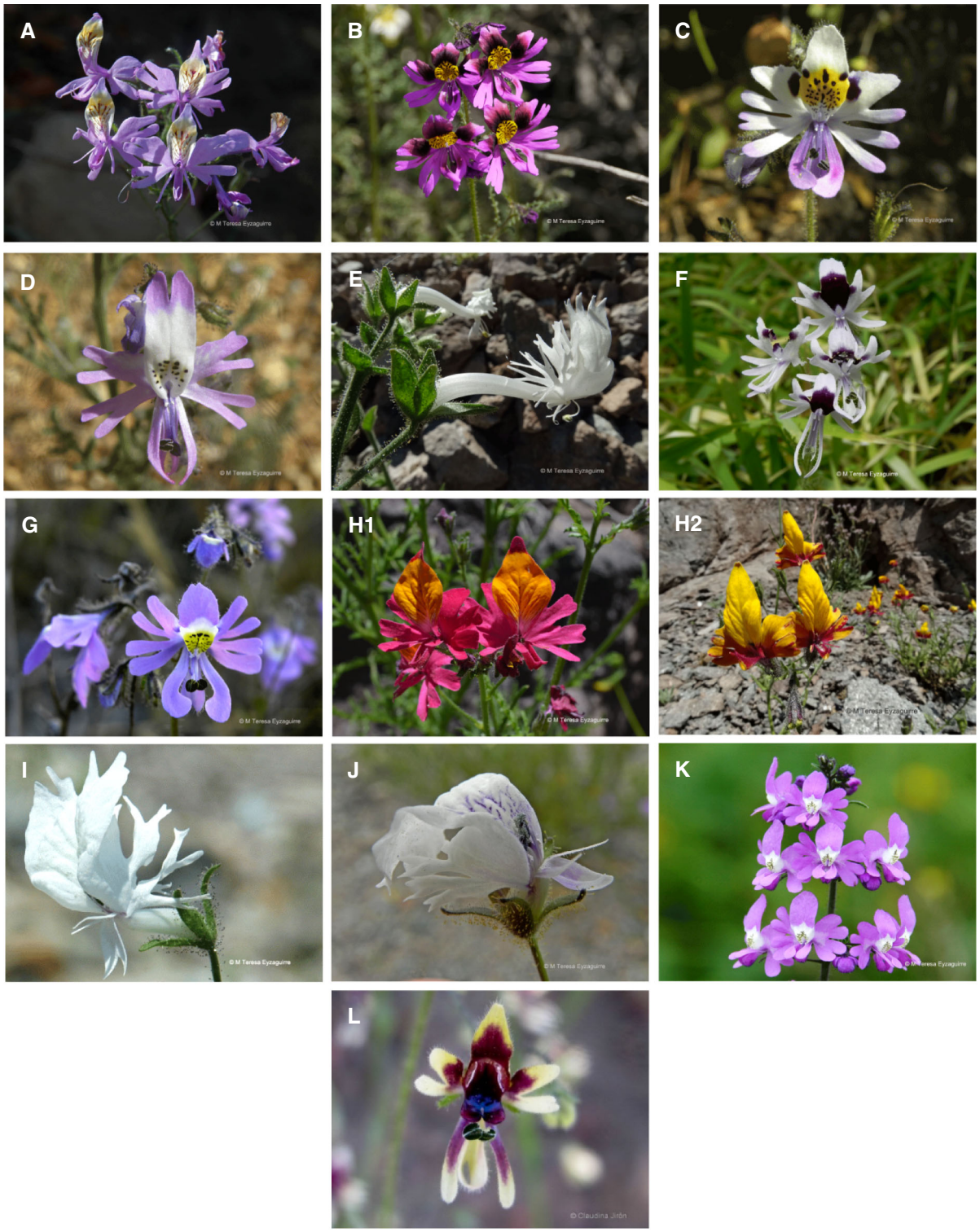


Fig. 3 Pictures of the *Schizanthus* species. **A** *S. hookeri* Gillies ex Graham; **B** *S. litoralis* Phil.; **C** *S. tricolor* Grau and Gronbach; **D** *S. alpestris* Poepp. ex. Benth.; **E** *S. integrifolius* Phil.; **F** *S. pinnatus* Ruiz & Pav.; **G** *S. porrigens* Graham; **H1** *S. grahamii* Gill. ex Hooker var. *grahamii*; **H2** *S. grahamii* Gill. ex Hooker var. *coccinea*; **I** *S. candidus* Lindl.; **J** *S. lacteus* Phil.; **K** *S. laetus* Phil.; **L** *S. parvulus* Sudzuki (Fundación RA Philippi)

Table 1 Alkaloids identified in *Schizanthus*

Alkaloids	MW	Formula	Compound	Species	References
1	139	$C_8H_{13}NO$	Tropinone	*S. tricolor*	Humam et al. (2007)
2	141	$C_8H_{15}NO$	Hygrine	*S. grahamii*	Christen et al. (2009)
				S. hookeri	Jordan et al. (2006)
				S. tricolor	Humam et al. (2007)
3	141	$C_8H_{15}NO$	3α-Hydroxytropane or	*S. alpestris*	Gambaro et al. (1982)
			3α-Tropine	*S. hookeri*	San Martin et al. (1980) and Gambaro et al. (1982, 1983)
				S. grahamii	Gambaro et al. (1982) and San Martin et al. (1987)
				S. litoralis	Gambaro et al. (1982)
				S. pinnatus	De la Fuente et al. (1988)
				S. tricolor	Humam et al. (2007)
4	143	$C_8H_{17}NO$	Hygrolines	*S. grahamii*	San Martin et al. (1987) and Christen et al. (2009)
				S. hookeri	San Martin et al. (1980), Gambaro et al. (1983) and Jordan et al. and (2006)
				S. tricolor	Humam et al. (2007)
				S. litoralis	Muñoz et al. (1996)
5	157	$C_8H_{15}NO_2$	3α,6β-Dihydroxytropane or 3α,6β-Tropanediol	*S. grahamii*	Christen et al. (2009)
				S. hookeri	Gambaro et al. (1982, 1983)
				S. litoralis	Gambaro et al. (1982)
6	183	$C_{10}H_{17}NO_2$	3α-Acetoxytropane	*S. tricolor*	Humam et al. (2007)
7	223	$C_{13}H_{21}NO_2$	3α-Senecioyloxytropane	*S. grahamii*	San Martin et al. (1987)
				S. hookeri	Gambaro et al. (1982)
				S. litoralis	Gambaro et al. (1982)
				S. tricolor	Humam et al. (2007)
8a	224	$C_{13}H_{24}N_2O$	Cuscohygrine (or *N*-methylpyrrolidinylhygrine) isomer **a**	*S. grahamii*	Christen et al. (2009)
				S. tricolor	Jordan et al. (2006)
8b	224	$C_{13}H_{24}N_2O$	Cuscohygrine (or *N*-methylpyrrolidinylhygrine) isomer **b**	*S. grahamii*	Christen et al. (2009)
				S. tricolor	Jordan et al. (2006)
9	239	$C_{13}H_{21}NO_3$	3α-Hydroxy-6β-angeloyloxytropane	*S. alpestris*	Gambaro et al. (1982)
				S. grahamii	Gambaro et al. (1982) and Bieri et al. (2006a, b, c)
				S. hookeri	San Martin et al. (1980) and Gambaro et al. (1982,1983)
				S. litoralis	Gambaro et al. (1982)
				S. pinnatus	De la Fuente et al. (1988)
				S. porrigens	Muñoz and Cortes (1998)
				S. tricolor	Humam et al. (2007)
10	239	$C_{13}H_{21}NO_3$	3α-Hydroxy-6β-senecioyloxytropane	*S. grahamii*	Bieri et al. (2006a, b, c)
				S. litoralis	Muñoz et al. (1996)
				S. tricolor	Humam et al. (2007)

Table 1 continued

Alkaloids	MW	Formula	Compound	Species	References
11	239	$C_{13}H_{21}NO_3$	3α-Hydroxy-6β-tigloyloxytropane	*S. grahamii*	Bieri et al. (2006a, b, c)
				S. hookeri	Gambaro et al. (1982, 1983)
				S. pinnatus	De la Fuente et al. (1988)
				S. tricolor	Humam et al. (2007)
12	239	$C_{13}H_{21}NO_3$	3α-Senecioyloxy-6β-hydroxytropane	*S. alpestris*	Gambaro et al. (1982)
				S. grahamii	Gambaro et al. (1982) and Bieri et al. (2006a, b, c)
				S. hookeri	San Martin et al. (1980) and Gambaro et al. (1982, 1983)
				S. litoralis	Gambaro et al. (1982) and Muñoz et al. (1996)
				S. pinnatus	De la Fuente et al. (1988)
				S. porrigens	Muñoz and Cortes (1998)
				S. tricolor	Humam et al. (2007)
13	253	$C_{13}H_{19}NO_4$	3α-Mesaconyloxytropane	*S. tricolor*	(Cretton, unpublished result)
14	255	$C_{13}H_{21}NO_4$	3α,4β-Dihydroxy-6β-angeloyloxytropane	*S. tricolor*	Cretton et al. (2017)
15	267	$C_{14}H_{21}NO_4$	3α-Methylmesaconyloxytropane	*S. hookeri*	Jordan et al. (2006)
				S. tricolor	Humam et al. (2007)
16	267	$C_{14}H_{21}NO_4$	3α-Methylitaconyloxytropane	*S. tricolor*	Humam et al. (2007)
17	291	$C_{17}H_{25}NO_3$	4-Hydroxyphenylpropanoylhygroline	*S. hookeri*	Cretton et al. (2017)
18a	313	$C_{18}H_{19}NO_4$	*cis*-*N*-(4-Hydroxyphenethyl)ferulamides	*S. litoralis*	Muñoz et al. (1996)
18b	313	$C_{18}H_{19}NO_4$	*trans*-*N*-(4-Hydroxy-phenethyl)ferulamides	*S. litoralis*	Muñoz et al. (1996)
19	337	$C_{18}H_{27}NO_5$	3α-(*E*)-4-Hydroxy-senecioyloxy-6β-angeloyloxytropane (schizanthine N)	*S. tricolor*	Humam et al. (2011a, b)
20	337	$C_{18}H_{27}NO_5$	3α-(*E*)-4-Hydroxy-senecioyloxy-6β-senecioyloxytropane (schizanthine O)	*S. tricolor*	Humam et al. (2011a, b)
21	351	$C_{18}H_{25}NO_6$	3α-Mesaconyloxy-6β-senecioyloxytropane (schizanthine P)	*S. tricolor*	Humam et al. (2011a, b)
22	351	$C_{18}H_{25}NO_6$	3α-Itaconyloxy-6β-senecioyloxytropane	*S. tricolor*	(Cretton, unpublished result)
23	365	$C_{19}H_{27}NO_6$	3α-Methylmesaconyloxy-6β-tigloyloxytropane (schizanthine F)	*S. pinnatus*	De la Fuente et al. (1988)
				S. tricolor	Cretton et al. (2010)
24	365	$C_{19}H_{27}NO_6$	3α-Methylitaconyloxy-6β-tigloyloxytropane (schizanthine G)	*S. pinnatus*	De la Fuente et al. (1988)
25	365	$C_{19}H_{27}NO_6$	3α-Methylitaconyloxy-6β-angeloyloxytropane (schizanthine H)	*S. pinnatus*	De la Fuente et al. (1988)
				S. tricolor	Humam et al. (2007) and Cretton et al. (2010)
26	365	$C_{19}H_{27}NO_6$	3α-Methylmesaconyloxy-6β-angeloyloxytropane (schizanthine I)	*S. pinnatus*	De la Fuente et al. (1988)
				S. tricolor	Humam et al. (2007) and Cretton et al. (2010)
27	365	$C_{19}H_{27}NO_6$	3α-Methylmesaconyloxy-6β-senecioyloxytropane	*S. litoralis*	Muñoz et al. (1996)
				S. tricolor	Cretton et al. (2010)
28	365	$C_{19}H_{27}NO_6$	3α-Methylitaconyloxy-6β-senecioyloxytropane	*S. tricolor*	Cretton et al. (2010)
29	365	$C_{19}H_{27}NO_6$	3α-Methylcitraconyloxy-6β-angeloyloxytropane	*S. tricolor*	Cretton et al. (2010)

