Pergamon

Phytochemistry Vol. 49, No. 7, pp. 1989–1995, 1998
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Printed in Great Britain
0031-9422/98/$ - see front matter

PII: S0031-9422(98)00430-0

DISTRIBUTION AND TAXONOMIC SIGNIFICANCE OF CALYSTEGINES IN THE CONVOLVULACEAE†

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(*Received in revised form* 14 *May* 1998)

Key Word Index—*Aniseia*; *Argyreia*; *Bonamia*; *Calystegia*; *Convolvulus*; *Cuscuta*; *Dichondra*; *Erycibe*; *Evolvulus*; *Falkia*; *Hewittia*; *Ipomoea*; *Iseia*; *Jacquemontia*; *Lepistemon*; *Maripa*; *Merremia*; *Odonellia*; *Operculina*; *Porana*; *Stictocardia*; *Turbina*; Convolvulaceae; GC-MS; tropane alkaloids; calystegines; glycosidase inhibitors; chemotaxonomy.

Abstract—The GC-MS analysis of 65 convolvulaceous species from many, predominantly tropical provenances, belonging to 22 genera (9 tribes), revealed the occurrence of one to five calystegines in 30 species belonging to 15 genera (8 tribes). This indicates the chemotaxonomic significance of these glycosidase inhibiting polyhydroxy-nortropanes for the Convolvulaceae. © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

Calystegines, polyhydroxy-nortropanes with a tertiary hydroxyl group at the bicyclic ring bridgehead (aminoketal functionality), have been discovered as constituents of *Calystegia sepium* [2, 3], *Convolvulus arvensis* (both Convolvulaceae), and *Atropa belladonna* (Solanaceae) [2]. They were proposed as nutritional mediators in the plant rhizosphere (plant bacteria relationship). Moreover, they possess glycosidase inhibiting properties [4] comparable to polyhydroxy alkaloids of different structure, like e.g., the indolizidines swainsonine or castanospermine [5]. To date the structure of nine calystegines has been elucidated including three trihydroxy-nortropanes (A_3, A_5, A_6) [3, 6–8], four tetrahydroxy-nortropanes (B_1, B_2, B_3, B_4) [3, 7–10], and two pentahydroxy-nortropanes (C_1, C_2) [6, 8, 11, 12]. Moreover, a first glycoside could be identified as the 3-*O*-*β*-D-glucopyranoside of calystegine B_1 [13].

Whereas the occurrence of calystegines in the Solanaceae is documented for nine genera (*Atropa*, *Datura*, *Duboisia*, *Hyoscyamus*, *Mandragora*, *Nicandra*, *Physalis*, *Scopolia*, *Solanum*) [12, 14, 15], there is only one further report of a calystegine in the Convolvulaceae. Thus calystegine B_2 was identified in the seeds of *Ipomoea polpha* R. W. Johnson and another Australian *Ipomoea* sp. (Q6, aff. *calobra*, Weir Vine). These species are found to be toxic to animals [16].

In order to obtain more information on the occurrence and distribution of these compounds in the Convolvulaceae, we studied 65 species from 22 genera belonging to 9 tribes. The largest genus *Ipomoea*, divided into 13 sections [17], is represented by species from 7 sections. We chose samples of all plant organs and from many predominantly tropical provenances belonging to all continents except Australia.

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†Part 6 in the series "Phytochemistry and Chemotaxonomy of the Convolvulaceae". For Part 5, see Ref. [1]. Presented in part at the 13th Annual Meeting of the International Society of Chemical Ecology, 1996, Prague, Czech Republic (Abstract Book, p. 98), at the IOCD/CYTED International Joint Symposium, 1997, Panamá, Republic of Panama (Abstract Book L-5), and at the 45th Annual Congress of the Society for Medicinal Plant Research, 1997, Regensburg, Germany (Abstract Book E12).

