Tropane Alkaloids of *Datura innoxia* from Morocco

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Fifty three alkaloids were identified in the organs (roots, stems, leaves, flowers, and seeds) of *Datura innoxia* by GC/MS. Seventeen of them are reported for the first time for this species and one *nor*-derivative, 3-phenylacetoxynortropane (**28**), for the genus *Datura*. Furthermore, four new tropane esters were tentatively identified as 3-acetoxy-6,7-epoxytropane (acetylscopine) (**10**), 3-acetoxy-6-propionyloxy-7-hydroxytropane (**15**), 6,7-dehydro-3-phenylacetoxytropane (**25**), and 3-(2'-phenylpropionyloxy)-6,7-epoxynortropane (dihydroaponorscopolamine) (**37**) on the basis of their mass spectral data. Hyoscyamine (**44**) and scopolamine (**48**) figure as main alkaloids in the roots and aerial parts, respectively.

Key words: GC/MS, *Datura innoxia*, Solanaceae, Tropane Alkaloids

Introduction

The genus *Datura* belongs to the family Solanaceae and comprises about 20 species which grow worldwide (Evans *et al.*, 1972). One of them, *Datura innoxia* Mill., native to America, is now widely distributed in the warm regions of the world and is a noxious weed of cultivated cereal crops (Barguil *et al.*, 2006). This herbaceous annual, usually known in Morocco as "Chdek ej-jmel" (Bellakhdar, 1997), is an erect plant, pubescent, with leaf margins entire, white flowers, and spiny capsules which are filled with numerous yellowish-brown, kidney-shaped seeds (Barguil et *al.*, 2006).

This plant is very toxic, causing hallucinations and delirium, which can even lead to psychosis (Barguil *et al.*, 2006). It was used by the Moroccan people as medicinal plant for the treatment of asthma (Bellakhdar, 1997). The alkaloids of *Datura innoxia* have been extensively investigated (Witte *et al.*, 1987; Lounasmaa and Tamminen, 1993; Ionkova *et al.*, 1994; Berkov and Zayed, 2004; Doncheva *et al.*, 2006), but no data are available on the alkaloid composition of stems and flowers of this plant. Knowledge of the complete alkaloid pattern is of interest not only phytochemically, but also in relation to aspects of alkaloid biogenesis and metabolism. Further investigation of *D. innoxia* was undertaken in our laboratory by capillary gas chromatography (GC) and GC coupled with mass spectrometry (GC/MS) which has been used successfully for the identification of tropane alkaloids (Christen *et al.*, 1993). This is the first report on the determination of the alkaloid composition of *D. innoxia* growing in the subtropical climate of Morocco.

Material and Methods

Plant material

Datura innoxia was collected at the stage of opening of the first capsule in the north-west of Morocco, in the locality of Temara, situated near the sea, in April 2009. Voucher specimens were deposited at the herbarium of the Laboratory of Genetics and Biometry (LGB), Department of Biology, University Ibn Tofaïl, Kénitra, 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 chromatography/ mass spectrometry (GC/MS)

Alkaloid extraction was performed essentially as described by El Bazaoui *et al.* (2009). The GC/MS analysis was carried out on an Agilent 6890/MSD5975B (Agilent Technologies, Palo Alto, CA, USA) instrument operating in the electron impact

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(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 μm) 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 (Table I). In some cases, when no identical spectra were found, the structural type of the corresponding component was suggested only on the basis of its mass spectral fragmentation and retention data.

