**Pyrrolizidine alkaloid profiling of four Boraginaceae species from Northern Germany and implications for the analytical scope proposed for monitoring of maximum levels**

Inga Mädge1,2, Matthias Gehling1, Cindy Schöne1, Peter Winterhalter2, Anja These1,\*

1 Department Safety in the Food Chain, German Federal Institute for Risk Assessment, Berlin, Germany

2 Institute of Food Chemistry, Technische Universität Braunschweig, Braunschweig, Germany

\* Corresponding author. E-mail: [anja.these@bfr.bund.de](file:///C:\Users\lanea\AppData\Local\Microsoft\Windows\INetCache\IE\XY4CIB3A\anja.these@bfr.bund.de)

# Appendix A – Supplementary material

Table S1: PA profile of the plant species *Echium vulgare* is listed with mass spectrometric and structural properties of PAs and PANOs. For tentatively identified PAs and PANOs structural proposals and (trivial) names are listed; (trivial) names were only given if the proposed structure can be associated with alkaloids from literature.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **PA** | |  |  |  | **structure** | | |  |
| **no.** | **[M+H]+** | **sum formula** | **detected isomers** | **PA, corresponding PANO1 and isomer** | **content**2 **[mg/kg]** | **necine base**3 | **necic acid3 at** | | **references** |
| **C7 (R1)** | **C9 (R2)** |
| 1 | 238.1441 | C13H19NO3 | - | 7-tigloylretronecinea,c or | 0.9 | r | Tigl | H | El-Shazly et al. (1996a) |
| 7-angeloylretronecinea,c | Ang |
| 2 | 238.1441 | C13H19NO3 | - | 9-tigloylretronecinea or | 10.3 | r | H | Tigl | El-Shazly et al. (1996a) |
| 9-angeloylretronecinea or | Ang |
| 9-senecionyllretronecinea | Sen |
| 3 | 240.1596 | C13H21NO3 | - | 7-angeloylplatynecinea,b,c | 0.1 | p | Ang | H | Witte et al. (1993) |
| 4 | 300.1804 | C15H25NO5 | 1 | intermedine | 9.2 | r | H | (+)-Trach | Skoneczny et al. (2015) |
| lycopsamine | (-)-Vir |
| 5 | 316.1758 | C15H25NO6 | 1 | echimiplatinea | 256.0 | r | H | Ech | Boppré et al. (2005) |
| leptanthinea | (+)-Trach, 5'-OH |
| 6 | 340.1756 | C17H25NO6 | 1 | 7-angeloyl-9-(2,3-dihydroxybutyryl)retronecinea or | 3.1 | r | Ang | 2,3-dihydroxybutric acid | El-Shazly et al. (1996a) |
| 7-tigloyl-9-(2, 3-dihydroxybutyryl)retronecinea or | Tigl | 2,3-dihydroxybutric acid |
| isomera | *iso* |  |
| 7 | 358.1860 | C17H27NO7 | - | uplandicinea | 5.6 | r | Ac | Ech | El-Shazly et al. (1996a) |
| 8 | 358.1860 | C17H27NO7 |  | *PA not detected* |  |  |  |  |  |
|  |  | - | 5'-acetylechimiplatine NOa,b or | 0.2 | r | H | Ech, 5'-OAc |
| 5'-acetylleptanthine NOa,b | (+)-Trach, 5'-OAc |
| 9 | 382.1860 | C19H27NO7 | - | echiuplatinea | 45.6 | r | Ang | 3-hydroxyl-3-methylglutaric acid | Boppré et al. (2005) |
| 10 | 382.2231 | C20H31NO6 | 2 | echiumine Aa | 12.0 | r | Ang | (+)-Trach | Skoneczny et al. (2015) |
| echiumine Ba | *iso* | (+)-Trach/(-)-Vir |
| unknown isomera,b | *iso* | (+)-Trach/(-)-Vir |  |
| 11 | 398.2187 | C20H31NO7 | 1 | echimidine or | 785.6 | r | Ang | Ech | El-Shazly et al. (1996a) |
| echihumilinea or | Sen | Ech |
| echimidine isomer (tigloyl)a | Tigl | Ech |
| 12 | 398.2181 | C20H31NO7 | - | vulgarinea | 136.8 | r | H | (+)-Trach, 5'-OAng | Boppré et al. (2005) |
| 13 | 400.1968 | C19H29NO8 | - | 3'-acetyluplandicinea,b or | 0.1 | r | Ac | Ech, 3'-OAc |  |
| 5'-acetyluplandicinea,b | Ech, 5'-OAc |
| 14 | 400.2342 | C20H33NO7 | - | 7-(2-methylbutyryl)-9-echimidinylretronecinea,b | 6.1 | r | 2-methyl-butric acid | Ech | El-Shazly et al. (1996b) |
| 15 | 414.2122 | C20H31NO8 |  | *PA not detected* |  |  |  |  |  |
|  |  | 2 | 7-(4-hydroxy-*iso*)-9-echimidinylretronecine NOa,b | 0.4 | r | *iso* + O | Ech |
| unknown isomers NOa,b | Ech/(+)-Trach,5'-OH |
| 16 | 440.2288 | C22H33NO8 | - | 3'-acetylechimidinea | 2.7 | r | Ang | Ech, 3'-OAc | El-Shazly et al. (1996a) |
| 17 | 440.2272 | C22H33NO8 | - | 7-acetylvulgarinea | 4.7 | r | Ac | (+)-Trach, 5'-OAng | Boppré et al. (2005) |
| 18 | 480.2585 | C25H37NO8 | - | echivulgarinea | 50.7 | r | Ang | (+)-Trach, 5'-OH, 3'-OAng | Cairns et al. (2015) |