Table 1. Calystegine patterns in 65 convolvulaceous species; taxonomy according to [17, 29–31]

Tribus, Genus, Sectio, Species	Organ	Origin	A_3	A_5	B_1	B_2	B_3	B_4	C_1
Tribus Argyreiae (Choisy) Choisy									
Argyreia Lour.									
A. capitata (Vahl) Choisy	herbal material†	Chiang Mai, Thailand	–	–	•	•	–	–	–
	roots†		–	–	–	–	–	–	–
A. hookeri Clarke	leaves†	Java, Indonesia	–	–	•	–	–	•	•
A. mollis (Burm. f.) Choisy	herbal material†	Java, Indonesia	•	–	•	•	–	–	–
A. nervosa (Burm. f.) Bojer	herbal material†	Java, Indonesia	–	–	–	–	–	–	–
	roots†	Java, Indonesia	–	–	–	–	–	–	–
Stictocardia Hall. f.									
S. campanulata (L.) Merrill	leaves†	Panama City, Panama	•	–	–	•	•	–	–
	roots†	Panama City, Panama	–	–	–	•	–	–	–
Turbina Raf.									
T. abutiloides (H.B.K.) O'Donell	roots†	Guayaquil, Ecuador	•	–	•	•	–	–	–
T. corymbosa (L.) Raf.	leaves†	Campana, Panama	–	–	–	–	–	–	–
Tribus Convolvuleae (Choisy) Choisy									
Calystegia R. Br.									
C. macrostegia (Greene) Brumm.									
ssp. *cyclostegia*	herbal material†	California, U.S.A.	–	–	–	–	–	–	–
C. sepium (L.) R. Br.	leaves*	Berlin, Germany	•	•	•	•	•	–	–
	flowers*	Berlin, Germany	•	–	•	•	–	–	–
	root culture [2, 3]		•	–	•	•	–	–	–
Convolvulus L.									
C. arvensis L.	herbal material*	Berlin, Germany	•	•	–	•	•	•	–
	flowers*	Berlin, Germany	–	•	–	•	–	–	–
C. caput-medusae Lowe	herbal material*	Gran Canaria, Spain	•	–	•	•	–	–	–
C. chilensis Pers.	herbal material†	Viña del Mar, Chile	–	–	–	–	–	–	–
C. cneorum L.	roots‡	Mediterranean	•	•	•	•	–	–	–
C. sabatius Viv.									
ssp. *mauritanicus* (Boiss.) Murb.	flowers‡	Northwest Africa	•	–	•	•	–	–	–
Evolvulus L.									
E. argyreus Choisy	herbal material†	San Antonio, Ecuador	–	–	–	•	–	–	–
Jacquemontia Choisy									
J. corymbulosa Benth.	herbal material†	Guayaquil, Ecuador	–	–	–	–	–	–	–
J. paniculata (Burm. f.) Hall. f.									
var. *paniculata*	herbal material†	Java, Indonesia	–	–	–	–	–	–	–
J. pentantha (Jacq.) G. Don	herbal material†	Taboga Island, Panama	–	–	–	–	–	–	–
J. tamnifolia (L.) Griseb.	herbal material†	Guayaquil, Ecuador	–	–	–	–	–	–	–
J. tomentella (Miq.) Hall. f.	herbal material*	Java, Indonesia	–	–	–	–	–	–	–
J. reclinata House	herbal material*	Boca Raton Beach, FL, U.S.A.	–	–	–	–	–	–	–
Odonellia K. Rob.									
O. hirtiflora (Mart. et Gal.) K. Rob.	leaves†	Campana, Panama	–	–	–	–	–	–	–
Tribus Cresseae Benth. et Hook.									
Bonamia Thouars									
B. semidigyna (Roxb.) Hall. f.									
var. *semidigyna*	herbal material†	Toamasina, Madagascar	–	–	–	•	–	–	–
B. spectabilis (Choisy) Hall.	herbal material†	Morondava, Madagascar	–	–	–	•	–	•	–
Iseia O'Donell									
I. luxurians (Moric.) O'Donell	leaves*	Gamboa, Panama	–	–	•	•	–	–	–
	seeds*	Gamboa, Panama	–	–	•	•	–	–	–
Tribus Cuscuteae (Choisy) Choisy									
Cuscuta L.									
C. australis R. Br.	whole plant*	Java, Indonesia	–	–	–	–	–	–	–
C. europaea L.	whole plant*	Hamburg, Germany	–	–	–	–	–	–	–
C. palaestina Boiss.	whole plant*	Cyprus	–	–	–	–	–	–	–
C. approximata Bab.									
ssp. *episonchum* (Webb et Berth.) Feinbr.	whole plant*	Gran Canaria, Spain	–	–	–	–	–	–	–
	flowers*	Lanzarote, Spain	–	–	–	–	–	–	–
Tribus Dichondreae (Choisy) Choisy									
Dichondra Forst. et Forst.									
D. repens Forst. et Forst.	herbal material*	Concepción, Chile	•	–	–	•	•	–	–
	herbal material†	Concepción, Chile	•	–	–	•	•	–	–
Falkia L. f.									
F. repens L. f.	whole plant‡	Cape peninsula, South Africa	–	–	•	•	–	–	–
Tribus Erycibeae (Endl.) Hall. f.									
Erycibe Roxb.									
E. malaccensis Clarke	leaves*	Java, Indonesia	–	–	–	•	–	–	–
E. parvifolia Gagnep.	herbal material*	Java, Indonesia	–	–	–	•	–	–	–
Maripa Aubl.									
M. panamensis Hemsl.	leaves*	Soberanía, Panama	•	•	•	•	•	–	–
	fruits without seeds*	Soberanía, Panama	–	–	–	–	•	–	–
Tribus Ipomoeeae Hall. f.									
Ipomoea L.									
Sectio Pharbitis (Choisy) Griseb.									
I. eriocarpa R. Br.	herbal material*	Java, Indonesia	–	–	–	–	–	–	–

