Gas-Liquid Chromatography-Mass Spectrometry Investigation of Tropane Alkaloids in *Hyoscyamus albus* L. from Morocco

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Z. Naturforsch. **67c**, 461–465 (2012); received February 15/May 31, 2012

Thirty-four alkaloids were identified in the organs of *Hyoscyamus albus* L. by gas-liquid chromatography-mass spectrometry (GLC-MS). Eight new compounds for the roots, eleven for the stems, twelve for the leaves, nineteen for the flowers, and seven for the seeds were detected. The alkaloids 5-(2-oxopropyl)-hygrine (**8**) and phygrine (**20**) are new for this species and 3-(hydroxyacetoxy)tropane (**9**), 6,7-dehydro-3-phenylacetoxytropane (**15**), 3-(2'-phenylpropionyloxy)tropane (**17**), 6,7-dehydro-3-apotropoyloxytropane (**18**), 3-(3'-methoxytropoyloxy)tropane (**23**), and aponorscopolamine (**25**) are described for the first time for the genus *Hyoscyamus*. Hyoscyamine was the main alkaloid in the plant organs.

Key words: Tropane Alkaloids, *Hyoscyamus albus* L., Solanaceae, GLC-MS

Introduction

Hyoscyamus is one of the most important and largest genera of the family Solanaceae comprising about 84 genera and 3000 species (Yasin, 1985). One of them, *H. albus* L., is an herbaceous, yellowish-pale flowering annual which is widespread in the Mediterranean region (Feinbrun-Dothan, 1978; El-Shazly *et al.*, 1997).

This plant, also known in Morocco as "Sikran" (Bellakhdar, 1997), is one of the species very rich in tropane alkaloids (Zehra *et al.*, 1998; Kartal *et al.*, 2003; Bahmanzadegan *et al.*, 2009). The plant extracts are used in traditional medicine as an antiasthmatic and antispasmodic. On the other hand, "Sikran" is used by children as a hallucinogenic and sedative drug consumed alone or mixed with *Cannabis* and *Datura* (Bellakhdar, 1997). Previous chemical work on intact plants of *H. albus* has shown that this plant accumulates a number of tropane-derived alkaloids, mainly hyoscyamine and scopolamine (Parr *et al.*, 1990; Doerk-Schmitz *et al.*, 1994; El-Shazly *et al.*, 1997). In Morocco, the plant does not appear to have been investigated for its alkaloids.

As a part of our continuing investigation of Moroccan alkaloid-containing plants with pharmacological, toxicological, and chemotaxonomic properties (El Bazaoui *et al.*, 2009, 2011, 2012), we now report our findings on the alkaloid patterns of roots, stems, leaves, flowers, and seeds of *H. albus* L. plants.

Material and Methods

Plant material

Hyoscyamus albus L. was collected from its natural habitats in the District of Harhoura Temara Province of Morocco in April 2009. Voucher specimens were deposited at the herbarium of the Laboratory of Genetics and Biometry (LGB), Department of Biology, University Ibn Tofail, Kenitra, Morocco. Roots, stems, leaves, flowers, and seeds of the plant were air-dried in the shade for several days at room temperature and powdered.

Alkaloid extraction and gas-liquid chromatography-mass spectrometry (GLC-MS)

Alkaloid extraction was performed essentially as described by El Bazaoui *et al.* (2009). The GLC-MS analysis was carried out on an Agilent 6890/MSD5975B instrument (Agilent Tech-

nologies, Palo Alto, CA, USA) operating in the electron impact (EI) ionization mode at 70 eV, with MS transfer line temperature of 280 °C, ion source temperature of 230 °C, quadrupole temperature of 150 °C, and mass range of 30–500 amu. An HP-5MS column (Hewlett Packard, Palo Alto, CA, USA) (30 m × 0.25 mm × 0.25 m) was used. The flow rate of the carrier gas (He) was 1 ml/min. The temperature program was 60 to 300 °C, ramped at 4 °C/min and held at the final temperature for 10 min. Injector temperature was 270 °C. The injection was performed in the splitless mode, and the injected volume was 1 μl. The identities of the alkaloids were confirmed by comparing the measured mass spectral data with those obtained from the literature (see Table I for MS references).

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; Parr *et al.*, 1990; Doerk-Schmitz *et al.*, 1994; El-Shazly *et al.*, 1997; Berkov *et al.*, 2006; El-Shazly and Wink, 2008; El Bazaoui *et al.*, 2011, 2012) these methods were employed in the identification of major and minor alkaloids in the plant organs of Moroccan *H. albus* L.

