Tropane Alkaloids of *Hyoscyamus boveanus, H. desertorum, H. muticus* and *H. albus* from Egypt

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The alkaloid composition of *Hyoscyamus boveanus, H. desertorum, H. muticus* and *H. albus* was investigated by capillary GLC and GLC-mass spectrometry. Altogether 39 alkaloids of the tropane and pyrrolidine types were identified. Several alkaloids were found in the different species for the first time, e.g., 22 alkaloids in *H. boveanus*, 13 in *H. desertorum*, 24 in *H. muticus* and 4 in *H. albus*. 3β-Hyoscyamine, dehydrohyoscyamine, phyllalbine, 3α-phenylacetyltropane, phenylacetylscopine as well as phygrine and 2,3-diacetonyl-N-methylpyrrolidine are apparently new for the genus *Hyoscyamus*. Alkaloid profiles and alkaloid contents of flowers, fruits, leaves, stems and roots were determined and their chemoecological functions discussed.

Introduction

Our interest are plants inhabiting deserts and arid land in Egypt and the Near East and their strategies to defend themselves against herbivores, competing plants and microorganisms. Plant secondary metabolites play a prominent role in this context (Harborne, 1993; Wink 1993). Plants of the genus *Hyoscyamus* which are abundant in deserts and on arid land are especially rich in tropane alkaloids. Tropane alkaloids occur widely in the plant families Solanaceae and Erythroxylaceae, but minor occurrences have been reported from other families like Convolvulaceae, Cruciferae, Dioscoreaceae, Elaeocarpaceae, Euphorbiaceae, Orchidaceae, Proteaceae and Rhizophoraceae (Harborne and Baxter 1993). These alkaloids exhibit a wide range of pharmacological and toxic activities including antispasmodic, anticholinergic, mydriatic and antiemetic effects. In addition, they are used in the treatment of motion sickness and as a pre-operative medication to sedate and reduce secretions, produce amnesia and assist the induction of anaesthesia and reduce some of its side effects (Southon and Buckingham 1989; Harborne and Baxter 1993). Some of the pharmacological and toxic effects can be attributed to a modulation of muscarinic acetylcholine receptors (Schmeller *et al.*, 1995).

Twenty *Hyoscyamus* species are found in the Euro-Siberian, Mediterranean, Irano-Turanian, and Saharo-Arabian regions (Feinbrun-Dothan 1978). In Egypt at least seven *Hyoscyamus* species occur, of which four were studied in this project: *Hyoscyamus boveanus* (Dun. in DC.) Asch. et Schweinf. is a rare perennial herb. The whole plant including the inflorescence is spreadingly hairy. The flowers are white with purple blotches and stripes, filaments and anthers have cream colour (Täckholm 1974). *Hyoscyamus desertorum* (Asch. and Boiss) Täckh. is a rare annual, erect, yellowish-green herb with small, ovate to rhombic rarely cordate based leaves which are covered all over with densely glandular hairs and bright yellow flowers (Täckholm 1974; Feinbrun-Dothan 1978). *H. muticus* and *H. albus* are more widely distributed annual plants. According to a literature survey *H. boveanus* has not been investigated phytochemically. Few alkaloids were reported already from *H. desertorum* (Sabri *et al.*, 1973; Parr *et al.*,

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1990). *H. muticus* (Egyptian henbane) contains hyoscyamine and scopolamine (hyoscine) (Ahmed and Fahmy 1949; Balbaa *et al.*, 1964; Giri and Ahuja 1994) as well as other minor alkaloids detected by GLC-mass spectrometry (Parr *et al.*, 1990). Plants growing in Afghanistan showed hyoscyamine, *apo*atropine and hyoscine as major and *nor*atropine and *nor*hyoscine as minor components (Trease and Evans 1983). Since the alkaloid composition of *H. albus* has been studied extensively already (e.g., Parr *et al.*, 1990; Doerk-Schmitz *et al.*, 1994) plants from Egypt were included as a reference.

Since capillary GLC and GLC-MS are powerful tools for the rapid and sensitive analysis of tropane alkaloids (Karawya *et al.*, 1975; Hartmann *et al.*, 1986; Witte *et al.*, 1987; Parr *et al.*, 1990; Kubwabo *et al.*, 1993; Doerk-Schmitz *et al.*, 1994; Dupraz *et al.*, 1994; Gorinova *et al.*, 1994; Ionkova *et al.*, 1994; Todd *et al.*, 1994) these methods have been employed in order to identify major and minor alkaloids which may be of value for the chemoecology and chemotaxonomy of the genus *Hyoscyamus*. Alkaloidal contents and profiles of the different organs of the four *Hyoscyamus* species (*H. boveanus, H. desertorum*, *H. mutics* and *H. albus*) were documented and discussed under chemoecological aspects.

Material and Methods

Plant material

Stems, flowers, leaves and fruits of *Hyoscyamus boveanus* (Dun. in DC.) Asch. et Schweinf. were collected from Saint Catherine, Sinai in March 1995. *H. desertorum* (Asch. & Boiss.) Täckh. was collected from El-Arish, Sinai in April 1996. *H. muticus* L. was harvested from the surroundings of Suez city in March 1995. *H. albus* L. was collected from the experimental garden of the Faculty of Pharmacy, Zagazig University in April 1995. The identification of the plants was verified by Prof. Dr. N. El-Hadidi, Profesor of plant taxonomy, Faculty of Science, Cairo University. Voucher specimens are deposited in the Herbarium of the Department of Pharmacognosy, Zagazig University.