Table 1 (*continued*)

Tribus, Genus, Sectio, Species	Organ	Origin	A_3	A_5	B_1	B_2	B_3	B_4	C_1
I. setifera Poir.	leaves†	Algarrobo, Panama	•	–	•	•	–	–	–
	roots†	Algarrobo, Panama	–	–	–	•	–	–	–
Sectio Mina (Cerv.) Griseb.									
I. hederifolia L.	herbal material†	Ella, Sri Lanka	–	•	•	•	–	–	–
	seeds†	Ella, Sri Lanka	–	–	•	•	–	–	–
I. neei (Spreng.) O'Donell	herbal material†	Boquete, Panama	–	–	–	–	–	–	–
	roots†	Boquete, Panama	–	–	–	–	–	–	–
Sectio Calonyction (Choisy) Griseb.									
I. alba L.	herbal material†	Santo Domingo, Ecuador	–	•	•	•	–	–	–
	roots†	Santo Domingo, Ecuador	–	•	•	–	–	–	–
I. turbinata Lag.	herbal material†	Guayaquil, Ecuador	–	–	–	–	–	–	–
Sectio Batatas (Choisy) Griseb.									
I. batatas (L.) Lam.	root†	Boquete, Panama	•	–	•	–	•	–	–
(wild form, no cultivar)									
Sectio Eriospermum Hall. f.									
I. anisomeres Rob. et Bart.	herbal material†	Algarrobo, Panama	–	–	–	–	–	–	–
	roots‡	Algarrobo, Panama	–	–	–	–	–	–	–
I. batatoides Choisy	herbal material†	Campana, Panama	–	–	–	–	–	–	–
	roots†	Campana, Panama	–	–	–	–	–	–	–
I. eremnobrocha D. F. Austin	herbal material†	Campana, Panama	–	–	–	•	–	–	–
I. habeliana Oliver	roots†	Galapagos Islands	–	–	–	–	–	–	–
I. horsfalliae Hook.	leaves*	Java, Indonesia	–	–	–	–	–	–	–
I. reticulata O'Donell	herbal material†	Naranjal, Ecuador	–	–	–	–	–	–	–
I. umbraticola House	flowers†	Flamingo, Costa Rica	–	–	–	–	–	–	–
Sectio Orthipomoea									
I. cairica (L.) Sweet									
ssp. *brasiliensis*	flowers‡	tropical Africa	–	–	–	–	–	–	–
Sectio Erpipomoea Choisy									
I. aquatica Forssk.	herbal material†	Don Muang, Thailand	–	–	•	•	–	•	–
	roots†	Don Muang, Thailand	–	–	–	–	–	–	–
I. violacea L.	stems†	Boca Raton Beach, FL, U.S.A.	–	–	•	–	–	–	•
Lepistemon Blume									
L. binectariferum (Wall.) O. K.									
var. *borneense*	leaves†	Borneo, Malaysia	–	–	–	–	–	–	–
Tribus Merremieae D. F. Austin									
Aniseia Choisy									
A. martinicensis (Jacq.) Choisy	leaves†	Río Coclé Auxiliar, Panama	–	–	–	–	–	–	–
	fruits without seeds*	Río Coclé Auxiliar, Panama	–	–	–	–	–	–	–
Hewittia Wight et Arn.									
H. sublobata (L. f.) O. K.	fruits without seeds*	Chiang Mai, Thailand	–	–	–	–	–	–	–
