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Calystegines as chemotaxonomic markers in the Convolvulaceae ☆

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Abstract

An extended GC–MS study of 129 convolvulaceous species belonging to 29 genera (all 12 tribes) including the results of a previous survey (65 spp.) revealed the occurrence of one to six polyhydroxy alkaloids of the nortropane type (calystegines) in 62 species belonging to 22 genera of all tribes except the unique parasitic Cuscuteae. The large genus *Ipomoea* turned out to comprise calystegine-positive species in at least eight out of ten sections checked. The number of the calystegines used as reference compounds has been increased from seven (previous survey) to 11 (present study). Furthermore, the results concerning these additional four alkaloids could also be completed for all species of the previous survey. The plant material (epigeal vegetative parts and/or roots, flowers, fruits/seeds) was obtained from collections in the wild from a wide range of tropical, subtropical, and temperate locations of all continents as well as from cultivation in the greenhouse. All plant organs turned out to be potential locations for the occurrence of these metabolites though they are detectable often only in certain organs of a given species. Three genera (*Cuscuta*, *Operculina*, *Polymeria*) might have lost the ability to synthesize these plesiomorphic characters in the course of the evolution since the examination of several different organs and/or provenances of five species each failed to show calystegines as constituents. Nevertheless, the present data clearly demonstrate that the occurrence of calystegines is an almost consistent trait in the Convolvulaceae in principle, from basal to most advanced tribes.
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1. Introduction

Polyhydroxy alkaloids, e.g., the calystegines (nortropanes) bind specifically to the active sites of glyco-

sidases inhibiting the enzymes. Thus, they have aroused increasing interest as, e.g., potential antiviral, anticancer, and antidiabetic agents. From the ecological point of view such compounds may cause intoxications in cattle (Asano et al., 2000 and literature therein) and are also toxic for vertebrates, insects, and microbes (Fellows et al., 1989 and literature therein). Furthermore, they exhibit antifeedant effects against lepidopterans (Simmonds et al., 1990). However, it is not yet clear if there is a physiological role

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of polyhydroxy alkaloids in general and of calystegines in particular for the producing plant species itself (Dräger, 2004; Hoeke and Dräger, 2004).

The calystegines seem to be confined to the two major Solanales families, Convolvulaceae and Solanaceae, as well as to the unrelated Moraceae. To date the structures of 15 calystegines have been elucidated including five trihydroxynortropanes (A_3, A_5–A_8), six tetrahydroxy congeners (B_1–B_6), and two pentahydroxy derivatives (C_1, C_2) (see Fig. 1). Besides free calystegines, several glycosides could be isolated from solanaceous species. In addition, the N-methylated congeners of the calystegines B_2 and C_1, respectively, have been found again in solanaceous species (Asano et al., 2000; Dräger, 2004). The occurrence of calystegines in the family Solanaceae is documented in the literature for 15 genera (out of 92) covering 32 species (out of 2300) (Table 3). In the sister family Convolvulaceae these alkaloids were found in *Calystegia sepium* and *Convolvulus arvensis* (Tepfer et al., 1988), two further *Calystegia* spp. (Molyneux et al., 1995) and seven *Ipomoea* taxa (Asano et al., 2001). In our previous study on the occurrence and distribution of these compounds in the Convolvulaceae comprising 65 species (out of 1850) from 22 genera (out of 55) after all 30 species belonging to 15 genera were found to be calystegine-positive (Schimming et al., 1998).

Our present study has been extended considerably in several respects: (1) It comprises another 64 species including for the first time also Australian taxa with the consequence that all continents are represented now. (2) Again for the first time the rare genera *Cardiochlamys*, *Humbertia*, *Rapona* (all endemic to Madagascar), and *Hildebrandtia* (East Africa/Madagascar/Arabia) altogether classified as basal (Stefanović et al., 2003), as well as the more advanced genera *Polymeria* (endemic to Australia) and *Astripomoea* (endemic to Africa) are involved. (3) We have been able to include species from not yet investigated sections of the large genera *Ipomoea* and *Merremia*, respectively. (4) The number of species in certain genera which had turned out already in the previous study to be calystegine-positive has been increased disproportionately (the largest genus *Ipomoea* by additional 21 species, comprising now in total 38 species; the large genus *Convolvulus* by 13 (now in total: 18), the basal genus *Erycibe* by 3 (now in total: 5) in order to confirm these previous findings. (5) In our first study two genera (*Jacquemontia*, *Operculina*) did not show any occurrence of calystegines in different samples of several species. By including further species and/or organs of these genera we wanted to find out if these will support this previous finding or not. (6) Our former study was based only on seven calystegines whereas now we could integrate four additional congeners. This also means that we are able to enhance the results of the former 65 species by these additional polyhydroxylated alkaloids. Thus, we want to report on the occurrence/the lack of all these metabolites in 129 species belonging to 29 genera which include
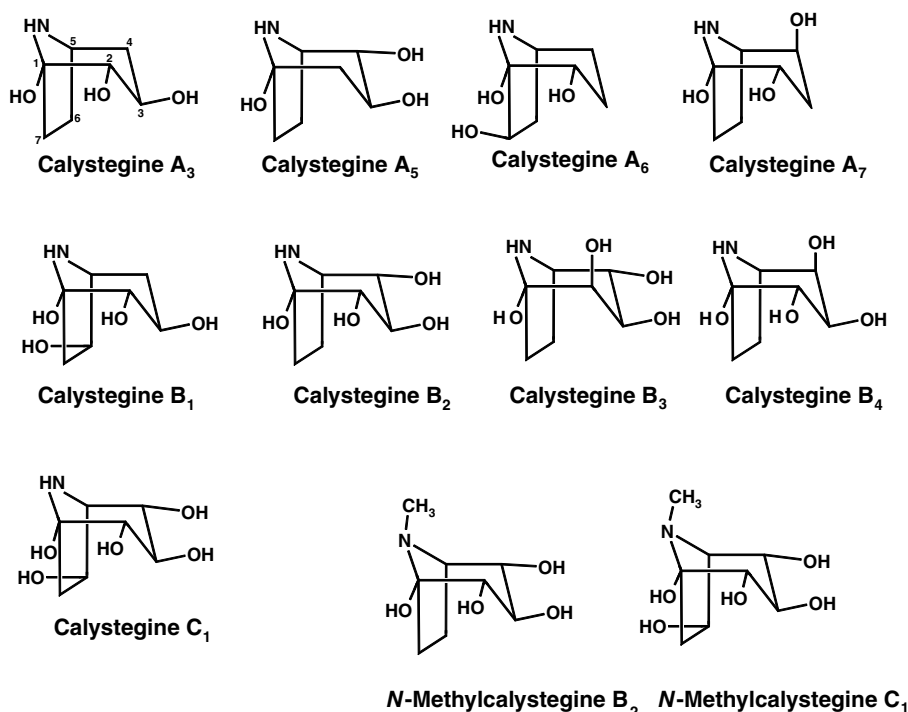