Alkaloid extraction

The plant material (except for roots of *H. muticus* and *H. albus*, dried material was employed) (5 g for each organ) was homogenized in a Ultra-Turrax in 100 ml 0.5 N HCl and left to stand for at least one hour. The acidic solution was washed with CH_2Cl_2, made alkaline with NH_4OH and extracted with CH_2Cl_2. The organic extracts were dried (anhyd. Na_2SO_4), filtered and concentrated under reduced pressure to yield alkaloid mixtures. Preperative thin-layer chromatography of *H. boveanus* alkaloid extract using Silica gel Merck F_{254}, CH_2Cl_2- MeOH-NH_4OH (25%), 100–15–1 and Dragendorff's reagent yielded the alkaloids scopolamine and hyoscyamine having R_f value of 0.84 and 0.63, respectively.

Tissue culture

Root cultures were established from *H. desertorum* seeds. Roots were subcultured in hormone-free Woody Plant (WP) liquid medium (McCown and Lloyd, 1982) with 3% sucrose for two weeks and subcultured. The harvested roots were homogenized in 0.5 N HCl with an Ultra-Turrax and left standing for 30 min. After filtration the filtrate was made alkaline with NH_4OH and applied to Extrelut column (Merck, Darmstadt). The alkaloids were eluted with CH_2Cl_2.

Capillary GLC analysis

A Carlo Erba ICU 600 gas chromatograph equipped with FID and Spectra Physics integrator. Column: DB1–30 W (J&W Scientific; 15m, 0.317 mm inner diameter). Conditions: carrier gas He (2 ml / min.); detector temp. 300 °C; injector temp. 250 °C; oven temp. program: initial 150 °C 2 min. isothermal, 150–250 °C at 15 min^1, 250–300 °C at 25 min^{-1}, 300 °C, 5 min isothermal. Retention indices (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 the Institute für Pharmazeutische Biologie, Heidelberg University. Hyoscyamine was used as an external standard for quantification.

GLC-MS analysis

A Carlo Erba Mega 5160 gas chromatograph equipped with a fused silica column (DB1, J&W Scientific; 30 m). The capillary column was directly coupled to a quadrupole mass spectrometer (Finnigan MAT 4515). A Finnigan MAT 4500, coupled with a Carlo Erba HRGC 4160 gas chromatograph equipped with OV1 fused silica column was also used. EI-mass spectra were recorded at 40 eV. Condition: injector 250°C; temp. program 70–300 °C, 6 °C /min or 150–300 °C, 6 °C /min. Split ratio 1 : 20; carrier gas He 50 kPa.

NMR measurements

^{1}H- and ^{13}C NMR spectra were recorded on a AC Bruker instrument in $CDCl_3$, at 300 and 75 MHz, respectively.

Scopolamine **35** ^{1}H NMR: (300 MHz, $CDCl_3$), δ 7.19–7.37 (5Ar. H, *m*), 5.01 (1H, *t*, *J* = 5.4 Hz, H-3), 4.15 (1H, *dd*, *J* = 2, 8.5 Hz, H-9), 3.76 (2H, *m*, CH_2OH), 3.36 (1H, *d*, *J* = 3 Hz, H-6), 3.11 (1H, *s*, *J* = 1.92 Hz, H-5), 2.97 (1H, *q*, *J* = 2 Hz, H-1), 2.64 (1H, *d*, *J* = 2.97 Hz, H-7), 2.44 (3H, *s*, N-CH_3), 2.08 (2H, *m*, H-2 and H-4 axial), 1.55 (1H, *d*, *J* = 15.3 Hz, H-2 equ.), 1.32 (1H, *d*, *J* = 15.5 Hz, H-4 equ.). ^{13}C NMR (75 MHz, $CDCl_3$), δ 57.80 (C-1), 30.95 (C-2), 66.83 (C-3), 30.77 (C-4), 57.93 (C-5), 56.30 (C-6), 55.85 (C-7), 42.22 (N-CH_3), 71.83 (C-9), 54.24 (C-10), 63.98 (CH_2-OH), 135.61 (C-1'), 128.97 (C-2'), 128.03 (C-3'), 127.95 (C-4'), 128.03 (C-5'), 128.97 (C-6') ^{1}H- and ^{13}C NMR spectra were identical with those reported by Muhtadi and Hassan (1990).

Hyoscyamine **30** ^{1}H NMR: (300 MHz, $CDCl_3$), δ 7.23–7.33 (5 Ar. H, *m*), 5.05 (1H, *t*, *J* = 5 Hz, H-6), 4.17 (1H, *ddd*, *J* = 6, 2, 6 H-9), 3.79 (2H, *m*, CH_2OH), 3.25 (1H, *m*, H-1), 3.13 (1H, *m*, H-5), 2.34 (3H, *s*, N-CH_3), 1.2–2.42 (8H, *m*, H-2, H-4, H-6 and H-7). ^{13}C NMR (75 MHz, $CDCl_3$), δ 60.26 (C-1), 35.46 (C-2), 66.98 (C-3), 35.26 (C-4), 63.36 (C-5), 24.93 (C-6), 24.42 (C-7), 39.60 (N-CH_3), 172.03 (C-9), 54.32 (C-10), 64.02 (CH_2-OH), 135.31 (C-1'), 128.93 (C-2'), 128.09 (C-3'), 127.82 (C-4'), 128.09 (C-5'), 128.93 (C-6') ^{1}H- and ^{13}C NMR spectra were identical with those reported by Muhtadi (1994).

