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On-line structure characterization of pyrrolizidine alkaloids in *Onosma stellulatum* and *Emilia coccinea* by liquid chromatography–ion-trap mass spectrometry

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Abstract

On-line structure characterization of pyrrolizidine alkaloids in two various plant species (*Onosma stellulatum* W.K., family Boraginaceae and *Emilia coccinea* Sims., family Compositae) was performed by a newly elaborated RP-HPLC ion trap MS method with atmospheric pressure chemical ionization (APCI) interface. Different PAs (*N*-oxides, free bases, otonecine alkaloids) isolated were separated on Waters XTerra C_{18} column using a gradient elution. The combination of a CE-SPE with multiple isolation and fragmentation steps for specific masses in ion trap MS detector enabled fast and sensitive analysis of various types of PAs (*N*-oxides and free bases). In *O. stellulatum*, spectra 12 various types of structures (13 different alkaloids) have been determined for the first time: leptanthine-*N*-oxide, lycopsamine-*N*-oxide, heliospathuline, lycopsamine, trachelanthamine-*N*-oxide, dihydroechinatine, leptanthine, heliospathuline-*N*-oxide, 7-acetylintermedine, uplandicine, echimidine and echimidine-*N*-oxide. In *E. coccinea*, the following types of PAs were found: platyphylline-*N*-oxide, platyphylline (three stereoisomers with the same MS^n spectrum), ligularidine, neoligularidine, neosenkirkine and also previously reported senkirkine. The method elaborated can be applied in the structural analysis of PAs in newly examined plant materials or food products but further analysis is needed to determine the stereochemistry in details.

Keywords: Pyrrolizidine alkaloids; *Onosma stellulatum*; *Emilia coccinea*

1. Introduction

Pyrrolizidine alkaloids (PAs) play an important role as naturally occurring carcinogens [1]. They are mainly distributed in *Boraginaceae*, *Compositae* and *Fabaceae* plant families [1,2]. They are derived from 1-hydroxymethylpyrrolizidine (necine) and can be either saturated or non-saturated in 1,2-position. The naturally occurring PAs are mono- or diesterified open-ring alkaloids, dicarboxylic acids closed-ring alkaloids, the *N*-oxides of the free bases or diesters from otonecine. Mainly in developing countries, characteristic liver diseases such as cirrhosis and primary tumors with high mortality occurred due to occasional or continued consumption of medicinal plants (bush-teas) [2]. For this reason, it is necessary to monitor the concentration of toxic PAs. As their occurrence is widely distributed it is still important to develop rapid methods of their quantitative and qualitative analyses in plant samples or food products.

For toxic PAs, spectrophotometric colour reactions were elaborated [3–6], to give a colour adduct. The same conditions can be applied to detection of PAs on TLC plates [7–9].

For the analysis of PAs in natural samples GC and HPLC methods are mainly used due to their sensitivity. In capillary GC methods, flame ionization (FID) [10–12] and more often mass spectrometry (MS) detectors [10,13–22] are used. Sometimes GC tandem MS method was applied [16,23]. Gas chromatography matrix-isolation Fourier transform infrared spectroscopy (GC-MI-FT-IR) was used

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for the identification of PAs from comfrey roots [24]. Although GC methods are often applied they revealed some disadvantages including thermal decomposition of labile *N*-oxides and sometimes also diesterified alkaloids. For these reasons HPLC methods achieved more consideration.

For these purposes RP phases such as C_{18} [25–27], C_8 [28], cyano-bonded [29] and amino-bonded phase [25] were elaborated. Due to the problem of peak asymmetry ion-pair RP-HPLC is also advised [20,30]. LC–MS analysis of macrocyclic PAs in *Senecio* spp. using thermospray interface was previously reported [28,31]. Lin et al., [27] analysed various types of macrocyclic PAs with both in-source (collision induced dissociation in the collision cell) or out-source HPLC–MS or HPLC–MS–MS using electrospray interface and quadrupole instrument. Atmospheric pressure chemical ionization (APCI) HPLC–MS method was also reported for the determination of macrocyclic PAs in honey [32]. Ndjoko et al. [31] applied LC–NMR technique to distinguish *E* and *Z* isomers of senecionine in plant extracts.

