Pyrrolizidine alkaloids in honey bee products

Kempf, M.[1], Beuerle, T.[2], Reinhard, A.[1,2],
von der Ohe[3], W., von der Ohe[3], K. and Schreier, P.[1]

[1] Lehrstuhl für Lebensmittelchemie, Universität Würzburg, Am Hubland, 97074 Würzburg
[2] Institut für Pharmazeutische Biologie, Technische Universität Braunschweig, Mendelssohnstrasse 1, 38106 Braunschweig
[3]LAVES Institut für Bienenkunde Celle

Introduction

Pyrrolizidine alkaloids (PA) comprise about 370 different structures. Their occurrence is limited to only five plant families: the Asteraceae (Senecioneae and Eupatorieae), the Boraginaceae, the Apocynaceae, the genus *Crotalaria* within the Fabaceae and certain genera of the Orchidaceae [1]. PA are consisting of two building blocks, a basic structure (necine base), mostly retronecine, which is esterified by acids (necine acids), resulting in five different PA types [1] (Fig. 1). PA occur in two major forms, a tertiary form and the corresponding *N*-oxide; PA containing a 1,2-double bond are pre-toxins and metabolically activated by the action of hepatic P-450 enzymes to acute toxic and genotoxic pyrroles [2].

Humans are directly exposed to these toxins by consumption of herbal medicine, herbal teas, dietary supplements or food containing PA-plant material. Secondary exposure was reported for food, where the upstream food chain was contaminated with PA, such as milk [3], eggs [4] or honey [5, 6].

There are several examples worldwide where PA containing plants caused severe intoxications to humans and livestock [7]. Besides the acute toxic effects, the genotoxic and tumorigenicity potential of PA was demonstrated in some eukaryotic model systems [7]. In the ´International Programme on Chemical Safety (IPCS)´ the WHO has evaluated PA and concluded that their presence as contaminants in food is a thread to human health and safety [8]. In pharmaceuticals, the use of these plants is regulated by the German Federal Health Bureau to a total PA intake of 1 µg per day for a six-week period per year, or, if six weeks are surpassed, the level is reduced to 0.1 µg total PA content per day with exclusion of pregnant or lactating women for which zero exposure is recommended [9]. Other countries have established similar policies or are in process of establishing regulations.

With regard to food the DFG-Senate Commission on Food Safety (SKLM) passed an opinion that […] *The existing data base dealing with the content of PA in honey collected from PA-containing plants* […] *as well as the data base dealing with the exposure of consumers to PA are judged to be inadequate* […]. Furthermore they reminded that […] *The main goal of future research should be the careful analytical determination of the PA content of honey and pollen* […] [10].

Initiated by this opinion, the already proven PA transfer from plants to honey has found particular attention, and reliable tools for selective and quantitative determination of PA in bee products are necessary.

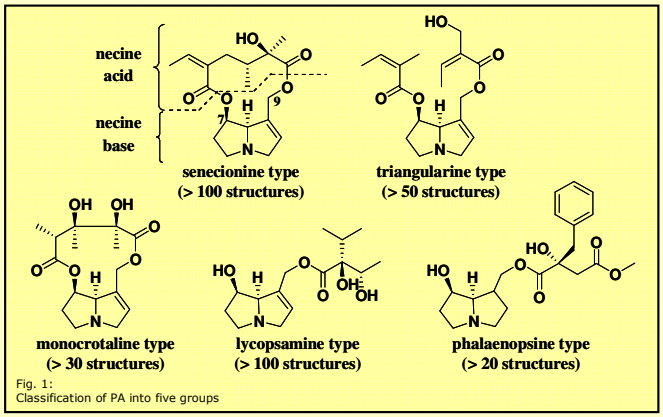

Fig. 1:
Classification of PA into five groups

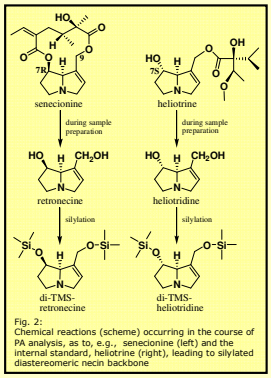

Fig. 2:
Chemical reactions (scheme) occurring in the course of PA analysis, as to, e.g., senecionine (left) and the internal standard, heliotrine (right), leading to silylated diastereomeric necin backbone