Results and Discussion

Alkaloid identification

Alkaloid contents and alkaloid profiles of *Hyoscymus boveanus*, *H. desertorum H. muticus*, and *H. albus* were analysed by GLC and GLC-MS (Tables I, II, III; Fig. 1). Most alkaloids were identified on basis of their retention indices (RI), mass spectra and comparison with reference samples, a few alkaloids were identified only tentatively, since they were only present in minute amounts which prevented their isolation and full structure elucidation.

Hyoscyamine and scopolamine were the main constituents in all examined *Hyoscyamus* species (Table III). The average ratio between hyoscyamine and scopolamine was 13 : 1. in *H. boveanus*, 0.8 : 1. in *H. desertorum*, 44.5 : 1. in *H. muticus*, and 1.8 : 1. in *H. albus*.

23 alkaloids were detected in *Hyoscyamus boveanus*. The major alkaloids were isolated and identified by ^{1}H and ^{13}C NMR analyses as hyoscyamine and scopolamine. Other tropane bases were detected in smaller quantities and identified as tropinone, 3α-tropine, 3β-tropine, scopoline, scopine, *nor*phenylacetoxytropane, 3α-phenylacetyltropane, *nor*apotropine, *apo*atropine, *apo*scopolamine, *nor*hyoscyamine, *nor*scopolamine, 4'-hydroxylittorine, 6S-hydroxyhyoscyamine, and 6*R*-hydroxyhyoscyamine. In addition, the biosynthetic precursor *N*, *N*, *N'*, *N'*-tetramethyl-1,4-butanediamine (*N*, *N*-tetramethylputrescine) and pyrrolidine alkaloids such as hygrine, cuscohygrine, *N*-methylpyrrolidinylcuscohygrine A and 2,3-diacetonyl-N-methylpyrrolidine were found in trace amounts.

17 alkaloids were detected in the intact plant and root cultures of *H. desertorum*. Of these, hyoscyamine, scopolamine, 3α-tropine and 6-hydroxyhyoscyamine had been described previously (Sabri *et al.*, 1973; Parr *et al.*, 1990). The remaining 13 alkaloids tropinone, 3β-tropine, scopoline, 2',4-*N*-methylpyrrolidinlylhygrine, 2',3- *N*-methylpyrrolidinlylhygrine, cuscohygrine, 3α-phenylacetyltropane, *apo*atropine, 6-hydroxy-3-phenylacetyltropane, phenylacetylscopine, *apo*scopolamine and littorine are new for *H. desertorum*. The alkaloid pattern of root cultures of *H. desertorum* was almost identical with that of roots of intact plants, confirming the finding that roots are a site of alka-

Table I. Alkaloids identified in *H. boveanus, H. desertorum, H. muticus* and *H. albus* by GLC and GLC-MS. + = alkaloid present, – = not detected

No.	Alkaloid	*H. boveanus* (aerial parts)	*H.desertorum* (roots)	*H. muticus* (roots)	*H. albus* (roots)
1	*N,N*-Tetramethylputrescine‡	+	–	+	+
2	*Nor*hygrine	–	–	–	F
3	Hygrine	+	–	+	+
4	Cyclotropine*	+	–	+	+
5	Tropinone†*	+	+	+	+
6	3α-Tropine	+	+	+	+
7	3β-Tropine†	+	+	+	+
8	Scopoline (Oscine) †*	+	+	+	+
9	Scopine*	+	–	+	F
10	3α-Acetoxytropane*	–	–	+	+
11	3β-Acetoxytropane*	–	–	+	–
12	2,5-Diacetonyl-N-methylpyrrolidine*	+	–	+	–
13	2',4-*N*-methylpyrrolidinylhygrine †*	–	+	+	+
14	2',3-*N*-methylpyrrolidinylhygrine †*	–	+	+	+
15	3α-Tigloyloxytropane*	–	–	+	+
16	Cuscohygrine†	+	+	+	+
17	*Nor*phenylacetyltropane*	+	–	F	–
18	3α-Phenylacetyltropane†*	+	+	+	+
19	*Nor*apoatropine	+	–	–	F
20	*Apo*atropine†‡	+	+	+	+
21	Phygrine*	–	–	+	–
22	Phenylacetylscopine†‡	–	+	–	F
23	Dehydrohyoscyamine‡	–	–	–	F
24	6-hydroxy-3-phenylacetyltropane†	–	+	–	–
25	*Apo*scopolamine†*	+	+	+	+
26	*N*-methylpyrrolidinyl- cuscohygrine A*	+	–	+	+
27	*Nor*hyoscyamine	+	–	F	F
28	Littorine†*	–	+	+	+
29	Hyoscyamine	+	+	+	+
30	*N*-methylpyrrolidinyl-cuscohygrine B*	–	–	+	+
31	6-Hydroxy*apo*atropine*	–	–	+	+
32	3β-Hyoscyamine*	–	–	+	–
33	*Nor*scopolamine	+	–	+	F
34	Scopolamine	+	+	+	+
35	4'-Hydroxylittorine†*	+	H	+	–
36	6S-Hydroxyhyoscyamine*	+	+	+	+
37	6R-Hydroxyhyoscyamine*	+	–	+	+
38	Phyllalbine (vanillyltropine)*	–	–	+	–
39	Unknown 1†*		–	+	–