Table 1 continued

Alkaloids	MW	Formula	Compound	Species	References
30	365	$C_{19}H_{27}NO_6$	3α-Methylcitraconyloxy-6β-senecioyloxytropane	*S. tricolor*	Cretton et al. (2010)
31	371	$C_{19}H_{33}NO_6$	1-Methyl-2-(1-methyl-2-pyrrolidinyl)-ethyl-6-deoxy-3-*O*-angeloyl-α-galactopyranoside	*S. integrifolius*	Muñoz et al. (1994)
32	379	$C_{20}H_{29}NO_6$	3α-Ethylmesaconyloxy-6β-senecioyloxytropane (schizanthine A)	*S. pinnatus*	Ripperger (1979)
33	379	$C_{20}H_{29}NO_6$	3α-Ethylmesaconyloxy-6β-tigloyloxytropane (schizanthine K)	*S. pinnatus*	De la Fuente et al. (1988)
34	379	$C_{20}H_{29}NO_6$	3α-Ethylitaconyloxy-6β-angeloyloxytropane (schizanthine L)	*S. pinnatus*	De la Fuente et al. (1988)
35	379	$C_{20}H_{29}NO_6$	3α-Ethylitaconyloxy-6β-tigloyloxytropane (schizanthine M)	*S. pinnatus*	De la Fuente et al. (1988)
36	413	$C_{23}H_{27}NO_6$	3α-Methylmesaconyloxy-6β-cinnamoyloxytropane	*S. litoralis*	Muñoz et al. (1996)
37	474	$C_{26}H_{38}N_2O_6$	Ditropane diester of mesaconic acid (Schizanthine C)	*S. grahamii*	San Martin et al. (1987)
38	474	$C_{26}H_{38}N_2O_6$	Ditropane diester of itaconic acid	*S. litoralis*	Muñoz et al. (1996)
39	490	$C_{26}H_{38}N_2O_7$	Ditropane diester of mesaconic acid (Schizanthine D)	*S. grahamii*	San Martin et al. (1987)
40	490	$C_{26}H_{38}N_2O_7$	Ditropane diester of itaconic acid (Schizanthine E)	*S. grahamii*	San Martin et al. (1987)
41	490	$C_{26}H_{38}N_2O_7$	Ditropane diester of itaconic acid (Schizanthine Y)	*S. porrigens*	Muñoz and Cortes (1998)
42	490	$C_{26}H_{38}N_2O_7$	Ditropane diester of mesaconic acid (Schizanthine Z)	*S. porrigens*	Muñoz and Cortes (1998)
43	572	$C_{31}H_{44}N_2O_8$	Ditropane diester of mesaconic acid (Schizanthine B)	*S. pinnatus*	Ripperger (1979)
44	572	$C_{31}H_{44}N_2O_8$	Ditropane diester of mesaconic acid (Schizanthine X)	*S. grahamii*	Muñoz et al. (1991)
45	638	$C_{35}H_{46}N_2O_9$	2-{[(3α-Hydroxytropo-6β-yl)oxy]carbonyl}2-methyl-3-{[((6β-angeloyloxy)-3α-yl)oxy]-carbonyl}-4-phenylcyclobutanecarboxylic acid (Grahamine A)	*S. grahamii*	Cretton et al. (2011)
46	638	$C_{35}H_{46}N_2O_9$	2-{[(3α-Hydroxytropo-6β-yl)oxy]carbonyl}-2-methyl-3-{[((6β-tigloyloxy)-3α-yl)oxy]-carbonyl}-4-phenyl-cyclobutanecarboxylic acid (Grahamine B)	*S. grahamii*	Cretton et al. (2011)
47	638	$C_{35}H_{46}N_2O_9$	1-Methyl-2-{[(3α-hydroxytropo-6β-yl)oxy]carbonyl}-4-{[((6β-angeloyloxy)-3α-yl)oxy]carbonyl}-3-phenyl-cyclobutanecarboxylic acid (Grahamine C)	*S. grahamii*	Cretton et al. (2011)
48	777	$C_{43}H_{63}N_3O_{13}$	1,2-*bis*{[(3α-Hydroxytropo-6β-yl)oxy]carbonyl}-2-methyl-3-{[((6β-angeloyloxy)-3α-yl)oxy]carbonyl}-4-phenylcyclobutane carboxylate (Grahamine D)	*S. grahamii*	Cretton et al. (2011)
49	871	$C_{48}H_{61}N_3O_{12}$	Grahamine	*S. grahamii*	Hartmann et al. (1990)
50	889	$C_{48}H_{63}N_3O_{13}$	1-{[(3α-Mesaconyloxy-tropo-6β-yl)oxy]carbonyl}-2-{[(3α-hydroxytropo-6β-yl)oxy]carbonyl}-2-methyl-3-{[((6β-angeloyloxy)-3α-yl)oxy]carbonyl}-4-phenylcyclobutane carboxylate (Grahamine E)	*S. grahamii*	Cretton et al. (2011)

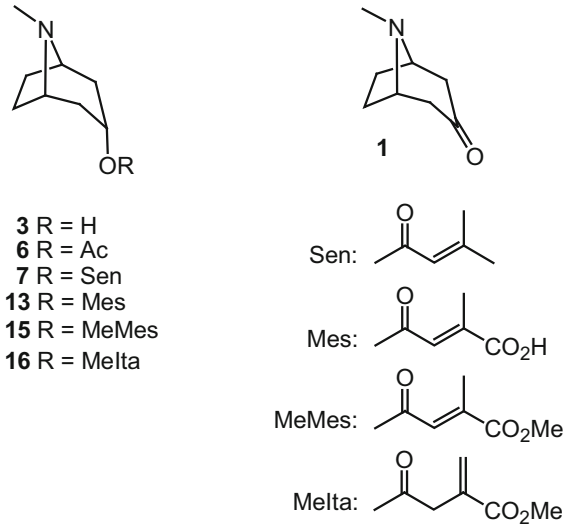


Fig. 4 *meso*-Tropane alkaloids from *Schizanthus*

4-hydroxyphenylpropanoylhygroline (**17**) has recently been isolated by centrifugal partition chromatography (Cretton et al. 2017). The compound arose from hygroline A and 4-hydroxyphenylpropanoic acid.

Schizanthus litoralis Phil.

This is an annual herb of up to 50 cm high, sticky to the touch due to its glandular trichomes, has doubly pinnatifid leaves of 4 to 8 cm in length, the upper ones being smaller and often entire. The zygomorphic flowers are divided into violet segments with yellow patches and a dark line at the division between the upper lobes. It grows principally in the littoral zone of the Coquimbo and Valparaiso regions (Fig. 3B) of Chile and is known as the butterfly of the coast. The five alkaloids 3α-hydroxytropane (**3**), 3α,6β-dihydroxytropane (**5**), 3α-senecioyloxytropane (**7**), 3α-hydroxy-6β-angeloyloxytropane (**9**), and 3α-senecioyloxy-6β-hydroxytropane (**12**) were identified, although no information from which part of the plant the compounds were isolated (Gambaro et al. 1982) has been provided. In a later study the leaves of this species (Muñoz et al. 1996), provided the isomeric mixture of hygrolines A and B (**4**), 3α-hydroxy-6β-senecioyloxytropane (**10**), compound **12**, *cis*- (**18a**) and *trans*-*N*-(4-hydroxyphenylethyl)ferulamides (**18b**), which strictly speaking should not necessarily be considered as alkaloids, 3α-methylmesaconyloxy-6β-senecioyloxytropane (**27**), 3α-

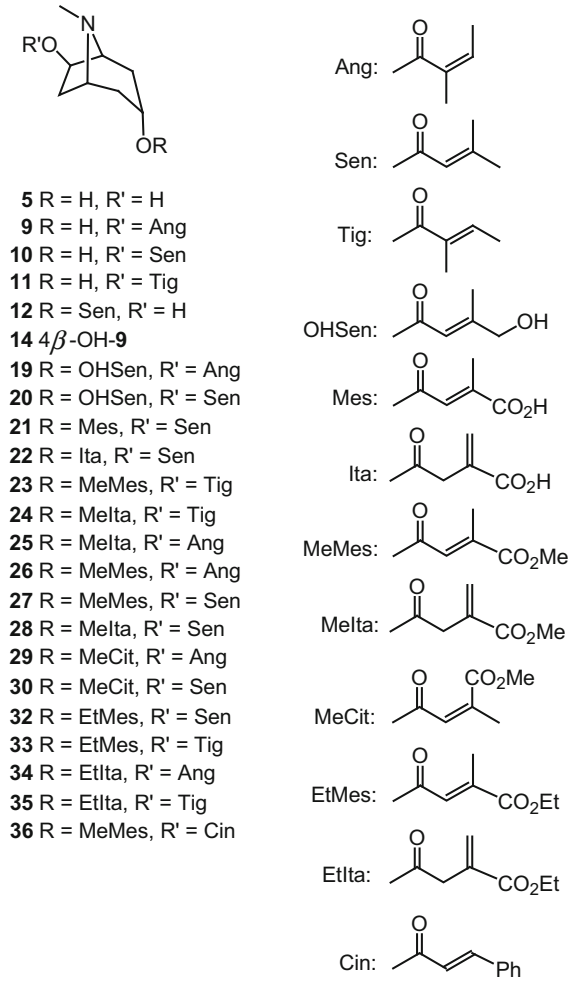


Fig. 5 3α,6β-Dihydroxytropane derived alkaloids from *Schizanthus*

methylmesaconyloxy-6β-cinnamoyloxytropane (**36**), and the ditropane diester of itaconic acid **38**. The characterization of the latter was based on spectroscopic data. The electron impact mass spectrum (EI-MS) showed the well-established fragmentation pattern of a 3,6-diacyloxytropane ester, while ^{1}H-NMR data revealed the presence of itaconic acid and one angelic ester residues. Two triplets at δ 5.04 and 5.07 ppm showed that two tropane skeletons were linked to a C-3α ester and the third ester was positioned at C-6β as an angelate. The ester linkages to the tropane moieties followed from an HMBC NMR measurement.

