Tropane and Pyrrolidine Alkaloids from *Convolvulus lanatus* Vahl

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The alkaloid composition of *Convolvulus lanatus* was investigated by GLC and GLC-MS. Altogether, thirteen tropane and pyrrolidine alkaloids were identified on the basis of mass spectral data and/or Kovats retention indices. Twelve of them have been recorded for the first time in this plant and some of these alkaloids were identified for the first time in the genus *Convolvulus*. Cuscohygrine is the major constituent. Qualitative and quantitative differences of root and aerial parts are discussed.

Key words: Convolvulus lanatus, Tropinone, Pyrrolidine Alkaloids

Introduction

Convolvulus is a genus of about 250 species of flowering plants in the bindweed family Convolvulaceae, with a cosmopolitan distribution (Feinbrun-Dothan, 1978). This species occurs in many temperate and subtropical regions.

Alkaloids, flavonoids, coumarins, sterols, saponins, resin glycosides, tannins and stilbene derivatives have been isolated from plants of this genus (Wagner *et al*., 1983; Rizk, 1986; Southon and Buckingham, 1989; Noda *et al*., 1990; 1992; Glasby, 1991; Molyneux *et al*., 1993; Todd *et al*., 1995; El-Fiky *et al*., 1996; Jenett-Siems *et al*., 1998; Dawidar *et al*., 2000; Menemen *et al*., 2002).

Many *Convolvulus* species are known for their medicinal utilization and exhibit interesting biological properties such as purgative, CNS disturbing and antidepressant, antioxidant, hypoglycemic, antinociceptive, anticancer, anti-ulcerogenic and antidiarrhoeal activities (Shabana *et al.*, 1990; Noda *et al*., 1990, 1992; Sairam *et al*., 2001; Meng *et al*., 2002; Parihar and Hemnani, 2003; Atta and Mouneir, 2004; Atta and El-Sooud, 2004; Kumar, 2006; Dhingra and Valecha, 2007). Calystegins (polyhydroxytropanes) isolated from *Convolvulus arvensis* are known as potent glycosidase inhibitors (Molyneux *et al*., 1993). In addition, some pharmacological and toxic effects of tropane and pyrrolidine alkaloids can be attributed to the modulation of muscarinic acetylcholine receptors (Schmeller *et al*., 1995).

In Egypt, the genus *Convolvulus* is represented by about 20 species (Boulos, 2000). *Convolvulus lanatus* is a common spiny shrub, which grows wild in Egyptian desert and arid lands. The plant has not been thoroughly studied for its alkaloid content with modern analytical methods. Since 1983, only the alkaloid cuscohygrine has been isolated from this plant (Hilal *et al*., 1983).

As a part of continuous investigation of Egyptian plants containing alkaloids and having pharmacological, toxicological and chemotaxonomic properties (El-Shazly *et al.*, 1996a, b, c, 1997, 1998, 1999, 2000, 2005; El-Shazly, 2002; El-Shazly and Wink, 2003), we now report our findings on the alkaloid constituents of the root and aerial parts of *C. lanatus*.

We were able to unambiguously identify most of alkaloids in this plant and have found a number of tropane and pyrrolidine compounds for the first time in the studied species and in genus *Convolvulus*.

Material and Methods

Plant material

Flowering plants of *Convolvulus lanatus* Vahl (syn. *C. forsskaolii* Delile, *C. el-arishensis* Boulos) (Boulos, 2000) were collected from a sandy area near Bilbeis, Sharkeya province, Egypt, in April 2004. The identity of the plant has been kindly verified by Prof. Dr. H. Abdel Baset, Faculty of Science, Zagazig University, Zagazig, Egypt. A

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voucher specimen was deposited in the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt.