All reported alkaloids are new for *H. boveanus*; † = new for *H. desertorum*; * = new for *H. muticus*; ‡ = new for *H. albus;* F = not in the root, but in the fruits; H = Detected only in root culture.

loid biosynthesis in *Hyoscyamus* (Liebig and Schütte, 1985). The profile differed only quantitatively: hyoscyamine accounted for 59.7%, scopolamine for 30.1%, and 6S-hydroxyhyoscyamine for 9.99%; traces of 4'-hydroxylittorine were only found in the root culture but not in roots of the intact plant (Table I). In contrast to a previous report (Parr *et al.*, 1990) we obtained no evidence for *nor*hygrine, hygrine and 3α-acetoxytropane.

Previous studies on *H. muticus* had revealed the presence of 11 alkaloids (Ahmed and Fahmy 1949; Balbaa *et al.*, 1964; Johne *et al.* 1975; Trease and Evans 1983; Parr *et al.*, 1990; Giri and Ahuja 1994). Our analysis, however, produced 34 alkaloids, 24 of which are reported in this species for the first time (Table I). Ten of the previously reported alkaloids were confirmed but not the presence of *nor*hygrine.

Table II. RI and Mass spectral data of the alkaloids of *H. boveanus, H. desertorum, H. muticus* and *H. albus.*

No.	Alkaloid	RI	M^+	characteristic ion (abundance%)	Ref.
1	*N,N*-Tetramethylputrescine	994	144(15)	129(8), 100(5), 98(9), 84(11), 71(28), 58(100), 45(18), 42(14).	1,2
2	*Nor*hygrine	1050	127(8)	112(5), 84(12), 70(100), 68(10), 56(15), 43(25).	
3	Hygrine	1060	141(6)	98(4), 84(100), 82(10), 70(7), 55(5), 43(10), 42(18).	3,4,5,6
4	Cyclotropine	1137	139(100)	124(5), 110(48), 96(49), 83(19), 82(22), 68(80), 67(31), 54(25), 43(39), 42(60).	7
5	Tropinone	1154	139(18)	110(15), 96(30), 83(23), 82(100), 81(45), 68(13), 55(15), 42(40).	3,4,8
6	3α-Tropine	1167	141(47)	124(43), 113(25), 96(86), 83(72), 82(100), 67(14), 57(20), 55(15), 42(38).	3,4,5
7	3β-Tropine	1185	141(32)	124(30), 113(16), 96(61), 83(52), 82(100), 67(10), 57(13), 55(12), 42(30).	3,4,5
8	Scopoline	1254	155(75)	138(10),126(30),110(15), 96(100), 81(45), 70(25), 57(30), 42(50).	3,4
9	Scopine	1285	155(25)	138(9), 112(16), 110(25), 94(18), 82(10), 70(11), 68(15), 57(44), 42(100).	3,4
10	3α-Acetoxytropane	1308	183(23)	140(10), 124(100), 96(18), 95(12), 94(35), 83(28), 82(47), 67(15), 43(22), 42(21).	3,4,5,9
11	3β-Acetoxytropane	1317	183(22)	140(2), 124(100), 96(20), 95(17), 94(39), 83(46), 82(54), 67(24), 56(15), 43(20).	4,5
12	2,5-Diacetonyl-N-methylpyrrolidine*	1442	197(2)	182(1), 140(100), 98(10), 96(15), 84(3), 82(90), 43(28), 42(11).	6
13	2',4-N-Methylpyrrolidinylhygrine	1567	224(7)	167(6), 152(10), 139(5), 124(5), 110(5), 84(100), 82(10), 42(16).	3,4,5,11
14	2',3-N-Methylpyrrolidinylhygrine	1578	224(5)	167(5), 152(10), 139(4), 124(4), 110(4), 84(100), 82(8), 42(15).	3,4,5,11
15	3α-Tigloyloxytropane	1645	223(9)	208(13), 140(19), 125(15), 124(100), 96(25), 95(16), 94(28), 83(35), 82(10), 55(17), 42(17).	3,4,5
16	Cuscohygrine	1650	224(0.1)	223(0.5), 209(4), 140(10), 98(8), 84(100), 83(10), 82(8), 42(20).	3,4,5,9
17	Norphenylacetyltropane	1928	245(4)	119(10), 110(100), 98(12), 97(20), 91(20), 84(25), 83(12), 67(10), 55(8).	
18	3α-Phenylacetyltropane	1936	259(20)	140(10), 124(100), 94(20), 91(19), 83(20), 82(25), 67(10), 55(5).	3,4,5,10
19	Norapoatropine	2015	257(10)	110(100), 103(15), 83(10), 82(10), 80(15), 68(20), 67(12), 55(5).	3
20	Apoatropine	2020	271(22)	140(10), 124(100), 103(15), 96(30), 95(21), 83(20), 82(26), 67(10).	3,4,5
21	Phygrine	2043	280(0.1)	265(4), 222(4), 196(7), 154(8), 140(35), 84(100), 82(31), 55(5), 42(14).	6
22	Phenylacetylscopine	2045	273(35)	207(10), 154(40), 138(77), 136(52), 108(57), 94(100), 91(74), 84(18), 83(20), 82(22), 81(28), 67(15), 57(15), 42(40).	
23	Dehydrohyoscyamine*	2112	287(30)	138(51), 122(80), 121(77), 103(15), 95(28), 94(100), 81(38).	
24	6-Hydroxy-3-phenylacetyltropane	2115	275(3)	231(2), 140(20), 122(12), 95(73), 94(100), 91(20), 80(14), 55(19).	4
25	Aposcopolamine	2130	285(54)	154(55), 138(80), 136(54), 120(15), 108(65), 103(75), 94(100), 77(27), 68(12), 57(11), 42(37).	4,5
26	N-Methylpyrrolidinyl-cuscohygrine A	2165	307(4)	292(2), 224(8), 182(8), 167(10), 152(7), 139(6), 126(6), 110(11), 98(8), 97(8), 85(8), 84(100), 83(8), 82(9), 42(8).	4
27	Norhyoscyamine	2167	275(2)	121(2), 111(8), 103(6), 91(5), 82(8), 81(8), 80(18), 68(15), 67(11).	4,10
28	N-Methylpyrrolidinyl-cuscohygrine B	2176	307(3)	292(1), 224(7), 182(7), 167(10), 152(6), 139(5), 126(5), 110(14), 98(9), 97(9), 84(100), 83(8), 42(10).	4
29–30	Hyoscyamine / Littorine	2170	289(20)	142(1), 140(8), 125(10), 124(100), 103(5), 96(8), 95(7), 94(16), 83(15), 82(18), 67(7).	3,4,5