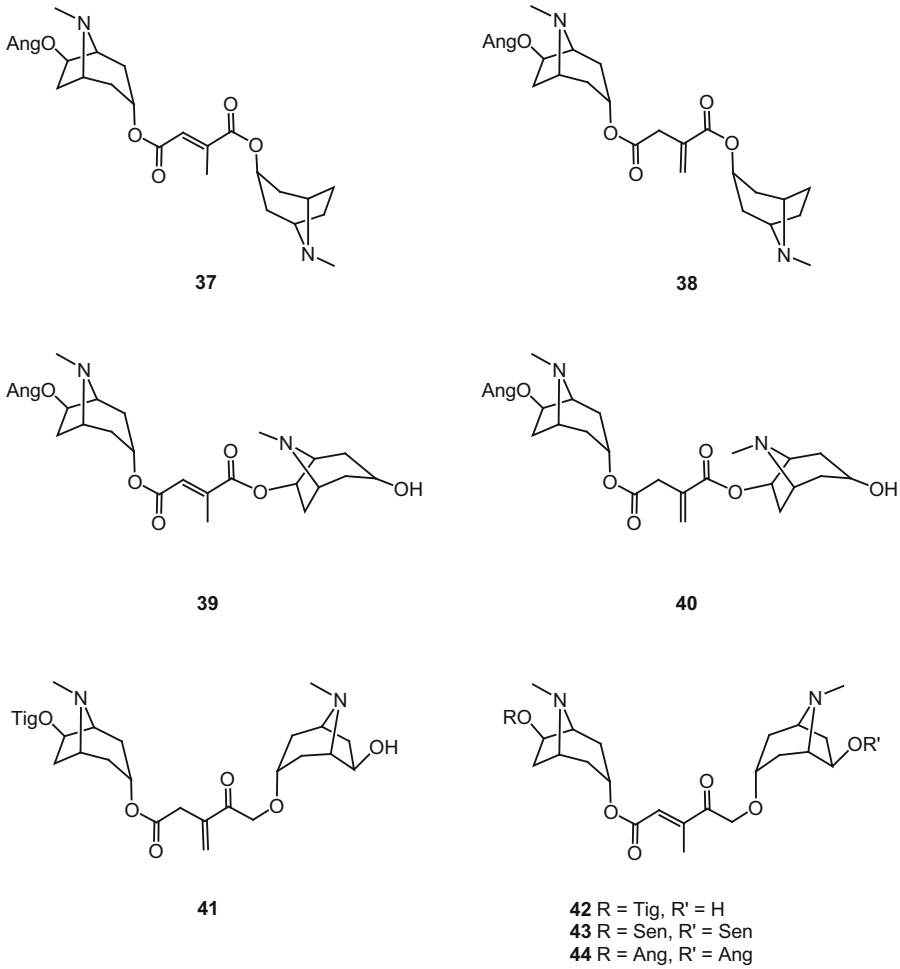


Fig. 6 Alkaloids from *Schizanthus* containing two tropane units

Schizanthus tricolor Grau and Gronbach

This is an annual herb of 60 cm high with zygomorphic lilac flowers having a yellow trimmed, deep purple center. It grows between 800 and 1400 m in the central Valparaiso and Santiago Metropolitan regions of Chile (Fig. 3C).

This plant is a factory of molecules providing one half of all known constituents of the genus since 26 alkaloids have been isolated and characterized from this species. A GC–MS study (Humam et al. 2007) of the aerial parts allowed the identification of 17 alkaloids by means of their retention indices, fragmentation pattern, and reference material when available. Among them, tropinone (**1**), a biosynthetic precursor of the tropane alkaloids in the Solanaceae family, its reduction product 3α-hydroxytropane (**3**), hygrine (**2**), hygrolines (**4**), 3α-acetoxytropane (**6**), 3α-senecioyloxytropane (**7**), compounds **9**–**12**, 3α-methylmesaconyloxytropane (**15**), 3α-methylitaconyloxytropane (**16**), schizanthine H (**25**), and schizanthine I (**26**). Later on, alkaloids **19**–**21** have been isolated (Humam et al. 2011a) and named schizanthines N, O and P, respectively. The AC of the tropane moiety (3*R*,6*R* or 3*S*,6*S*) has been determined by electronic circular dichroism (ECD) spectroscopy (Humam et al. 2008, 2011a). Schizanthines N (**19**) and P (**21**) are the first tropane alkaloids with a hydroxysenecioyl ester. In addition, schizanthine P was shown to be the 3α-mesaconyl ester of **10**. The isolation and structure elucidation or identification of further seven new isomeric tropane alkaloids with a molecular mass of 365 Da were later reported (Cretton et al. 2010), namely 3α-methylmesaconyloxy-6β-tigloyloxytropane or schizanthine F (**23**), the already isolated compounds **25** and **26**, 3α-

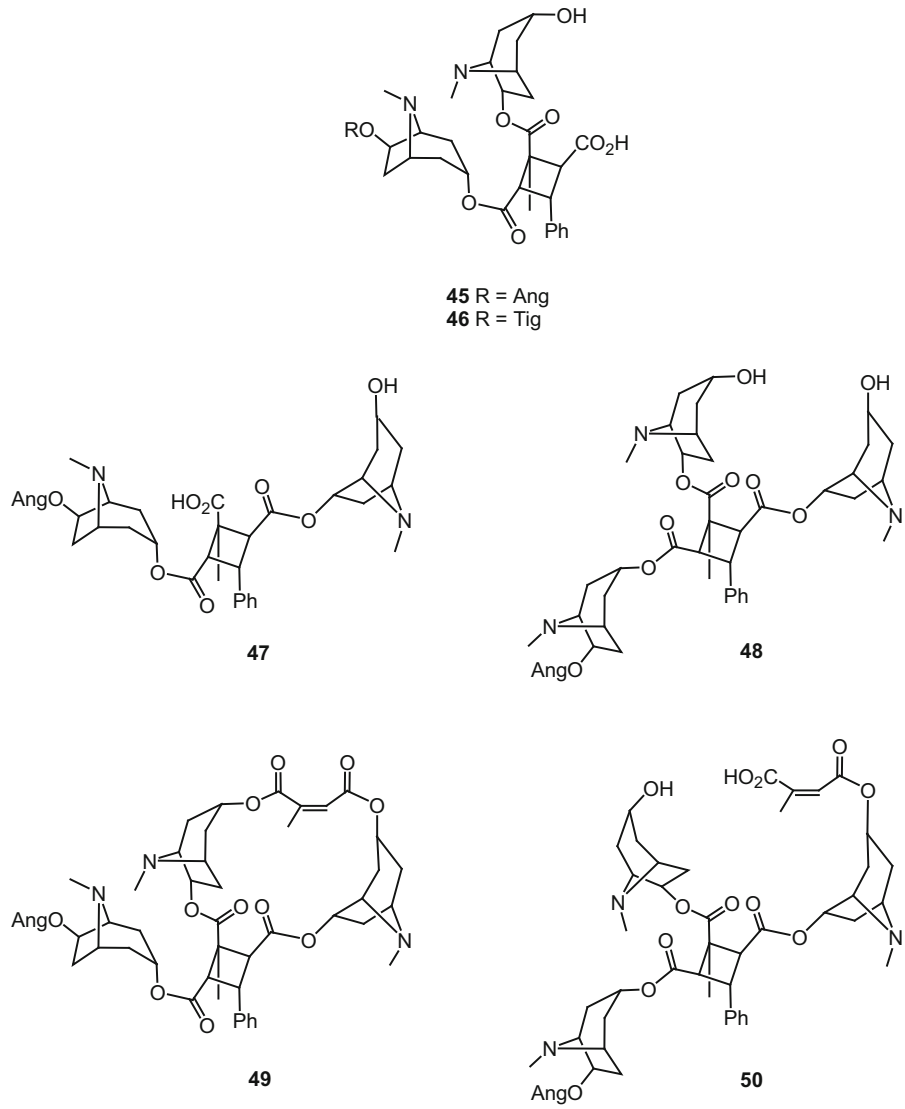


Fig. 7 Alkaloids from *Schizanthus* containing a cyclobutane skeleton

methylmesaconyloxy-6β-senecioyloxytropane (**27**), 3α-methylitaconyloxy-6β-senecioyloxytropane (**28**), 3α-methylcitraconyloxy-6β-angeloyloxytropane (**29**), and 3α-methylcitraconyloxy-6β-senecioyloxytropane (**30**). The new structures were established by ^{1}H- and ^{13}C-NMR including HSQC, HMBC, COSY, and NOESY experiments, as well as by UV, IR and MS. Among them, compounds **28**–**30** were new tropane alkaloids. Recently, the isolation and identification of 3α,4β-dihydroxy-6β-angeloyloxytropane (**14**) from the aerial parts of this species was published (Cretton et al. 2017). The authors used HRESIMS, 1D- and 2D-NMR (HSQC, HMBC, COSY, and NOESY), IR, UV, and GC–MS for this purpose. This was the first time that a 3,4-dihydroxytropane alkaloid is found in the Solanaceae family. In a recent, yet unpublished, investigation of the aerial parts of this species, one of us (S.C.) isolated the two new alkaloids 3α-mesaconyloxytropane (**13**) and 3α-itaconyloxy-6β-senecioyloxytropane (**22**) by semi-preparative HPLC.

Schizanthus alpestris Poepp. ex. Benth.