Results and Discussion

GC/MS is a useful and reliable method for rapid separation and identification of complex mixtures of tropane alkaloids (Witte *et al*., 1987; Ionkova *et al*., 1994; El Bazaoui *et al.*, 2011). Fifty-three alkaloids from the extracts of *D. innoxia* (Moroccan origin) were identified by GC/MS (Table I). The characteristic alkaloids of *D. innoxia* are tropanol esters of a range of acids. Most of the alkaloids listed are, to our knowledge, hitherto unknown for *D. innoxia*. Newly detected alkaloids are 6,7-dehydrotropine (**2**), cyclotropine (**3**), methylecgonine (**11**), 3,6-diacetoxytropane (**14**), 3-tigloyloxy-6,7-epoxytropane (**21**), 3β-hydroxy-6β-tigloyloxytropane (**24**), 3-phenylacetoxynortropane (**28**), 3-(2'-phenylpropionyloxy)tropane (**29**), 3-(2'-phenylpropionyloxy) nortropane (**30**), 6,7-dehydro-3-apotropoyloxytropane (**31**), 3-phenylacetoxy-6,7-epoxytropane (**36**), 3-(2'-phenylpropionyloxy)-6,7-epoxytropane (dihydroaposcopolamine) (**38**), 6,7-dehydro-3-tropoyloxytropane (**39**), 3-(3'-methoxytropoyloxy)tropane (**40**), norhyoscyamine (**45**), 4'-hydroxylittorine (**49**), and methylscopolamine (**47**), which was previously characterized in genetically transformed root cultures of this species (Ionkova *et al*., 1994). These alkaloids have been previously reported for *D. stramonium* species (El Bazaoui *et al*., 2011) except norhyoscyamine (**45**) which has been identified in *D. ceratocaula* (Berkov, 2003).

Alkaloids **16** and **19**, **23** and **24** appeared as double peaks in GC/MS with identical mass spectra. They are isomeric tropine and pseudotropine esters (Witte *et al*., 1987). The stereochemistry of these alkaloids could not be established solely by MS data. It was suggested on the basis of their retention data reported in the literature. Generally in Solanaceae, 3α-isomers of the homologous tropine esters occur in considerably higher amount than the 3β-isomers (Witte *et al.*, 1987; Robins and Walton, 1993).

One *nor*-derivative, **28**, was identified as 3-phenylacetoxynortropane with a $[M]^+$ at *m/z* 245 and a base peak at *m/z* 110, an alkaloid described for the first time for the genus *Datura*. Previously it has been reported as a constituent of the genus *Hyoscyamus* (El-Shazly *et al*., 1997) and *Erythroxylum* (Al-Said *et al*., 1986; Christen *et al*., 1993, 1995).

Four new alkaloids were also detected. The first was identified as 3-acetoxy-6,7-epoxytropane (**10**, Fig. 1). Its EI mass spectrum exhibited the fragmentation pattern typical for esters of scopine (6,7-epoxytropine) (Vitale and Acher, 1995). The $[M]^+$ at *m/z* 197 corresponds to the molecular formula $C_{10}H_{15}NO_3$ with a base peak at *m/z* 94. The ions at *m/z* 154 ($[M]^+ - 43$; C_2H_3O), 138 ($[M]^+ - 59$; $C_2H_3O_2$), and 43 (C_2H_3O) indicate the attachment of the acetate moiety at C-3. In the absence of other spectroscopic data, it was

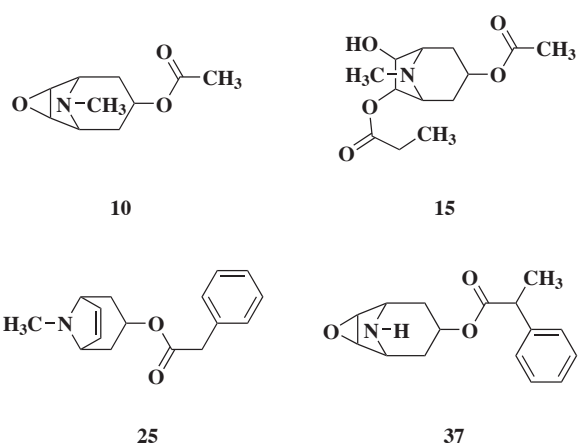

Fig. 1. Chemical structures of 3-acetoxy-6,7-epoxytropane (**10**), 3-acetoxy-6-propionyloxy-7-hydroxytropane (**15**), 6,7-dehydro-3-phenylacetoxytropane (**25**), and 3-(2'-phenylpropionyloxy)-6,7-epoxynortropane (**37**).