Table II. (continued).

No.	Alkaloid	RI	M^+	characteristic ion (abundance%)	Ref.
31	6-Hydroxyapoatropine	2200	287(27)	243(15), 140(27), 103(20), 95(70), 94(100), 77(8), 42(12).	3,4,10
32	3β-Hyoscyamine	2210	289(30)	125(9), 124(100), 96(5), 95(5), 83(10), 82(22), 67(7), 57(7).	7
33	Norscopolamine	2280	289(5)	140(10), 138(13), 124(43), 123(50), 122(100), 106(14), 103(17), 94(45), 80(40).	3,4
34	Scopolamine	2290	303(25)	154(41), 138(90), 137(20), 136(45), 120(15), 108(53), 103(23), 94(100), 81(19), 57(10), 42(35).	3,4,5
35	4'-Hydroxylittorine	2315	305(12)	142(3), 140(3), 124(100), 107(4), 96(10), 94(11), 82(8).	4
36	6S-Hydroxyhyoscyamine	2342	305(16)	261(17), 140(40), 95(80), 94(100), 82(6), 57(4), 42(10).	3,4,10
37	6R-Hydroxyhyoscyamine	2360	305(14)	261(14), 140(38), 95(82), 94(100), 82(7), 57(3), 42(12).	3,4,10
38	Phyllalbine (Vanillyltropine)*	2360	291(20)	151(14), 125(11), 124(100), 123(14), 108(5), 83(28), 82(40), 67(12), 55(8), 42(33).	12,13
39	Unknown 1	2865	414(20)	399(5), 358(10), 331(5), 292(30), 290(30), 272(4), 247(10), 142(30), 140(15), 124(50), 94(10), 84(100), 67(3).	5

1 = Johne *et al.* (1975); 2 = Veith *et al.* 1971; 3 = Ionkova *et al.* (1994); 4 = Doerk-Schmitz *et al.* (1994); 5 = Witte *et al.* (1987); 6 = Basey *et al.* (1992); 7 = Bachmann P. (personal communication); 8 = Blossey *et al.* (1964); 9 = Kubwabo *et al.* (1992); 10 = Hartmann *et al.* (1986); 11 = Jenett-Siems *et al.* (1996); 12 = Gorinova *et al.* (1994); 13 = Parello *et al.* (1963).
* Tentatively identified by GLC-MS.

Table III. Alkaloid profiles (major compounds only) of various parts of *Hyoscyamus boveanus, H. desertorum. H. muticus,* and *H. albus* (total alkaloids = 100%).