Alkaloid extraction

The dried plant material (root and aerial parts, 100 g each) were separately extracted twice with 0.5 M HCI (1 l) through homogenization by Ultraturrax treatment and left to stand for 1 h. The resulting extracts were defatted with CH_2CI_2, made alkaline with NH_4OH and extracted with CH_2CI_2, The organic extracts were dried (anhydrous Na_2SO_4), filtered and concentrated under reduced pressure to yield alkaloid mixtures. The yield was 0.06% for the root and 0.01% for the aerial parts.

Capillary GLC analysis

Carlo Erba ICU 600 gas chromatograph equipped with an FID and Spectra Physics integrator. Column: OV1 (15 m, 0.317 mm i. d., 0.25 μm film thickness). Condition: carrier gas, He (2 ml/min); detector temperature, 300 °C; injector temperature, 250 °C; oven temperature program: intial 100 °C for 2 min, isothermal, 100–250 °C at 15 °C min^{-1}, 250–300 °C at 25 °C min^{-1}, 300 °C for 5 min, isothermal. Retention index (RI): Kovats indices (Kovats, 1958) were calculated with respect to a set of co-injected even-numbered hydrocarbons (C_{10}–C_{28}). Each RI was subjected to a library search by comparison with reference RI values stored in a data base of Institut für Pharmazie und Molekulare Biotechnologie, Universität Heidelberg, Germany. Cuscohygrine was used as external standard for quantification.

GLC-MS analysis

Carlo Erba Mega 5160 gas chromatograph equipped with a fused silica column (DB1, 30 m). The capillary column was directly coupled to a quadrupole mass spectrometer (Finnigan MAT 4515). Condition: injector temperature, 250 °C; temperature program, 70–300 °C at 6 °C min^{-1}; split ratio, 1:20; carrier gas, He (1 ml/min^{-1}). EI-mass spectra were recorded at 70 eV.

Results and Discussion

Since capillary GLC and GLC-MS are powerful tools for the rapid and sensitive analysis of tropane and pyrrolidine alkaloids (Witte *et al.*, 1987; Doerk-Schmitz *et al.*, 1993; Ionkova *et al.*, 1994; Todd *et al.*, 1995; El-Shazly *et al.*, 1997) these methods have been employed in order to identify major and minor alkaloids in the root and aerial parts of *C. lanatus*.

A previous report on *C. lanatus* revealed the presence of the pyrrolidine alkaloid cuscohygrine (Hilal *et al.*, 1983). In addition to the previously reported cuscohygrine, another twelve pyrrolidine derivatives and tropinone were detected and identified (Tables I and II). Ten alkaloids could be unequivocally identified by direct comparison (MS, RI) with authentic alkaloids or by comparison with literature data (Blossey *et al.*, 1964; Witte *et al.*, 1987; Basey *et al.*, 1992; Gorinova *et al.*, 1994; Kubwabo *et al.*, 1993; Doerk-Schmitz *et al.*, 1993; Ionkova *et al.*, 1994; Jenett-Siems *et al.*, 1996; Brachet *et al.*, 1997; El-Shazly *et al.*, 1997) namely: hygrine (**1**), tropinone (**2**), 5-(2-oxopropyl)-hygrine (**3**), 5-(2-hydroxypropyl)-hygrine (**4**), 2′,4-*N*-methylpyrrolidinylhygrine (**6**), 2′,3-*N*-methylpyrrolidinylhygrine (**7**), cuscohygrine (**8**), phygrine (**11**), *N*-methylpyrrolidinylcuscohygrine A (**12**) and *N*-methylpyrrolidinylcuscohygrine B (**13**) (Fig. 1). The alkaloid pattern of both root and aerial parts were also investigated (Table I).

Table I. Alkaloid distribution in the root and aerial parts of *Convolvulus lanatus* as determined by GLC (total alkaloid 100%).