Merremia Dennst.									
M. aurea (Kell.) O'Donell	flowers†	Baja California, Mexico	–	–	–	–	–	–	–
M. cissoides (Vahl) Hall. f.	roots†	Morondava, Madagascar	•	–	•	–	–	–	–
M. medium (L.) Hall.	herbal material†	Fort Dauphin, Madagascar	–	–	–	–	–	–	–
	roots†	Fort Dauphin, Madagascar	–	–	–	–	–	–	–
M. quinquefolia (L.) Hall. f.	roots†	Guayaquil, Ecuador	–	–	•	•	–	–	–
M. tuberosa (L.) Rendle	herbal material†	Canary Islands, Spain	–	–	–	–	–	–	–
	fruits without seeds*	Canary Islands, Spain	–	–	–	–	–	–	–
	seeds*	Canary Islands, Spain	–	–	–	–	–	–	–
M. umbellata (L.) Hall. f.	herbal material†	Guayaquil, Ecuador	–	–	•	•	–	–	–
Operculina S. Manso									
O. pteripes (G. Don) O'Donell	roots†	San Lorenzo–San Juan, Panama	–	–	–	–	–	–	–
O. riedeliana (Oliv.) Ooststr.	fruits without seeds*	Java, Indonesia	–	–	–	–	–	–	–
O. triquetra (Vahl) Hall. f.	leaves†	Los Angeles, Panama	–	–	–	–	–	–	–
O. turpethum (L.) S. Manso	seeds*	Morondava, Madagascar	–	–	–	–	–	–	–
Tribus Poraneae									
Porana Burm. f.									
P. volubilis Burm. f.	fruits*	Singapore	–	–	•	•	–	–	–

*plant material collected in the wild.
†cultivated in the greenhouse of the Institut für Pharmazie II (Pharmazeutische Biologie), Freie Universität Berlin.
‡cultivated in the Botanischer Garten, Freie Universität Berlin, Germany.

RESULTS AND DISCUSSION

We focussed on the occurrence of seven calystegines (A_3, A_5, B_1, B_2, B_3, B_4, C_1), which were identified by GC-MS analysis with authentic samples as references. An extraction procedure based on the methods developed by Dräger [18] and Hohenschutz *et al.* [19] was used. Since all these compounds are highly water-soluble, they cannot be isolated by conventional alkaloid separation techniques. Ion-exchange chromatography was therefore extensively employed for separating this alkaloid fraction from neutral and acidic compounds present in the extract. In order to achieve suitably volatile derivatives for GC-MS, the silylation method of Fleet *et al.* [20] was used. This procedure leads to trimethylsilyl substitution at the hydroxyl groups, leaving the secondary amino group unsubstituted.