Alkaloid	*H.boveanus*				*H. desertorum*					*H. muticus*					*H. albus*				
	Fl	Fr	L	S	Fl	Fr	L	S	R	Fl	Fr	L	S	R	Fl	Fr	L	S	R
3α-Phenylacetyltropane	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	5.05	tr	tr	tr	1.35	5.84	1.94	1.57	tr
Apoatropine	2.16	3.11	2.22	3.73	tr	tr	tr	tr	tr	4.78	11.65	2.55	3.69	3.85	4.57	5.95	4.91	3.08	2.72
Norhyoscyamine	2.28	tr	3.40	tr	–	–	–	–	–	tr	tr	tr	tr	–	1.72	tr	1.33	0.66	–
Hyoscyamine	87.71	90.53	87.21	90.82	43.06	34.87	33.23	50.55	58.14	93.16	80.52	96.43	93.98	93.35	56.75	49.44	57.88	70.05	74.6
Scopolamine	9.58	6.36	6.29	4.90	56.94	60.88	66.77	49.45	38.10	2.06	2.78	1.02	2.33	2.80	35.61	38.77	33.94	24.50	22.6
6S-hydroxy hyoscyamine	0.27	tr	0.88	0.55	tr	4.25	tr	tr	3.76	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
Alkaloid content*	0.93	1.58	0.30	0.59	0.41	0.40	0.22	0.46	0.32	1.25	1.02	1.90	1.59	0.72	0.56	0.75	0.28	0.21	0.33

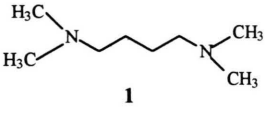
Fl = flowers, Fr = fruits, L = leaves, S = stems, R = roots; tr= trace amounts; – =not detected.
* mg/100 mg w/w dried plant material except the roots of *H.muticus* and *H.albus* by weight of fresh material.

About 30 alkaloids were detected in the roots and aerial parts of the Egyptian *H. albus*. *N, N, N', N'*-tetramethyl-1,4-butanediamine, *apo*atropine and phenylacetylscopine were reported for the first time in this plant. The occurrence of dehydrohyoscyamine which had been reported (Trease and Evans, 1983) as intermediate in the biosynthesis of scopolamine via 6-hydroxyhyoscyamine was also confirmed. 3β-acetoxytropane, 3α- and 3β-propionyloxytropane, 3-*iso*butyryloxytropane, 3β-tigloyloxy*nor*tropane and tigloidine previously reported in *H. albus* cultivated in Germany (Doerk-Schmitz *et al.*, 1994) were not detected in our plant sample.

Hygrine and *nor*hygrine had been found in transformed roots of *Hyoscyamus desertorum, H. muticus, H. albus* and *H. pusillus* (Parr *et al.* 1990). In the present study *nor*hygrine was detected in the aerial parts of *H. albus* and hygrine in those of *H. boveanus* as well as in the roots of *H. muticus* and *H. albus*.

Some minor alkaloids could be only tentatively identified by GLC-MS; since material was limited

the isolation of these minor components was not possible. Alkaloid **12** has a M^+ at *m/z* 197 and 56 mass units more than that of hygrine. Comparison of mass spectra with data previously reported (Basey *et al.*, 1992) implied that alkaloid **12** is 2,5-diacetonyl-N-methylpyrrolidine. This alkaloid was detected in the aerial parts of *H. boveanus* and in the root of *H. muticus*. Alkaloid **23** was found in the aerial parts of *H. albus*. It displayed M^+ at 287, being 2 units lower than that of hyoscyamine (M^+ 289); in addition an intense peak at *m/z* 122 (80%) indicates the presence of a double bond. Compound **23** was tentatively identified as dehydrohyoscyamine but the position of double bond needs further elucidation. It should be recalled that 6,7-dehydrohyoscyamine has been described as an intermediate in the biosynthesis of scopolamine via 6-hydroxyhyoscyamine (Trease and Evans 1983). Phygrine (**21**) (RI 2043) was detected in root extracts of *H. muticus*. The identification of this compound is based on comparison of its mass spectra with those reported in the literature (Basey *et* al., 1992). Traces of littorine (**30**) (a structural isomer of hyoscyamine) co-eluted with hyoscyamine (**29**). The identification of this alkaloid was suggested by an informative fragment ion



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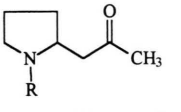
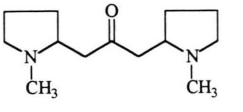


R = H **2**
R = CH_3 **3**



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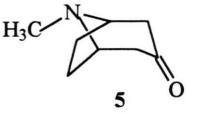

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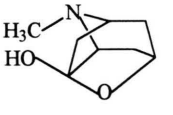

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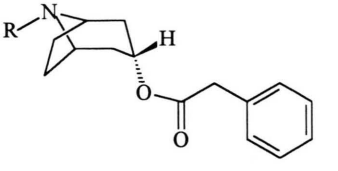
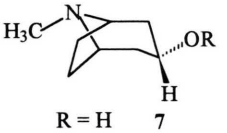
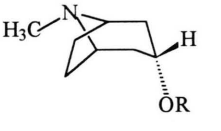


R = H **17**
R = CH_3 **18**



R = H **7**
R = Ac **11**



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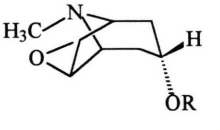
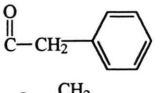
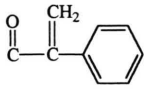