1 PANO is presented in the form of corresponding PA, *exception if no PA was detected*. 2 sum of PA and PANO 3 The abbreviations of the necine bases and necic acids are described in figure 2a and 2b. a This is tentatively identified. b This represents a new PA/PANO for the the species *Echium vulgare*. c The PANO was not detected.

Table S2: PA profile of the plant material *Symphytum* spp. is listed with mass spectrometric and structural properties of PAs and PANOs. For tentatively identified PAs and PANOs structural proposals and (trivial) names are listed; (trivial) names were only given if the proposed structure can be associated with alkaloids from literature.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **PA** | |  |  |  | **structure** | | |  |
| **no.** | **[M+H]+** | **sum formula** | **detected isomers** | **PA, corresponding PANO1 and isomer** | **content**2 **[mg/kg]** | **necine base**3 | **necic acid3 at** | | **references** |
| **C7**  **(R1)** | **C9**  **(R2)** |
| 19 | 300.1807 | C15H25NO5 | 1 | intermedine | 256.9 | r | H | (+)-Trach | El-Shazly and Wink (2014) |
| lycopsamine | (-)-Vir |
| 20 | 316.1755 | C15H25NO6 |  | *PA not detected* |  |  |  |  |  |
|  |  | 1 | echimiplatine NOa,b | 18.6 | r | H | Ech |
| leptanthine NOa,b | (+)-Trach, 5'-OH |
| 21 | 342.1913 | C17H27NO6 | - | 7-acetyllycopsamine | 4.0 | r | Ac | (-)-Vir | El-Shazly and Wink (2014) |
| 22 | 382.2264 | C20H31NO6 | 1 | symlandinea or | 23.8 | r | Ang | (-)-Vir |
| symphytinea or | Tigl | (-)-Vir |
| symviridinea | Sen | (-)-Vir |
| 23 | 398.2173 | C20H31NO7 |  | *PA not detected* |  |  |  |  |
|  |  | - | echimidine NO | 53.9 | r | Ang | Ech |

1 PANO is presented in the form of corresponding PA, *exception if no PA was detected*. 2 sum of PA and PANO 3The abbreviations of the necine base and necic acids are described in figure 2a and 2b. a This is tentatively identified. b This represents a new PA/PANO for the genus *Symphytum*.

Table S3: PA profile of the plant species *Cynoglossum officinale* is listed with mass spectrometric and structural properties of PAs and PANOs. For tentatively identified PAs and PANOs structural proposals and (trivial) names are listed; (trivial) names were only given if the proposed structure can be associated with alkaloids from literature.