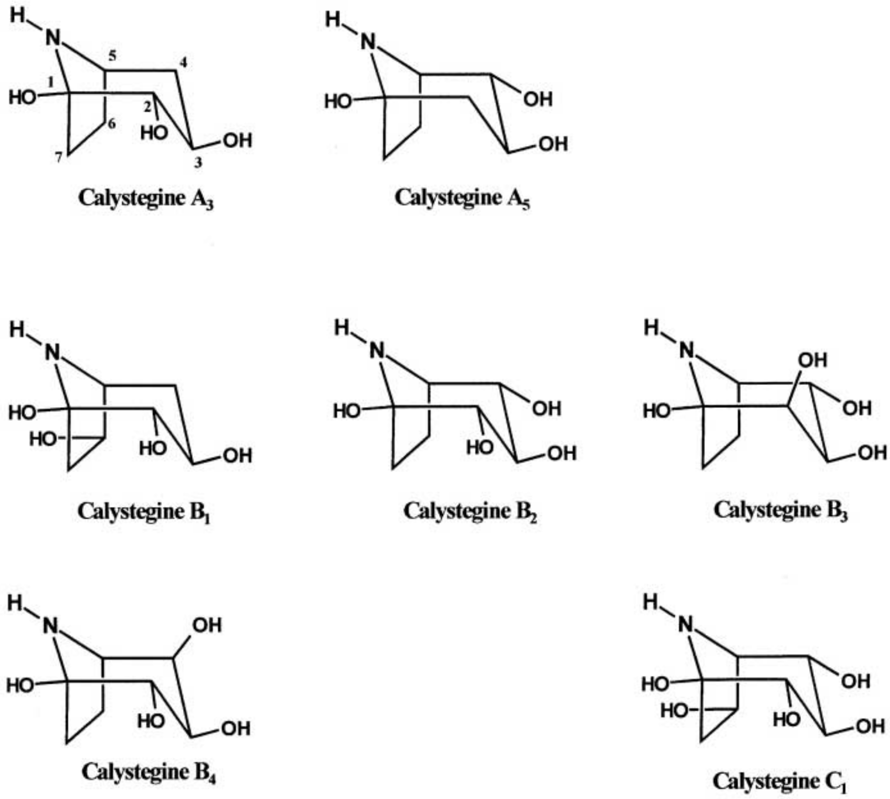

Fig. 1. Structures of the tri-, tetra-, and pentahydroxy-nortropanes included in this study.

Calystegines (Fig. 1) turned out to be common convolvulaceous metabolites and may be detectable in all plant organs (Table 1). Although calystegines could be detected in only 30 species (out of 65 examined), these plants belong to 15 genera (out of 22 examined) and to 8 tribes (out of 9 examined). Of these 30 "positive" species, 22 contained two or three calystegines. However, in three samples as many as five analogues were detectable (*Calystegia sepium*, *Convolvulus arvensis*, *Maripa panamensis*). Only one calystegine could be detected in five species (*Bonamia semidigyna*, *Erycibe malaccensis*, *E. parvifolia*, *Evolvulus argyreus*, *Ipomoea eremnobrocha*). The qualitatively dominant compound of the A series was calystegine A_3, detected in 43% of the 30 "positive" species followed by A_5 (20%). In the B series, calystegine B_2 exhibited the highest occurrence (86%) followed by B_1 (70%), B_3 (20%), and B_4 (13%). Calystegine C_1 was found in less than 1% of the species. All plant extracts showing no calystegines (cf. Table 1) have been re-examined once, starting with the extraction of the plant material, in order to verify the negative result.