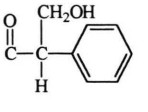
R = H **9**



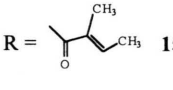
R = **22**



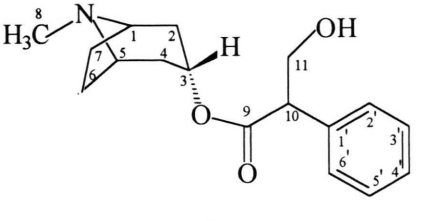
R = **25**



R = **34**



R = **15**



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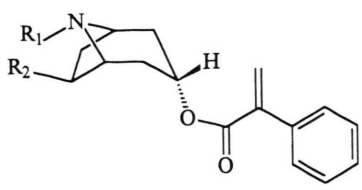
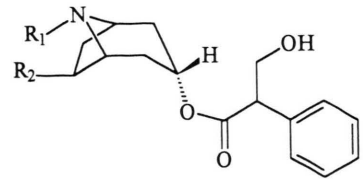
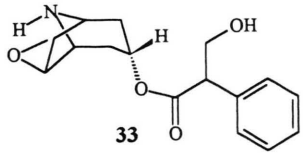


$R_1 = H$ $R_2 = H$ **19**
$R_1 = CH_3$ $R_2 = H$ **20**
$R_1 = CH_3$ $R_2 = OH$ **31**



$R_1 = H$ $R_2 = H$ **27**
$R_1 = CH_3$ $R_2 = (s)\ OH$ **36**
$R_1 = CH_3$ $R_2 = (R)\ OH$ **37**



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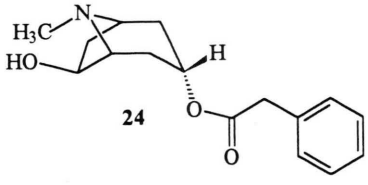

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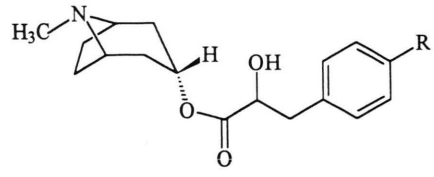
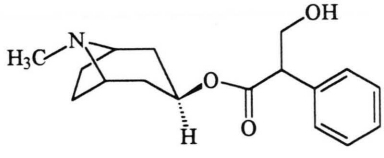


R = H **30**
R = OH **35**



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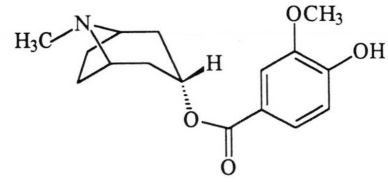

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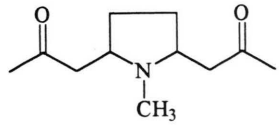

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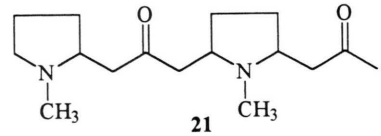

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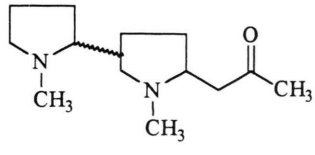

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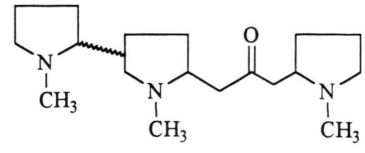

26, 28

Fig. 1. Structures of tropane alkaloids found in *Hyoscyamus* species.

at *m/z* 142 as compared to *m/z* 140 in hyoscyamine. Thus, a small amount of littorine may be masked under the main alkaloid peak representing hyoscyamine. The incomplete separation of both compounds under our GLC condition is in agreement with a previous reports (Witte *et al.*, 1987; Bachmann *et al.*, 1991; Ionkova *et al.*, 1994). The molecular ion of **35** *m/z* 305 is 16 mass units higher than that of littorine indicating the presence of an additional hydroxyl group. Comparing RI and the mass spectrum with those reported in the literature we assume that **35** is 4'-hydroxylittorine, an alkaloid that has previously been reported from *H. albus* (Doerk-Schmitz *et al.*, 1994). 3β-Hyoscyamine (**32**) (RI 2210) was identified in the alkaloid extracts of *H. muticus* based on the similarity of its mass fragmentation pattern to that of hyoscyamine (RI 2170), and its delayed retention time in GLC (Bachmann *et al.*, 1991). The mass spectrum of alkaloid **38** showed a molecular ion at *m/z* 291, a base peak at *m/z* 124 and an ion at *m/z* 151 which is characteristic for the acid moiety of phyllalbine (Parello *et al.*, 1963; Gorinova *et al.*, 1994). The GLC-MS of the alkaloids of aerial parts of *H. boveanus* and *H. muticus* revealed an alkaloid (RI 1928) just in front of 3α-phenylacetyltropane. It displayed a molecular ion at *m/z* 245 and a fragmentation pattern (base peak *m/z* 110) characteristic for *nor*phenylacetyltropane (**17**). Alkaloid **22** (RI 2045) showed a mass fragmentation pattern typical for scopine esters. RI value, a molecular ion at *m/z* 273 and the fragment ion at *m/z* 138 indicated that it is phenylacetylscopine. Traces of an unknown alkaloid (RI 2865) were detected in *H. muticus*. The mass spectrum of alkaloid **39** has previously been reported as an unknown compound in *Datura innoxia* (Witte *et al.*, 1987). The base peak at *m/z* 84 suggests an N-methylpyrrolidinyl moiety and the ion at *m/z* 124 a tropane nucleus.

