Alkaloids of *Datura ceratocaula*

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Thirty-six alkaloids were identified in the organs of *Datura ceratocaula* by GC/MS. Thirty-three of them have not been previously reported for the species. Furthermore, a new tropane ester was tentatively identified as 3-(3′-formyloxytropoyloxy)tropane on basis of its mass spectral fragmentation. Hyoscyamine was the main alkaloid in the plant organs.

Key words: Datura ceratocaula, GC-MS, Tropane Alkaloids

Introduction

Datura ceratocaula Ort. (*Solanaceae*) is an aquatic, hollow-stemmed, prostrate, creeping plant known by the Mexicans as the narcotic *torna-loca* or maddening plant. This species is the connecting link between herbaceous daturas and brugmansias (Griffin and Lin, 2000). Only a few alkaloids have been reported for *D. ceratocaula* (Beresford and Woolley, 1974; Lounasmaa and Taminnen, 1993; Griffin and Lin, 2000). Knowledge of the complete alkaloid pattern is of interest not only phytochemically, but also in relation to aspects of alkaloid biogenesis, metabolism and application in the plant biotechnology. Recent investigations of genera *Datura* and *Brugmansia* with modern analytical methods, namely GC-MS, demonstrated that tropane alkaloid-containing plants generally have a large number of alkaloids which are not detected by other methods (Witte *et al.*, 1987; Brachet *et al.*, 1997).

Materials and Methods

Plant material

Plants of *D. ceratocaula* were grown from seeds (received from the Botanical Garden of Nijumegen, The Netherlands) at the greenhouse of the Institute of Botany – Bulgarian Academy of Sciences. A voucher specimen (CO-667) is deposited in the herbarium of the Institute of Botany-BAS.

Alkaloid extraction

Plant samples were dried at 50 °C and macerated in 3% H_2SO_4 for 2 h at room temperature. The supernatants were made alkaline with 25% NH_4OH (pH 9–10) and applied to Extrelut (Merck) columns. The alkaloids were eluted by CH_2Cl_2 (6 ml/1 g Exrelut) and the extracts were evaporated to dryness. Thus obtained residues were resolved in CH_3OH for the further analysis.

Gas chromatography/mass spectra (GC-MS)

GC-MS measurements were performed after Berkov and Philipov (2002). The identities of the alkaloids were confirmed by comparing the measured data with those of authentic compounds, or with data obtained from the literature. 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.

Quantification of alkaloids

For quantification, an FID-detector was used after a calibration with known amounts of hyoscyamine and scopolamine standards. GC was performed on a Hewlett Packard 5890 equipped with a HP-1 column (30 m × 0.25 mm × 0.25 μm). The flow rate of the carrier gas (N_2) was 0.8 ml/min^{-1} and the splitting ratio was 1:100. The temperature program was 150–270 °C at 6°/min^{-1} and held at the final temperature for 15 min. The flame ioniza-

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tion detector was used at 300 °C and the injector temperature was 280 °C.

Results and Discussion

In the present study a GC-MS procedure was applied for the identification of alkaloids in the plant organs of *D. ceratocaula.* More than 40 compounds in the alkaloid fractions showed the characteristic mass spectral fragmentation of the tropane alkaloids and their metabolites. Of these, 36 alkaloids were identified (Table I). Up to now, 3-hydroxy-6-(2′-methylbutiryloxy)tropane (**13**), atropine (± hyoscyamine), hyoscyamine (**25**) and scopolamine (**31**) have been reported for the species (Beresford and Woolley, 1974; Lounasmaa and Taminnen, 1993; Griffin and Lin, 2000). Two our knowledge, 33 bases are reported for the first time for *D. ceratocaula.* Furthermore, one novel alkaloid was identified.

The characteristic alkaloids of *D. ceraticaula* are tropanol esters of a range of acids. Alkaloids **8** and **10**, **14** and **16**, **19** and **20** appeared as double peaks in CG-MS with identical mass spectra. They are

Table I. Identified alkaloids from *Datura ceratocaula* presented as% of the total ion current[a].