This herb of 20 to 40 cm high with pinnatisect leaves has small lilac flowers with the middle segment of the rectangular upper lip almost elliptical, always lobed at the apex and white and dark spots in the center. It grows in the Atacama and Coquimbo regions, between

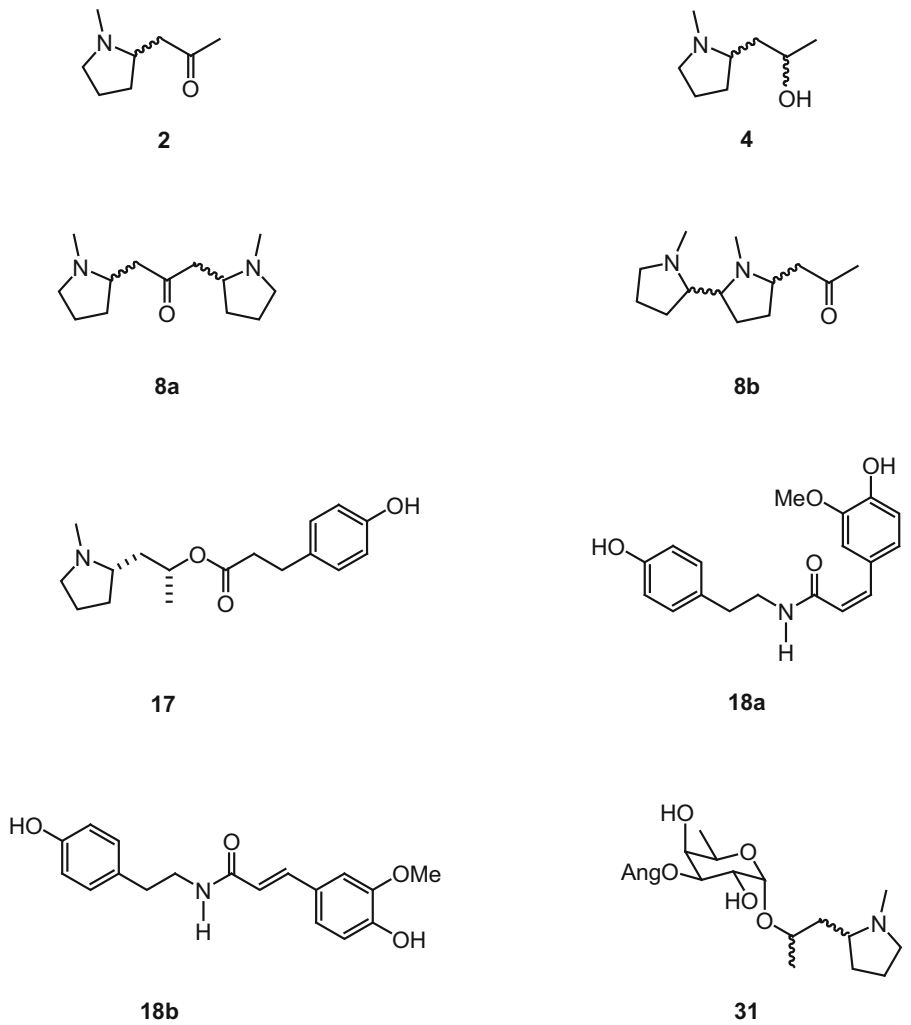
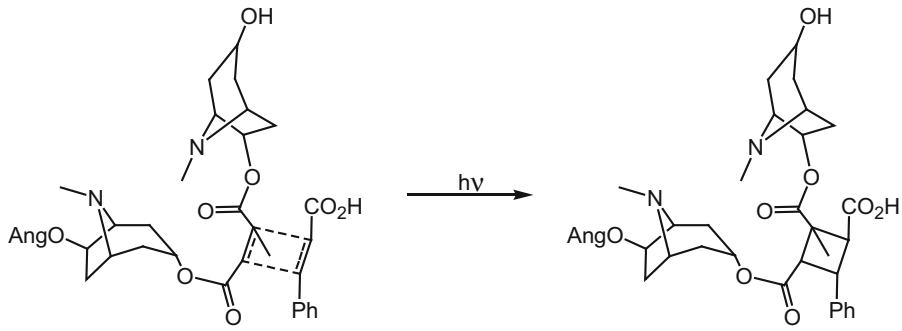


Fig. 8 Pyrrolidine alkaloids and isomeric amides from *Schizanthus*

900 and 2900 m of altitude (Fig. 3D). This species has been very little studied from a phytochemical point of view. Preliminary investigation (Gambaro et al. 1982) showed the presence of 3α-hydroxytropane (**3**), 3α-hydroxy-6β-angeloyloxytropane (**9**), and 3α-senecioyloxy-6β-hydroxytropane (**12**). Other alkaloids have been detected but not yet identified, so further studies are necessary.

Schizanthus integrifolius Phil.

Annual species of 60 to 80 cm high with white flowers, long arched tube and doubly divided petals. It grows in the Atacama and Coquimbo regions between 600 and 2700 m (Fig. 3E). One paper only (Muñoz et al. 1994) reported on the chemical investigation of this species indicating that from an ethanol leaf extract 1-methyl-2-(1-methyl-2-pyrrolidinyl)-ethyl 6-deoxy-3-*O*-angeloyl-α-galactopyranoside (**31**) was isolated and identified by 1D and 2D NMR spectroscopy. The AC of the sugar and hygroline moieties could not be determined because insufficient material. This is the only glycosylated alkaloid identified in the genus *Schizanthus* so far. The presence of a hygroline glycoside in this species and the absence of tropane alkaloids could represent a link with external genera such as *Nicotiana* and could be considered as a case of primitivism (Peña and Muñoz 2002).



39 + Cinnamic acid **44**

Fig. 9 Photoinduced [2 + 2] cycloaddition biosynthetic pathway for grahamines

Schizanthus pinnatus Ruiz & Pav.

Popularly known as butterfly flower, this annual herb of up to 60 cm high with bipennatisect leaves has white, pink or violet flowers arranged in inflorescences. It is native to Chile, where it is widely distributed in the hills of coastal areas of the Andes between 50 and 1400 m, growing from the south of the Coquimbo region to the region of Los Lagos (Fig. 3F). This species has been genetically manipulated for many years and is a common ornamental plant in Europe and the USA (Walter 1969). From a methanol leaf and stem extract two tropane alkaloids have been isolated and identified as schizanthines A (**32**) and B (**43**) (Ripperger 1979). The former is 3α-ethylmesaconyloxy-6*β*-senecioyloxytropane and in the latter the dicarboxylic mesaconic acid is linking the two tropanol monomers (**43**). Later on, seven other 3α,6*β*-dihydroxytropane derivatives: schizanthines F (**23**), G (**24**), H (**25**), I (**26**), K (**33**), L (**34**), and M (**35**) (De la Fuente et al. 1988) together with 3α-hydroxytropane (**3**), 3α-hydroxy-6*β*-angeloyloxytropane (**9**), 3α-hydroxy-6*β*-tigloyloxytropane (**11**), and 3α-senecioyloxy-6*β*-hydroxytropane (**12**) were isolated. Schizanthines A, B, G, K, L and M have only been identified in this species so far.

Schizanthus porrigens Graham

This is an annual large herbaceous plant of up to 80 cm high, with pink, purple or white flowers. The upper petal is yellow-stained with black spots. The pinnatisect leaves are covered with numerous glandular trichomes. It grows in the hills of coastal areas of the Coquimbo and Valparaiso regions, as well as in the metropolitan region of Santiago, at altitudes between 250 and 300 m (Fig. 3G). Four tropane alkaloids have been isolated and characterized from the leaves (Muñoz and Cortes 1998). Two of them are the monoesterified tropane derivatives, namely 3α-hydroxy-6*β*-angeloyloxytropane (**9**) and 3α-senecioyloxytropan-6*β*-hydroxytropane (**12**). The two other compounds are the ditropane alkaloids schizanthine Y (**41**) and Z (**42**), the former carrying an itaconic acid residue, instead of the mesaconic acid residue for **42**, between the two tropane skeletons.

Schizanthus grahamii Gill. ex Hooker

This annual or biennial plant of 30 to 70 cm grows in the Andes in the regions of Valparaiso and Biobío, between 700 and 2500 m. It is glandulous-pubescent, with pinnatisect leaves up to 11 cm long. Southern populations of this species display red flowers (*var. grahamii*, Fig. 3H1), while northern populations have pink-purple flowers or partially yellow flowers with reduced lateral sections (*var. coccinea*, Fig. 3H2) (Pérez 2011). Native to Chile, it is also cultivated as ornamentals elsewhere.

Twenty alkaloids have been detected or isolated from the roots and the aerial parts: 3α-hydroxytropane (**3**), hygrolines A and B (**4**), 3α-senecioyloxytropane (**7**), 3α-hydroxy-6*β*-angeloyloxytropane (**9**), and 3α-senecioyloxytropan-6*β*-hydroxytropane (**12**) have been identified more than three decades ago (Gambaro et al. 1982; San Martin et al. 1987). Hygrine (**2**), 3α,6*β*-dihydroxytropane (**5**), and cuscohygrine (or *N*-methylpyrrolidinylhygrine) (**8**) have been detected

and identified in the stem-bark by combining capillary gas chromatography with a selective nitrogen phosphorus detector and MS (Christen et al. 2009). Hygrine, as an example of a pyrrolidine alkaloid, is the biosynthetic precursor of pharmacologically important tropane alkaloids. The presence of **4** was also confirmed. Furthermore, the four positional and configurational tropane isomers **9–12** have been identified by combining capillary gas chromatography equipped with a selective nitrogen phosphorus detector coupled with a mass spectrometer and a Fourier transform infrared spectrometer (Christen et al. 2009). Identification of 3α-senecioyloxy-6β-hydroxytropane (**12**) has been further confirmed by injection of the pure compound previously isolated from *S. pinnatus* (De la Fuente et al. 1988). The AC of the four 3α,6β-tropanediol monoesters **9–12** has been assigned as (3*R*,6*R*) using density functional theory (DFT) calculations in combination with experimental vibrational circular dichroism (VCD) and comparison with the spectra of similar tropane alkaloids (Reina et al. 2010). The AC of 3α-hydroxy-6β-senecioyloxytropane (**10**) has also been determined by ^{1}H-NMR anisochrony induced by the Mosher chiral auxiliary reagents (−)-(*R*)- and (+)-(*S*)-α-methoxy-α-trifluoromethylphenylacetyl chlorides (Humam et al. 2011b).

Three ditropanol diesters of mesaconic acid, named schizanthines C (**37**) and D (**39**), and of itaconic acid named schizanthine E (**40**) have also been isolated (San Martin et al. 1987). They were characterized essentially by MS and the well-established fragmentation pattern of 3,6-diacyloxytropane derivatives, as well as by ^{1}H- and ^{13}C-NMR. The signals of diagnostic value were the chemical shift and multiplicity of the olefinic protons of the corresponding esters and the position and multiplicities of the skeletal protons at the point of attachment of the ester residues to the tropane scaffolds. Schizanthine X (**44**), another tropanol mesaconic acid derived diester, has been isolated from the leaves (Muñoz et al. 1991) and its structure determined by spectroscopic methods.