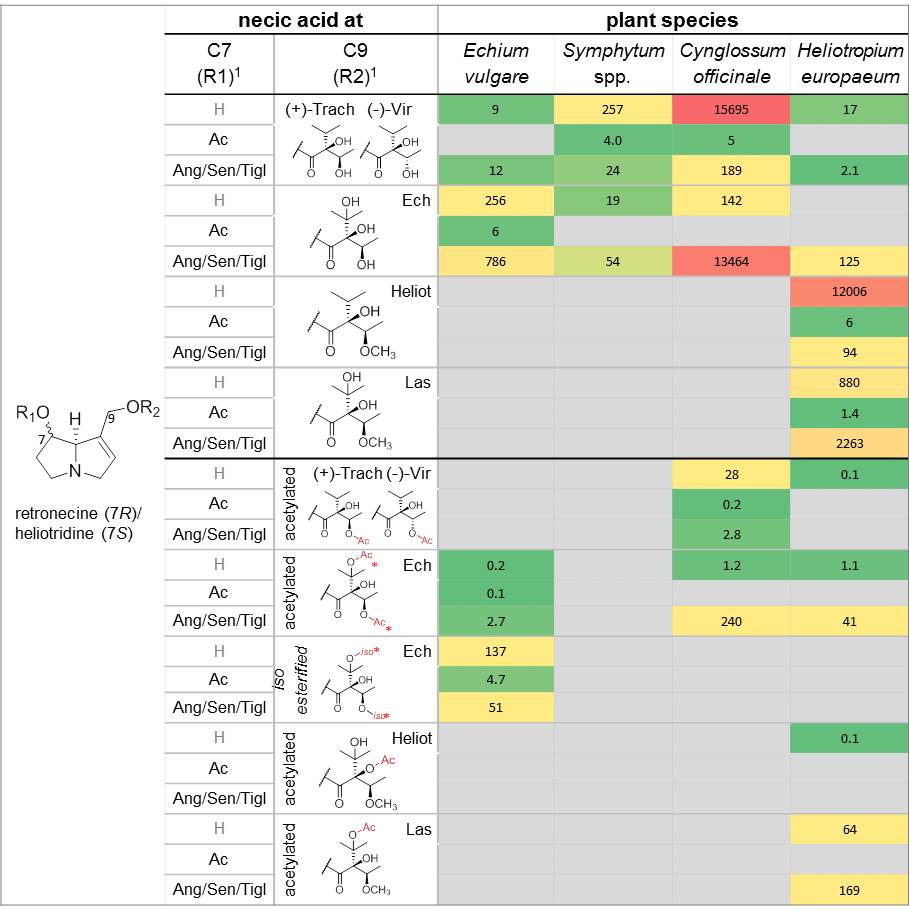
|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **PA** | |  |  |  | **structure** | | |  |
| **no.** | **[M+H]+** | **sum formula** | **detected isomers** | **PA, corresponding PANO1 and isomer** | **content**2 **[mg/kg]** | **necine base**3 | **necic acid3 at** | | **references** |
| **C7**  **(R1)** | **C9**  **(R2)** |
| 24 | 238.1431 | C13H19NO3 | - | 7-angeloylheliotridinea or | 121.0 | h | Ang | H | van Dam et al. (1995) |
| 7-tigloylheliotridinea | Tigl | El-Shazly et al. (1996c) |
| 25 | 286.2011 | C15H27NO4 | 1 | trachelanthamine | 2457.3 | t | - | (+)-Trach | van Dam et al. (1995) |
| viridiflorinea | (-)-Vir |
| 26 | 298.1649 | C15H23NO5 |  | *PA not detected* |  |  |  |  |  |
|  |  | - | unknown PANOa,b | 0.2 | h | H | saturated (+)-Trach/(-)-Vir |
| 27 | 300.1807 | C15H25NO5 | 1 | rinderine | 15695.2 | h | H | (+)-Trach | van Dam et al. (1995) |
| echinatine | (-)-Vir |
| 28 | 302.1957 | C15H27NO5 | 1 | floridiminea,b | 15.3 | t | - | (-)-Vir, 5'-OH | Reina et al. (1997) |
| unknown isomera,b | (+)-Trach, 5'-OH |  |
| 29 | 302.1962 | C15H27NO5 |  | *PA not detected* |  |  |  |  | Reina et al. (1998a) |
|  |  | - | megalanthonine NOa,b | 48.5 | p | H | (-)-Vir |
| 30 | 314.1959 | C16H27NO5 | 1 | unknown PAa,b | 4.9 | h | H | C8H16O4 |  |
| 31 | 316.1758 | C15H25NO6 | 1 | 5'-hydroxyrinderinea,b | 141.9 | h | H | (+)-Trach, 5'OH | Shimshoni et al. (2015) |
| unknown isomera,b | Ech |  |
| 32 | 328.1753 | C16H25NO6 | - | unknown PAa,b | 7.6 | h | H | C8H14O5 |  |
| 33 | 342.1911 | C17H27NO6 | - | 3'-acetylechinatinea | 28.4 | h | H | (-)-Vir, 3'-OAc | van Dam et al. (1995) |
| 34 | 342.1917 | C17H27NO6 | - | 7-acetylechinatinea | 5.0 | h | Ac | (-)-Vir | van Dam et al. (1995) |
| 35 | 356.2068 | C18H29NO6 |  | *PA not detected* |  |  |  |  |  |
|  |  | - | unknown PANOa,b | 0.1 | h | C3H6O2 | (+)-Trach/(-)-Vir |  |