Fig. 1. Structure of the calystegines and *N*-methylcalystegines included in this study.

members of all 12 convolvulaceous tribes recently established on the basis of molecular cladistic phylogenetic analysis (Stefanović et al., 2002), afterwards correctly aligned with the traditional phylogenetic point of view (Stefanović et al., 2003) (Table 1).

There are a few reports on the co-occurrence of both, calystegines and swainsonine, a polyhydroxylated indolizidine alkaloid well-known from certain fabaceous species, in the seeds of two Australian *Ipomoea* taxa, *I. polpha* and *I.* sp. Q6 (aff. *calobra*, Weir Vine), the latter supposed to be a subspecies of the former (Molyneux et al., 1995) and from the leaves of a pantropical toxic *Ipomoea* species of American origin, *I. carnea* ssp. *fistulosa* (de Balogh et al., 1998; Asano et al., 2001; Haraguchi et al., 2003). Therefore swainsonine as well as its congener castanospermine have been also integrated in our present study.

2. Results and discussion

Since polyhydroxylated alkaloids cannot be isolated by conventional alkaloid separation techniques, ion-exchange chromatography was employed for separating this polar alkaloid fraction from neutral and acidic constituents of the extract (Hohenschutz et al., 1981; Dräger, 1995). In order to achieve volatile derivatives necessary for GC–MS, the silylation method of Fleet et al. (1990) was used. This procedure leads to trimethylsilyl substitution at the hydroxyl groups leaving the secondary amino group unsubstituted. The compounds have been identified by combined gas-chromatographic and mass spectrometric analysis (GC–MS) comparing retention times and fragmentation patterns with authentic samples. In the majority of the cases samples of epigeal vegetative parts and roots have been investigated. Since calystegines are supposed to accumulate predominantly in meristematic tissues (Dräger et al., 1995; Keiner et al., 2000; Scholl et al., 2001) fresh young parts of the organs were preferred as far as available.

Calystegines turned out to be common convolvulaceous metabolites and may occur in all plant organs though this is not always the case (Table 1). They were detected unequivocally in 62 out of 129 species belonging to 22 out of 29 genera included in this study. The calystegine-positive species belong to 11 out of 12 tribes recognized for this family (Stefanović et al., 2003). Three genera with at least five species each showed a high percentage of positive species (*Argyreia* 6 out of 7; *Calystegia* 4 out of 5; *Erycibe* 5 out of 5). In the case of *Calystegia* data of two species taken from the literature are involved in 5 (Table 1). Other genera with at least four species included yielded 50:50 results: *Bonamia* 2 out of 4; *Convolvulus* 10 out of 18; *Ipomoea* 22 out of 42; *Merremia* 3 out of 7. In the case of the largest genus *Ipomoea* data of five species from the lit-

erature are involved in the numbers (22 and 42, respectively): four species which have not been included in our study and in addition *I. pes-caprae* which has been negative in our study (for details see Table 1). This genus turned out to comprise calystegine-positive species in at least eight of ten sections checked. For the remaining two sections (Leptocallis, Orthipomoea) only one and two species, respectively, have been available. Thus, it is equivocal if these sections are calystegine-negative in general. In the case of *Merremia* the four sections involved in this study seem to contain calystegine-positive species in contrast to the closely related genus *Operculina* (see below). *Jacquemontia* has been the strangest genus since the assay of 20 samples taken from altogether six species resulted in 19 negative findings; only one sample turned out to be positive: the roots of *J. tamnifolia* with at least five calystegines. This is especially remarkable because the aerial parts from two provenances and the fruits of this species were also negative.

The number of compounds in calystegine-positive species (a few taken from the literature included; see above and Table 1) varied between one and six. Only one compound each could be detected in the samples of 24% of these species, followed by two and three compounds each (22% of the species each), four (20%), five (9%), and six compounds (3%). The tetrahydroxylated alkaloids B_2 and B_1 turned out to be the most frequent compounds (90% and 68% of the positive species, respectively) followed by the trihydroxynortropane A_3 (38%) and the pentahydroxylated congener C_1 (26%). Interestingly, calystegine B_2 is also in the lead concerning the sister family Solanaceae (Dräger, 2004). The calystegines A_5, B_3, and B_4 displayed a minor frequency in our study ranging from 16% to 20% (Table 2). Calystegine A_6, characterized by the lack of a hydroxy group at C-3, could only be discovered in *Stictocardia mojangensis*. Neither its A_7 congener as well as the *N*-methyl derivatives of B_2 and C_1, respectively, nor the indolizidine type polyhydroxy alkaloids castanospermine and swainsonine could be identified in any of our samples. Thus, the surprising occurrence of swainsonine in three *Ipomoea* taxa documented in the literature (see Section 1) remains unique for the family Convolvulaceae. Recently, the question whether the serious livestock poisonings by *I. carnea* ssp. *fistulosa* (Zakir et al., 1989; Srilatha et al., 1997; de Balogh et al., 1998) is due to the single effect of swainsonine or to a combination of effects caused by swainsonine and calystegines might have been answered: The lysosomal β-glycosidase inhibitory effect of calystegines seems to have little risk of inducing intoxication of livestock suggesting that the toxic principle of this species is represented by swainsonine rather than by calystegines (Haraguchi et al., 2003; Ikeda et al., 2003). Therefore the lack of swainsonine in all these numerous calystegine-positive as well as calystegine-negative species of the present study may explain