Alkaloid	Aerial parts	Root
Hygrine (**1**)[a]	–	tr[c]
Tropinone (**2**)[a]	–	tr
5-(2-Oxopropyl)-hygrine (**3**)[a,b]	–	tr
5-(2-Hydroxypropyl)-hygrine (**4**)[a,b]	tr	4.66
2,5-Di-(2-hydroxypropyl)-*N*-methylpyrrolidine (**5**)[a,b]	–	tr
2′,4-*N*-Methylpyrrolidinylhygrine (**6**)[a,b]	5.47	3.07
2′,3-*N*-Methylpyrrolidinylhygrine (**7**)[a,b]	6.19	4.70
Cuscohygrine (**8**)	80.75	72.86
2-[2-Hydroxy-3-(*N*-methyl-2-pyrrolidinyl)-propanyl]-*N*-methylpyrrolidine (**9**)[a,b]	3.39	2.83
Isomer of **9** (**10**)[a,b]	4.20	7.69
Phygrine (**11**)[a]	tr	tr
N-Methylpyrrolidinylcuscohygrine A (**12**)[a,b]	tr	4.19
N-Methylpyrrolidinylcuscohygrine B (**13**)[a,b]	tr	tr
Total alkaloids[d]	0.01	0.06

[a] New for *C. lanatus*.
[b] First report in genus *Convolvulus*.
[c] tr, trace.
[d] In mg/100 mg dried plant material.