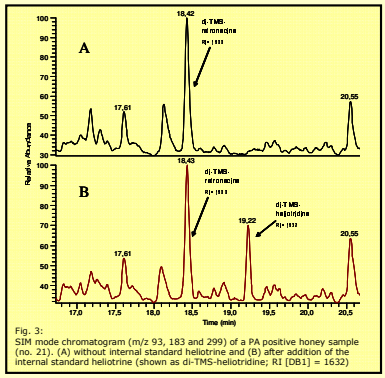

Fig. 3:
SIM mode chromatogram (m/z 93, 183 and 299) of a PA positive honey sample (no. 21). (A) without internal standard heliotrine and (B) after addition of the internal standard heliotrine (shown as di-TMS-heliotridine; RI [DB1] = 1632)

Method

To analyse PA, we elaborated a new method consisting of strong cation exchange solid-phase extraction (SCX-SPE), two reduction steps followed by silylation and subsequent capillary gas chromatography-mass spectrometry (HRGC-MS) using SIM mode [11]. This procedure transfers the PA to their common skeletal structure, i.e. retronecine, and all different PA were detected in the form of a single sum parameter (di-TMS-retronecine). Heliotrine was used as internal standard. During the workup heliotrine is converted to heliotridine - a diastereomer to retronecine (Fig. 2). Both can be separated via HRGC (Fig. 3). The procedure was validated using extracts of *Senecio vernalis* as well as authentic standards of PA and their *N*-oxides.

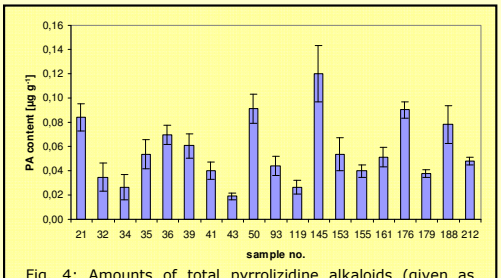

Fig. 4: Amounts of total pyrrolizidine alkaloids (given as retronecine-equivalents) in commercial honeys (n=216); only the honeys containing PA are listed [11]. Standard deviations are given (n=3).

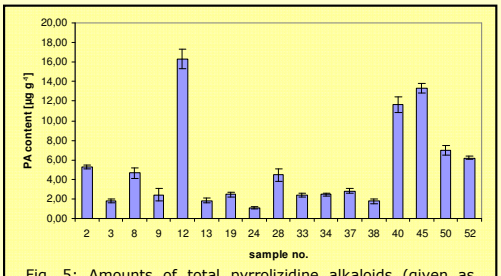

Fig. 5: Amounts of total pyrrolizidine alkaloids (given as retronecine-equivalents) in pollen products (n=55); only the samples containing PA are listed [12]. Standard deviations are given (n=4).

Conclusion

For the first time, the present method allows the sensitive and selective determination of toxic PA by measuring a sum parameter which reveals the toxic principle of the PA (1,2-double bond). The method is non-targeted and does not depend on additional information such as botanical origin, marker PA or the oxidation state of the PA (tertiary PA, *N*-oxides). The values determined especially in pollen products provoke the discussion of an international regulation of PA in food.

Results and Discussion

Our purpose was to generate a dataset on PA contamination for retail honeys and pollen products available on the German/European market. We did not apply any selection criteria on the samples. No additional information, such as apiarist interrogations about the habitats of the bee colonies or pollen analyses were available beforehand. The samples were purchased from various supermarkets in Germany and other European countries, as well as from internet stores. The new developed method was applied to 216 commercially available floral honey samples [11] and, after some modification, to 55 pollen products available as food supplements [12].