Table 1

Calystegine patterns in 135 convolvulaceous species; tribal taxonomy based on molecular cladistic phylogenetic analysis by Stefanović et al. (2002, 2003), and traditional anatomic morphological features concerning the infragenetic classification of *Ipomoea* and *Merremia* (Austin, 1998)

Tribus, genus, species	Plant organ[a,b]	Origin	A_3	A_5	B_1	B_2	B_3	B_4	C_1	Prec.
Tribus Humbertieae (Pichon) Stefanovic & D.F. Austin										
Humbertia Comm. ex. Lam.										
H. madagascariensis Lam.	lea (2 prov.: 2.0, 7.2)	Madagascar	•	–	–	–	–	•	–	n.d.
Tribus Cardiochlamyeae Stefanovic & D.F. Austin										
Cardiochlamys Oliv.										
C. madagascariensis Oliv.	lea (0.35)	Madagascar	–	–	•	•	–	•	–	n.d.
Tribus Erycibeae (Endl.) Hallier f.										
Erycibe Roxb.										
E. macrophylla Hallier f.	lea (2.2)	Java/Indonesia	–	–	•	•	•	–	?	n.d.
E. malaccensis C.B.Clarke	lea (3.1)	Java/Indonesia	–	–	–	•	–	–	–	n.d.
E. micrantha Hallier f.	lea (2.1)	Java/Indonesia	•	–	•	•	–	–	•	1
E. parvifolia Hallier f.	aep	Java/Indonesia	–	–	–	•	–	–	–	n.d.
E. rheedii Blume	lea (3.0)	Java/Indonesia	–	–	–	–	–	–	•	n.d.
Tribus Dichondreae s.l. (Choisy) Choisy										
Dichondra J.R. Forst. & G. Forst.										
D. micrantha Urb.	aep (9.0)	Chile	•	–	–	•	•	•	–	1,2
D. sericea Sw.	aep (2.0)	Chile	–	–	–	•	–	–	–	1,2
Falkia L. f.										
F. repens L. f.	aep/ro	South Africa	–	–	•/•	•/•	–	–	–	1,2,4
Porana Burm. f.										
P. volubilis Burm. f.	lea/fr (1.5)	Singapore	?/ –	–	–/•	?/•	–/•	–	•/–	n.d.
Rapona Baill.										
R. tiliifolia (Bak.) Verdc.	lea (1.0)/fr (2.0)	Madagascar	–/•	–	–/•	–/•	–/•	–	–	n.d.
Tribus Cresseae s.l. Benth. & Hook.										
Bonamia Hallier f.										
B. dietrichiana Hallier f.	aep (7.0)	Qld., Australia	–	–	–	–	–	–	–	n.d.
B. semidigyna (Roxb.) Hallier f. var. *semidigyna*	aep	Madagascar	–	–	–	•	–	–	–	1,2,4
B. spectabilis (Choisy) Hallier f.	aep	Madagascar	–	–	–	•	–	•	–	1,2,3,4
B. trichantha Hallier f.	lea	Panama	–	–	–	–	–	–	–	2,3,4
Evolvulus L.										
E. argyreus Choisy	aep	Ecuador	–	–	–	•	–	–	–	2,4
E. glomeratus Choisy cv. 'Blue Days'	aep	Nursery	–	–	–	–	–	–	–	1,2,4
E. nummularius L.	aep (3.0)	N. T., Australia	–	–	–	–	–	–	–	1,2
Hildebrandtia Vatke										
H. austinii Staples	st ♂ (0.4)/♀(0.2)	Madagascar	–	–	–	–	–	–	–	n.d.
H. promontorii Deroin	aep ♂ (1.4)/♀(0.3)	Madagascar	–	–	–	–	–	–	–	n.d.
H. valo Deroin	aep ♂ (6.4)/♀(0.4)	Madagascar	•/–	–	–	•/–	–	–	–	n.d.
dto. ♀	fr (2.0)	Madagascar	–	–	–	•	•	–	–	n.d.
Tribus Maripeae Webb. & Berth.										
Maripa Aubl.										
M. panamensis Hemsl.	lea/peric/flo (2.1)	Panama	•/–/–	–	•/–/•	•/–/•	•/•/–	–	–	1,2

Tribus Jacquemontieae Stefanovic & D.F. Austin
***Jacquemontia* Choisy**

J. corymbulosa Benth.	aep/fr (3.8)	Ecuador	–	–	–	–	–	–	–	–
J. paniculata (Burm. f.) Hallier f. var. *paniculata*	aep (2 prov.)	Java/Indonesia	–	–	–	–	–	–	–	–
J. pentantha (Jacq.) G. Don	aep (4 prov.)/ro	Panama	–	–	–	–	–	–	–	**2**
Dto.	flo (2.0)/ fr. (4 prov.)	Panama	–	–	–	–	–	–	–	**2**
J. reclinata House	aep (5.0)	Florida, USA	–	–	–	–	–	–	–	n.d.
J. tamnifolia (L.) Griseb.	aep (2 prov.)/fr/ro	Ecuador	–/–/●	–/–/●	–/–/●	–/–/●	–/–/?	–/–/?	–/–/●	**1,2**
J. tomentella (Miq.) Hallier f.	lea (1.1)	Indonesia	–	–	–	–	–	–	–	n.d.