In the paper presented here the on-line structure characterization of pyrrolizidine alkaloids in two plant species (*Onosma stellulatum* W.K., family Boraginaceae and *Emilia coccinea* Sims., family Compositae) was performed by newly elaborated RP-HPLC ion trap MS method with atmospheric pressure chemical ionization (APCI) interface. Various types of PAs (*N*-oxides and free bases) occurring in small amounts could be fast and efficiently analysed.

2. Experimental

2.1. Chemicals and reagents

The standards of senkirkine, senecionine and seneciphylline were obtained from Carl Roth (Karlsruhe, Germany). Retrorsine, retrorsine-*N*-oxide and monocrotaline were purchased from Sigma Chemical (St. Louis, MO, USA). Lichrolut SCX (500 mg, 3 ml) strong cation-exchange solid-phase extraction columns were from Merck (Darmstadt, Germany). Methanol, acetonitrile and 25% ammonia (each solvent was of HPLC gradient grade) were obtained from J.T. Baker (Gross-Gerau, Germany). Tartaric acid was of analytical grade and purchased from POCh Gliwice, Poland.

Herbs of *O. stellulatum* W.K. and *E. coccinea* Sims. were harvested from the garden of Department of Pharmacognosy with Medicinal Plants Laboratory, Medical University of Lublin, on August 2001. The second part of the herb of *E. coccinea* Sims. was collected from the Botanical Garden of the Maria Curie-Skłodowska University of Lublin, on September 2000. The plant species were identified by Mss Maria Być. A voucher specimen of each plant is deposited at the herbarium of the Department of Pharmacognosy with Medicinal Plants Laboratory, Medical University of Lublin.

Samples of plant materials were dried at room temperature and powdered.

2.2. Extraction of plant materials and the samples clean-up by cation-exchange solid-phase extraction (CE-SPE)

A 2 g sample of each powdered plant material was extracted with 100 ml of hot 1% tartaric acid solution in methanol for 2 h. Twenty milliliters of aliquots of each extract were purified and alkaloids isolated by previously described cation-exchange solid-phase extraction (CE-SPE) [30]. PAs were eluted from the cartridge with 15 ml of methanol–10% ammonia (3:1, v/v). The residues after CE-SPE were reconstituted in 2 ml of methanol for HPLC analysis.

2.3. High-performance liquid chromatography–diode array–ion trap mass spectrometric assay (HPLC–DAD–IT MS) of the alkaloids

The HPLC–DAD–IT MS experiments were performed using Agilent 1100 Series HPLC–LCQ trap mass and ion trap MS^n (Finningan LCQ) detector. The analytical column was a 5 μm XTerra C_{18} (Waters, USA), 150 × 4.6 mm i.d. The column was held at 25 °C, and the mobile phase flow rate was 1.0 ml/min. A two-pump gradient program was applied: reservoir A contained 15 mM ammonia water solution, and reservoir B contained 100% acetonitrile. The injection volume was 20 μl (samples; in triplicate). The following gradient program was used: 0–20 min: linear gradient from 5 to 50% B; 20–25 min: isocratic at 50% B; 25–28 min: linear gradient from 50 to 100% B; 28–33 min: isocratic at 100% B; 33–36 min: linear gradient from 100 to 5% B.

The total time of analysis was 36 min.

Finningan LCQ detector was equipped with an atmospheric pressure chemical ionization (APCI) interface as the ionization source, which was operated under the following conditions: sheat gas, N_2 with flow of 70 l/min, capillary temperature, 150 °C; APCI vaporizer temperature, 400 °C; source voltage, 6 kV; source current, 5 μA; capillary voltage, 46 V; tube lens offset, 55 V; multipole 1 offset, −1.50 V; multipole 2 offset, −6.50 V; inter-multipole lens voltage, −18 V; trap DC offset voltage, −10 V, source induced dissociation (SID) 10 V. Full-scan mass spectra (150–500 amu) were recorded every 2 s in the positive ion mode. The protonated molecule $[M + H]^+$ of PAs and *N*-oxides were chosen as the parent ions for isolation and fragmentation. For MS^n experimental conditions were as follows: He CID pressure, 0.5 mTorr; relative collision energy, 38%; fragmentation time, 200 ms. The spectra were acquired every second and the window was reduced to 50–500 amu.