The alkaloid patterns detected in roots, stems, leaves, flowers, and seeds of the intact plant are shown in Table I. Some of the alkaloids listed are, to our knowledge, hitherto unknown for the genus *Hyoscyamus.* Newly detected alkaloids are 3-(hydroxyacetoxy)tropane (**9**), 6,7-dehydro-3-phenylacetoxytropane (**15**), 3-(2'-phenylpropionyloxy)tropane (dihydroapoatropine) (**17**), 6,7-dehydro-3-apotropoyloxytropane (**18**), 3-(3'-methoxytropoyloxy)tropane (**23**), and aponorscopolamine (**25**) (Fig. 1) which were previously described for the genus *Datura* (El Bazaoui *et al.*, 2011, 2012). The alkaloids 5-(2-oxopropyl)-hygrine (**8**) and phygrine (**20**) are new for this species (Fig. 2). Previously they had been reported for *H. muticus* species (El-Shazly *et al.*, 1997). Furthermore, eight new compounds for the *H. albus* roots, eleven for the stems, twelve for the leaves, nineteen for the flowers, and seven for the seeds were determined by GLC-MS.

Compounds **12** and **14** appeared as double peaks in the GLC-mass spectra with identical mass spectra. They are isomeric tropine and pseudotropine esters (Witte *et al.*, 1987; El Bazaoui *et al.*, 2011, 2012).

Previous studies on *H. albus* intact plants had revealed the presence of thirty-eight alkaloids (El-Shazly *et al.*, 1997; Parr *et al.*, 1990; Doerk-Schmitz *et al.*, 1994). Of these, twenty-six were confirmed in our analysis but not the presence of *N,N*-tetramethylputrescine, norhygrine, 3α-acetoxytropane, 3β-acetoxytropane, 3α-propionyloxytropane, 3β-propionyloxytropane, 3-isobutyryloxytropane, 3β-tigloyloxynortropane, norapoatropine, norhyoscyamine, norscopolamine, and littorine.

Occurrence of cyclotropine (**2**) and apoderivates like apohyoscyamine (**19**), aposcopolamine (**26**) and 6-hydroxyapohyoscyamine (**30**) may be artifacts from the isolation or detection procedures by GLC-MS as discussed elsewhere (Witte *et al.*, 1987; Christen *et al.*, 1990; Parr *et al.*, 1990; Robins *et al.*, 1990; Ionkova *et al.*, 1994; Brachet *et al.*, 1997; Berkov, 2003; Jenett-Siems *et al.*, 2005; El Bazaoui *et al.*, 2012). The existence of nor-derivatives, like aponorscopolamine (**25**), detected in the aerial parts (seeds) of the species, is probably due to demethylation of the corresponding tropane (Robins and Walton, 1993; Doerk-Schmitz *et al.*, 1994; Berkov *et al.*, 2005).

The alkaloid mixtures of the plant organs were dominated by hyoscyamine (**29**) (Table I). The highest relative percentage of **29** in the alkaloid mixtures was found in the seeds – 80.4% of the total alkaloids. Respectively, the lowest percentage was found in the roots – 63.8% of the total alkaloids. Alkaloid profiles of the roots were more complex, because roots are the site of tropane biosynthesis. This coincides with results obtained for *Datura stramonium* L. and *D. innoxia* Mill. (El Bazaoui *et al.*, 2011, 2012). Some of these alkaloids are known precursors in the biosynthesis of tropane alkaloids, *e. g.* tropinone (**3**), tropine (**4**), and pseudotropine (**5**), whereas *N*-methylpyrrolidinylhygrine A (**10**), *N*-methylpyrrolidinyl-hygrine B (**11**), cuscohygrine (**13**), *N*-methylpyrrolidinylcuscohygrine A (**27**), and *N*-methylpyrrolidinylcuscohygrine B (**28**) are products of side reactions of the biosynthetic pathway (Lounasmaa, 1988; Christen *et al.*, 1993, 1995; Brachet *et al.*, 1997; Berkov, 2003; El Bazaoui *et al.*, 2012). The 12 minor alkaloids represented more than 1% of total alkaloids.

Like the roots, the stems, leaves, flowers, and seeds also exhibited a high range of alkaloids – 23

Table I. Alkaloids identified in *Hyoscyamus albus* L. plant parts presented as percentage of total ion current[a].