However, on the basis of this work, a "negative" sample should not necessarily be interpreted as generally lacking calystegines. The stage of development of such "negative" species may lead to concentrations of these alkaloids which are below the detection limit because, e.g. metabolism has already happened. It also should be mentioned that the observed concentrations, in general, are very low also in the "positive" samples. The detection limits of the procedure used in this study 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.

To date, calystegine C_1 has been isolated only from *Morus alba* [11], belonging to the Moraceae family which is taxonomically not related to the Solanales. A first occurrence of C_1 in this order was reported by Molyneux [14], based on the analysis of *Ipomoea carnea* and *I.* sp. Q6 (aff. *calobra*). In our study this pentahydroxylated nortropane was found in two further species: *Argyreia hookeri* and *Ipomoea violacea*.

Inconsistent results on *C. arvensis* have been reported: Tepfer *et al.* could detect calystegines [2], whereas Todd *et al.* did not [21], presumably due to the fact that silylation had been omitted. Since we could detect several calystegines in the herbal material as well as in the flowers, there is no doubt any longer concerning the presence of these compounds in this species.

However, the wide distribution of calystegines apparently indicates an important taxonomic significance for the Convolvulaceae, as it does for the Solanaceae (Table 2). Thus, it is of chemotaxonomic interest as a further common feature of the

Table 2. Distribution of the calystegines in the Convolvulaceae examined in this study compared with the Solanaceae [12, 14, 15]

	Convolvulaceae			Solanaceae		
	total	examined for calystegines	calystegines found	total	examined for calystegines	calystegines found
Species	2000	65	30	2600	25	19
Genera	55	22	15	90	13	9
Tribes	9	9	8	7	7	6

two most important Solanales families. In order to answer the question whether, in addition, there are any intra-familiar aspects of relevancy, e.g., certain genera or *Ipomoea* sections lacking calystegines, further investigation is needed. Nevertheless, it should be noticed that neither six *Jacquemontia* species nor four *Operculina* species, showed any occurrence of a calystegine. Furthermore, the four species of the parasitic genus *Cuscuta*, with its extremely reduced habit and metabolism, did not show any occurrence of calystegines, too. This seems to be remarkable since *Cuscuta* is the only genus of the Cuscuteae tribe, often separated as a family of its own by several authors. Thus, species of all 8 tribes of the Convolvulaceae family *sensu stricto* turned out to synthesize calystegines.

Since tropan-3β-ol (pseudotropine) and tropan-3-one (tropinone) are supposed to be precursors of the calystegines in the Solanaceae [18, 22], it should be emphasized that the co-occurrence of both, hydrophilic calystegines as well as lipophilic tropan-3β-ol and its derivatives in many convolvulaceous species [21, 23, 24], apparently supports tropan-3β-ol as being a common calystegine precursor also in this family.

Remarkably, calystegines are even synthesized in species which contain alkaloids structurally diverging from these nortropanes. This is true for *Stictocardia campanulata*, the leaves of which contain ergot alkaloids [25] and calystegines, but also for *Ipomoea alba* (herbal material: indolizidines [26] and calystegines), *I. hederifolia* (seeds: pyrrolizidines [27] and calystegines), and *Merremia quinquefolia* (roots: pyrrolizidines, pyrrolidides [28], and calystegines).

EXPERIMENTAL

Plant material

The origins collected and the localities of the plants are summarized in Table 1. Voucher specimens of all plants are deposited in the Institut für Pharmazie II (Pharmazeutische Biologie), Freie Universität Berlin. The following species have been identified from voucher specimens by Professor Dr. Austin, Florida Atlantic University, Boca Raton, FL: *Ipomoea anisomeres*, *I. batatas*, *I. batatoides*, *I. eremnobrocha*, *Jacquemontia reclinata*.