3. Results and discussion

3.1. PAs separation by RP-HPLC

PAs separation has been optimized using a charge transfer sorbent (XTerra C_{18}) and alkaline mobile phase (gradi-

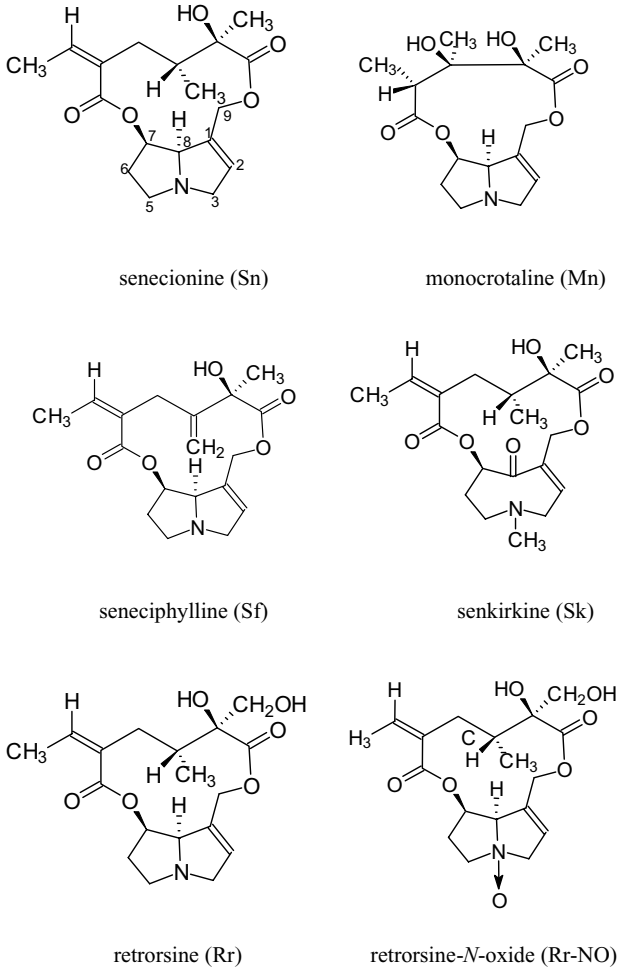

Fig. 1. Chemical structures of pyrrolizidine alkaloid (PAs) standards investigated.

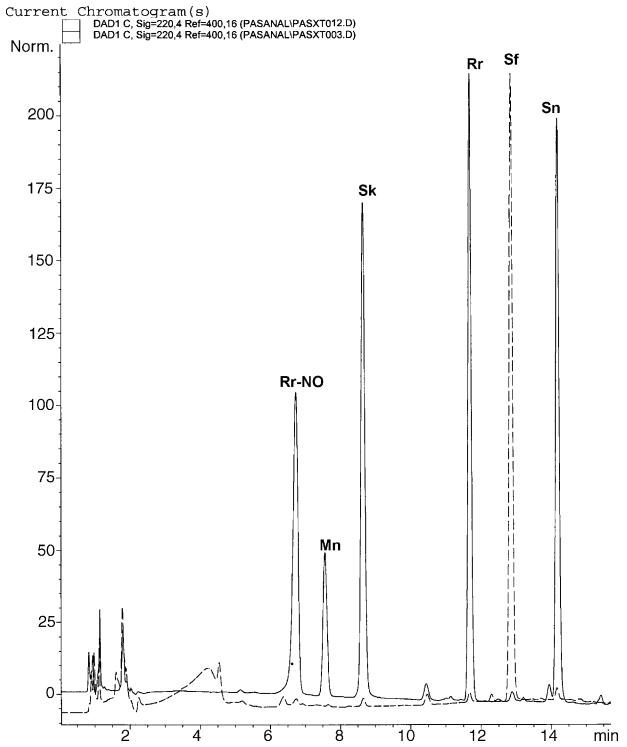

Fig. 2. RP-HPLC separation of the alkaloid standards. Stationary phase: Waters XTerra C_{18}, 250 × 4.6 mm i.d.; d_p = 5 μm. Mobile phase: gradient of acetonitrile in 15 mM ammonia solution (see the text). Flow rate: 1.0 ml/min; column temperature: 25 °C; injection volume: 10 μl. UV trace recorded at 220 nm. The concentrations injected: (for retrorsine, retrorsine-*N*-oxide, senkirkine, senecionine and seneciphylline were about 80 μg/ml, and for monocrotaline 40 μg/ml). Abbreviations used: Rr, retrorsine; Rr-NO, retrorsine-*N*-oxide; Sf, seneciphylline; Sn, senecionine; Sk, senkirkine; Mn, monocrotaline.