Compounds	*RT* [min]	M^+/base ion (*m/z*)	roots	stem	leaves	flowers	> seeds	MS Ref.
Tropine (**1**)	2.51	141/82	0.20	0.13	–	–	–	Witte *et al.* (1987)
Pseudotropine (**2**)	2.60	141/82	0.24	0.20	–	–	–	Witte *et al.* (1987)
3-Acetoxytropane (**3**)	3.26	183/124	–	–	–	–	0.62	Witte *et al.* (1987)
Methylecgonine (**4**)	4.80	199/82	0.14	0.18	–	–	–	Berkov *et al.* (2002)
3-Acetoxy-6-hydroxytropane (**5**)	5.04	199/94	< 0.1	–	–	–	–	Ionkova *et al.* (1994)
N-Methylpyrrolidinyl-hygrine A (**6**)	5.84	224/84	< 0.1	–	–	–	–	Witte *et al.* (1987)
N-Methylpyrrolidinyl-hygrine B (**7**)	5.92	224/84	< 0.1	–	–	–	–	Witte *et al.* (1987)
3α-Tigloyloxytropane (**8**)	6.76	223/124	1.55	0.44	–	–	–	Witte *et al.* (1987)
Cuscohygrine (**9**)	6.81	224/84	< 0.1	–	–	–	–	Witte *et al.* (1987)
3β-Tigloyloxytropane(**10**)	6.83	223/124	0.28	–	–	–	< 0.1	Witte *et al.* (1987)
3,6-Diacetoxytropane (**11**)	7.98	241/94	< 0.1	0.13	–	–	–	Parr *et al.* (1990)
3-Hydroxy-6-(2′methylbutyryloxy)tropane (**13**)	8.14	113/241	–	0.12	–	–	–	Witte *et al.* (1987)
3(α?)-Tigloyloxy-6-hydroxytropane (**14**)	9.48	239/94	2.87	2.29	–	–	–	Witte *et al.* (1987)
3-Hydroxy-6-tigloyloxy tropane (**15**)	9.65	239/113	1.01	0.67	2.64	–	–	Witte *et al.* (1987)
3(*β*?)-Tigloyloxy-6-hydroxytropane (**16**)	9.78	239/94	< 0.1	< 0.1	–	–	–	Witte *et al.* (1987)
3-Tigloyloxy-6-propionyloxy-7-hydroxy-tropane (**17**)	10.84	311/94	1.82	2.48	2.19	2.16	1.41	Berkov *et al.* (2002)
Phenylacetoxytropane (**18**)	11.22	259/124	< 0.1	0.28	–	< 0.1	< 0.1	Ionkova *et al.* (1994)
3(α?)-Tigloyloxy-6,7-dihydroxytropane (**19**)	12.13	255/94	4.20	7.09	–	–	–	Witte *et al.* (1987)
3(*β*?)-Tigloyloxy-6,7-dihydroxytropane (**20**)	12.32	255/94	1.8	2.72	–	–	–	Witte *et al.* (1987)
Apohyoscyamine (**21**)	12.49	271/124	1.62	2.96	1.00	1.98	3.25	Witte *et al.* (1987)
Aponorhyoscyamine (**22**)	12.80	257/110	< 0.1	0.98	–	0.36	–	Ionkova *et al.* (1994)
Aposcopolamine (**23**)	14.12	285/94	–	0.24	–	–	0.42	Witte *et al.* (1987)
Littorine (**24**)	14.71	289/124						–
Hyoscyamine (**25**)	14.71	289/124	26.28	44.19	34.39	49.08	50.62	Witte *et al.* (1987)
Norhyoscyamine (**26**)	14.94	275/110	0.1	3.12	–	–	–	Ionkova *et al.* (1994)
3,6-Ditigloyloxytropane (**27**)	15.02	321/94	0.78	–	–	–	–	Witte *et al.* (1987)
6-Hydroxyapohyoscyamine (**28**)	15.19	287/94	2.21	1.53	–	–	< 0.1	Ionkova *et al.* (1994)
3-(3′-Formyloxytropoyloxy)tropane (**29**)[b]	15.30	317/124	–	–	< 0.1	0.36	< 0.1	–
3-(3′-Acetoxytropoyloxy)tropane (**30**)	16.08	331/124	–	–	1.05	0.32	4.09	Philipov and Berkov (2002)
Scopolamine (**31**)	16.43	303/94	2.15	6.26	9.15	19.31	4.23	Witte *et al.* (1987)
3-(2′-Hydroxytropoyloxy)tropane (**32**)	16.88	305/124	1.02	–	–	–	–	Philipov and Berkov (2002)
3,6-Ditigloyloxy-7-hydroxytropane (**33**)	16.97	337/94	6.42	1.24	1.92	0.29	–	Witte *et al.* (1987)
7-Hydroxyhyoscyamine (**34**)	17.28	305/94	1.16	0.36	0.1	–	–	Ionkova *et al.* (1994)
6-Hydroxyhyoscyamine (**35**)	17.55	305/94	7.82	2.39	–	0.58	2.01	Ionkova *et al.* (1994)
6-Tigloyloxyhyoscyamine (**36**)	22.08	387/94	0.18	0.28	0.89	–	–	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 is not a true quantification but can be used for comparison between the samples which is the objective of this work.

[b] EIMS 70 eV, *m/z* (rel. int.): 317 [M^+] (10.3), 272 (3.3), 140 (6.5), 124 (100), 103 (8.1), 94 (21.95), 82 (18.7), 67 (11.4), 55 (7.3), 42 (8.2).

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 as the 3β-isomers (Witte *et al.*, 1987; Robins and Walton, 1993).

Methylecgonine (**4**), an unusual alkaloid for *Solanaceae*, was identified in the roots of *D. ceratocaula.* This alkaloid have not been detected in the intact plants of *Solanaceae.* Previously it was characterized in genetically transformed root cultures of *D. stramonium* (Berkov *et al.*, 2003). The structure of this compound was confirmed by a comparison of its mass spectrum with those of the reference compound from database NIST 98. C-2 substituted tropanes are chemotaxonomic marker for family *Erythroxylaceae* (Griffin and Lin, 2000).