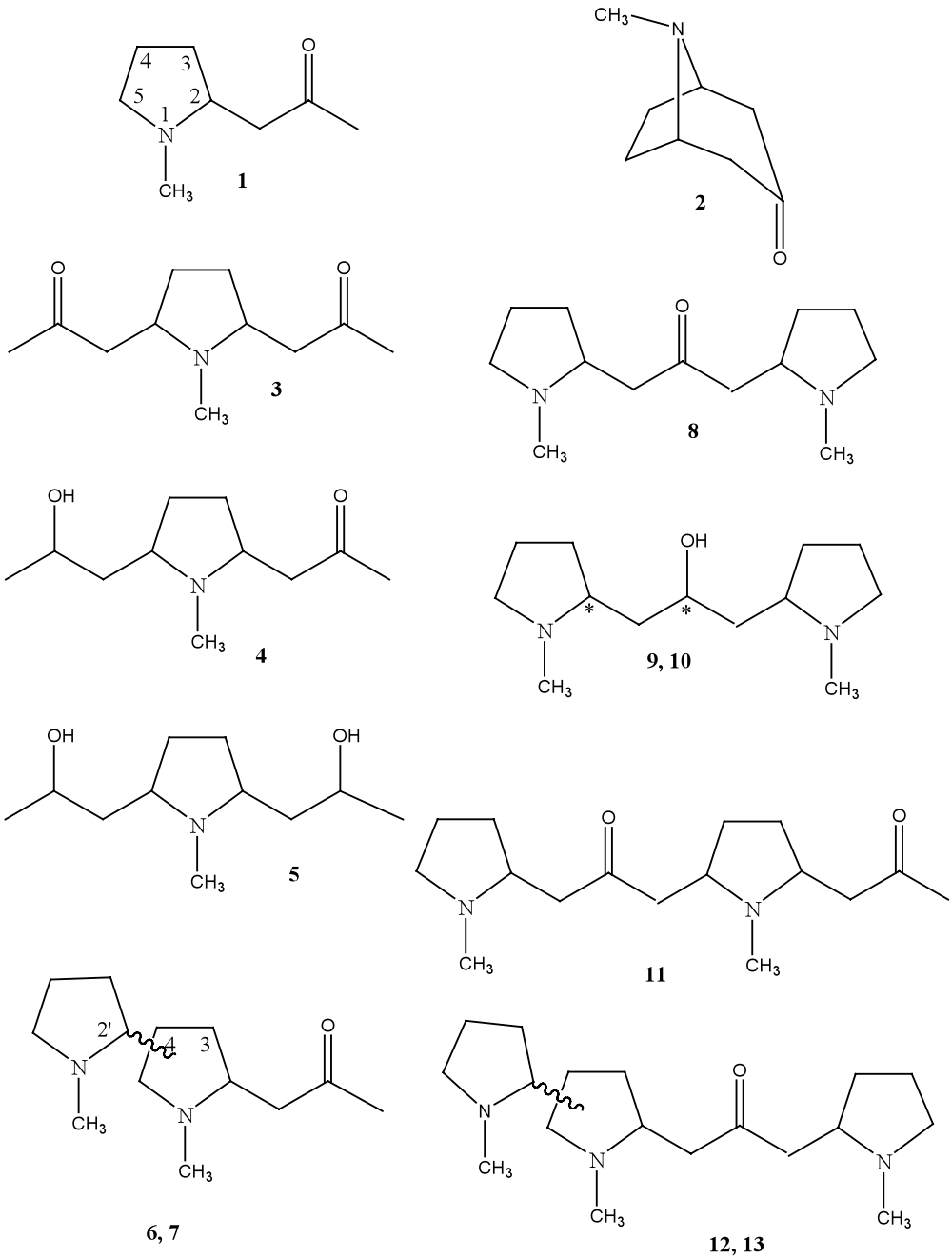

Fig. 1. Structures of tropinone and pyrrolidine alkaloids from *Convolvulus lanatus*.

Alkaloid **5** showed a similar mass fragmentation pattern to that of **4**. Its [M^+] at *m/z* 201 (two mass units higher than **4** and four mass units higher than **3**), corresponds to $C_{11}H_{23}NO_2$. The base peak at *m/z* 142 is probably due to the loss of one of the hydroxypropyl moieties at C2 or C5. The quite high intensity fragment at *m/z* 84 is typical of an *N*-methylpyrrolidinyl ion providing good evidence for the loss of the two hydroxypropyl moieties at C2 and C5. Thus, based on mass fragmentation

Table II. GLC-MS data for tropane and pyrrolidine alkaloids found in *Convolvulus lanatus*.

Compound	RI[a]	[M$^+$]	Characteristic ions (abundance, %)	Ref.[b]
1	1060	141	98(4), 85(5), 84(100), 82(10), 70(6), 43(11), 42(23)	1, 2, 3
2	1153	139	116(5), 110(6), 96(30), 83(17), 82(100), 81(40), 55(20), 42(50), 41(15)	3, 4
3	1440	197	140(80), 98(8), 96(17), 83(8), 82(100), 43(39), 42(13)	5, 6
4	1451	199	142(47), 141(10), 140(100), 98(11), 96(17), 84(25), 83(11), 82(98), 57(8), 43(28), 42(19)	6
5	1478	201	143(10), 142(100), 98(10), 96(10), 84(30), 80(14), 57(8), 45(12), 43(8), 42(14)	
6	1570	224	167(3), 152(7), 139(3), 124(4), 110(4), 98(3), 84(100), 83(6), 82(10), 70(4), 43(6), 42(11)	3, 7
7	1580	224	224(3), 167(5), 152(9), 139(3), 124(5), 110(5), 98(3), 84(100), 83(5), 82(9), 70(3), 43(5), 42(12)	3, 7
8	1650	224	209(5), 140(15), 99(9), 98(15), 97(8), 85(10), 84(100), 83(23), 82(18), 70(5), 55(5), 44(15), 42(28)	3, 8
9	1682	226	211(1), 170(1), 152(2), 128(3), 110(2), 98(5), 85(8), 84(100), 69(3), 58(2), 43(8), 42(8)	
10	1695	226	211(0.5), 170(0.5), 128(4), 110(2), 98(4), 85(7), 84(100), 70(2), 42(8)	
11	2045	280	154(5), 140(40), 124(2), 109(4), 96(12), 84(100), 83(15), 82(50), 81(12), 70(6), 57(11), 43(23)	3, 5
12	2163	307	167(3), 123(2), 107(2), 93(2), 84(100), 82(16), 59(5), 43(12), 42(13)	3, 9
13	2175	307	167(2), 97(3), 91(3), 84(100), 83(15), 82(24), 69(3), 57(6), 55(9), 43(13), 42(6)	3, 9