| 36 | 358.1860 | C17H27NO7 | - | 3'-acetyl-5-hydroxyrinderinea,b or | 1.2 | h | H | (+)-Trach,5'-OH,3'-OAc |  |
| unknown isomera,b | (-)-Vir,5'-OH, 3'-OAc/ Ech, 3'-OAc |
| 37 | 382.2230 | C20H31NO6 | 1 | 7-angeloylrinderinea | 189.2 | h | Ang | (+)-Trach | El-Shazly et al. (1996c) |
| 7-angeloylechinatinea | (-)-Vir |
| 38 | 384.1970 | C19H29NO7 | - | 3', 7-diacetylrinderinea,b or | 0.2 | h | Ac | (+)-Trach, 3'-OAc |  |
| 3', 7-diacetylechinatinea,b | (-)-Vir, 3'-OAc |
| 39 | 386.1809 | C18H27NO8 |  | *PA not detected* |  |  |  |  |  |
|  |  | - | unknown PANOa,b | 0.8 | h | H | C10H16O7 |
| 40 | 398.2184 | C20H31NO7 | - | heliosupine | 13464.3 | h | Ang | Ech | Crout (1967) |
| 41 | 414.2122 | C20H31NO8 |  | *PA not detected* |  |  |  |  |  |
|  |  | 2 | 7-(4-hydroxyl-*iso*)-9-echimidinyl heliotridine NOa,b | 3.3 | h | *iso* + O | Ech |
| unknown isomers NOa,b | (+)-Trach/(-)-Vir, 5'-OH |
| 42 | 424.2330 | C22H33NO7 |  | *PA not detected* |  |  |  |  |  |
|  |  | - | 7-angeloyl-3-acetylrinderine NOa,b or | 2.8 | h | Ang | (+)-Trach, 3'-OAc |
| 7-angeloyl-3-acetylechinatine NOa,b | (-)-Vir, 3'-OAc |
| 43 | 432.2244 | C20H33NO9 | 1 | unknown PAa,b | 0.4 | h | *iso* + O2H2 | Ech |  |
| unknown PAa,b | (+)-Trach, 5'-OH |
| 44 | 440.2285 | C22H33NO8 | - | 3'-acetylheliosupinea | 240.0 | h | Ang | Ech, 3'-OAc | Resch and Meinwald (1982) |
| 45 | 456.2228 | C22H33NO9 |  | *PA not detected* |  |  |  |  |  |
|  |  | 2 | 7-(4-hydroxyl-*iso*)-9-3'-acetyl-echimidinyl heliotridine NOa,b | 0.3 | h | *iso* + O | Ech, 3'-OAc |
| unknown isomers NOa,b | (+)-Trach/(-)-Vir, 5'-OH, 3'-OAc |
| 46 | 468.2592 | C24H37NO8 |  | *PA not detected* |  |  |  |  |  |
|  |  | - | unknown PANOa,b | 0.2 | h | *iso* | (+)-Trach/(-)-Vir, 3'-OC4H6O2 |
| 47 | 484.2541 | C24H37NO9 |  | *PA not detected* |  |  |  |  |  |
|  |  | - | unknown PANOa,b | 0.5 | h | *iso* | Ech, 3'-OC4H6O2 |