Table I. Alkaloids identified in *Datura innoxia* plant parts presented as percentage of total ion current[a].

Alkaloid	RT[b] [min]	$[M]^+$/base ion (*m/z*)	Roots	Stems	Leaves	Flowers	Seeds	Reference
Hygrine (**1**)	09.04	141/84	0.2	-	-	-	-	Witte *et al.* (1987)
6,7-Dehydrotropine (**2**)	10.51	139/94	<0.1	<0.1	-	-	-	Blossey *et al.* (1964)
Cyclotropine (**3**)	11.39	139/68	<0.1	<0.1	-	-	-	Jenett-Siems *et al.* (2005)
Tropinone (**4**)	12.28	139/82	<0.1	<0.1	-	-	<0.1	Blossey *et al.* (1964)
Tropine (**5**)	12.54	141/82	0.9	0.6	0.1	0.3	0.2	Witte *et al.* (1987)
Pseudotropine (**6**)	13.14	141/82	<0.1	<0.1	-	-	0.3	Witte *et al.* (1987)
Scopoline (**7**)	15.56	155/96	0.3	0.4	0.6	0.9	0.2	Ionkova *et al.* (1994)
Scopine (**8**)	16.76	155/42	0.1	0.1	0.2	0.7	<0.1	Ionkova *et al.* (1994)
3,6-Dihydroxytropane (**9**)	19.61	157/113	0.7	<0.1	-	-	-	Gambaro *et al.* (1983)
3-Acetoxy-6,7-epoxytropane (**10**)[c]	20.54	197/94	-	0.1	-	-	-	-
Methylecgonine (**11**)	22.26	199/82	0.2	0.3	0.3	<0.1	-	Berkov *et al.* (2003)
3-(Hydroxyacetoxy)tropane (**12**)	22.50	199/124	0.3	-	-	-	-	Robins *et al.* (1990)
3-Acetoxy-6-hydroxytropane (**13**)	23.07	199/94	0.1	0.1	-	-	-	Ionkova *et al.* (1994)
3,6-Diacetoxytropane (**14**)	25.62	241/94	<0.1	-	-	-	-	Parr *et al.* (1990)
3-Acetoxy-6-propionyloxy-7-hydroxytropane (**15**)[c]	26.75	271/94	-	0.1	-	-	-	-
3α-Tigloyloxytropane (**16**)	27.01	223/124	0.4	-	-	-	-	Witte *et al.* (1987)
Cuscohygrine (**17**)	27.13	224/84	<0.1	-	-	-	-	Witte *et al.* (1987)
3-Hydroxy-6-isobutyryloxytropane (**18**)	27.17	227/113	<0.1	-	-	-	-	Christen *et al.* (1990)
3β-Tigloyloxytropane (**19**)	27.33	223/124	<0.1	-	-	-	-	Witte *et al.* (1987)
3-Isovaleroyloxy-6-hydroxytropane or 3-(2'-methylbutyryloxy)-6-hydroxytropane (**20**)	29.44	241/94	0.1	-	-	-	-	Berkov and Zayed (2004)
3-Tigloyloxy-6,7-epoxytropane (**21**)	30.02	237/94	<0.1	-	-	-	-	El Bazaoui *et al.* (2011)
3-Tigloyloxy-6-hydroxytropane (**22**)	32.20	239/94	0.7	<0.1	<0.1	<0.1	-	Witte *et al.* (1987)
3α-Hydroxy-6β-tigloyloxytropane (**23**)	32.25	239/113	0.4	<0.1	<0.1	<0.1	-	Witte *et al.* (1987)
3β-Hydroxy-6β-tigloyloxytropane (**24**)	32.50	239/113	-	0.3	0.1	<0.1	-	Doncheva *et al.* (2006)
6,7-Dehydro-3-phenylacetoxytropane (**25**)[c]	33.60	257/94	-	<0.1	<0.1	<0.1	<0.1	-
3-Tigloyloxy-6-propionyloxy-7-hydroxytropane (**26**)	34.50	311/94	-	-	<0.1	0.2	-	Berkov *et al.* (2003)
3-Phenylacetoxytropane (**27**)	34.89	259/124	0.4	0.3	0.1	0.2	0.3	El Bazaoui *et al.* (2009)
3-Phenylacetoxynortropane (**28**)[d]	35.03	245/110	-	<0.1	<0.1	<0.1	-	Al-Said *et al.* (1986)
3-(2'-Phenylpropionyloxy)tropane (**29**)	35.32	273/124	-	<0.1	-	<0.1	-	Doncheva *et al.* (2004)
3-(2'-Phenylpropionyloxy)nortropane (**30**)	35.41	259/110	-	<0.1	-	<0.1	-	Doncheva *et al.* (2004)
6,7-Dehydro-3-apotropoyloxytropane (**31**)	35.87	269/94	<0.1	<0.1	0.1	<0.1	<0.1	El Bazaoui *et al.* (2011)
3-Tigloyloxy-6,7-dihydroxytropane (**32**)	36.55	255/94	0.3	<0.1	-	1.6	-	Witte *et al.* (1987)
Apohyoscyamine (**33**)	36.99	271/124	2.3	10.1	0.3	0.3	3.6	El Bazaoui *et al.* (2009)
Norapoatropine (**34**)	37.02	257/110	1.3	<0.1	0.3	0.8	-	Evans and Ramsey (1981)
3-Phenylacetoxy-6,7-epoxynortropane (**35**)	37.46	259/122	-	-	<0.1	<0.1	-	Berkov *et al.* (2005)
3-Phenylacetoxy-6,7-epoxytropane (**36**)	37.63	273/94	0.2	0.6	0.5	1.4	0.4	Vitale and Acher (1995)
3-(2'-Phenylpropionyloxy)-6,7-epoxynortropane (**37**)[c]	37.84	273/122	-	-	-	<0.1	-	-
3-(2'-Phenylpropionyloxy)-6,7-epoxytropane (**38**)	38.07	287/94	-	-	-	0.1	-	Freitas *et al.* (1996)
6,7-Dehydro-3-tropoyloxytropane (**39**)	39.10	287/94	0.1	-	0.4	0.4	0.1	Blossey *et al.* (1964)