Extraction

Plant material (5–10 g dry wt.; *Cuscuta europaea*, *C. palaestina*: 2.5 g; *Dichondra repens*, wild material: 2.5 g; *Erycibe malaccensis*: 3.1 g, *Ipomoea habeliana*: 3.0 g; *Jacquemontia tomentella* 1.1 g; *Porana volubilis*: 1.5 g) was homogenized and extracted three times with 50% MeOH (1:10). After centrifugation, the supernatant was evaporated to 1 ml, mixed with skin powder, filtered, and applied to a column of strongly acidic cation exchange resin (Dowex 50 WX8, 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., three quarters of which were used for TLC, and one quarter was used for GC-MS, respectively.

TLC

The following system was employed for preliminary screening: silica gel 60 F_{254} (0.2 mm), MeOH–$CHCl_3$ 32% aq. NH_3–H_2O (46:50:1:3), developed twice. Calystegines of the A-group: R_f 0.49–0.52; calystegines of the B-group: R_f 0.41–0.51; calystegine C_1: R_f 0.33. The polyhydroxylated N-containing compounds were identified as yellowish–brown spots with $AgNO_3$/NaOH dip reagents [2].

GC

GC was performed under the following conditions: capillary column coated with the methyl silicone stationary phase DB1, 30 m $\times$ 0.25 mm, temp.-program: 160° isotherm for 2 min, 160–240° at 5° 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.

Silylation

A quarter of the dry plant extract was dissolved in pyridine. The soln was mixed with hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS, 4:1) and kept at *ca.* 50° for 15 min. *n*-Octadecane (50 μl, c = 200 ng/μl) was added as an internal standard. RR_ts (*n*-octadecane) (Table 3) were used to identify the calystegines present in the extracts.

Table 3. RR_ts and fragmentation patterns in GC-MS of pure silylated calystegines

Compound	RR_t	m/z (rel. int.)
Tri-TMSi-calystegine A_3	0.90	360 (6), 286 (33), 285 (33), 244 (47), 170 (48), 156 (100)
Tri-TMSi-calystegine A_5	0.85	360 (6), 286 (14), 285 (18), 259 (48), 204 (63), 156 (100)
Tetra-TMSi-calystegine B_1	1.28	448 (1), 373 (6), 244 (50), 129 (51)
Tetra-TMSi-calystegine B_2	1.55	448 (1), 373 (2), 259 (25), 217 (100), 170 (10), 156 (13), 129 (6)
Tetra-TMSi-calystegine B_3	1.26	448 (1), 373 (3), 284 (14), 259 (29), 217 (99), 156 (18), 129 (8)
Tetra-TMSi-calystegine B_4	1.16	448 (1), 373 (1), 284 (9), 259 (7), 244 (15), 217 (100), 170 (9), 156 (12), 129 (5)
Penta-TMSi-calystegine C_1	1.98	373 (4), 347 (8), 258 (6), 244 (5), 217 (100), 129 (7)

Acknowledgements—The authors are indebted to Mr. Andreas Kannegießer (Institut für Pharmazie I, Freie Universität Berlin) for recording the GC-MS spectra as well as Mrs. Elisabeth Bäumel-Eich, Berlin, Profesora Dr Magalis Bittner Berner, Departamento de Botanica, Universidad de Concepción, Chile, and Mr. Alex Fernando Espinosa, Universidad de Panamá, for essential support in exploring and collecting the plant material. Furthermore, the authors are grateful to Professor Dr Werner Greuter and Mr. Christoph Schiers, Botanischer Garten, Freie Universität Berlin, for providing the plant material of *Falkia repens* and *Convolvulus cneorum* and supporting the cultivation of further species in the area of the Botanical Garden. Finally, we thank Professor Dr Daniel F. Austin, Florida Atlantic University, Boca Raton, FL, U.S.A., for the determination of certain species (see Experimental), Dr Macki Kaloga, Institut für Pharmazie II, Freie Universität Berlin, for discussions concerning MS data, and Dr Jim Greatorex, Norges Landbrukshøgskole, Institutt for Tekniske Fag, Ås, Norway, for linguistic help in the preparation of this manuscript.

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