HPLC–MS/MS Profiling of Tryptophan-Derived Alkaloids in Food: Identification of Tetrahydro-β-carbolinedicarboxylic Acids

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A method for selective detection of 1,2,3,4-tetrahydro-β-carbolinecarboxylic acids (THCCs) was developed based on electrospray ionization–tandem mass spectrometry coupled to liquid chromatography (HPLC–ESI–MS/MS). Low-energy collision-induced dissociation (CID) led to characteristic fragment ions due to neutral loss of 73 amu. Subsequently, constant neutral loss scanning was used for substructure specific screening of THCCs in food samples. Detection limits for HPLC–ESI–MS/MS analysis of THCCs applying neutral loss experiments were established at 100 ng mL^{-1} (ca. 2.5 pmol on column). Application of this MS/MS method enabled us to detect THCC derivatives derived from Pictet–Spengler condensation of tryptophan with α-oxo acids. Subsequently, diastereomeric 1,2,3,4-tetrahydro-β-carboline-1,3-dicarboxylic acid **3a/b**, 1-methyl-1,2,3,4-tetrahydro-β-carboline-1,3-dicarboxylic acid **4a/b**, and 1-(2′-carboxyethyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid **5a/b** were identified in alcoholic beverages, seasoning sauces, yeast extract, and fruit products for the first time. Most food samples under study contained **3a/b** and **4a/b** in significant amounts. **5a/b** was identified in soy sauce, worcestershire sauce, seasoning sauce, and yeast extract. Due to the excellent selectivity of tandem mass spectrometry coeluting tetrahydro-β-carboline derivatives could be identified unequivocally by HPLC–ESI–MS/MS.

Keywords: *Tetrahydro-β-carboline; electrospray ionization; tandem mass spectrometry; neutral loss scanning*

INTRODUCTION

1,2,3,4-Tetrahydro-β-carboline-3-carboxylic acid derivatives (THCCs) are readily formed by Pictet–Spengler condensation of tryptophan with various aldehydes or α-oxo acids (Harvey et al., 1941). THCCs have been demonstrated to inhibit monoamine oxidase, to alter the re-uptake of biogenic amines, and to interfere with benzodiazepine receptors (McKenna and Towers, 1984; Rommelspacher et al., 1994). Because of their neuropharmacological effects these alkaloids have attracted much concern; thus, their participation in pathogenesis of alcoholism and psychiatric disorders is under investigation (Rommelspacher et al., 1984; Spies et al., 1995).

Structures of THCCs can vary widely depending on both indole amine derivatives and carbonyl compounds involved in Pictet–Spengler condensation reaction. However, only 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid **1** and 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid **2a/b** have been demonstrated to occur in considerable amounts in smoked and cured meat products and food samples like wine, beer, soy sauce (Wakabayashi et al., 1983), cheese, yoghurt, and bread (Bosin et al., 1986; Adachi et al., 1991; Papavergou and Clifford, 1992; Herraiz, 1996). Besides the widespread occurrence of β-carbolines **1** and **2a/b**, condensation products of tryptophan with glycolaldehyde (Sen et al., 1995), hexanal (Arai et al., 1971), pyridoxal (Argoudelis, 1994), and isobutyraldehyde (Herraiz et al., 1993) have been described, but until now knowledge of the presence of THCCs in food samples has been rather limited. For example, no information is available on products formed by Pictet–Spengler condensation of tryptophan with α-oxo acids commonly found in several food samples. One likely explanation is the labile character of the corresponding tetrahydro-β-carbolinedicarboxylic acids which makes isolation and derivatization difficult. In order to clarify the role of these polar tryptophan derivatives in food, a reliable analytical method for detection and determination of THCCs in complex matrices was required.

Thus, we report an approach based on HPLC–ESI–MS/MS for substructure specific detection of novel THCC derivatives in food samples. In addition, results obtained by analysis of tetrahydro-β-carboline-1,3-dicarboxylic acids in alcoholic beverages, seasoning sauces, yeast extract, and fruit products are presented.