[a] Tentatively identified by GLC-MS.
[b] 1, Witte *et al.*, 1987; 2, Ionkova *et al*., 1994; 3, El-Shazly *et al*., 1997; 4, Blossey *et al*., 1964; 5, Basey *et al*., 1992; 6, Brachet *et al*., 1997; 7, Jenett-Siems *et al*., 1996; 8, Kubwabo *et al*., 1993; 9, Doerk-Schmitz *et al*., 1993.

and biogenic consideration, alkaloid **5** was tentatively identified as 2,5-di-(2-hydroxypropyl)-*N*-methylpyrrolidine. The structure of **5** has not been found from plant material so far.

The mass spectra of alkaloids **9** and **10** were very similar, having RI values of 1682 and 1695, respectively. Both compounds showed [M$^+$] at *m/z* 226 (2 mass units higher than cuscohygrine) indicating that they could be an isomeric form of these alkaloids. Considering the chiral carbon atoms at C2 in the pyrrolidine ring and/or at C2′ in the chain, it is reasonable to assume that the two compounds represent the two possible isomers of 2-[2-hydroxy-3-(*N*-methyl-2-pyrrolidinyl)-propanyl]-*N*-methylpyrrolidine (hydroxy instead of carbonyl in cuscohygrine), but the structure of the two compounds could not be unequivocally elucidated by MS alone. But we did not have enough material to carry out a NMR analysis.

Although, most of these alkaloids are well known from other genera of Convolvulaceae, Solanaceae and Erythroxylaceae, some of the alkaloids listed were, to our knowledge, hitherto unknown in the genus *Convolvulus*. With the exception of cuscohygrine, all compounds mentioned here have been detected for the first time in *C. lanatus*. Our results contribute to the chemoecology and chemotaxonomy of the genus *Convolvulus*.

Atta A. H. and Mouneir S. M. (2004), Antidiarrhoeal activity of some Egyptian medicinal plant extracts. J. Ethnopharmacol. **92**, 303–309.

Atta A. H. and El-Sooud K. A. (2004), Antinociceptive effect of some Egyptian medicinal plant extracts. J. Ethnopharmacol. **95**, 235–238.

Basey K., McGaw B. A., and Wooley J. G. (1992), Physgrine, an alkaloid from *Physalis* species. Phytochemistry **31**, 4173–4176.

Blossey E. C., Budzikiewicz H., Ohashi M., Fodor G., and Djerassi C. (1964), Mass spectrometry in structural and stereochemical problems – XXXIX, Tropane alkaloids. Tetrahedron **20**, 585–595.

Boulos L. (2000), Flora of Egypt, Vol. 2. Al Hadara Publishing, Cairo, Egypt, p. 243.

Brachet A., Munoz O., Gupta M., Veuthey J., and Christen P. (1997), Alkaloids of *Erythroxylum lucidum* stem-bark. Phytochemistry **46**, 1439–1442.

Dawidar A. M., Ezmirly S. T., Abdel-Mogib M., El-Dessouki Y., and Angawi R. F. (2000), New stilbene carboxylic acid from *Convolvulus hystrix*. Pharmazie **55**, 848–849.

Dhingra D. and Valecha R. (2007), Evaluation of the antidepressant-like activity of *Convolvulus pluricaulis* choisy in the mouse forced swim and tail suspention tests. Med. Sci. Monit. **13**, BR155–161.

Doerk-Schmitz K., Witte L., and Alfermann A. W. (1993), Tropane alkaloid patterns in plants and hairy roots of *Hyoscyamus albus*. Phytochemistry **35**, 107–110.

El-Fiky F. K., Abdel-Kader M. S., and Aboul-Ela M. A. (1996), Constituents of *Convolvulus lanatus* Vahl with antiviral and cytotoxic activity. Alexandria J. Pharm. Sci. **10**, 25–29.