ent 15 mM of ammonia in acetonitrile). The applied gradient procedure was suitable for APCI interface. None post-column splitting or buffer addition was necessary. The various types of alkaloids (Fig. 1) could be separated as shown in Fig. 2. The PAs showed retention times between 5 and 15 min. The order of elution was quite different to those determined in our previous ion-pair HPLC conditions [30] and was as follows: retrorsine-*N*-oxide < monocrotaline < senkirkine < retrorsine < seneciphylline < senecionine. Already high amounts of standard injected (800 ng) caused only slight tailing for retrorsine-*N*-oxide, but other alkaloids shown symmetrical peaks. In contrary to previously mentioned ion pair HPLC method [30] and using acidified mobile phases in RP mode [25–27] in our method both the separation and the peak asymmetry factors were suitable. The method presented here should be considered for screening studies of various types of PAs found usually in plant samples.

3.2. DAD, total ion current (TIC) and selected ion monitoring (SIM) chromatograms

In case of *O. stellulatum*, the protonated PAs molecules (with characteristic maximum at 220 nm [30]) for *m/z* at 300, 302, 316, 318, 332, 342, 358, 398 and 414 have been detected, whereas for *E. coccinea* (collected from two different localizations) the protonated compounds have been measured at *m/z* 338, 354, 366 and 408. In SIM chromatograms *N*-oxide structure could be easily assigned, as the intensive peaks were both for protonated molecule and for the one lower of 16 units.

3.3. Chromatographic and MS date of the alkaloids determined

Retention times, MS^1 spectra comprising molecular weight assessment and proposed types of structures according to optimized RP-HPLC IT procedure are summarised in Table 1. In the extract from herb of *O. stellulatum* thirteen different alkaloids were determined (**I1**–**I14**). The following order of elution for different types of PAs has been found according to the increase of retention factor: leptanthine-

Table 1
Chromatographic and MS date of the alkaloids determined by RP-HPLC-IT MS method

Compound[a]	Retention time (min)	MS^1 (relative intensity)	Common name	References
I1	14.58	$[M + H]^+$ 292.06 (100%)	Not-identified	
I2	6.44	$[M + H]^+$ 300.29 (100%)	Isolycopsamine/heliospathuline/tesselatine	[33–35,38–39]
I3	8.58	$[M + H]^+$ 300.21 (100%)	Intermedine/lycopsamine (or stereoisomer)	[33–35]
I4	6.35	$[M + H]^+$ 302.35 (100%); $[M + H - O]^+$ 286.30 (1%)	Trachelanthamine-*N*-oxide (or stereoisomer)	[33–35]
I5	10.84	$[M + H]^+$ 302.30 (100%)	Dihydroechinatine/dihydrolycopsamine (or stereoisomer)	[34–35]
I6	3.87	$[M + H]^+$ 316.25 (100%)	Leptanthine (or stereoisomer)	[40]
I7	4.90	$[M + H]^+$ 316.31 (100%); $[M + H - O]^+$ 300.30 (6.2%)	Heliospathuline-*N*-oxide (or stereoisomer)	[33–35,38–39]
I8	5.64	$[M + H]^+$ 316.28 (100%); $[M + H - O]^+$ 300.20 (2.9%)	Lycopsamine-*N*-oxide/intermedine-*N*-oxide	[13,34–35]
I9	2.09	$[M + H]^+$ 332.24 (100%); $[M + H - O]^+$ 316.30 (25.9%)	Leptanthine-*N*-oxide (epimer)	[40]
I10	2.83	$[M + H]^+$ 332.28 (100%); $[M + H - O]^+$ 316.20 (6.4%)	Leptanthine-*N*-oxide (or stereoisomer)	[40]
I11	12.88	$[M + H]^+$ 342.24 (100%)	7-Acetylintermedine/7-acetyllycopsamine (or stereoisomer)	[34–35]
I12	9.84	$[M + H]^+$ 358.15 (100%)	Uplandicine (or stereoisomer)	[41–42]
I13	15.15	$[M + H]^+$ 398.29 (100%)	Heliosupine/echimidine/hydroxymioscorpine (or stereoisomer)	[34–35]
I14	10.26	$[M + H]^+$ 414.36 (100%); $[M + H - O]^+$ 398.10 (1.2%)	Echihumiline-*N*-oxide/echimidine-*N*-oxide (or stereoisomer)	[37]
K1	4.66	$[M + H]^+$ 268.04 (100%)	Not-identified	
K2	15.82	$[M + H]^+$ 338.27 (100%)	Neoplatyphylline (or stereoisomer)	[27,43]
K3	8.85	$[M + H]^+$ 354.36 (100%); $[M + H - O]^+$ 338.30 (6.4%)	Platyphylline-*N*-oxide/hadiensine-*N*-oxide (or stereoisomer)	[27]
K4	9.32	$[M + H]^+$ 366.23 (100%)	Senkirkine	[27]
K5	14.30	$[M + H]^+$ 408.32 (100%)	Ligularidine/12-acetylneosenkirkine	[27,36]
L1	4.62	$[M + H]^+$ 268.09 (100%)	Not-identified	
L2	15.62	$[M + H]^+$ 338.34 (100%)	Platyphylline (or stereoisomer)	[27,43]
L3	16.00	$[M + H]^+$ 338.27 (100%)	Neoplatyphylline (or stereoisomer)	[27,43]
L4	8.82	$[M + H]^+$ 354.30 (100%); $[M + H - O]^+$ 338.40 (6%)	Platyphylline-*N*-oxide/hadiensine-*N*-oxide (or stereoisomer)	[27]
L5	9.31	$[M + H]^+$ 366.37 (100%)	Senkirkine	[27]
L6	10.58	$[M + H]^+$ 366.32 (100%)	Neosenkirkine	[27]
L7	14.14	$[M + H]^+$ 408.19 (100%)	Neoligularidine/12-acetylsenkirkine (or stereoisomer)	[27,36]
L8	14.30	$[M + H]^+$ 408.30 (100%)	Ligularidine/12-acetylneosenkirkine (or stereoisomer)	[27,36]