Tribus Cuscuteae (Choisy) Choisy
***Cuscuta* L.**

C. approximata Bab. ssp. *episonchum* Webb. & Berth.	aep/flo	Canary Islands	–	–	–	–	–	–	–	n.d.
C. australis R.Br.	aep	Java/Indonesia	–	–	–	–	–	–	–	**2,4**
C. europaea L.	aep (2.5)	Germany	–	–	–	–	–	–	–	n.d.
C. palaestina Boiss.	aep (2.5)	Cyprus	–	–	–	–	–	–	–	n.d.
C. sp. (on *Launaea arborescens*, Asterac.)	aep	Canary Islands	–	–	–	–	–	–	–	n.d.

Tribus Aniseieae Stefanovic & D.F. Austin
***Aniseia* Choisy**

A. martinicensis (Jacq.) Choisy	lea/ peric (5.0)/ro	Panama	–/–/●	–	–/–/●	–/–/●	–	–	–	**1,2,4**

***Iseia* O'Donell**

I. luxurians (Moric.) O'Donell	lea/ro/flo/se	Panama	–	–	●/–/–/●	●/?/–/●	–	–	●/–/–/●	**2,4**

***Odonellia* K. Rob.**

O. hirtiflora (Mart. & Gal.) K. Rob.	lea/ro	Panama	–	–	–	–	–	–	–	**1**

Tribus Convolvuleae (Choisy) Choisy
***Calystegia* R.Br.**

C. japonica Choisy[c]	ro	Japan	●	–	●	●	–	–	–	n.d.
C. macrostegia ssp. *cyclostegia* (House) Brummitt	aep	California, USA	–	–	–	–	–	–	–	**1,2**
C. sepium R.Br.	lea/flo	Germany	●/●	●/–	●/●	●/●	●/–	–	–	**1,3,4**
C. silvatica (Kit.) Griseb.	lea/flo/ro	Poland	●/–/●	–/–/●	●/●/●	●/●/●	–	●/?/●	–	**2,3,4**
C. soldanella (L.) Roem. & Schult.[c]	whole parts	Japan	–	●	●	●	●	–	–	n.d.

***Convolvulus* L.**

C. arvensis L.	aep/flo white/flo pink	Germany	●/–/●	●/●/●	–/●/–	●/●/●	●/–/●	●/–/●	–/?/-	**1,2,3**
C. caput-medusae Lowe	aep	Canary Islands	●	–	●	●	–	–	–	**1,2**
C. chilensis Pers.	aep	Chile	–	–	–	–	–	–	–	**1,2,3**
C. clementii Domin	aep	Qld., Australia	–	–	–	?	–	?	–	–
C. cneorum L.	ro	Mediterranean	●	●	●	●	–	–	–	**1,2,3,4**
C. demissus Choisy	ro	Chile	–	–	–	–	–	–	–	**1,2,4**
C. elongatus Willd.	aep/ro (5.0)	Canary Islands	●/●	–	●/●	●/?	–	–/●	–	**1,2,3,4**
C. floridus L.f.	aep	Canary Islands	–	–	–	–	–	–	–	**1,2,3,4**
C. glandulosus (Webb.) Hallier	aep	Canary Islands	–	–	●	●	–	–	–	**1,2,3**
C. graminetinus (R.Br.) Spreng.	lea (2.5)	Qld., Australia	–	–	–	●	–	?	–	**1,2**
C. humilis Jacq.	aep (5.0)	Mediterranean	●	–	●	●	–	–	–	n.d.
C. kilimandschari Engl.	aep	Tansania	–	–	–	–	–	–	–	**2**
C. lopezsocasii Svent.	aep	Canary Islands	–	–	–	–	–	–	–	**1,2,3,4**

(continued on next page)

Table 1 (*continued*)