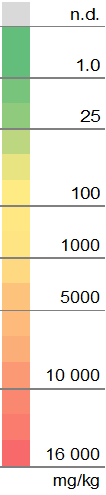
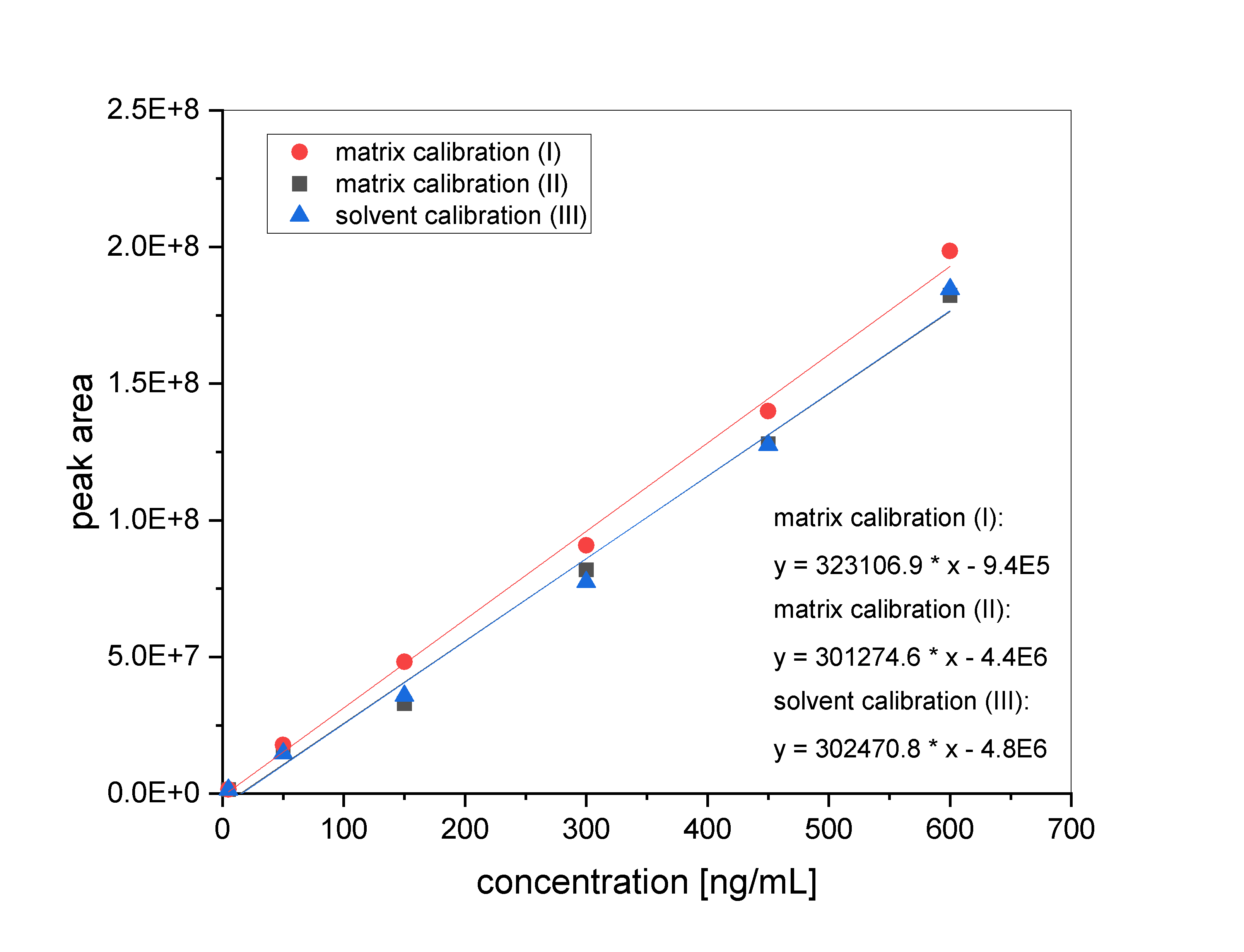
1 PANO is presented in the form of corresponding PA, *exception if no PA was detected*. 2 sum of PA and PANO. 3The abbreviations of the necine bases and necic acids are described in figure 2a and 2b. a This is tentatively identified. b This represents a new PA/PANO for the species *Cynoglossum officinale*.