Stationary phase: Waters XTerra C_{18}, 250 × 4.6 mm i.d.; d_p = 5 μm. Mobile phase: gradient of acetonitrile in 15 mM ammonia solution (see the text). Flow rate: 1.0 ml/min; column temperature: 25 °C; injection volume: 20 μl. APCI-(+) interface was applied. The protonated molecule $[M + H]^+$ of PAs and *N*-oxides were chosen as the parent ions for isolation and fragmentation.

[a] Compounds determined in herbs of *O. stellulatum* (**I1**–**I14**), *E. coccinea* (**K1**–**K5** and **L1**–**L8**) from two places.

N-oxides (two epimers) > leptanthine > heliospathuline-*N*-oxide > lycopsamine-*N*-oxide > trachelanthamine-*N*-oxide > heliospathuline > lycopsamine > uplandicine > echihumiline-*N*-oxide > dihydroechinatine > 7-acetylintermedine > heliosupine. In the herb of *E. coccinea* collected from the Botanical Garden of the Maria Curie-Skłodowska University of Lublin four PAs (**K2**–**K5**) have been identified and included platyphylline-*N*-oxide, senkirkine, ligularidine and neoplatyphylline according to the increase of retention factor. For the second part of the plant species collected from the garden of Department of Pharmacognosy with Medicinal Plants Laboratory, Medical University of Lublin six different alkaloids were established (**L2**–**L7**). Besides platyphylline-*N*-oxide, senkirkine, neoligularidine and neoplatyphylline additionally stereoisomers of senkirkine (neosenkirkine probably) and platyphylline have been detected.

3.4. MS^n fragmentation pathways of PAs

For the compound with $[M + H]^+$ 302 saturated necine moiety is confirmed by series peaks in MS^2 spectrum with *m*/*z* 96, 122 and 140. The peak with *m*/*z* 286 is noted due to cleavage of *N*-oxide bond, so there is the *N*-oxide of the saturated necine. The fragments with *m*/*z* 258, 240 are created after fragmentation of viridifloric or trachelanthic acid residues esterified with the saturated necine *N*-oxide. The structure

of the compound **I4** in *O. stellulatum* has been established as typical for trachelanthamine-*N*-oxide or its stereoisomer [33–35].