Tribus, genus, species	Plant organ[a,b]	Origin	A$_3$	A$_5$	B$_1$	B$_2$	B$_3$	B$_4$	C$_1$	Prec.
C. sabatius Viv. ssp. *mauritanicus* (Boiss.) Murb.	aep (5.0)/flo (5.0)	N. W. Africa	•/•	–	•/•	•/•	–/?	–	•/–	**1,2**
C. sagittatus Thunb.	aep	Zimbabwe	–	–	–	?	–	–	–	**1,2,3**
C. scoparius L.f.	aep (5.0)	Canary Islands	–	–	–	–	–	–	–	–
C. subauriculatus (Burch.) Linding.	aep	Canary Islands	–	–	•	•	–	–	–	**2**
C. tricolor L. ssp. *tricolor*	flo	Andalusia/Spain	–	•	•	•	–	–	?	**1,2**
Polymeria R.Br.										
P. ambigua R.Br.	aep (2 prov.)/fr	Qld., Australia	–	–	–	–	–	–	–	**1**
P. calycina R.Br.	aep	Qld., Australia	–	–	–	–	–	–	–	**1**
P. longifolia Lindl.	aep (2 prov.)/ro	Qld., Australia	–	–	–	–	–	–	–	**2**
P. marginata Benth.	aep/ro	Qld., Australia	–	–	–	–	–	–	–	–
P. pusilla R.Br.	aep/fr	Qld., Australia	–	–	–	–	–	–	–	–
Tribus "Merremieae" D.F. Austin										
Hewittia Wight & Arn.										
H. sublobata (L.f.) Kuntze	peric	Thailand	–	–	–	–	–	–	–	**1**
Merremia Dennst.										
Tuberosa allies ("section")										
M. aurea (Kell.) O'Donell	aep (5.0)/flo/ro (5.0)	Mexico	–/–/•	–	•/–/•	•/–/–	–	–	–	**1**
M. tuberosa (L.) Rendle	aep/fr	Canary Islands	–	–	–	–	–	–	–	**1**
Sectio Cissoides										
M. cissoides (Vahl) Hallier f.	ro	Madagascar	?	–	?	–	–	–	–	**1,2**
M. quinquefolia (L.) Hallier f.	ro	Ecuador	–	–	?	–	–	–	–	**1,2,3**
Vitifolia allies ("section")										
M. dissecta (Jacq.) Hallier	lea/flo (2 prov.)	Bahamas	–	–	–	–/•/–	–	–	–	**1,2,3**
Sectio Xanthips										
M. umbellata (L.) Hallier f.	aep/ro	Ecuador	–	–	•/–	•/–	–	–	–	**2,4**
M. pterygocaulos (Steud. ex Choisy) Hallier f.	aep + flo	Madagascar	–	–	–	–	–	–	?	**1,3**
Operculina Silva Manso										
O. aequisepala (Domin) R.W. Johnson	lea/peric (3.9)	Qld., Australia	–	–	–	–	–	–	–	**1**
O. pteripes (G. Don) O'Donell	ro	Panama	–	–	–	–	–	–	–	n.d.
O. riedeliana (Oliv.) Ooststr.	aep (2 prov.)/fr	Qld./Australia	–	–	–	–	–	–	–	**1,2**
O. triquetra (Vahl) Hallier f.	aep	Panama	–	–	–	–	–	–	–	n.d.
O. turpethum (L.) Silva Manso	se/ro	Madagascar	–	–	–	–	–	–	–	n.d.
Xenostegia D.F. Austin & G.Staples										
X. medium (L.) D.F. Austin & G. Staples[d]	aep/fr/ro	Madagascar	–	–	–	–	–	–	–	–
Tribus Ipomoeeae Hallier f.										
Argyreia Lour.										
A. androyensis Deroin	lea (0.8)	Madagascar	–	–	•	•	–	•	•	n.d.
A. capitata (Vahl) Choisy	aep/ro	Thailand	–	–	•/–	•/–	–	–	–	**1,2,3**
A. hookeri Clarke	lea	Java, Indonesia	–	–	•	–	–	•	•	**1**
A. mollis (Burm. f.) Choisy	aep/ro	Madura, Indon.	•/•	–	•/•	•/•	–/•	–/?	–/•	**2**
A. nervosa (Burm. f.) Boj.	aep/flo/se/ro	Java, Indonesia	–	–	–	–	–	–	–	**1,2**
A. onilahiensis Deroin	lea (1.3)	Madagascar	–	–	•	•	–	•	•	n.d.
A. vahibora Deroin	lea (3.4)	Madagascar	–	–	•	•	–	•	•	n.d.

Astripomoea Meeuse

A. malvacea (G. Klotz) Meeuse	aep/root bark	Uganda	–	–	–	–	–	–	–	1
Ipomoea L.										
Sectio Calonyction										
I. alba L.	aep/flo (2.8)/se/ro	Ecuador	–/•/–/–	•/•/–/•	•/•/•/•	•/•/•/–	–/–/•/–	–/–/?/-	–/–/•/–	1,2,3,4
I. turbinata Lag.	aep/ro	Ecuador	–	–	–	–	–	–	–	1,2
Sectio Eriospermum										
I. anisomeres Rob. & Bartl.	aep/flo (0.9)/se (5.0)/ro	Panama	–	–	–	–	–	–	–	1,2,3,4
I. batatas (L.) Lam. (cultivar)	aep/ro	Mexico	–	–/•	•/•	•/•	•/–	–	?/ ?	1,2,3,4
I. batatas (L.) Lam. (wild form)	ro	Panama	•	–	•	–	•	–	–	1,2,4
I. batatas (L.) Lam. var. edulis Makino[c]	aep	Japan	–	–	•	•	–	–	–	n.d.
I. batatoides Choisy	aep/ro	Panama	–	–	–	–	–	–	–	2
I. cairica (L.) Sweet	lea/flo (2 prov.)	Argentina	–	–	–	–/•	–	–	–	1,2,3,4
I. carnea Jacq.[c]	aep	Japan	–	–	•	•	–	–	–	1
I. carnea Jacq. ssp. fistulosa (Mart. ex Choisy) D.F. Austin	flo (2.0)	Panama	•	–	•	•	?	•	•	n.d.
I. carnea Jacq. ssp. fistulosa (Mart. ex Choisy) D.F. Austin[e]	lea	Mozambique	–	–	–	•	–	–	•	n.d.
I. eremnobrocha D.F. Austin	ro	Panama	–	–	–	?	?	–	–	2,4
I. habeliana Oliv.	ro (3.0)	Galapagos	–	–	–	–	–	–	–	n.d.
I. horsfalliae Hook.	lea	Java/Indonesia	–	–	–	–	–	–	–	n.d.
I. mauritiana Jacq.	flo (2.0)	Cameroon	–	–	–	–	–	–	–	1
I. regnellii Meisn.	aep/ro	Ecuador	–	–	–	–	–	–	–	1,2
I. reticulata O'Donell	aep/ro	Ecuador	–	–	–	–	–	–	–	1
I. squamosa Choisy	aep/flo (2.2)/ro (5.0)	Panama	–	–	–/•/•	–/•/•	–	–	–	1,2,3
I. trifida (H.B.K.) G. Don	aep/flo/ro	Panama	–	–	–/–/•	–/–/•	–	–	–	1,2,3
I. tuxtlensis House	flo (0.5)	Panama	–	–	–	?	–	–	–	–
I. umbraticola House	flo (5.0)	Costa Rica	–	–	–	–	–	–	–	n.d.
Sectio Erpipomoea										
I. aquatica Forssk.	aep/ro	Thailand	•/–	–	•/–	•/–	?/–	•/–	–	1,2,3,4
I. asarifolia Roem. & Schult.	lea/flo	Panama	–	–	–	–	–	–	–	1,2,3,4
I. graminea R.Br.	aep (4.0)	N. T., Australia	–	–	–	–	–	–	–	n.d.
I. obscura Ker.[c]	whole parts	Japan	–	–	•	•	•	•	•	n.d.
I. pes-caprae (L.) R.Br.[c]	aep	Japan	–	–	–	•	–	–	–	n.d.
I. pes-caprae (L.) R.Br. ssp. pes-caprae	aep	Madagascar	–	–	–	–	–	–	–	2
I. polpha R.W. Johnson[f]	se	N. T., Australia	–	–	–	•	–	–	•	n.d.
I. sp. Q6 [aff. calobra][f]	se	Qld., Australia	–	–	–	•	–	–	–	n.d.
I. stolonifera (Cyr.) Gmelin[g]	aep/flo	Florida, USA	–	–	–	•/•	–	–	–	n.d.
I. trichosperma Blume	aep/ro	Java/Indonesia	–	•/•	–	–	–	–	–	2,4
I. violacea L. [syn.: I. tuba (Schlecht.) G. Don]	aep/buds/flo/ro	Florida, USA	–	–	•/–/?/-	–/–/•/?	–/–/?/-	–	•/–/–/–	1,2,3,4
Sectio Involucratae										
I. involucrata Beauv.	aep/flo/ro	Tanzania	–	–	?/•/–	?/–/–	–/•/–	–	–/?/-	1
Sectio Ipomoeae										
I. wightii (Wall.) Choisy	aep (5.0)	Zimbabwe	–	–	•	–	–	–	–	1
Sectio Leptocallis										
I. capillacea G. Don	aep (0.6)	Panama	–	–	–	–	–	–	–	n.d.