Alkaloid	RT[b] [min]	M^+/base peak (*m/z*)	Content (%)					MS reference[c]
			Roots	Stems	Leaves	Flowers	Seeds	
Hygrine (**1**)	09.04	141/84	1.7	0.1	<0.1	<0.1[e]	-	1, 2 , 3, 4
Cyclotropine (**2**)[d]	11.39	139/68	<0.1	<0.1[e]	<0.1[e]	<0.1[e]	<0.1[e]	2, 5, 6
Tropinone (**3**)	12.28	139/82	0.1	<0.1	<0.1	<0.1[e]	<0.1	2, 3, 7
Tropine (**4**)	12.54	141/82	2.0	0.6	0.4	1.4[e]	0.6	1, 2, 8
Pseudotropine (**5**)	13.14	141/82	<0.1	0.2	0.4	1.2[e]	0.1	1, 2, 8
Scopoline (**6**)	15.56	155/96	<0.1	<0.1[e]	<0.1[e]	<0.1[e]	<0.1	2 ,4 , 8
Scopine (**7**)	16.76	155/42	<0.1	-	<0.1[e]	<0.1[e]	<0.1	2, 4, 8
5-(2-Oxopropyl)-hygrine (2,5-diacetonyl-*N*-methylpyrrolidine) (**8**)[f]	21.55	197/82	0.2[e]	<0.1[e]	<0.1[e]	<0.1[e]	-	3, 9, 10
3-(Hydroxyacetoxy)tropane (**9**)[g]	22.50	199/124	0.3[e]	0.1[e]	-	-	-	11
N-Methylpyrrolidinyl-hygrine A (**10**)	24.8	224/84	0.3	<0.1[e]	<0.1[e]	<0.1[e]	-	1, 2, 3
N-Methylpyrrolidinyl-hygrine B (**11**)	25.09	224/84	0.5	<0.1[e]	<0.1[e]	<0.1[e]	-	1, 2, 3
3*α*-Tigloyloxytropane (**12**)	27.01	223/124	<0.1	-	-	-	-	1, 2, 8
Cuscohygrine (**13**)	27.13	224/84	4.4	0.5	0.6	0.2[e]	-	1, 2, 3, 8
3*β*-Tigloyloxytropane (**14**)	27.33	223/124	<0.1	-	-	-	-	1, 12
6,7-Dehydro-3-phenylacetoxytropane (**15**)[g]	33.60	257/94	<0.1[e]	-	<0.1[e]	<0.1[e]	<0.1[e]	13
3-Phenylacetoxytropane (**16**)	34.89	259/124	2.9	1.3	1.0	3.7	1.6	2, 14
3-(2'-Phenylpropionyloxy)tropane (dihydroapoatropine) (**17**)[g]	35.32	273/124	<0.1[e]	-	-	-	<0.1[e]	15
6,7-Dehydro-3-apotropoyloxytropane (**18**)[g]	35.87	269/94	<0.1[e]	<0.1[e]	<0.1[e]	<0.1[e]	<0.1[e]	6
Apohyoscyamine (**19**)[d]	36.99	271/124	8.1	4.2	4.0	6.1	8.4	2, 8, 14
Phygrine (**20**)[f]	37.33	280/84	<0.1[e]	-	-	-	-	2, 3, 9
3-Phenylacetoxy-6,7-epoxytropane (**21**)	37.63	273/94	0.5[e]	0.2[e]	0.5[e]	0.8[e]	0.5	2, 16
6,7-Dehydrohyoscyamine (**22**)	39.10	287/94	<0.1[e]	0.1[e]	<0.1[e]	<0.1[e]	<0.1	2, 7, 6
3-(3'-Methoxytropoyloxy)tropane (**23**)[g]	39.24	303/124	-	-	-	-	0.2[e]	6
3-Phenylacetoxy-6-hydroxytropane (**24**)	39.34	275/94	0.7	0.4[e]	0.2[e]	<0.1[e]	-	2, 11, 12
Aponorscopolamine (**25**)[g]	39.35	271/122	-	-	-	-	<0.1[e]	17
Aposcopolamine (**26**)[d]	39.49	285/94	1.1	0.7[e]	1.5	2.0[e]	0.9[e]	1, 2, 8, 12
N-Methylpyrrolidinyl-cuscohygrine A (**27**)	39.62	307/84	1.2	-	-	-	-	2, 3, 12
N-Methylpyrrolidinyl-cuscohygrine B (**28**)	39.82	307/84	1.6	-	-	-	-	2, 3, 12
Hyoscyamine (atropine) (**29**)	40.59	289/124	63.8	77.8	70.2	66.3	80.4	2, 8, 14
6-Hydroxyapohyoscyamine (**30**)[d]	41.25	287/94	1.0	0.9	0.9[e]	0.3[e]	0.1	1, 2
Scopolamine (**31**)	43.21	303/94	4.2	9.1	16.6	16.5	6.4	1, 2
4'-Hydroxylittorine (**32**)	43.69	305/124	0.6	-	-	-	-	2, 12
7-Hydroxyhyoscyamine (**33**)	44.50	305/94	1.3	0.6	2.2	0.3[e]	0.1	4, 8, 2, 12
6-Hydroxyhyoscyamine (**34**)	44.79	305/94	2.0	2.6	0.8	0.5	0.2	4, 8, 2, 12