In MS^2 spectrum of echimidine-*N*-oxide or its stereoisomers [37] for the protonated molecule **I14** $[M + H]^+$ 414 the following fragmentation peaks are substantial: *m/z* 398 (N–O cleavage); 396, 370, 356, 353, 352, 338 (echimidinic acid); 298 [angelic acid (or stereoisomer) esterified at C_7]; 254 (after McLafferty rearrangement), 236, 220, 136, 120 [unsaturated necine *N*-oxide with angelic acid (or stereoisomer) at C_7]. For the compound **I13** fragmentation ions lower of 16 Da to those determined for **I14** confirmed the type of echimidine or its stereoisomers [34–35].

Platyphylline-*N*-oxide (or its stereoisomers) structure (**K3**, **L4**) [43] from the extract of *E. coccinea* has been established on the following fragmentation sequence: *m/z* 156, 140, 122 (the saturated necine); 338, 294 (*N*-oxide of the saturated necine esterified with dicarboxylic acid); 336, 326, 308, 293 (cleavage of CO and CO_2 molecules from macrocyclic diester) [27]. Further fragments (238, 222) confirmed the structure of macrocyclic acid.

In plant extracts from *E. coccinea*, three various compounds **K2**, **L2**, **L3** with molecular weight of 16 Da lower than platyphylline-*N*-oxide and about 0.2 min differences in retention times has been determined. They have been assigned as platyphylline and its stereoisomers [27,43].

For the otonecine–PAs (**K5**, **L7**, **L8**) characteristic 'otonecine' peaks in MS^2 (*m/z* 168, 150, 122) [27] and MS^3 (93) are visible. Lack of the fragment $[M + H - OH]^+$ at *m/z* 391 together with 348, 366, 365, 349, 330 suggested the esterification at C_{12} with acetic acid. Further peaks at *m/z* 338, 320 are typical for macrocyclic acetylester. As the relative intensity of the fragments at *m/z* 168, 150, 122 was different to senkirkine we suggested rather the structure of ligularidine or its stereoisomers [36]. In the extracts from *E. coccinea* two different stereoisomers of ligularidine have been found.

In case of compound **I2** with $[M + H]^+$ 300, the major fragmentation ion in MS^2 was at *m/z* 156 corresponding to the structure of protonated retronecine (or stereoisomer). Loss of C_2H_4O (44 Da) and $C_2H_4O_2$ (62 Da) was typical for residues of trachelanthic or viridifloric acid, and peaks at *m/z* 94, 120, 136 are typical for an unsaturated necine moiety. As ion at *m/z* 156 was more abundant than 138, the type of structure of heliospathuline, tesselatine or isolycopsamine was suggested [33–35,38–39].

For the compound **I3** with the same molecular weight as for **I2** fragmentation ion at *m/z* 138 was the most abundant in MS^2 spectrum. Other peaks were similar. There-