Table 1 (continued)

Tribus, genus, species	Plant organ[a,b]	Origin	A3	A5	B1	B2	B3	B4	C1	Prec.
Sectio Mina										
I. hederifolia L.	aep/se	Sri Lanka	–	•/–	•/•	?/ •	–	–	–	–
I. hederifolia L., sub nom. Quamoclit angulata Boj.[c]	aep	Korea	–	–	•	•	–	–	–	n.d.
I. lobata Thell.	aep (5.0)/flo (3.0)	Nursery (Berlin)	–	–	•/•	•/•	–	–	•/–	–
I. neei (Spreng.) O'Donell	lea/flo (3.1)/ro (5.0)	Panama	–	–	–	–	–	?/–/–	–	–
Sectio Orthipomoea										
I. plebeia R.Br.	aep/ro (8.0)	Qld./Australia	–	–	–	–	–	–	–	1,2
I. tenuirostris Steud. ex Choisy	lea	Malawi	–	–	–	–	–	–	–	1
Sectio Pharbitis										
I. eriocarpa R.Br.	aep/ro	Indonesia	–	–	?/–	•/•	–	?/–	–	2,3,4
I. indica (Burm. f.) Merr.	aep	Panama	–	–	–	–	–	–	–	1
I. nil (L.) Roth[c]	whole parts	Japan	–	–	–	–	–	–	–	n.d.
I. nil (L.) Roth	lea	Tanzania	–	–	–	–	–	–	–	1,2
I. purpurea (L.) Roth	flo (5.0)	Bot. Gd. Berlin	–	–	–	–	–	–	–	1,2,4
I. setifera Poir.	lea/ro	Panama	?/–	–	•/–	•/•	–	–	–	1,2
Sectio Tricolores										
I. chiriquiensis Standl.	aep	Panama	–	–	•	•	–	–	–	2,4
I. tricolor Cav. cv. 'Heavenly Blue'	flo (5.0)	Nursery	–	–	–	•	–	–	–	1,2,4
Lepistemon Blume										
L. binectariferum (Wall.) Kuntze var. borneense	lea	Malaysia	–	–	–	–	–	–	–	n.d.
L. urceolatum (R.Br.) F. Muell.	lea	Qld./Australia	–	–	–	?	–	–	•	1,3
Stictocardia Hallier f.										
S. campanulata (L.) Merrill	lea/ro	Panama	•/–	–	–	•/•	–	–	–	1,2,4
S. mojangensis D.F. Austin & Eich[h]	ro	Madagascar	•	?	•	•	–	–	–	1,2
Turbina Raf.										
T. abutiloides (H.B.K.) O'Donell	ro	Ecuador	•	–	•	•	–	–	–	1
T. corymbosa (L.) Raf.	lea	Panama	–	–	–	–	–	–	–	4

•: detected; –: not detected; ?: equivocal; n.d.: not determined; Prec.: biogenetic precursor(s) determined by GC–MS measurements: **1**: 3-oxotropane (tropinone), **2**: 3β-hydroxytropane (pseudotropine), **3**: 3-oxonortropane (nortropinone), **4**: 3β-hydroxynortropane (norpseudotropine).

[a] *Abbreviations*: aep: aerial parts (vegetative); flo: flowers; fr: fruits; lea: leaves; peric: pericarp; ro: roots; se: seeds; st: stems; prov.: provenances.

[b] 10 g dry wt., unless otherwise indicated (in parentheses).

[c] Species not involved in this study, data taken from Asano et al., 2001.

[d] sub nom. *Merremia medium* (L.) Hallier f. in Schimming et al. (1998).

[e] Species not involved in this study, data taken from de Balogh et al., 1998.

[f] Species not involved in this study, data taken from Molyneux et al., 1995.

[g] Species not involved in this study, Dräger, personal communication.

[h] In addition: A6.