Among the honeys 94 were from Europe, 34 from Central- and South-America, 6 from USA/Canada, and 22 from Australia and New Zealand. Another 60 samples had no regional identification or were of dubious origin. These 60 samples were mostly mixtures of different proveniences labeled as "mixture from honeys of non EC-countries" or "mixture from honeys of EC-/non EC-countries". Within these 216 honeys under study 19 samples (9%) contained PA in the range from 0.019 to 0.120µg g^{-1} (Fig. 4). The average PA contamination was 0.056 µg g^{-1}. Seven out of the 19 PA-positive honeys found in our study were labeled as "non EC-countries", five were labeled as "EC-/non EC-countries", three from New Zealand, three from Central-/South America and one sample from Canada, respectively. Two honeys, including the sample with the highest amount of PA in our study (no. 145), were labeled as borage honey (New Zealand). The other 17 PA containing samples were without any conspicuous declaration. From the 94 samples which were labeled as European origin no honey was tested PA-positive [11].

Among the 55 pollen products 33 were from Europe, four from USA and Mexico, one from New Zealand, one from Asia, as well as 16 samples of not specified origin, respectively. Within these 55 pollen products 17 samples (31%) revealed a PA contamination in the range from 1.08 to 16.35 µg g^{-1} (Fig. 5). Nine out of the 17 PA-positive pollen products were from European (Spain/5, Rumania/2, Italy/1 and France/1) and the remaining eight of not specified origin, respectively. As shown by additional pollen analysis, all PA-positive pollen products exhibited a significant amount of PA-plant pollen (mostly *Echium* spp.) [12].

The per-capita consumption of honey in Europe is regarded to be 1.3g/day [13] (the worldwide highest amount). Neglecting the part of the "non-honey eaters" this level increases to 3.9g/day [13, 14]. According to the suppliers, the recommended intake for pollen products is about 10g (1-2 table spoons per day). Taking into account the genotoxic potential of the PA, it would be reasonable to reconsider also the honey results with respect to an usual amount consumed by a single person per day (2 table spoons equals 20g).

One can calculate a total PA intake for a single person by assuming a "normal" daily consumption dose of 20g for honey and 10g for pollen. In this case all but one sample tested positive for PA will exceed the limit of the current regulations of the German Federal Health Bureau for herbal pharmaceutical products (1.0 µg PA/day, restricted to a maximum of six weeks per year [9]). Especially pollen where almost one third of the samples showed PA contamination the levels of a single daily dose are in average 100 times higher as the limit for phytopharmaceuticals.

Literatur:
[1] Hartmann, T., Witte, L., in Alkaloids: Chemical and Biological Perspectives, vol. 9, Pergamon Press, Oxford, 1995.
[2] Williams, L. et al. (2002) Toxicology and Applied Pharmacology 182, 98-104.
[3] Panter, K.E., James, L.F. (1990) J. Anim. Sci. 68, 892-904.
[4] Edgar, J. A., Smith, L.W. (2000) ACS Symposium Series 745, 118-128.
[5] Deinzer, M. L. et al. (1977) Science 195, 497 –499.
[6] Culvenor, C.C.J. et al. (1981) J. Agric. Food Chem. 29, 956-960.
[7] Fu, P.P. et al. (2004) Drug Metabolism Reviews 36, 1-55.
[8] WHO, IPCS Environmental health criteria 80, Pyrrolizidine alkaloids, 1988.
[9] German Federal Health Bureau, Bundesanzeiger, 1992, June 17, 4805.
[10] DFG-Senate Commission on Food Safety (SKLM), Opinion on PA, 08.11.2002
[11] Kempf, M. et al. (2008) Mol. Nutr. Food Res., 52, 1193-1200.
[12] Kempf, M. et al. (2009) Mol. Nutr. Food Res. submitted.
[13] WHO, Food Safety Programme, WHO: Geneva, 1998 (WHO/FSF/FOS98.3).
[14] MAFF U.K., in: Food Surveillance Information Sheet 52, 1995.

This work was supported by the Deutsche Forschungsgemeinschaft (DFG), Bonn, project No. SCHR 211/22-1/23-1 and BE 3200/1-1/3-1.