[a] The area of GC/MS peaks depends not only on the concentration of the corresponding compounds but also on the intensity of their mass spectral fragmentation, so the data given in the table are not a true quantification but can be used for comparison between the samples, which is the objective of this work.
[b] Retention time.
[c] References: 1, Witte *et al.* (1987); 2, El-Shazly *et al.* (1997); 3, El-Shazly and Wink (2008); 4, Ionkova *et al.* (1994); 5, Jenett-Siems *et al.* (2005); 6, El Bazaoui *et al.* (2011); 7, Blossey *et al.* (1964); 8, Zayed and Wink (2004); 9, Basey *et al.* (1992); 10, Brachet *et al.* (1997); 11, Robins *et al.* (1990); 12, Doerk-Schmitz *et al.* (1994); 13, El Bazaoui *et al.* (2012); 14, El Bazaoui *et al.* (2009); 15, Doncheva *et al.* (2004); 16, Vitale and Acher (1995); 17, Evans and Ramsey (1981).
[d] Alkaloids might be artifacts from the isolation and GLC-MS analysis.
[e] New alkaloid for this organ.
[f] New for *H. albus* species.
[g] Alkaloid described for the first time for the genus *Hyoscyamus*.

for the stems, 24 for the leaves, 24 for the flowers, and 21 for the seeds. Hyoscyamine (**29**) as well as other compounds listed in Table I contributed about 1% or more of the total alkaloids – 4 for the stems, 5 for the leaves, 6 for the flowers, and 3 for the seeds.

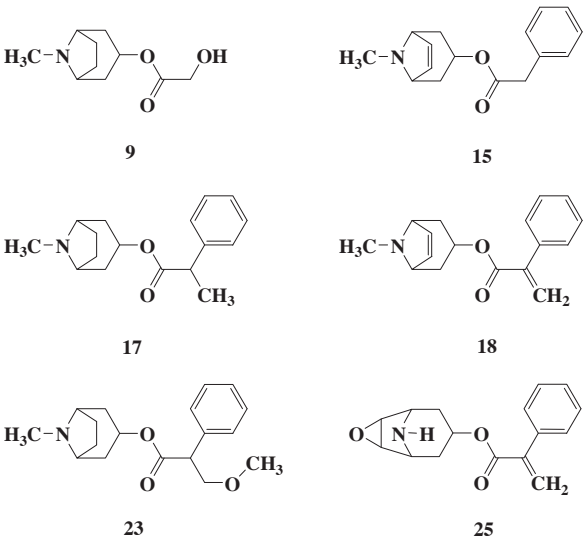

Fig. 1. Chemical structures of the alkaloids described for the first time for the genus *Hyoscyamus:* 3-(hydroxyacetoxy)tropane (**9**), 6,7-dehydro-3-phenylacetoxytropane (**15**), 3-(2'-phenylpropionyloxy)tropane (**17**), 6,7-dehydro-3-apotropoyloxytropane (**18**), 3-(3'-methoxytropoyloxy)tropane (**23**), aponorscopolamine (**25**).

It is interesting to note the presence of C-3'-substituted tropoyloxytropanes in the seeds – 0.2% for 3-(3'-methoxytropoyloxy)tropane (**23**). These alkaloids have recently been identified as constituents in the plants and their biogenesis is unknown (El Bazaoui *et al*., 2011, 2012).

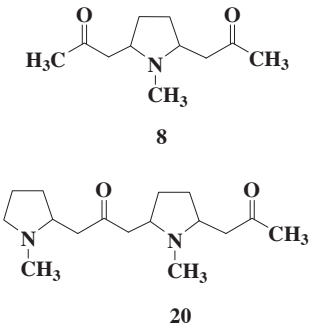

Fig. 2. Chemical structures of the new compounds isolated from *Hyoscyamus albus* L. species: 5-(2-oxopropyl)-hygrine (**8**), phygrine (**20**).

Acknowledgements

The authors are thankful to Dr. A. Moutmir for his assistance. Furthermore, they gratefully acknowledge Prof. A. Taleb (Agronomic and Veterinary Institute, Hassan II, Rabat, Morocco) for plant identification.

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