Table I continued.

Alkaloid	RT[b] [min]	$[M]^+$/base ion (*m/z*)	Roots	Stems	Leaves	Flowers	Seeds	Reference
3-(3'-Methoxytropoyloxy)tropane (**40**)	39.24	303/124	0.1	-	-	-	-	El Bazaoui *et al.* (2011)
3-Phenylacetoxy-6-hydroxytropane (**41**)	39.34	275/94	0.2	-	-	-	-	Robins *et al.* (1990)
Aponorscopolamine (**42**)	39.35	271/122	0.1	-	<0.1	0.1	0.1	Evans and Ramsey (1981)
Aposcopolamine (**43**)	39.49	285/94	1.6	18.4	9.5	13.5	5.3	Witte *et al.* (1987)
Hyoscyamine (atropine) (**44**)	40.59	289/124	56.5	31.9	5.3	18.2	37.7	El Bazaoui *et al.* (2009)
Norhyoscyamine (**45**)	40.78	275/110	-	<0.1	0.1	<0.1	-	El-Shazly *et al.* (1997)
6-Hydroxyapoatropine (**46**)	41.25	287/94	2.6	1.2	0.8	<0.1	0.1	Witte *et al.* (1987)
Methylscopolamine (**47**)	42.10	317/94	<0.1	-	-	0.1	-	Ionkova *et al.* (1994)
Scopolamine (**48**)	43.21	303/94	13.2	35.2	79.5	60.0	51.3	Witte *et al.* (1987)
4'-Hydroxylittorine (**49**)	43.69	305/124	0.3	-	-	-	-	Doerk-Schmitz *et al.* (1994)
3,6-Ditigloyloxy-7-hydroxytropane (**50**)	44.32	337/94	9.4	-	-	-	-	Witte *et al.* (1987)
7-Hydroxyhyoscyamine (**51**)	44.50	305/94	1.9	1.1	0.4	-	0.1	Ionkova *et al.* (1994)
6-Hydroxyhyoscyamine (**52**)	44.79	305/94	3.3	0.5	0.5	-	0.3	Ionkova *et al.* (1994)
3-Tropoyloxy-6-tigloyloxytropane (**53**)	51.51	387/94	<0.1	-	-	-	-	Witte *et al.* (1987)