Table 2
Abundancy and combination of calystegines in 69 species which unequivocally contained calystegines plus seven species taken from literature according to Table 1

A_3	A_5	A_6	A_7	B_1	B_2	B_3	B_4	C_1
26 spp.	11 spp.	1 sp.	–	47 spp.	62 spp.	14 spp.	14 spp.	18 spp.
=38%	=16%	< 2%	–	=68%	=90%	=20%	=20%	=26%

A group only		B group only		C group only		A + B		B + C		A + B + C
1 sp.		27 spp.		2 spp.		23 spp.		10 spp.		6 spp.
< 2%		=39%		=3%		=33%		=15%		=9%

why these species have never come to scientific attention by intoxications (except those which contain ergoline alkaloids) in contrast to *I. carnea* and the two Australian *Ipomoea* taxa.

3-Oxotropane (tropinone), 3β-hydroxytropane (pseudotropine), and 3β-hydroxynortropane (norpseudotropine) are consecutive candidates as precursors in the biosynthesis of calystegines (Scholl et al., 2001; Dräger, 2004). Therefore we included the results concerning these putative precursors (last column in Table 1) for as many species as possible dependent on the availability of additional plant material (98 out of 129 species). In addition we checked the occurrence of 3-oxonortropane, another potential precursor. Since these four alkaloids are adequately lipophilic they could be analyzed directly by GC–MS. In the vast majority of cases in which calystegines could be detected and also data for the putative precursors are available we found a co-occurrence of both groups of alkaloids (48 out of 50 species = 96%). This finding strongly supports the assumption that such compounds are real precursors. Furthermore, though two species (4%) displayed the polyhydroxy alkaloids whereas no putative precursor could be found in any checked organ of the corresponding species in the present study, also these cases are explainable: In principle the lipophilic putative precursors are present mainly in the roots. However, in these two precursor-negative cases no roots have been available in this study but only small amounts of aerial parts (*Ipomoea hederifolia*, *I. lobata*). This assumption is supported by the fact that 3-oxotropane (tropinone) had been found already in the roots of *Ipomoea hederifolia* in a previous study of our group (Jenett-Siems, 1996).

On the other hand, there have been 40 species lacking calystegines though showing at least one precursor. This may be interpreted in different ways depending on the specific situation: (1) a certain species is able to synthesize lipophilic, biogenetically basal tropanes but unable to transform them into the hydrophilic congeners due to the lack of corresponding enzymes, e.g. *Argyreia nervosa* (four organs/two provenances checked), *Astripomoea malvacea* (three organs checked), *Ipomoea plebeia* (two organs checked); (2) an accidentally unfortunate selection of the stage of plant development has been carried out; (3) only one or two organs of this spe-

cific plant have been analyzed with calystegine-negative results whereas these hydrophilic metabolites might have been stored in another organ not checked. There are different examples in which one organ contained the precursors and another one the polyhydroxy alkaloids, e.g. *Argyreia capitata*, *Iseia luxurians*. Thus, it might be also that this is the case in, e.g. *Evolvulus nummularius*, *Hewittia sublobata*, *Merremia pterygocaulos*. Besides the 62 positive species (plus five further *Ipomoea* spp. and two further *Calystegia* spp., respectively, from the literature) there has been some evidence for the occurrence of calystegines in samples of further seven species; however, it has been impossible to reproduce the results due to the lack of sufficient amounts of the corresponding plant material. These ambiguous taxa have been two *Convolvulus* spp. (*C. clementii*, *C. sagittatus*), three *Merremia* spp. (*M. cissoides*, *M. pterygocaulos*; *M. quinquefolia*), and two *Ipomoea* spp. (*I. neei*, *I. tuxtlensis*), all of them belonging to genera with several other unequivocally calystegine-positive species. In such cases a question mark is set for the corresponding compound in Table 1.

The five species of the monotypic tribe Cuscuteae turned out to be generally calystegine-negative. Apart from *Cuscuta* only two further genera, *Operculina* (five out of 20 recognized species checked; tribe ''Merremieae'') as well as *Polymeria* (five out of seven recognized species checked; tribe Convolvuleae) might have lost the ability to synthesize these plesiomorphic metabolites since all samples from different organs of both genera (12 samples each) did not show any calystegine. Moreover, the genus *Polymeria* might have lost the ability for the synthesis of biogenetically basal tropanes to a large extent since only three out of the 12 samples resulted in the detection of a very low concentration of 3β-hydroxytropane (pseudotropine). Two other genera found also calystegine-negative comprising only two recognized species each (*Odonellia*, tribe Aniseieae; *Xenostegia*, tribe ''Merremieae'') were represented in this study with only one species each and two or three samples, respectively. These limited data do not justify the assumption that these two genera are calystegine-free in principle. This is also true for the monotypic genus *Hewittia* (tribe ''Merremieae'') since only one sample is included here as well as for *Astripomoea* (tribe Ipomoeeae) because

only one out of 12 recognized species with three samples could be integrated.

Anyhow, species which did not show any calystegine in our study may not be regarded necessarily as calystegine-negative. The stage of development of such "negative" species at the time of its harvest may have led to concentrations of these alkaloids below the detection limit though this is very low. It turned out to be 0.6 μg/g dry wt. (B_1) and 1.5 μg/g dry wt. (A_3, B_4, C_1), respectively, by comparison with standard solutions. As a rule 10 g (dry wt.) of the corresponding plant organ have been used; however, sometimes only much lower amounts have been available which are indicated in such cases (Table 1). Even very small amounts may show unequivocally calystegine-positive results, e.g., leaves of *Cardiochlamys madagascariensis* (0.3 g dry wt.), leaves of *Argyreia androyensis* (0.8 g dry wt.), and fruits of *Porana volubilis* (1.5 g dry wt.). In other cases much higher amounts may not be sufficient.

Thus, e.g., 10 g aerial parts of *I. pes-caprae* did not show any calystegine in our study whereas the same organs from another provenance contained B_1 (Asano et al., 2001). Of course, this could also be a problem of chemotypes. All this demonstrates that samples of certain species found negative do not prove the absolute inability of a species to synthesize these metabolites. That has been the reason why we tried to check as many samples of particularly "negative" species as possible in order to verify the negative result.