El-Shazly A. (2002), Pyrrolizidine alkaloid profiles of some *Senecio* species from Egypt. Z. Naturforsch. **57c**, 429–439.

El-Shazly A. and Wink M. (2003), Tetrahydroisoquinoline and β-carboline alkaloids from *Haloxylon articulatum* (Cav.) Bunge (Chenopodiaceae). Z. Naturforsch. **58c**, 477–480.

El-Shazly A., Sarg T., Ateya A., Abdel Aziz A., El-Dahmy S., Witte L., and Wink M. (1996a), Pyrrolizidine alkaloids in *Echium setosum* and *Echium vulgare*. J. Nat. Prod. **59**, 310–313.

El-Shazly A., Sarg T., Ateya A., Abdel Aziz A., Witte L., and Wink M. (1996b), Quinolizidine alkaloids of *Argyrolobium uniflorum*. Pharmazie **51**, 768–772.

El-Shazly A., Ateya A., Witte L., and Wink M. (1996c), Quinolizidine alkaloid profiles of *Retama raetam*, *R. sphaerocarpa* and *R. monosperma*. Z. Naturforsch. **51c**, 301–308.

El-Shazly A., Tei A., Witte L., El-Domiaty M., and Wink M. (1997), Tropane alkaloids of *Hyoscyamus boveanus, H. desertorum, H. muticus* and *H. albus* from Egypt. Z. Naturforsch. **52c**, 729–739.

El-Shazly A., El-Domiaty M., Witte L., and Wink M. (1998), Pyrrolizidine alkaloids in members of Boraginaceae from Sinai (Egypt). Biochem. Syst. Ecol. **26**, 619–639.

El-Shazly A., Abdel Aal M., and Wink M. (1999), Pyrrolizidine alkaloids from *Echium rauwolfii* and *Echium horridium*. Z. Naturforsch. **54c**, 295–300.

El-Shazly A., Ateya A., and Wink M. (2000), Quinolizidine alkaloid profiles of *Lupinus albus albus*, *L. hartwegii* and *L. densiflorus*. Z. Naturforsch. **56c**, 21–30.

El-Shazly A., Dorai G., and Wink M. (2005), Alkaloids of *Haloxylon salicornicum* (Moq.) Bunge ex Boiss. (Chenopodiaceae). Pharmazie **60**, 949–952.

Feinbrun-Dothan N. (1978), Flora Palaestina, Vol. 3. Jerusalem Academic Press, pp. 162–163.

Glasby J. S. (1991), Dictionary of Plants Containing Secondary Metabolites. Taylor and Francis, London, p. 86.

Gorinova N. I., Velcheva M. P., Dyulgerov A. S., and Atanassov A. I. (1994), Tropane alkaloids in cell cultures of *Physochlaina orientalis*. Fitoterapia **65**, 452–456.

Hilal S. H., Haggag M. Y., Soliman F. M., and El-Kashorey E. A. (1983), Phytochemical study and biological screening of *Convolvulus lanatus* Vahl. Egypt J. Pharm. Sci. **24**, 139–148.

Ionkova I., Witte L., and Alfermann A. W. (1994), Spectrum of tropane alkaloids in transformed roots of *Datura innoxia* and *Hyoscyamus* × *györffyi* cultivated *in vitro*. Planta Med. **60**, 382–384.

Jenett-Siems K., Henrici A., Tofern B., Bufacchi-Richter A., Kaloga M., Witte L., Hartmann T., and Eich E. (1996), Occurrence and distribution of hygrines and tropanes in the convolvulaceous genus *Merremia* including the report on merredissine, a novel tropane alkaloid. 24. Congress on Medicinal Plants Research, Prag, Abstract book, p. 128.