Six cyclobutane-centered tropane alkaloids named grahamines have been isolated from the aerial parts of this species (Cretton et al. 2011; Hartmann et al. 1990). These molecules are characterized by two or three acylated 3α,6β-dihydroxytropane moieties attached to a phenylcyclobutane skeleton. Grahamine (**49**) was the first tritropane alkaloid, which was isolated in 1990 (Hartmann et al. 1990). It contains three esterified 3α,6β-dihydroxytropane moieties with two mesaconate units, one cinnamoyl residue and one angelate. Five other new grahamines **45–48**, **50**, named grahamines A-E, respectively, carrying the same central cyclobutane scaffold, have been isolated and characterized from the aerial parts of this species (Cretton et al. 2011). Three of them, grahamines A (**45**), B (**46**), and C (**47**), have two tropane moieties, while grahamines D (**48**) and E (**50**) have three tropane units attached to the cyclobutane skeleton. Alkaloid **46**, named grahamine B, is very similar to **45**, the only difference being a tiglate instead of the angelate, while **47** is a positional isomer of grahamine A (**45**). The two tropane moieties are attached to carbonyl groups that are substituents on vicinal carbons of the cyclobutane in **45**, while they are attached to non-vicinal atoms in **47**. Alkaloid **48** possesses three tropane moieties attached to carbonyl groups on the cyclobutane ring, and an angeloyl group is attached to one tropane

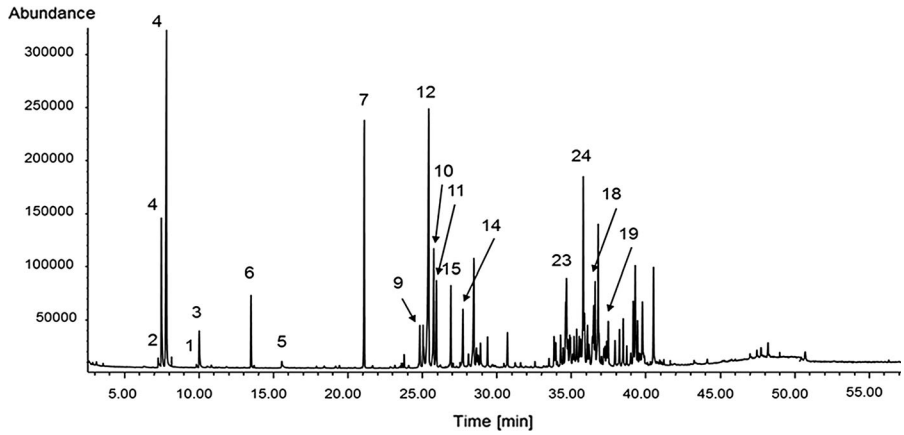


Fig. 10 GC-MS analysis of the aerial parts of *Schizanthus tricolor*. Column: HP5-MS 30 m × 0.25 mm i.d., 0.25 µm; carrier gas: He; ionization voltage: 70 eV; Oven temperature program: 70 °C (1 min) to 285 °C (15 min) at 5°/min; injection: splitless mode (1 µL); transfer line set at 280 °C

moiety. Compound **50** possesses also three tropanes, all observed to be cofacial by NOESY experiment as in **48**, while in **47** only two are cofacial. A mesaconate in **50** is the spacer of two tropanes.

From a biogenetic point of view, it is hypothesized that the cyclobutane derivatives could be biosynthesized in a [2 + 2] photocatalyzed cycloaddition process, and as depicted in Fig. 9, schizanthine X (**44**) could be formed by cyclization between the double bonds of the mesaconyl unit of schizanthine D (**39**) and a molecule of cinnamic acid. This hypothesis is supported by the absence of cyclobutane-containing alkaloids in the roots. Numerous cyclobutane-containing alkaloids have been isolated from terrestrial and marine organisms (Dembitsky 2008, 2014; Sergeiko et al. 2008). The ditropane alkaloids mooniines A and B, carrying a cyclobutane moiety, have been isolated from the leaves of *Erythroxylum moonii* (Rahman et al. 1998) and from coca leaves collected in various regions of South America (truxillines) (Moore et al. 1994).

Currently phytochemical unstudied *Schizanthus* species

For these four species only a botanical description is provided.

Schizanthus candidus Lindl.

Annual herbaceous plant of 30 to 60 cm high, with pinnatifid linear leaves and white flowers. The lower lip has the middle lobe divided into two acuminate flat segments, whereas the upper lip is two-lobed. It grows in the coastal zone of the Atacama region at altitudes between 20 and 720 m (Fig. 3I).

Schizanthus lacteus Phil.

Annual plant of 20 to 70 cm high, whose flowers are white, rarely pink, with the upper petal 5-fold divided and the lower lip 3-fold divided. The plant is covered of glandulous trichomes. It grows on the coast in the Antofagasta region between Paposo and Taltal at altitudes between 180 and 800 m (Fig. 3J).

Schizanthus laetus Phil.

Annual plant of 30 to 50 cm high with pinnatisect leaves and violet flowers with the superior middle petal stained with white, yellow, and purple dark spots at the base. It grows between Alto Punta de Lobos in the Tarapaca region, and Tocopilla and Paposo in the Antofagasta region between 50 and 800 m (Fig. 3K).

Schizanthus parvulus Sudzuki

Annual plant of 25 to 75 cm high covered with glandular trichomes. The plant presents few small pinnatisect leaves, and the flowers are the smallest in this genus (7–10 mm), they are purple with white or yellow ends. It grows in the Choapa province of the Coquimbo region between 200 and 1000 m (Fig. 3L).

Extraction, separation and analysis of the alkaloids from *Schizanthus*

Comprehensive reviews on the extraction, separation and analysis of tropane alkaloids from plant material, as well as from biological matrices have been published (Christen et al. 2008, 2013; Draeger 2002). Therefore only a brief overview of the methods and analytical techniques used for the identification of the alkaloids from *Schizanthus* is presented hereafter.

Extraction

Typically, the dried, powdered plant material (roots, aerial parts, stem-bark, leaves, etc.) is suspended under agitation in alcoholic solvents (MeOH or EtOH) for a few hours at temperature of ca. 50 °C. Sometimes it may be worthwhile to remove the fats and waxes from the ground plant material with petroleum ether or *n*-hexane prior to the extraction with polar solvents. The lack of selectivity of such extraction methods usually requires further cleanup procedures before analysis. Liquid–liquid extraction (LLE) with immiscible solvents or solid phase extraction (SPE) using conventional sorbents (e.g. C-18, diatomaceous earth) is commonly used for this purpose. The crude residue is dissolved in acidic aqueous solution and washed with dichloromethane. The aqueous phase is alkalinized to ca. pH 11–12 with NH_4OH or dil. NaOH and further extracted with dichloromethane. At this pH range, the free tropane alkaloid bases are nonionized

and largely soluble in organic solvents, particularly in dichloromethane and chloroform. SPE is often preferred to LLE because it is faster, uses less solvent and avoids the formation of undesirable emulsions that are often encountered in LLE.

Methods of separation and analysis

Thin-layer chromatography

Despite the advance of hyphenated analytical techniques, where very efficient separation instruments are coupled online with detectors generating spectral information, thin-layer chromatography is still the initial screening method well suited for plant extracts to confirm the presence or absence of alkaloids in specific fractions of interest. The development of high-performance thin-layer chromatography (HPTLC) (Reich and Schibli 2006), and the possibility to couple it with MS generates interest for unequivocal substance identification, as well as for the identity and quality control of botanical materials in general, and of medicinal plants in particular. HPTLC-MS is a complement to GC and HPLC, which is cost effective and very simple to apply. Dragendorff's reagent (potassium bismuth iodide) is the most frequently used reagent for the TLC detection of highly branched secondary amines and tertiary amines in general and alkaloids in particular, producing orange-red color spots on the plates when alkaloids are present. Alkaloids from *S. litoralis* (Muñoz et al. 1996), grahamine **49** from *S. grahamii* (Hartmann et al. 1990), as well as schizanthines A (**32**) and B (**43**) from *S. pinnatus* (Ripperger 1979) have been detected initially by TLC. A preliminary TLC examination of the dichloromethane and methanol extracts of the aerial parts of *S. tricolor* revealed the presence of a large number of alkaloids (Humam et al. 2007).

Gas chromatography

With the advent of capillary column and the coupling with MS, GC has evolved as a method of choice for the analysis of thermally stable and volatile enough alkaloids. Currently, rapid identification of tropane alkaloids is routinely accomplished by GC–MS. The fragmentation pathway of these compounds under the conventional and standardized electron impact (EI) ionization mode is very well known and together with the retention index, their identification is facilitated. Furthermore, databases are commercially available even though exploitable MS data can be found for the most common tropane alkaloids in the scientific literature. In addition (Humam et al. 2007), the alkaloid profile of the aerial parts of *S. tricolor* by GC–MS has been established (Fig. 10).

Using this method, seventeen alkaloids have been readily detected on-line. The corresponding identification has been carried out by comparison of their experimental EI-MS with those of reference compounds, by their retention indices, as well as with literature data. Many of them have been described for the first time in this species. Among chromatographically well separated alkaloids, compounds **9**–**12**, **15**, **16**, **19**, **20**, **25**, and **26** showed very similar MS but slightly different retention indices and have thus been determined as configurational isomers characteristic of the genus *Schizanthus*. The unambiguous distinction between tigloyl, angeloyl, or senecioyl moieties on the sole base of their fragmentation pattern was not possible. Therefore, their identification by GC–MS have been considered as tentative. Fortunately reference compounds or reference MS were available for 15 of the detected alkaloids. One new compound, 3α-methylitaconyloxytropane (**16**) has been identified and a synthesis was completed (Humam et al. 2007) to assign its structure.

Capillary GC combined with a selective nitrogen phosphorus detector (GC-NPD), GC–MS, and Fourier transform infrared (GC–FTIR) detector have been used to analyse the stem bark of *S. grahamii* (Christen et al. 2009). The alkaloid extract consisted mainly of nine nitrogen containing compounds and among them, four configurational and positional tropane alkaloid isomers, namely 3α-hydroxy-6β-angeloyloxytropane (**9**), 3α-hydroxy-6β-senecioyloxytropane (**10**), 3α-hydroxy-6β-tigloyloxytropane (**11**), and 3α-senecioyloxy-6β-hydroxytropane (**12**) with molecular ion peak at *m/z* 239 were evidenced. The distinction between angeloyl, senecioyl, and tigloyl derivatives was difficult based only on MS information and the data obtained by GC-NPD and GC–MS were insufficient for unambiguous identification, although the latter method allowed to position the substituents at C-3 or C-6. An unequivocal distinction between these isomers has been obtained through GC–FTIR on a megabore (0.53 µm i.d.) column with a thick film.

Indeed, angelates are characterized by two absorption bands near 1232 and 1156 cm^{-1} with a lower intensity for the former band, while the corresponding tiglate absorptions present specific bands at 1258 and 1140 cm^{-1} with an inverted intensity ratio. To confirm that isomer **11** was a tigloyl derivative, its IR spectrum was compared with a tigloyloxytropane reference alkaloid, namely tigloidine, which corroborated the attribution. Additionally, the retention indices of the alkaloids recorded on four stationary phases have been found to be different and help in the identification process. The same four isomers have been baseline separated by very fast GC-FID (Bieri et al. 2006a). To drastically reduce the analysis time, very short narrow-bore columns have been used. Experimental parameters including stationary phase, temperature, high split flow, internal column diameter, and optimal practicable gas velocity have been determined, which allowed isothermal analysis and separation of the four alkaloids in less than 6 s.