[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] Mass spectral data of new tropane alkaloids. EI-MS (70 eV), *m/z* (rel. int. %):

197 (45) $[M]^+$, 154 (25), 138 (43), 136 (36), 120 (10), 108 (69), 97 (38), 94 (100), 57 (30), 43 (70), 42 (96), 41 (27).

271 (7) $[M]^+$, 228 (1), 212 (5), 171 (2), 155 (10), 138 (8), 94 (100), 96 (9), 95 (57), 81 (14), 75 (91), 59 (5), 57 (6), 43 (14), 42 (15).

257 (20) $[M]^+$, 138 (31), 122 (54), 121 (46), 120 (17), 95 (27), 94 (100), 91 (41), 81 (45), 65 (16).

273 (5) $[M]^+$, 140 (7), 124 (31), 123 (43), 122 (100), 106 (23), 105 (60), 94 (39), 80 (53), 79 (22), 77 (25), 67 (15).

[d] Alkaloid described for the first time for the genus *Datura*.

not possible to establish the configuration of the substituent at C-3.

The second new alkaloid was identified as 3-acetoxy-6-propionyloxy-7-hydroxytropane (**15**, Fig. 1). Its mass spectrum is characteristic of a diester of 3,6,7-trihydroxytropane. The $[M]^+$ at *m/z* 271 corresponds to the molecular formula $C_{13}H_{21}NO_5$. Since the base peak was at *m/z* 94, it was considered that the alkaloid had the ester function attached to C-3. The ions at *m/z* 228 ($[M]^+ - 43$; C_2H_3O) and 212 ($[M]^+ - 59$; $C_2H_3O_2$), 43 (C_2H_3O), and 59 ($C_2H_3O_2$) indicate an acetoxy group. The position of this group at C-3 is determined by the ion at *m/z* 171 formed by the cleavage of the 1,2- and 4,5-carbon bonds of the tropane nucleus (Witte *et al*., 1987). The cleavage of the 1,7- and 5,6-carbon bonds of the tropane nucleus gives rise to the 3-acetyloxy-*N*-methylpyridinium ion at *m/z* 155 ($[M^+]$ – HOC(7)HC(6) $HOCOC_2H_5$), which indicates the attachment of the acetyl group at position 3 (Blossey *et al*., 1964), propionyl and hydroxy groups at C-6 and C-7, respectively. Ions at *m/z* 228 and 212 correspond to a 6-propionyloxy-7-hydroxy-substituted tropane nucleus (Berkov *et al*., 2003). The peak at *m/z* 57 confirms the propionyl moiety. In the absence of other spectroscopic data, it was not possible to establish the configuration of the substituents at C-3, C-6, and C-7.