Jenett-Siems K., Mann P., Kaloga M., Siems K., Jakupovic J., Witte L., and Eich E. (1998), Tropane alkaloids with a unique type of acyl moiety from two *Convolvulus* species. Phytochemistry **49**, 1449–1451.

Kovats E. (1958), Gas-chromatographische Charakterisierung organischer Verbindungen. Teil I: Retentionsindices aliphatischer Halogenide, Alkaloide, Aldehyde and Ketone. Helv. Chim. Acta **41**, 1915–1932.

Kubwabo C., Rollmann B., and Tilquin B. (1993), Analysis of alkaloids from *Physalis peruviana* by capillary GC, capillary GC-MS, and GC-FtIR. Planta Med. **59**, 161–163.

Kumar V. (2006), Potential medicinal plants for CNS disorders: an overview. Phytother Res. **20**, 1023–1035.

Menemen Y., Williams C. A., and Jury S. L. (2002), Flavonoid patterns in *Convolvulus* L. (Convolvulaceae) species from Morocco. Pak. J. Bot. **34**, 291–295.

Meng X. L., Riordan N. H., Casciari J. J., Zhu Y., Zhong J., Gonzalez M. J., Miranda-Massari J. R., and Riordan H. D. (2002), Effects of a high molecular mass *Convolvulus arvensis* extract on tumor growth and angiogenesis. P. R. Health Sci. J. **21**, 323–328.

Molyneux R. J., Pan Y. T., Goldmann A., Tepfer D. A., and Elbein A. D. (1993), Calystegins, a novel class of alkaloid glycosidase inhibitors. Arch. Biochem. Biophys. **304**, 81–88.

Noda N., Kogetsu H., Kawasaki T., and Miyahara K. (1990), Scammonins I and II, the resin glycosides of radix Scammoniae from *Convolvulus scammonia*. Phytochemistry **29**, 3565–3569.

Noda N., Kogetsu H., Kawasaki T., and Miyahara K. (1992), Scammonins VII and VIII, two resin glycosides from *Convolvulus scammonia*. Phytochemistry **31**, 2761–2766.

Parihar M. S. and Hemnani T. (2003), Phenolic antioxidants attenuate hippocampal neuronal cell damage against kainic acid induced excitotoxicity. J. Biosci. **28**, 121–128.

Rizk A. M. (1986), The Phytochemistry of the Flora of Qatar. Scientific and Applied Research Center, University of Qatar, pp. 96–98.

Sairam K., Rao C. V., and Goel R. K. (2001), Effects of *Convolvulus pluricaulis* Chois on gastric ulceration and secretion in rats. Indian J. Exp. Biol. **39**, 350–354.

Schmeller T., Sporer F., Sauerwein M., and Wink M. (1995), Binding of tropane alkaloids to nicotinc and muscarinic acetylcholine receptors. Pharmazie **50**, 493–495.

Shabana M. M., Mirhom Y. W., Genenah A. A., Aboutabl E. A., and Amer H. A. (1990), Study into wild Egyptian plants of potential medicinal activity. Ninth communication: hypoglycemic activity of some selected plants in normal fasting and alloxanised rats. Arch. Exp. Veterinarmed. **44**, 389–394.

Southon I. W. and Buckingham J. (eds.) (1989), Dictionary of Alkaloids. Chapman and Hall, London.

Todd F. G., Stermitz F. R., Schultheis P., Knight A. P., and Traub-Dargatz J. (1995), Tropane alkaloids and toxicity of *Convolvulus arvensis*. Phytochemistry **39**, 301–303.

Wagner H., Schwarting G., Varijen J., Bauer R., Hamdard M. E., El-Faer M. Z., and Beal J. (1983), Chemical constituents of the Convolvulaceae-Resin IVI. Planta Med. **49**, 154–157.

Witte L., Müller K., and Arfmann H. A. (1987), Investigation of the alkaloid pattern of *Datura innoxia* plants by capillary gas-liquid-chromatography-mass spectrometry. Planta Med. **53**, 192–197.