Table S4: PA profile of the plant species *Heliotropium europaeum* is listed with mass spectrometric and structural properties of PAs and PANOs. For tentatively identified PAs and PANOs structural proposals and (trivial) names are listed; (trivial) names were only given if the proposed structure can be associated with alkaloids from literature.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **PA** | |  |  |  | **structure** | | |  |
| **no.** | **[M+H]+** | **sum formula** | **detected isomers** | **PA, corresponding PANO1 and isomer** | **content**2 **[mg/kg]** | **necine base**3 | **necic acid3 at** | | **references** |
| **C7 (R1)** | **C9 (R2)** |
| 48 | 238.1438 | C13H19NO3 | - | 7-angeloylheliotridinea,b | 2.2 | h | Ang | - | Crowley and Culvenor (1959) |
| 49 | 284.1857 | C15H25NO4 | - | supininea | 1.8 | s | - | (+)-Trach | Culvenor (1954) |
| 50 | 284.1856 | C15H25NO4 | - | unknown PAa,b,c | 6.7 | t | - | saturated (+)-Trach/ (-)-Vir |  |
| 51 | 286.2011 | C15H27NO4 | - | trachelanthamineb or | 4.7 | t | - | (+)-Trach | van Dam et al. (1995) |
| viridiflorinea,b | (-)-Vir |
| 52 | 298.1647 | C15H23NO5 | - | unknown PAa,b | 0.7 | h | H | saturated (+)-Trach/ (-)-Vir |  |
| 53 | 298.1645 | C15H23NO5 | - | unknown PAa,b | 1.9 | h | H | saturated (+)-Trach/ (-)-Vir |  |
| 54 | 298.2007 | C16H27NO4 | 1 | heleurinea,c | 12.5 | s | - | Heliot | Culvenor (1954) |
| unknown isomera,b,c |  |  |
| 55 | 300.1801 | C15H25NO5 | 1 | rinderine | 16.5 | h | H | (+)-Trach | Shimshoni et al. (2015) |
| echinatine | (-)-Vir |
| 56 | 312.1806 | C16H25NO5 | - | unknown PAa,b | 4.2 | h | H | saturated Heliot | **(Culvenor CC and Smith 1969)** |
| 57 | 314.1961 | C16H27NO5 | 1 | heliotrine | 12005.7 | h | H | Heliot | Culvenor et al. (1954) |
| unknown isomera,b |  |  |
| 58 | 316.1749 | C15H25NO6 | - | 5'-hydroxyrinderinea | 21.8 | h | H | (+)-Trach, 5'-OH | Shimshoni et al. (2015) |
| 59 | 328.2118 | C17H29NO5 |  | *PA not detected* |  |  |  |  |  |
|  |  | - | unknown PANOa,b | 3.1 | h | H | Heliot + CH2 |
| 60 | 330.1906 | C16H27NO6 | - | europine | 879.7 | h | H | Las | Culvenor et al. (1954) |
| 61 | 342.1911 | C17H27NO6 |  | *PA not detected* |  |  |  |  | Shimshoni et al. (2015) |
|  |  | - | 3'-acetylrinderine NOa | 0.1 | h | H | (+)-Trach, 3'-OAc |
| 62 | 342.1911 | C17H27NO6 |  | *PA not detected* |  |  |  |  |  |
|  |  | - | unknown PANOa,b | 1.7 | h | CH2O2 | Heliot |
| 63 | 356.2068 | C18H29NO6 |  | *PA not detected* |  |  |  |  |  |
|  |  | - | 2'-acetylheliotrine NOa,b | 0.1 | h | H | Heliot, 2'-OAc |
| 64 | 356.2068 | C18H29NO6 |  | *PA not detected* |  |  |  |  |  |
|  |  | - | 7-acetylheliotrine NOa,b | 5.5 | h | Ac | Heliot |