A peak with *RT* – 14.71 showed the characteristic fragmentation pattern of hyoscyamine. Occurrence of a small ion at *m/z* 142 suggests presence of littorine (Witte *et al.*, 1987; Brachet *et al.*, 1997). This alkaloid is well known positional isomer of hyoscyamine. Another ion at *m/z* 271 indicates dehydratation of the hyoscyamine (Brachet *et al.*, 1997). Consequently, both alkaloids are presented in the samples. These two compounds were difficult to distinguish by CG-MS. Also, it is well known that the natural product accumulated in *Datura* roots is (*l*)-hyoscyamine which racemates during extraction procedures. Since GC-MS used does not separate the (*d*)- and (*l*)- forms it was impossible to investigate this problem further and I have referred to the product as hyoscyamine throughout.

Alkaloid **29** showed M^+ at *m/z* 317 corresponding to a molecular formula $C_{18}H_{23}NO_4$. The base peak at *m/z* 124 together with the ion at *m/z* 140 (M^+ – 177) suggests a C-3 substitution. The molecular ion of **29** is with 28 mass units higher then those of hyoscyamine (**25**, M^+– 289). Ions at *m/z* 272 and *m/z* 103 are characteristic for the tropoyl group of hyoscyamine. Fragment at *m/z* 272 (M^+ – 45; CHO_2) indicates formyltropoyl ester group at C-3. Furthermore, the *RT* of alkaloid **29** is near to the *RT* of 3-(3′-acetoxytropoyloxy)tropane. On the basis of its mass spectral fragmentation alkaloid **29** was identified as 3-(3′-formyloxytropoyloxy)tropane – a new tropane alkaloid (Fig.1).

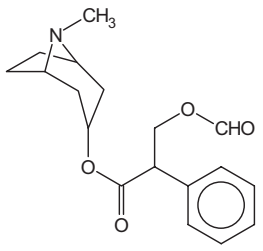

Fig. 1. 3-(3′Formyloxytropoyloxy)tropane (**29**).

The rest of alkaloids have been identified according their fragmentation pattern reported in the literature as indicated in Table I. Occurrence of *apo*-derivates as apohyoscyamine and aposcopolamine, may be artifacts from the isolation and CG procedures as discussed elsewhere (Witte *et al.*, 1987; Ionkova *et al.*, 1994).

The alkaloid spectrum of *D. ceratocaula* is organ dependent (Table I). Hyoscyamine (**25**) was the dominant compound in the alkaloid mixtures of the plant organs. The highest relative percentage of **25** in the alkaloid mixtures was found in the seeds – 50.62% of the total alkaloids. Respectively, the lowest percentage was found in the roots – 26.28%. The roots, as the site of alkaloid synthesis, exhibit a greater variety of compounds and contain as well as the tropane alkaloids, and several alkaloids of pyrrolidine type. Some of these alkaloids are known precursors in the biosynthesis of tropane alkaloids, *e. g.* tropine (**1**) and pseudotropine (**2**) whereas N-methylpyrrolidinyl-hygrine A (**6**), N-methylpyrrolidinyl-hygrine B (**7**) and cuscohygrine (**9**) are products of side-reactions of the biosynthetic pathway. In the roots, other thirteen compounds have a percentage contribution more than 1% of the total alkaloids.

Like the roots, the stem also exhibit a high range of alkaloids. Hyoscyamine (**25**) as well as other ten compounds listed in have a percentage contribution at a level of 1% or more of the total alkaloids.

Leaves, flowers and seeds have poorer alkaloid mixtures as compared with roots and stem. Their alkaloid mixtures are dominated by the end products of the biosynthetic pathway of tropanes – hyoscyamine (**25**) and scopolamine (**31**). The highest relative percentage of scopolamine – 19.31% was found in the alkaloid mixture of the flowers. With the exception of hyoscyamine, only six alka-

loids have a percentage contribution more than 1% of total alkaloids.

It is interesting to note the presence of C-3′ substituted tropoyloxytropanes in the aerial parts and their accumulation in the seeds – 4.09% for 3-(3′-acetoxytropoyloxy)tropane. These alkaloids are recently identified as constituents in the plants and their biogenesis is unknown (Philipov and Berkov, 2002).

3-Hydroxy-6-(2′-methylbutyryloxy)tropane (**13**), which has been found as a major alkaloid in the aerial parts (Beresford and Woolley, 1974) was detected only in the stem as a minor component of the alkaloid mixture (0.12%).

Quantitative investigation of hyoscyamine and scopolamine revealed that *D. ceratocaula* accumulates relatively less hyoscyamine and scopolamine as compared with other *Datura* species (Table II). This result is in agreement with a previous report (Beresford and Woolley, 1974). The highest hyoscyamine content was found in the leaves (0.47 mg/g DW) while the highest scopolamine content was in the flowers (0.34 mg/g DW).

Table II. Hyoscyamine and scopolamine content (mg/g of DW) in *D. ceratocaula* organs.

	Hyoscyamine	Scopolamine
Root	0.16	0.07
Stem	0.42	0.20
Leaves	0.47	0.29
Flowers	0.41	0.34
Seeds	0.25	0.07

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