EXPERIMENTAL PROCEDURES

Apparatus. Chromatographic separation was performed by an Applied Biosystems 140b pump. For sample injection a SunChrom Triathlon autosampler (BAI, Bensheim, Germany) was used. HPLC–ESI–MS/MS analysis was performed utilizing a TSQ 7000 tandem mass spectrometer system equipped with an ESI interface (Finnigan MAT, Bremen, Germany). Data acquisition and evaluation were conducted on a DEC 5000/33 (Digital Equipment, Unterföhring, Germany) with ICIS 8.1 software (Finnigan MAT, Bremen, Germany). NMR spectra were acquired with a Bruker WM 400 spectrometer calibrating the chemical shifts utilizing the solvent signal (DMSO: 2.58 ppm for ^{1}H-NMR, 39.7 ppm for ^{13}C-NMR. MeOH: 3.5 ppm for ^{1}H-NMR, 49.0 ppm for ^{13}C-NMR) as reference.

Reagents. Water, acetonitrile, both of HPLC gradient grade, L-tryptophan, and trifluoroacetic acid (spectroscopic grade) were from Merck (Darmstadt, Germany). Glyoxylic acid, pyruvic acid, and α-oxoglutaric acid were purchased from Sigma (Steinheim, Germany). Formaldehyde, acetaldehyde, and succinic semialdehyde were obtained from Aldrich (Deisenhofen, Germany). L-[Indole-D_5]tryptophan was from Cambridge Isotope Laboratories (Andover, MA). All chemicals were of analytical purity. Membrane filters of pore size 0.2 μm were from Ziemer (Mannheim, Germany). C18 cartridges (200 mg, 3 mL) were from Varian (Harbor City, MD). Food samples were purchased at local markets.

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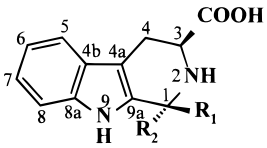
Table 1. 1,2,3,4-Tetrahydro-β-carboline-3-carboxylic Acids Identified in Food Samples



compd no.	R_1	R_2
1	H	H
2a[a]	CH_3	H
2b[a]	H	CH_3
3a[a]	H	COOH
3b[a]	COOH	H
4a[b]	CH_3	COOH
4b[b]	COOH	CH_3
5a[a]	CH_2-CH_2-COOH	H
5b[a]	H	CH_2-CH_2-COOH

[a] Assignment of absolute configuration was based on the observation that racemization at C3 is negligible while working at room temperature in aqueous media (Bailey et al., 1993). [b] Assignment of absolute configuration is exchangeable.

Reference Compounds. Synthesis of 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid **1** and 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid **2a/b** was based on the procedure of Jacobs and Craig (1936). Absolute configurations of the resulting diastereomers (1*S*,3*S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid **2a** and (1*R*,3*S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid **2b** were assigned according to published data (Yamada and Akimoto, 1969). The identity of **2a/b** was confirmed by ESI−MS, ESI−MS/MS, and NMR spectroscopy.

*1,2,3,4-Tetrahydro-β-carboline-1,3-dicarboxylic Acid (**3a/b**).* 2.5 mM L-tryptophan was dissolved in 7.5 mL of water. After addition of 1 mL of 0.5 M sulfuric acid (pH 2) and 5 mM glyoxylic acid, the reaction mixture was allowed to stand at room temperature for 2 days (Harvey et al., 1941). The precipitate consisting of the major diastereomer *cis*-1,2,3,4-tetrahydro-β-carboline-1,3-dicarboxylic acid **3b** was collected by filtration, washed with water, and dried. Both lyophilization and preparative HPLC of the *trans*-configurated isomer failed as the compound rapidly decomposed on concentration. Thus, the remaining filtrate containing a mixture of both diastereomers was concentrated by C18 solid phase extraction and eluted with CD_3OD containing 0.1% deuterated trifluoroacetic acid (TFA) (v/v) prior to NMR analysis of the *trans*-isomer. Relative configurations were determined by ^{13}C-NMR spectroscopy (Cox and Cook, 1995). Assignment of NMR signals (Table 1) was confirmed by DEPT experiments. ESI−MS, $[M + H]^+$ *m*/*z* 261; ESI−MS/MS (15 eV, 2.0 mTorr Ar), *m*/*z* 217, 188, 145. Data for *cis*-1,2,3,4-tetrahydro-β-carboline-1,3-dicarboxylic acid **3b**: ^{1}H-NMR (400 MHz, DMSO-d_6), 10.79 (s, 1H, H-9), 7.57 (d, 1H, H-5), 7.52 (d, 1H, H-8), 7.15 (dd, 1H, H-7), 7.05 (dd, 1H, H-6), 4.99 (s, 1H, H-1), 4.29 (dd, 1H, H-3), 3.28 (dd, 1H, H-4_{ax}), 3.09 (m, 1H, H-4_{eq}); $J_{5,6} = J_{7,8} = 8$ Hz, $J_{6,7} = 7$ Hz, $J_{3,4ax} = 5$ Hz, $J_{3,4eq} = 12$ Hz, $J_{4ax,4eq} = 16$ Hz; ^{13}C-NMR (100 MHz, CD_3OD + deuterated TFA), 170.70 and 167.73 (COOH and COOH′), 138.75 (C-8a), 127.09 (C-9a), 124.04 (C-4b), 120.96 (C-7), 119.08 (C-6), 118.35 (C-5), 112.92 (C-8), 107.80 (C-4a), 56.40, 56.10 (C-1 and C-3), 23.28 (C-4). Data for *trans*-1,2,3,4-tetrahydro-β-carboline-1,3-dicarboxylic acid **3a**: ^{13}C-NMR (100 MHz, CD_3OD + deuterated TFA), 54.70, 54.25 (C-1 and C-3), 22.79 (C-4).