| 65 | 358.1853 | C17H27NO7 | - | 3'-acetyl-5'-hydroxyrinderinea,b or | 1.1 | h | H | (+)-Trach, 5'-OH, 3'-OAc |  |
|  | unknown isomera,b | (-)-Vir,5'-OH, 3'-OAc/ Ech, 3'-OAc |
| 66 | 358.1860 | C17H27NO7 |  | *PA not detected* |  |  |  |  |  |
|  |  | - | unknown PANOa,b | 0.3 | h | CH2O2 | Las |
| 67 | 370.2224 | C19H31NO6 |  | *PA not detected* |  |  |  |  |  |
|  |  | - | unknown PANOa,b | 0.2 | h | C3H6O2 | Heliot |
| 68 | 372.2035 | C18H29NO7 | - | 7-acetyleuropinea,b | 1.4 | h | Ac | Las | Reina et al. (1998b) |
| 69 | 372.2017 | C18H29NO7 | - | 5'-acetyleuropinea | 63.7 | h | H | Las, 5'-OAc | Shimshoni et al. (2015) |
| 70 | 382.2211 | C20H31NO6 | - | 7-angeloylrinderinea,b or | 2.1 | h | Ang | (+)-Trach | El-Shazly et al. (1996c) |
| 7-angeloylechinatinea,b or | Ang | (-)-Vir |
| 7-senecioylrinderinea,b | Sen | (+)-Trach |
| 71 | 396.2337 | C21H33NO6 | - | 7-angeloylheliotrinea | 94.0 | h | Ang | Heliot | Tosun and Tamer (2004) |
| 72 | 398.2164 | C20H31NO7 | 1 | heliosupine | 125.0 | h | Ang | Ech | Shimshoni et al. (2015) |
| unknown isomera,b | *iso* | Ech |  |
| 73 | 412.2316 | C21H33NO7 | - | lasiocarpine | 2263.4 | h | Ang | Las | Drummond (1951) |
| 74 | 428.2279 | C21H33NO8 |  | *PA not detected* |  |  |  |  |  |
|  |  | 3 | 7-(4-hydroxyl-*iso*)-9-lasiocarpic heliotridine NOa,b | 6.5 | h | *iso* + O | Las |
| unknown isomers NOa,b |
| 75 | 440.2272 | C22H33NO8 | - | 3'-acetylheliosupinea | 40.6 | h | Ang | Ech, 3'-OAc | Shimshoni et al. (2015) |
| 76 | 454.2435 | C23H35NO8 | 1 | 5'-acetyllasiocarpinea | 169.4 | h | Ang | Las, 5'-OAc | Culvenor et al. (1975) |
| isomera | *iso* | Las, 5'-OAc | Shimshoni et al. (2015) |

1 PANO is presented in the form of corresponding PA, *exception if no PA was detected*. 2 sum of PA and PANO. 3The abbreviations of the necine bases and necic acids are described in figure 2a and 2b. a This is tentatively identified. b This represents a new PA/ PANO for the species *Heliotropium europaeum*. cThe PANO was not detected.

****Figure S1. Heatmap of 1,2-unsaturated PA profiles of *E. vulgare*, *Symphytum* spp., *C. officinale* and *H. europaeum*. The upper part shows PAs esterified with a monocarboxylic acid at C9 and the bottom part shows PAs esterified with a highly branched necic acid at C9. Stereochemistry of necine bases is not considered. 1 The abbreviations of the necic acids are described in figure 2b. \* 3'O- or 5'O- esterification is possible.

****Figure S2. Matrix effect evaluation for the pyrrolizidine alkaloid heliotrine. Plot of curve I (*Hypochaeris radicata*), II (hay) and III (solvent).

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