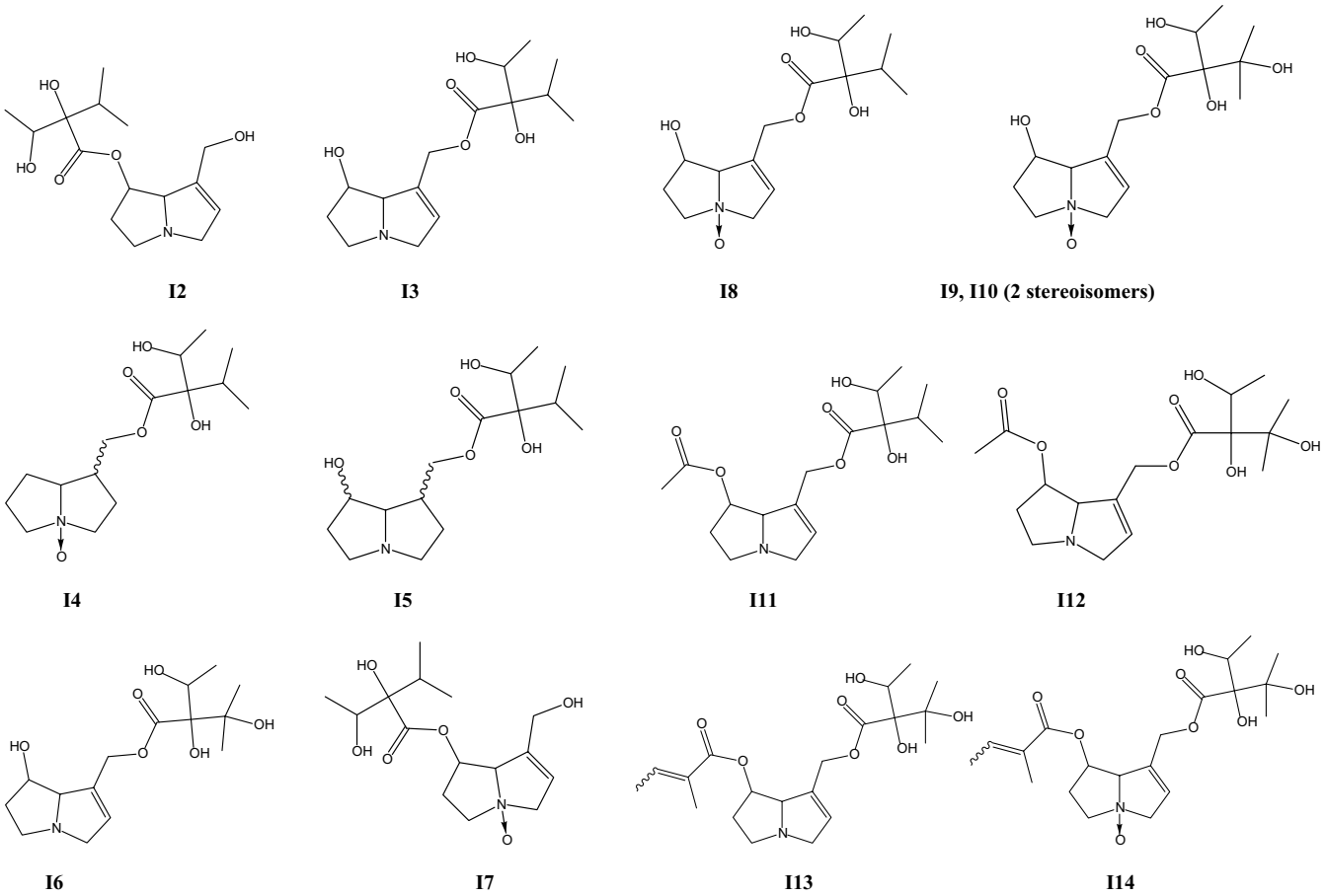

Fig. 3. Structural types of PAs determined in herb of *O. stellulatum* W.K. on the basis of on-line RP-HPLC-IT MS (see Section 2).

fore, lycopsamine type of structure has been assumpted [33–35].

Compound **I5** with $[M + H]^+$ 302 revealed similarity in MS^2 spectrum to trachelanthamine-*N*-oxide regarding peaks at *m*/*z* 284, 258, 240, 122, 96. A lack of peak at 286 excluded the *N*-oxide structure, and *m*/*z* 140 was more abundant. Therefore, this type of structure has been assigned as dihydroechinatine or its stereoisomers [34–35].

For the compound **I6** with $[M + H]^+$ 316, a leptanthine type of structure has been assumpted [40] by loss of the following fragments: C_2H_4O (44 Da) and $C_2H_4O_2$ (62 Da) together with C_3H_6O (58 Da) and $C_3H_8O_2$ (76 Da) (characteristic for echimidinic acid fragmentation). The peak at *m*/*z* 156 was formed after McLafferty rearrangement and possesses the structure of a protonated 1-methylene-2,7-dihydroxypyrrolizidine. Major fragmentation ion in MS^2 spectrum occurred after loss of H_2O molecule (*m*/*z* 298). Further ions were typical for the unsaturated necine with free-OH group at C_7 (*m*/*z* 138, 120, 94).

In the MS^1 and MS^2 spectra of compound **I7** fragments were of typically higher of 16 Da to those measured for compound **I2**. The structure of this compound, therefore, seems to be a heliospathuline-*N*-oxide (or stereoisomers) [33–35,38–39].

Similarily to lycopsamine type of alkaloid (**I3**) for compound **I8** the *N*-oxide structure could be established [13,34–35]. Again fragments higher of 16 Da were measured. Peak at *m*/*z* 300 was formed after cleavage of N–O bond.

Compounds **I9** and **I10** with $[M + H]^+$ 332 possess a type of structure typical for leptanthine-*N*-oxide [40]. In the MS^1 and MS^2 spectra a peak at *m*/*z* 316 corresponding to *N*-oxide structure occurs; other peaks were of 16 Da higher to those determined for the free base **I6**.