Capillary electrophoresis

Capillary electrophoresis (CE) coupled with UV and MS detection has emerged as a powerful analytical method for the determination of tropane alkaloids in plant material as well as in pharmaceutical formulations due to the ionizable properties of the compounds of interest. CE applications focusing on tropane alkaloids in different matrices have been reviewed (Aehle and Draeger 2010; Christen et al. 2008, 2013). The main advantages of CE are its high separation efficiency, short analysis time, low cost and the possibility of on-line coupling with electrospray ionization mass spectrometry (CE-ESIMS) allowing molecular structural information. Several modes of CE have been developed, namely capillary zone electrophoresis (CZE), micellar electrokinetic chromatography (MEKC), non-aqueous capillary electrophoresis (NACE), etc.

NACE appears to be ideally suited for online coupling with MS due to the high volatility and surface tension of various typically used organic solvents. Only one paper dealing with the analysis of tropane alkaloids from *Schizanthus* sp. has been published (Humam et al. 2005). It combined NACE with both, UV and MS detection to separate four isomeric tropane alkaloids isolated previously from *S. grahamii*, *S. hookeri*, *S. pinnatus*, and *S. litoralis*, namely 3α-hydroxy-6β-angeloyloxytropane (**9**), 3α-hydroxy-6β-senecioyloxytropane (**10**), 3α-hydroxy-6β-tigloyloxytropane (**11**), and 3α-senecioyloxy-6β-hydroxytropane (**12**). An electrolyte consisting of 1 M trifluoroacetic acid and 25 mM ammonium trifluoroacetate in a methanol:ethanol (40:60, v/v) mixture provided full separation of the four positional isomers in a fused-silica capillary. The volatile background electrolyte could be used for peak detection via UV and MS detection. The UV detector has been used for alkaloid quantification, and MS confirmed the structural identity of the isomers. CE-ESIMS measurements have been carried out in the positive ionization mode and performed with a single quadrupole using a CE-MS adapter kit from Agilent technologies. The required sheath liquid was a water:isopropanol (50:50, v/v) mixture containing 0.1% formic acid.

Liquid chromatography

Liquid chromatography is the separation technique which has been used most frequently for the analysis of tropane alkaloids over the last 30 years. Numerous HPLC methods have been published and concern mainly the analysis of hyoscyamine, its racemate atropine, scopolamine, and their metabolites (Aehle and Draeger 2010; Christen et al. 2008, 2013). Reversed phase C-18 columns are commonly employed for these analyses. Normal phases have high selectivity towards compounds that are chemically similar but physically different like isomers. However, these phases have some drawbacks causing peak tailing or irreversible adsorption and are rarely used for the separation of alkaloids. Alternatively, porous graphitic carbon stationary phase has hydrophobic properties and possesses a rigid and planar surface together with functions capable of strong charge-transfer interactions. The latter are mainly caused by the polarizable surface of the graphite contributing largely to the driving force enabling separation of closely related basic metabolites. Very few articles dealing with the separation and analyses of the alkaloids of the genus *Schizanthus* have been published so far. The four isomeric tropane alkaloids **9–12** isolated from *S. grahamii* have been successfully analyzed and baseline separated by capillary LC–MS on a HyperCarb capillary graphitic carbon column (Bieri et al. 2006b). Different chromatographic parameters such as the mobile phase composition, the concentration and

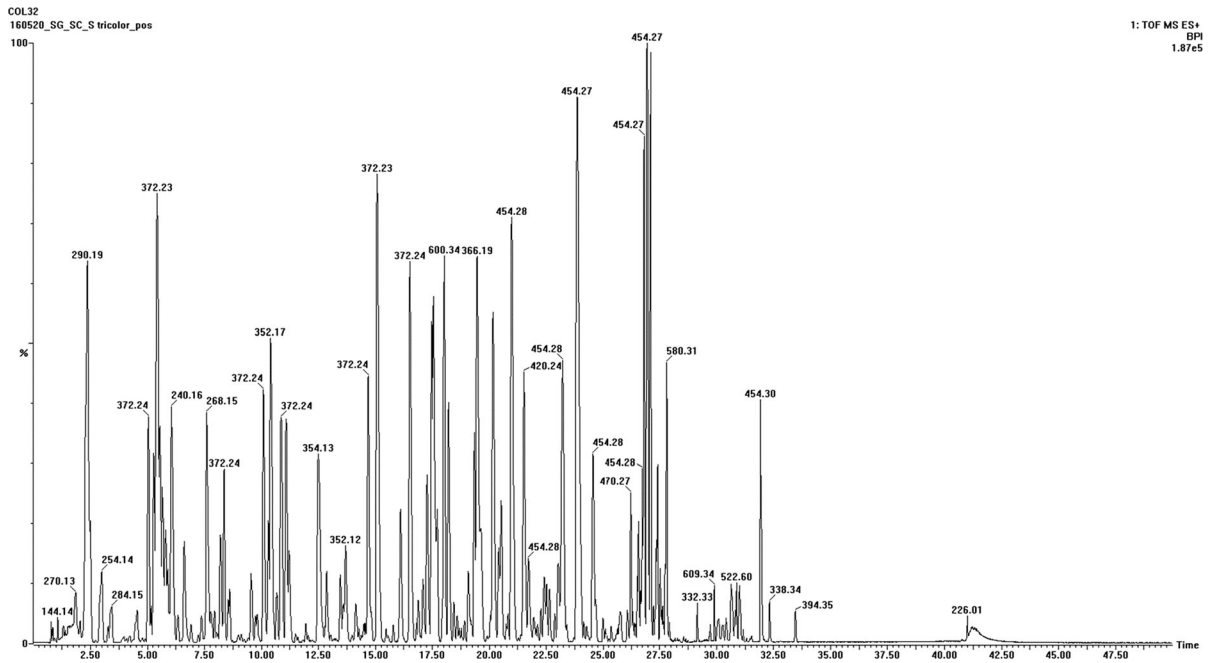


Fig. 11 UPLC-TOFMS chromatogram of the aerial parts of *Schizanthus tricolor*. Column: Acquity BEH C-18 UPLC 150 × 2.1 mm i.d., 1.7 µm; mobile phase A: MeCN-0.1% HCOOH, B: H_2O-0.1% HCOOH; Gradient: 2% A to 35% A (40 min) and 98% A (2 min). Flow rate 0.4 mL/min at 40 °C; Injection volume: 1 µL. ESI conditions: capillary voltage 2800 V, cone voltage 40 V, source temperature 120 °C, desolvation temperature 300 °C, cone gas flow 20 L/h, desolvation gas flow 600 L/h. Detection performed in positive ionization mode with a *m/z* range of 100–1300 Da and a scan time of 0.5 s in the W-mode

nature of the acidic or basic modifiers, the pH and the temperature have been evaluated. The optimized conditions allowed an outstanding selectivity towards the four alkaloids when an isocratic elution with 0.1% (v/v) formic acid in 30% MeOH at a flow rate of 4 µL/min at 60 °C was used.

UV detection mainly at 220 nm is often employed for tropane alkaloids possessing chromophore groups. However, some tropane or pyrrolidine alkaloids have low or even no UV absorption meaning that other detection modes are required like an evaporative light scattering detector or the universal refractive index detector.

The introduction of ultra-high performance liquid chromatography (UHPLC), together with significant advances in instrumentation and column technology allowed to significantly improving the separation power, resolution, speed and sensitivity. Gradient elution with water–methanol or water–acetonitrile mixture in acidic pH is generally used. UHPLC offers high-throughput analyses with reduced column lengths and reduced particle diameters, leading to dereplication avoiding the tedious isolation of already known compounds. The coupling of HPLC with mass spectrometry (HPLC–MS), in particular tandem mass spectrometry (MS^2 or MS^n) resulted in a powerful analytical tool for qualitative and quantitative determination of drugs. In particular, liquid chromatography-ion-trap mass spectrometry (LC-ITMS) and LC-time-of-flight mass spectrometry (LC-TOFMS) (Fig. 11) are powerful technologies particularly useful to study fragmentation patterns and to determine the elemental composition of analyzed compounds.

Using semi-preparative LC-UV (220 nm) equipped with a Symmetry Prep C-18 column using acidic (pH 3) methanol and then a radial compression analytical NovaPak C-18 column with isocratic acidic 16% methanol, it was possible (Humam et al. 2011a) to isolate schizanthines N (**19**), O (**20**), and P (**21**) from the aerial parts of *S. tricolor*. By means of two successive semi-preparative HPLC–UV (228 nm) separations on a LiChroprep Si 60 (250 × 8 mm, 5 µm) column and on a LiChroprep C-18 (250 × 8 mm, 5 µm) column, it was also possible

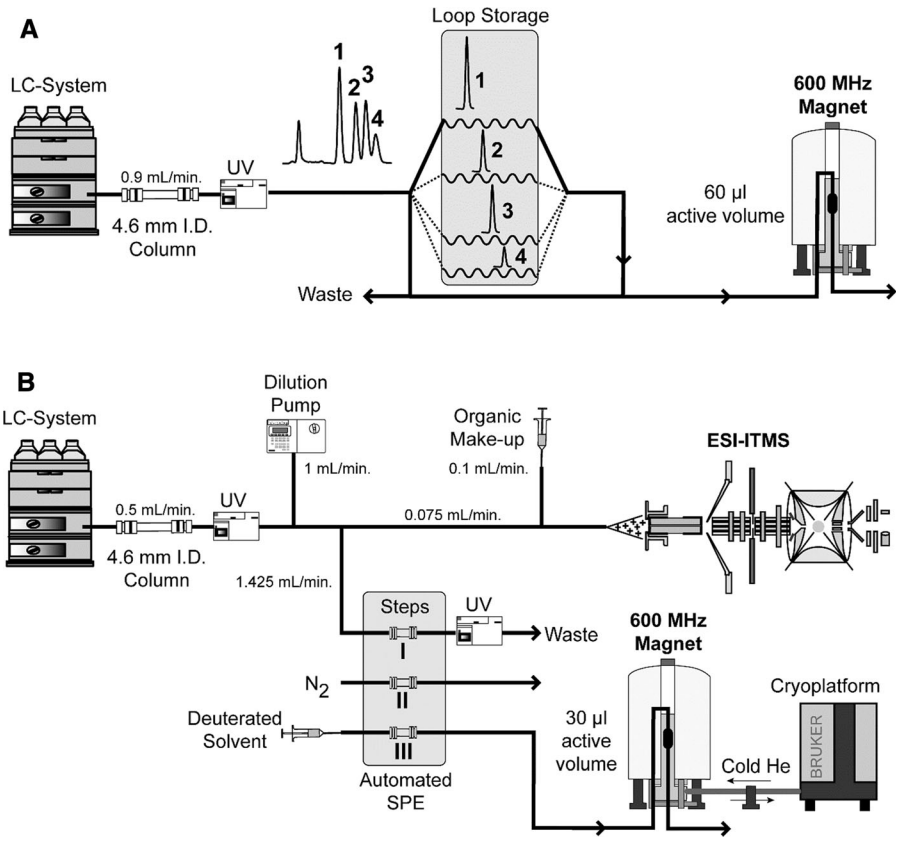


Fig. 12 Hyphenated HPLC-NMR approaches showing **a** peak sampling unit using loop storage and **b** peak trapping onto SPE cartridges with parallel MS and cryo-cooled NMR detection