The present data clearly show that the occurrence of calystegines is an almost consistent trait in the Convolvulaceae in principle, from basal to most advanced tribes. It may be assumed that this is also the case in the sister family Solanaceae though its data are still limited (Table 3). Anyhow, the broad occurrence of these plesiomorphic characters in the Convolvulaceae involves limitations concerning their significance for infrafamilial taxonomic relationships. However, in the case of certain infrageneric relationships (*Cuscuta*, *Operculina*, *Polymeria*) the lack of these metabolites might be of relevance. Furthermore, it is obvious that the calystegines A_3 and A_5, respectively, could be detected rather frequently in the closely related genera *Convolvulus* and *Calystegia* (both together A_3: 9 out of 14 calystegine-positive spe-

cies; both together A_5: 7 out of 14) but turned out to be rather rare in the genus *Ipomoea* (A_3 as well as A_5: 4 each out of 22 calystegine-positive species).

3. Experimental

3.1. Plant material

The plant material was obtained from collections in the wild as well as from cultivation in the greenhouse of the Institut für Pharmazie (Pharmazeutische Biologie), Freie Universität Berlin. For details concerning the organs used for analysis and the origin of the plants see Table 1. Voucher specimens are deposited in the herbarium of this institute.

3.2. Extraction of the plant material

The plant material used for the analysis of the calystegines (usually 10 g dry wt.; for exceptions see Table 1) was homogenized and extracted three times with 50% MeOH (1:10). Seeds (ground) were defatted with petrol ether before the hydrophilic extraction. After centrifugation of the hydrophilic extract the supernatant was evaporated to 1 ml, mixed with skin powder, filtered, and applied to a column of strongly acidic cation exchange resin (Dowex 50WX8, 3 ml gel per 5 g dry wt.) for purification and accumulation. After washing the column with 5 bed volumes of water to remove non-binding contaminants, the bound compounds were eluted with 5 bed volumes of 3.5% aq. NH_3. The extract was evaporated again to 1 ml vol per 5 g dry wt., one quarter of which was used for GC–MS.

The plant material used for the analysis of the lipophilic tropane alkaloids (putative precursors) was extracted according to a procedure published already previously (Jenett-Siems et al., 1998).

3.3. Silylation

The dry plant extract according to 3.2 was dissolved in pyridine (50 μl). The solution was mixed with hexamethyldisilazane (HMDS; 40 μl) and trimethylchlorosilane

Table 3
Occurrence of calystegines in the two major Solanales families: Convolvulaceae *versus* Solanaceae

	Convolvulaceae			Solanaceae		
	Total	Examined	Found	Total[c]	Examined[d]	Found[d]
Species	1850	135	69	2300	38	32
Genera	55[a]	29	22	92	18	15
Tribes	12[b]	12	11	21	9	7

[a] Austin (1998).
[b] Stefanović et al. (2003).
[c] Hunziker (2001).
[d] Asano et al. (2000), Bekkouche et al. (2001), Dräger (2004), Watson et al. (2001).

Table 4
RR^{t}s and fragmentation patterns in GC–MS of pure silylated calystegines, *N*-methylcalystegines, castanospermine, and swainsonine[a]

Compound	RR$_t$	*m/z*, rel. int. %
Tri-TMSi-calystegine A$_6$	0.76	375 [M]$^+$ (100), 359 (13), 311 (3), 288 (18), 260 (90), 259 (39), 244 (36), 229 (4), 198 (8), 170 (50), 156 (7)
Tri-TMSi-calystegine A$_7$	0.69	375 [M]$^+$ (5), 374 (17), 360 (32), 307 (2), 267 (69), 266 (100), 250 (44), 222 (7), 182 (49), 170 (51), 156 (73)
Tetra-TMSi-*N*-methyl calystegine B$_2$	1.65	477 [M]$^+$ (12), 462 (19), 388 (73), 374 (5), 305 (8), 273 (100), 258 (68), 243 (8), 217 (17), 186 (59), 172 (85), 171 (84), 133 (13), 131 (23), 129 (8)
Penta-TMSi-*N*-methyl calystegine C$_1$	1.98	565 [M]$^+$ (−), 470 (3), 458 (12), 360 (42), 345 (7), 331 (2), 272 (17), 259 (30), 217 (12), 170 (12), 133 (3)
Tri-TMSi-swainsonine	1.04	389 [M]$^+$ (5), 388 (6), 375 (4), 300 (5) 260 (29), 217 (15), 185 (100), 170 (24), 157 (14), 143 (45)
Tetra-TMSi-castanospermine	1.40	477 [M]$^+$ (−), 388 (3), 386 (2), 314 (4), 288 (15), 260 (30), 170 (6), 128 (11), 116 (13)

[a] For MS data of the tri-TMSi-calystegines A$_3$, A$_5$, the tetra-TMSi-calystegines B$_1$–B$_4$, and the penta-TMSi-calystegine C$_1$, see Schimming et al. (1998).

(TCMS, 10 μl) and kept at ca. 50 °C for 15 min. *n*-Octadecane (solved in *n*-hexane, 50 μl, *c* = 200 ng/μl) was added to the supernatant as an internal standard. RR$_t$s (*n*-octadecane) were used to identify the calystegines present in the extract (Table 4 and Schimming et al., 1998).

3.4. GC–MS analysis

GC used for the calystegínes was performed under the following conditions: capillary column coated with the methyl silicone stationary phase DB1, 30 m × 0.25 mm, temp.-program 160 °C isotherm for 2 min, 160–240 °C at 5 $^\circ$ min^{-1}. Carrier gas and flow: He at 1 ml min^{-1}. Inj. vol.: 1 μl; split ratio: 1:10. EI-MS: ionization energy 70 eV.

The procedure used for lipophilic tropane alkaloids has been carried out as described previously (Doerk et al., 1991).

Acknowledgements

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