Although most of these alkaloids are known constituents in some genera of the Solanaceae, the alkaloids phyllalbine, 3β-hyoscyamine, dehydrohyoscyamine, *nor*phenylacetyltropane, phenylacetylscopine, phygrine, and 2,5-diacetonyl-N-methylpyrrolidine are reported for the first time in the genus *Hyoscyamus*.

*Nor*phenylacetyltropane, *nor*apoatropine and *nor*hyoscyamine were identified in the aerial part of the species studied, but not in their roots. However, *nor*scopolamine was detected both in the aerial parts of *H. albus*, *H. boveanus* and in the roots of *H. muticus*. We suggest that the demethylated alkaloids derive from methylated precursors which are transported from the roots into the aerial parts.

As shown in Table I, the alkaloid composition of the roots of the plants was found to be more complex than that of the aerial parts reflecting the fact that tropane alkaloids are synthesized in the roots and translocated to the shoots (Liebisch and Schütte 1985). However, since the roots of *H. boveanus* were not available during this work and only its aerial parts were studied, the overall number of identified alkaloids was comparably smaller than those of the other species.

Alkaloid contents and profiles

Alkaloid contents and alkaloid profiles in different organs of *Hyoscyamus* plants are shown in Table III. We have recently analyzed aerial parts of *H. albus* from Leucate (southern France) which contained 4-hydroxylittorine as an additional alkaloid. The alkaloid profile differed quantitatively to some degree from the Aegyptian plants (compare with Table III): Stems contained hyoscyamine (40.9% of total alkaloids), scopolamine (42.2%), 6S-hydroxyhyoscyamine (6.9%), α-tropine (6.2%), and β-tropine (3.9%). Fruit capsules (alkaloid content 0.39 mg/g d.w.) contained hyoscyamine (56.8%), scopolamine (24.2%), 6S-hydroxyhyoscyamine (5.7%), α-tropine (4.9%), β-tropine (5.5%) and apoatropine (2.9%). Seeds (alkaloid contents 0.43 mg/g d.w.) had hyoscyamine (41.2%), scopolamine (46.2%), 3α-phenylacetyltropane (3.1%), tropinone (6.8%), and aposcopolamine (2.3%).

Because of the scarcity of foodplants in deserts, especially desert plants had to develop strategies during evolution to cope with insect and vertebrate herbivores. On Sinai, we observed that *Hyoscyamus boveanus* plants were not eaten by camels, goats or locusts which were abundant in the area and had access to the plants, suggesting that *Hyoscyamus* must possess potent chemical defences.

We assume that tropane alkaloids serve as chemical defence compounds against insects and vertebrate herbivores, since they exhibit substan-

tial toxicity and deterrence towards animals (Detzel and Wink, 1993; Wink, 1993; Wink *et al.*, 1998). It is likely that the inhibition of muscarinic acetylcholine receptors (mAChR) is the main cause for animal toxicity. It should be recalled that tropane alkaloids bind muscarinic AChR with high affinity (IC_{50} values are between 0.002 and 0.005 μM); they also bind to adrenergic and serotonin receptors, albeit less actively (IC_{50} for atropine: $alpha_1$ R= 6.1 μM, $alpha_2$ R= 10.1 μM; serotonin R = 6.0 μM; IC_{50} for scopolamine: $alpha_1$ R= 113 μM, $alpha_2$ R= 359 μM; serotonin R = 168.0 μM; Schmeller *et al.*, 1995). Is it possible to infer from these data a relevance of tropane alkaloids for chemical defence in *Hyoscyamus*?

Alkaloid contents of the four *Hyoscyamus* species range between 0.22 to 1.9% dry weight (Table III) with comparably high values in flowers and fruits which are important for reproduction and survival. The two major alkaloids are hyoscyamine and scopolamine, which showed the strongest activities in our previous neuroreceptor binding studies (Schmeller *et al.*, 1995). Apoatropine, norhyoscyamine, 6S-hydroxyhyoscyamine and 3-phenylacetyltropane are important minor alkaloids; also these alkaloids affect mACh receptors albeit with 10 times (apoatropine, 6S-hydroxyhyoscyamine) or 100 times (norhyoscyamine) weaker IC_{50} values (Schmeller *et al.*, 1995). Since hyoscyamine and scopolamine are the most active tropane alkaloids known from nature, it is plausible that both compounds figure as the main constituents in most solanaceous plants which produce tropane alkaloids.

Assuming an alkaloid concentration of 1 g/100 g dry weight (Table III), which is equivalent to approximately 100 mg/100 g fresh weight and a small herbivore with a body weight of 1000 g; if this animal would ingest 100 g of an alkaloid-producing plant it would ingest 100 mg of alkaloids. Suggesting that the alkaloids are completely resorbed and equally distributed in the body, we would obtain a concentration of 100 mg alkaloids/kg body weight. Taking a molecular weight of 289 for hyoscyamine, the alkaloid concentration in our herbivore would be 346 μM, which would be high enough to partially or completely block the binding of acetylcholine, serotonin or noradrenalin at their receptors. (IC_{50} values for hyoscyamine and scopalamine at mAChR are between 0.002 and 0.005 μM!). Considering alkaloid contents of the *Hyoscyamus* species studied (Table III), they appear sufficiently high to serve for chemical protection against insects and vertebrate herbivores. This would be in agreement with our observations in the field.

Acknowledgements

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