(Muñoz et al. 1996) to isolate seven new compounds from the leaves of *S. litoralis*, namely the ditropane diester of itaconic acid **38**, 3α-methylmesaconyloxy-6β-senecioyloxytropane (**27**), 3α-methylmesaconyloxy-6β-cinnamoyloxytropane (**36**), 3α-hydroxy-6β-senecioyloxytropane (**10**), and *cis*- (**18a**) and *trans*-*N*-(4-hydroxyphenylethyl)ferulamides (**18b**), together with alkaloids **4** and **12**. However, LC–MS and LC-UV cannot always provide unequivocal structural determination, particularly when isomeric compounds are present in a complex mixture such as crude plant extracts. In these cases, the coupling of LC with nuclear magnetic resonance (LC-NMR) represents a sophisticated and extremely powerful complementary technique to LC-UV–MS in phytochemical analysis for the detailed on-line structural assignment. In this context two fully automated LC-NMR approaches are published (Bieri et al. 2006c) namely the loop storage and the trapping approaches for the analysis of tropane alkaloids using a LC-UV–MS/SPE-NMR set-up to deal with the limited NMR sensitivity and overcome the short analysis times during on-flow measurements (Fig. 12). Both approaches have been applied to the separation of the four isomeric tropane alkaloids **9**–**12** isolated from the stem-bark of *S. grahamii*.

The first approach interfaced a porous graphitic carbon (PGC) column (125 × 4.6 mm, 5 µm) to NMR by means of a peak sampling unit with 36 loops allowing storage measurements of up to 36 peaks within the same HPLC run (Fig. 12A). The separation has been performed isocratically with deuterated water and 10% standard acetonitrile, both containing 0.1% formic acid at 0.9 mL/min. ^{1}H-NMR spectra have been recorded on a 600 MHz apparatus using a double solvent suppression pulse sequence irradiating both residual HOD:H_2O and acetonitrile signals.

Compared to the stop flow mode, the loop storage approach avoids interrupting the chromatographic process for NMR measurements. The second approach combined HPLC with parallel IT-MS detection for peak selection and NMR spectroscopy using SPE-NMR (Fig. 12B). The SPE cartridges were dried with a stream of nitrogen and analytes were sequentially eluted and directed to a cryogenically cooled flow-

probe with deuterated solvents. The choice of a cartridge packed with polymerized polystyrene-divinylbenzene (HySphere) was essential because it can influence the efficiency of the trapping process and can affect the quality of NMR spectra.

Complete ^{1}H NMR signals assignment of the tropane skeleton

A careful inspection of the tropane scaffold evidences the existence of a quite rigid system having an *N*-methylpiperidine whose ring is constituted by the N–C-1–C-2–C-3–C-4–C-5 atoms which is substituted by an ethylene fragment formed by the C-6–C-7 atoms. The substitution arrangement of the ethylene fragment must have the *axial–axial* orientation on the piperidine ring to allow the second ring to close. In turn, the *N*-methyl group adopts the *pseudo-equatorial* orientation on the tetrahedral nitrogen atom of the piperidine ring since in the *pseudo-axial* orientation a higher free energy of around 0.9 kcal/mol is expected due to 1,3-*axial-axial* interactions of the *N*-Me group and the 2β and 4β hydrogen atoms, as was in fact calculated (Muñoz et al. 2010) for the simplest diester of this family, (3*S*,6*S*)-diacetyloxytropane (**51**).

In order to gain experimental evidence for this rigid conformation, the ^{1}H-NMR spectrum of (3*R*,6*R*)-dibenzoyloxytropane (**52**) was measured under very good magnetic homogeneity conditions (Muñoz et al. 2012) since both the magnitudes of vicinal coupling constants and the existence of long-range coupling constants must be evidenced in detail for such a situation. The 13 vicinal coupling constant values are in agreement with calculated dihedral angle derived values estimated using the Altona software (Cerda-García-Rojas et al. 1990) and include some quite small values owing to hydrogen atoms having dihedral angles close to 90°. The three *gem* coupling constants are within expected values, while relevant long-range coupling constants found in this study (Muñoz et al. 2012) are: $^{4}J_{2\alpha,7\beta}$ = 1.37 Hz, $^{4}J_{2\beta,4\beta}$ = 2.21 Hz, and $^{4}J_{3\beta,5}$ = 1.02 Hz. It followed that four-bonds coupling constant values are larger than some three-bonds vicinal coupling constant values like $^{3}J_{1,2\beta}$ = 2.19 Hz, $^{3}J_{1,7\alpha}$ = 0.64 Hz, $^{3}J_{2\alpha,3}$ = 1.01 Hz, and $^{3}J_{3,4\beta}$ = 1.10 Hz, a situation that could, at first glance, complicate the signal assignment understanding. In addition, DFT calculations have shown to be able to predict tropane (Muñoz and Joseph-Nathan 2009, 2010) ^{1}H- and ^{13}C-NMR chemical shifts, a fact that nicely complement the coupling constants analysis.

Absolute configuration determination of 3,6-dihydroxytropane derivatives

As indicated earlier in the Introduction Section, a tropane having hydroxy or acyloxy substituents at C-3 and C-6, in addition to the C-3 and C-6 stereogenic centers possesses two additional stereogenic centers at C-1 and C-5. However, since the tropane skeleton is a rigid scaffold, which allows no isomerization at C-1 and C-5, it is not necessary to specify the AC of the four stereogenic centers, which means the 3*R*,6*R* or 3*S*,6*S* stereogenic descriptors suffice. In addition all tropane alkaloids isolated until now from *Schizanthus* species have the 3α,6β disposition of the substituents, which contributes to an easier evaluation of their AC. Two chiroptical methods, namely ECD and VCD, either in combination with DFT calculations, have been used for this task.

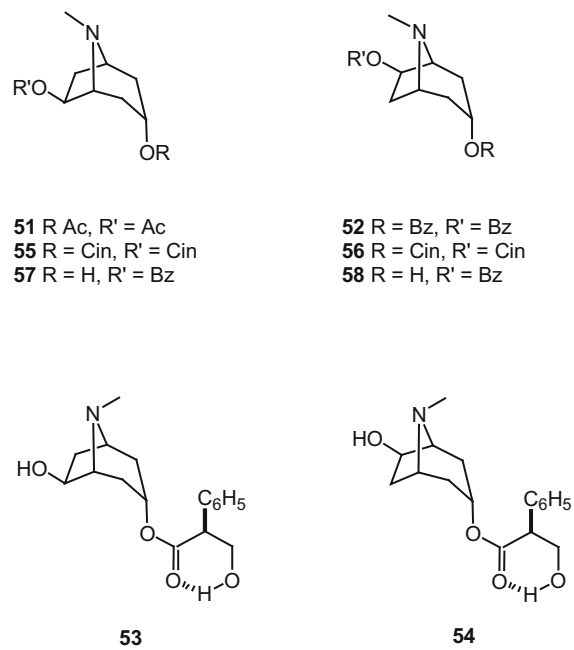
Regarding ECD, there are two papers addressing this methodology. The first of them (Humam et al. 2008) is a detailed study of 3α-(*E*)-4-hydroxy-senecioyloxy-6β-senecioyloxytropane (**20**), while in the second paper (Humam et al. 2011a), the latter alkaloid, also known as schizanthine O, together with 3α-(*E*)-4-hydroxysenecioyloxy-6β-angeloyloxytropane (**19**) or schizanthine N, and 3α-mesaconyloxy-6β-senecioyloxytropane (**21**) or schizanthine P, all isolated from *S. tricolor*, were studied. The three alkaloids possess two strong UV absorbing chromophores owing to their α,β-unsaturated esters and therefore the exciton coupling methodology in combination with time dependent density functional theory (TDDFT) calculations could be applied satisfactorily. Schizanthine O (**20**) showed (Humam et al. 2008) a negative Cotton effect around 230 nm in agreement with TDDFT calculations for the (3*R*,6*R*) enantiomer thereby establishing its AC. Similar observations for schizanthines N (**19**) and P (**21**) also established (Humam et al. 2011a) the same AC for them. Since all alkaloids isolated from *S. tricolor* share a common biogenetic origin, the 17 tropanes **6**, **7**, **9**–**13**, **15**, **16**, **22**, **23**, **25**–**30** have also the same AC, while the AC of **14** is (3*R*,4*R*,6*R*).

The determination of ECD spectra of tropane alkaloids like **19**–**21** is very convenient since the method requires a fraction of mg of the sample to provide a useful spectrum. However, in the absence of strong UV absorbing chromophores, VCD (Burgueño-Tapia and Joseph-Nathan 2015; Joseph-Nathan and Gordillo-Román 2015) turns out to be the method of choice since no chromophore at all is required, as even saturated chiral hydrocarbons can be studied. However a severe limitation of VCD is the required amounts of substance which, depending on the structure of the studied molecule, lies in the order of 5–10 mg.

The first tropane alkaloids that were studied by VCD, and that allowed to find out the suitability of this methodology, were the two 6β-hydroxyhyoscyamine diastereoisomers **53** and **54** (Muñoz et al. 2006) in which tropic acid is forming an ester with the hydroxy group at C-3. The DFT calculations allowed to conclude that levorotatory **53** has the (3*S*,6*S*,2′*S*) AC, while the dextrorotatory diastereoisomer is (3*R*,6*R*,2′*S*)-**54**, demonstrating that the contribution of the tropane moiety has a strong influence on the optical activity. The DFT results also showed an intramolecular hydrogen bond between the hydroxy and carbonyl groups of the tropic ester residue. This is relevant for successful DFT calculations because non-hydrogen bonded primary and secondary hydroxy groups should preferentially be avoided since they have a tendency to form intermolecular hydrogen bonds that complicate the calculation procedure. The four more stable conformers of **53** and **54**, which account for 82 and 86%, respectively, of the total conformational distribution, showed the *N*-Me group in the *syn* or *pseudo-axial* orientation due to formation of an intramolecular hydrogen bond between the 6β-hydroxy group and the electron pair of the nitrogen atom. It should be observed in Figs. 4, 5, 6 and 7 that the tropane scaffolds are drawn with the *equatorial* orientation of the *N*-methyl group, while in Fig. 1 the drawings show a *syn*- or *axial*-*N*-Me since the molecules are 6β-hydroxytropanes. In addition, one of the tropane skeletons of **41** is drawn as a *syn*-*N*-Me compound and, although not so represented due to simplicity of the drawings, the same methyl group orientation should be understood for 3α,6β-dihydroxytropane (**5**), for 3α-senecyoyloxy-6β-hydroxytropane (**12**), and for one of the tropane skeletons of **42**. In turn, the *N*-Me group in Fig. 13 is drawn as *axial* or *equatorial* according to the dominant conformational distribution due to the presence or absence of a 6β-hydroxy group.