*1-Methyl-1,2,3,4-tetrahydro-β-carboline-1,3-dicarboxylic Acid (**4a/b**).* **4a/b** was synthesized as described above using 5 mM pyruvic acid as carbonyl compound. The major diastereomer precipitated. The minor diastereomer was isolated by preparative HPLC (Eurospher 100 C18 column 16 × 250 mm, 5 μm; water−acetonitrile 8−2 containing 0.05% (v/v) TFA). NMR signal assignment (Table 1) was confirmed by DEPT, CH-COSY, and HMBC experiments. ESI-MS, $[M + H]^+$ *m*/*z* 275; ESI−MS/MS (15 eV, 2.0 mTorr Ar), *m*/*z* 258, 231, 202, 184, 159, 146, 130. Major diastereomer: ^{1}H-NMR (400 MHz, DMSO-d_6), 10.78 (s, 1H, H-9), 7.48 (d, 2H, H-5 and H-8), 7.12 (dd, 1H, H-7), 7.03 (dd, 1H, H-6), 4.17 (m, 1H, H-3), 3.22 (dd, 1H, H-4_{ax}), 2.96 (dd, 1H, H-4_{eq}), 1.81 (s, 3H, 1-CH_3); $J_{5,6} = J_{7,8} = 8$ Hz, $J_{6,7} = 7$ Hz, $J_{3,4ax} = 4$ Hz, $J_{3,4eq} = 12$ Hz, $J_{4ax,4eq} = 15$ Hz; ^{13}C-NMR (100 MHz, DMSO-d_6), 169.83 (COOH and COOH′), 136.30 (C-8a), 133.70 (C-9a), 125.92 (C-4b), 121.22 (C-7), 118.62 (C-6), 117.73 (C-5), 111.74 (C-8), 104.96 (C-4a), 55.75 (C-1), 52.15 (C-3), 24.49 (CH_3), 24.05 (C-4). Minor diastereomer: ^{1}H-NMR (400 MHz, DMSO-d_6), 11.16 (s, 1H, H-9), 7.49 (d, 1H, H-5), 7.41 (d, 1H, H-8), 7.13 (dd, 1H, H-7), 7.04 (dd, 1H, H-6), 4.15 (dd, 1H, H-3), 3.16 (dd, 1H, H-4_{ax}), 2.83 (dd, 1H, H-4_{eq}), 1.80 (s, 3H, 1-CH_3); $J_{5,6} = J_{7,8} = 8$ Hz, $J_{6,7} = 7$ Hz, $J_{3,4ax} = 4$ Hz, $J_{3,4eq} = 12$ Hz; $J_{4ax,4eq} = 15$ Hz; ^{13}C-NMR (100 MHz, DMSO-d_6), 172.64, 172.52 (COOH and COOH′), 136.50 (C-8a), 133.86 (C-9a), 125.81 (C-4b), 121.06 (C-7), 118.52 (C-6), 117.82 (C-5), 111.28 (C-8), 105.90 (C-4a), 60.19 (C-1), 54.08 (C-3), 25.07 (CH_3), 24.00 (C-4).