In the MS^2 spectrum of compound **I11** with $[M + H]^+$ 342, peaks at *m*/*z* 120 and 180 were predominate and together with ions at *m*/*z* 300, 282. This could be due to an esterification with acetic acid at C_7 of an unsaturated necine. The ions at *m*/*z* 298 and 297 were formed after previously mentioned fragmentation of viridifloric or trachelanthic acid at C_9. Therefore, the structure of this compound has been determined as 7-acetylintermedine [34–35] or its stereoisomers.

Lack of fragmentation ion $[M + H - O]^+$ in the MS^1 and MS^2 spectra of compound **I12** (molecular weight 357 Da) excluded the presence of a 7-acetylintermedine-*N*-oxide type of structure. Peaks at *m*/*z* 282, 296 were typical for echimidinic acid moiety, whereas those at *m*/*z* 298, 180 were characteristic for 7-acetate of an unsaturated necine. Together with an ion at *m*/*z* 198 (McLafferty rearrangement) an uplandicine type of alkaloid could be assumpted [41–42].

3.5. Structural types of the alkaloids investigated in the analysed samples

In herb of *O. stellulatum* (Fig. 3) on the basis of analysis of MS^n spectra 12 various types of structure (and 13 different alkaloids) have been determined for the first time and included: leptanthine-*N*-oxide (2 epimers), lycopsamine-*N*-oxide (the predominating compounds), heliospathuline, lycopsamine, trachelanthamine-*N*-oxide, dihydroechinatine, leptanthine, heliospathuline-*N*-oxide, 7-acetylintermedine, uplandicine, echimidine and echimidine-*N*-oxide.

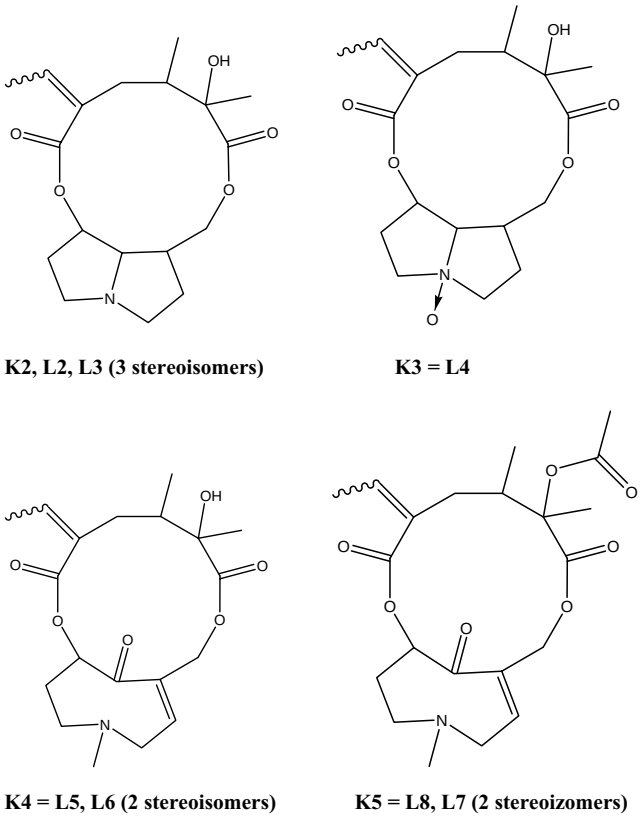

Fig. 4. Structural types of PAs determined in herb of *E. coccinea* Sims. harvested from two various localizations on the basis of on-line RP-HPLC-IT MS (see Section 2).

None substantial qualitative differences were found in *E. coccinea* (Fig. 4) collected from two different localizations. In this plant for the first time the following types of PAs were found: platyphylline-*N*-oxide (the predominating compound), platyphylline (three stereoisomers with the same MS^n spectrum), ligularidine, neoligularidine, neosenkirkine and also previously reported senkirkine.

4. Conclusions

The combination of a CE-SPE with multiple isolation and fragmentation steps for specific masses in ion trap MS detector enabled fast and sensitive analysis of various types of PAs (*N*-oxides and free bases) in small amounts.

On the basis of the analysis of the MS^n spectra diastereoisomeric PAs, their *N*-oxides, saturated as well as unsaturated, macrocyclic, mono- and di-esterified open-ring alkaloids have been found in the plants analysed for the first time.

The elaborated method can be applied in on-line analysis of PAs concerning their relative configuration, in plant materials or food products. To determine the exact stereochemistry further analysis is needed.

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