These results are in agreement with the VCD study (Muñoz et al. 2010), and its associated DFT calculation procedures, of (3*S*,6*S*)-diacetyloxytropane (**51**), which reveals that around 82% corresponds to the *equatorial*-*N*-Me group orientation, which is essentially the inverted proportion found for **53** and **54**. This study of **51** also revealed that the phase of some strong signals is conformational dependent on the orientation of the *N*-Me and acyl groups, while some other peaks, in particular those in the 1150–950 cm^{-1} region, are conformational independent in the individual conformers calculated for **51**, and in good agreement with the experimental spectrum. This suggested these bands were of diagnostic utility for the AC determination of the tropane derivative without the need to perform the laborious DFT calculations normally required for such purpose. Inspection of the same spectral region of (3*S*,6*S*,2′*S*)-hydroxyhyoscyamine (**53**) showed the same bands at approximately the same frequencies, while diastereoisomer **54** showed these bands with the inverted phases.

With the above knowledge in mind, the (3*R*,6*R*) AC of 3α-senecioyloxy-6β-hydroxytropane (**12**), and of a



51 R Ac, R' = Ac
55 R = Cin, R' = Cin
57 R = H, R' = Bz

52 R = Bz, R' = Bz
56 R = Cin, R' = Cin
58 R = H, R' = Bz

53 **54**

Fig. 13 Some tropane derivatives studied by chiroptical methods. For ester abbreviations see Fig. 5

mixture of 3α-hydroxy-6β-tigloyloxytropane (**11**) and 3α-hydroxy-6β-senecioyloxytropane (**10**), isolated from *S. grahamii*, was determined (Reina et al. 2010) by comparing the experimental spectra with DFT calculations, which in the case of the mixture corresponded to a weighted calculation of the individual spectra according to a 69:31 ratio of **11**:**10** quantified by ^{1}H-NMR signal integration. In the case of a 7:3 mixture of **12**:**9**, isolated from *S. pinnatus*, the AC followed from the experimental bands in the 1150–950 cm^{-1} region as compared with those for **11** and **10**. In addition, characteristic VCD bands for the AC determination of 3α,6β-dicinnamoyloxytropanes **55** and **56** (Muñoz et al. 2014) were found, thus avoiding the need of tedious DFT calculations. The generality of this simplified methodology, in which the phases of the signals in the 1150–950 cm^{-1} VCD spectral region suffice, was further complemented (Muñoz et al. 2016) using the two enantiomers of 3α-hydroxy-6β-benzoyloxytropane **57** and **58** revealing that diesters or monoesters at either position show similar vibrational band phases owing to the rigid tropane scaffold.

Biological activities

The genus *Schizanthus* is better known for its ornamental features rather than for its potential medicinal applications (Grau and Gronbach 1984; Peña and Muñoz 2002). However, ethnobotanical information reveals that some species of the genus are used in the central Chilean Andes by herdsmen and mountain climbers as stimulants and produce effects similar to those of coca leaves, which is confirmed by local inhabitants (Cocucci 1989; Muñoz 1986).

Some tropane alkaloids are known as muscarinic receptor antagonists. They block the action of the neurotransmitter acetylcholine on post-ganglionic cholinergic nerves of the parasympathetic nervous system, essentially by blocking its binding to muscarinic cholinergic receptors. Some of them, mainly semi- or synthetic compounds carrying a quaternary ammonium are widely used for their gastrointestinal, bronchial and genitourinary effects with no activity on the central nervous system (CNS). In addition, alkaloids carrying a tertiary amine have pharmacological activities on the CNS depending on their ability to penetrate the blood–brain barrier. One of them, cocaine, is a potent CNS stimulating agent very frequently sniffed as illicit drug of abuse. Atropinic drugs dilate the pupil (mydriasis) resulting in a loss of accommodation (cycloplegia). All anticholinergic compounds of the Solanaceae family share a common basic structure: the tropane skeleton. In the genus *Schizanthus* having a wide range of tropane bases, it is reasonable to assume that some of these alkaloids might have anticholinergic properties or might be precursors that give rise to related biological activities. However, very often, the small amounts of alkaloids isolated from this genus precluded further pharmacological investigations. Therefore, very few studies have been published on the pharmacological properties of this genus. There is a report (Morales et al. 2013) on the anticholinergic effects on the rat ileum of a purified alkaloid mixture extracted from *S. hookeri*. In this study ileal segments have been subjected to different concentrations (10^{-4}–10^{-2} mg/mL) of the alkaloid mixture and the contractile response of the ileum induced with increasing doses of carbachol ($5{\cdot}10^{-8}$–$8{\cdot}10^{-4}$ M) has been determined. The results have been compared with those obtained with atropine. The alkaloid mixture competitively antagonized the response to carbachol.

Schizanthines F (**23**) and I (**26**), 3α-methylmesaconyloxy-6β-senecioyloxytropane (**27**), and 3α-methylitaconyloxy-6β-senecioyloxytropane (**28**), as well as a mixture of **23**, **26**, and **27** isolated from the aerial parts of *S. tricolor* have been evaluated for in vitro antiplasmodial activity and cytotoxicity (Cretton et al. 2010). Isomers **23**, **26**, and **28** demonstrated weak inhibition of *Plasmodium falciparum* strain K_1 (resistant to chloroquine and pyrimethamine) with IC_{50} values of 22.8, 24.8, and 36.0 μM, respectively, compared to amodiaquine ($IC_{50} < 0.25$ μM) and displayed no cytotoxicity on MRC-65 SV2 cells (human fetal lung fibroblasts) ($CC_{50} > 64$ μM), while isomer **27** was inactive (IC_{50} 63.5 μM). The alkaloid mixture exhibited slightly higher activity (IC_{50} 17.0 μM) than the pure compounds, indicating a possible synergy between the different isomers or between one alkaloid and another non-alkaloid compound. Although the antiplasmodial activity was weak, this is the first time that an antiparasitic activity has been observed for tropane alkaloids.

Biotechnological approaches

Higher plants are valuable sources of diverse metabolites and many of them are used as pharmaceuticals. In this context, tropane alkaloids are particularly noteworthy. Furthermore, in recent years, the global pharmaceutical market has begun to show a preference for natural derived products. Therefore, the market for natural plant products has grown, and this is likely to continue. However, in the case of *Schizanthus* species, the production of plant-derived substances is limited by environmental, ecological, climatic conditions, hydric stress, and urban sprawl into spaces that were previously forested. Additionally, *Schizanthus* bases are characterized by very small alkaloid concentrations in the different parts of the plant. The study of alkaloids in *Schizanthus* is fairly recent and in vitro morphogenic aspects and regeneration of these species from cell cultures have been reported once only (Jordan et al. 2006). In this study, in vitro shoot and root organogenesis, plant regeneration and production of tropane alkaloids in *S. hookeri*, *S. grahamii*, and *S. tricolor* have been investigated. In vitro culture leading to the formation of calli and regenerated plantlets has been developed for *S. hookeri*. Green calli have been obtained with 2.69 μM 1-naphthaleneacetic acid (NAA) and 2.22 μM 6-benzylaminopurine (BA) forming new shoots (approx. 10 shoots/callus) after 60 days. Regeneration of plantlets in medium devoid of growth regulators has been obtained after 90–110 days of shoot subculture. One alkaloid only has been detected in the calli. MS fragmentation pattern suggested a 3,6-disubstituted tropane derivative with a molecular formula $C_{19}H_{27}NO_6$ esterified with tiglic, senecioic, or angelic acid, and methylmesaconic, or methylitaconic acid. However, in the absence of reference compound, its identification has not been possible. Regenerated plantlets of *S. hookeri* developed from in vitro callus and shoot cultures have been analyzed by GC–MS (Jordan et al. 2006). Ten alkaloids ranging from simple pyrrolidine derivatives **2** and **4** to tropane esters derived from angelic, senecioic, tiglic, or methylmesaconic acids have been detected by GC–MS. Six of them have been unambiguously identified as **2**, **4A**, **4B**, **11**, **12**, and **15**, of which **15** had not been described previously, and its identification has followed after comparison with a synthetic reference compound. This alkaloid has also been found later in the aerial parts of *S. tricolor* and identified by GC–MS (Humam et al. 2007). Four other alkaloids have also been detected but could not be identified due to the absence of reference material. One compound showing 321 Da in the MS, was a 3,6-disubstituted tropane alkaloid esterified with tiglic or senecioic acid. The other three alkaloids were 3,6-disubstituted isomers, showing 365 Da in the MS, esterified with tigloyl, senecioyl, or angeloyl, and methylmesaconyl, methylitaconyl, or methylcitraconyl moieties. The same growth conditions, as well as other growth regulator levels, have been tested to induce callus and root formation in *S. grahamii*. Root organogenesis and green calli appeared in the presence of 4.44 μM BA and 0.54 μM NAA. However, shoot formation did not occur. Only one alkaloid has been identified (281 Da) in the green calli. Its fragmentation pattern showed that it corresponds to a 3,6-disubstituted tropane alkaloid with acetic and tiglic, senecioic, or angelic residues as esterifying acids. The calli of *S. tricolor* showed less chloroplast formation turning brown within 1 month and were unable to recover in subculture under various levels of growth regulator combinations. Only one alkaloid, cuscohygrine or *N*-methylpyrrolidinylhygrine (**8**) has been detected.

Conclusion

Over 50 alkaloids have been isolated and identified in eight species of the genus *Schizanthus* between the period 1979–2017. The studied species are *S. alpestris*, *S. grahamii*, *S. hookeri*, *S. integrifolius*, *S. litoralis*, *S. pinnatus*, *S. porrigens*, and *S. tricolor*. In some cases GC–MS and (U)HPLC–MS profiles of the extracts showed the presence of a large number of nitrogen containing compounds ranging from simple pyrrolidine derivatives to highly complex tritropanes. From a chemotaxonomy point of view, the genus lies relatively far from the other genera of the Solanaceae family, probably due to the occurrence of di- and tritropane derivatives, which are only found in this genus. The presence of many tropane alkaloids, characteristic of the Solanaceae family to which the genus *Schizanthus* belongs, and whose pharmacological activities have not been studied so far, should encourage further research in this field. Despite the low amounts of alkaloids isolated from these species, pharmacological investigations on crude alkaloid

extracts should be undertaken. Furthermore, since it is well known that tropane alkaloids from other genera show relevant biological activities, the synthetic preparation of suitable amounts for such studies should not be ruled out. Regarding the absolute configuration of these tropanes, up to now all studies point to the (3*R*,6*R*) absolute configuration as determined by chiroptical methods like electronic and vibrational circular dichroism. The four species, *S. candidus*, *S. lacteus*, S. *laetus*, and *S. parvulus* have not yet been investigated and are awaiting that research groups spend some time to explore their chemical content.

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