The third new alkaloid **25** has $[M]^+$ at *m/z* 257 corresponding to the molecular formula $C_{16}H_{19}NO_2$ with a base peak at *m/z* 94. Its mass spectrum exhibits the fragmentation pattern typical for a 6,7-dehydrotropine ester (Blossey *et al*., 1964). The ions at *m/z* 138 ($[M]^+ - 119$; C_8H_7O), 122 ($[M]^+ - 135$; $C_8H_7O_2$), and 121 ($[M]^+ - 136$; $C_8H_8O_2$) indicate the presence of a phenylacetyl group at C-3. The phenylacetyl moiety is confirmed by the ion appearing at *m/z* 91 (C_7H_7) ascribed to a benzyl group (Vitale and Acher, 1995). On the basis of these MS data, the structure of **25** was tentatively determined as 6,7-dehydro-3-phenylacetoxytropane, a newly reported tropane alkaloid (Fig. 1). However, in the absence of other spectroscopic data, it was not possible to assign the configuration of the substituent at C-3.

The fourth new alkaloid has a molecular ion $[M]^+$ at *m/z* 273 corresponding to the molecular formula $C_{16}H_{19}NO_3$ and a base peak at *m/z* 122, which suggests a 6,7-epoxynortropane (norscopine) moiety with C-3 substitution. Additionally, the ions at *m/z* 140 ($[M]^+ - 133$; C_8H_9CO), 124 ($[M]^+ - 149$; $C_8H_9CO_2$), 105 (C_8H_9), and 77 (C_6H_5) indicate the presence of the 2-phenylpropionyl (dihydroapotropoyl) group at C-3. On the basis of these MS data, the structure was tentatively determined as 3-(2'-phenylpropionyloxy)-6,7-epoxynortropane (**37**, dihydroaponorscopolamine), a new tropane alkaloid (Fig. 1). In the absence of other spectroscopic data, it was not possible to establish the configuration of the substituent at C-3.

The remaining alkaloids were identified according to their fragmentation patterns reported in the literature as indicated in Table I. Occurrence of cyclotropine (**3**) and *apo*-derivates like apohyoscyamine (**33**) and aposcopolamine (**43**) may be artifacts from the isolation and GC procedures 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). The existence of *nor*-derivatives like compounds **28**, **30**, **34**, **35**, **37**, **42**, and **45** is probably due to demethylation of tropane (Robins and Walton, 1993; Doerk-Schmitz *et al.*, 1994; Berkov *et al*., 2005).

The alkaloid spectrum of *D. innoxia* is organ-dependent (Table I). Hyoscyamine (**44**) and scopolamine (**48**) figure as main alkaloids in the roots and aerial parts, respectively. The highest relative percentage of **48** in the alkaloid mixtures of the plant organs was found in the leaves – 79.5% of the total alkaloids. Respectively, the lowest percentage was found in the roots – 13.2%. The roots exhibited a greater variety of alkaloids than the aerial parts as they are the place of alkaloid biosynthesis (Witte *et al*., 1987; Doncheva *et al.*, 2006). Some of these alkaloids are known precursors in the biosynthesis of tropane alkaloids (Lounasmaa, 1988; Christen *et al*., 1993, 1995; Brachet *et al*., 1997; Berkov, 2003), *e.g.* 6,7-dehydrotropine (**2**), tropinone (**4**), tropine (**5**), and pseudotropine (**6**), whereas cuscohygrine (**17**) is a product of side reactions of the biosynthetic pathway. In the roots, other seven alkaloids contribute to more than 1% of total alkaloids.

Like the roots, the stems, leaves, and flowers also exhibit a large spectrum of alkaloids – 32 for the stems, 25 for the leaves, and 29 for the flowers. Scopolamine (**48**) as well as other compounds listed in Table I contribute to 1% or more of the total alkaloids – 5 for the stems, 2 for the leaves, and 4 for the flowers. Seeds have less diverse alkaloid mixtures as compared to the other organs.

Their alkaloid mixtures are dominated by the end products of the biosynthetic pathway of tropanes – hyoscyamine (**44**) and scopolamine (**48**), representing 37.7% and 51.3%, respectively, of the total alkaloids. With the exception of hyoscyamine and scopolamine, only two alkaloids contribute to more than 1% of total alkaloids.

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