*1-(2′-Carboxyethyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic Acid (**5a/b**).* Compound **5a/b** was synthesized by reaction of L-tryptophan and succinic semialdehyde. The major diastereomer *cis*-1-(2′-carboxyethyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid **5a** precipitated; the filtrate, a mixture of both diastereomers, was lyophilized prior to NMR analysis. Relative configuration of the diastereomers was determined by ^{13}C-NMR (Cox and Cook, 1995). Signal assignment (Table 1) was confirmed by CH−COSY experiments. ESI−MS, $[M + H]^+$ *m*/*z* 289; ESI−MS/MS (15 eV, 2.0 mTorr Ar), *m*/*z* 272, 216, 188, 130. Data for *cis*-1-(2′-carboxyethyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid **5a**: ^{1}H-NMR (400 MHz, CD_3OD), 7.69 (d, 1H, H-5), 7.57 (d, 1H, H-8), 7.36 (dd, 1H, H-7), 7.27 (dd, 1H, H-6), 5.02 (m, 1H, H-1), 4.59 (dd, 1H, H-3), 3.67 (dd, 1H, H-4_{ax}), 3.34 (dd, 1H, H-4_{eq}), 2.86 (m, 3H, H-2′ and H-$1'_{ax}$), 2.50 (m, 1H, H-$1'_{eq}$); $J_{5,6} = J_{7,8} = 8$ Hz, $J_{6,7} = 7$ Hz, $J_{3,4ax} = 5$ Hz, $J_{3,4eq} = 12$ Hz, $J_{4ax,4eq} = 16$ Hz; ^{13}C-NMR (100 MHz, DMSO-d_6 + deuterated TFA), 174.07, 170.53 (COOH and COOH′), 136.77 (C-8a), 129.64 (C-9a), 125.88 (C-4b), 122.35 (C-7), 119.54 (C-6), 118.47 (C-5), 111.77 (C-8), 105.67 (C-4a), 55.53, 53.42 (C-1 and C-3), 29.45 (CH_2-2′), 26.08 (CH_2-1′), 22.40 (C-4). Data for *trans*-1-(2′-carboxyethyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid **5b**: ^{13}C-NMR (100 MHz, DMSO-d_6 + deuterated TFA), 51.92, 51.34 (C-1 and C-3).

Analytical Procedures. *Sample Preparation.* Prior to any preconcentration step food samples were spiked with L-tryptophan-d_5 (10 μg mL^{-1}) for monitoring artifact formation during sample handling (Gutsche et al., 1996). Seasoning sauce, soy sauce, and worcestershire sauce were filtered through membrane filters of pore size 0.2 μm, and the resulting solutions were directly subjected to HPLC−ESI−MS/MS analysis. All other samples were concentrated by C18 solid phase extraction as follows: Aliquots of alcoholic beverages and fruit products (normally 10 mL) were diluted to 20 mL end-volume with distilled water. In the case of yeast extract and caramel color, the sample (1−10 g) was diluted to 20 mL with distilled water and centrifuged, and the supernatant was used for analysis. Each sample was adjusted to pH 1 by addition of 2 M hydrochloric acid and subjected to solid phase extraction (SPE) (Visiprep SPE vacuum manifold system; Supelco, Bellefonte, MD, U.S.A.). SPE was performed with C18 cartridges (200 mg) conditioned with 3 mL of methanol and 6 mL of water. After application of the acidified sample and washing with 3 mL of water adjusted to pH 1 with HCl, each column was eluted (0.2 mL/min) with 1 mL of methanol containing 1% TFA (v/v). The eluate was evaporated under a gentle stream of nitrogen and redissolved in 100 μL of aqueous (90 % (v/v)) acetonitrile.

Mass Spectrometric Analysis of Tetrahydro-β-carbolinecarboxylic Acids. Chromatographic separation for HPLC−ESI−MS/MS was performed on an Eurospher 100 C18 column (100 × 2.0 m i.d., 5 μm) (Knauer, Berlin, Germany) using a binary gradient. Solvent A was 0.05% TFA in water (v/v), solvent B was 0.05% TFA in acetonitrile (v/v). HPLC was programmed as follows: pressurizing with 50% B, equilibration time 5 min at 10% solvent B and linear gradient elution (0 min, 10% B; 20 min, 30% B; 30 min, 50% B). The flow rate was 200 μL min^{-1} and the injection volume was 5 μL, respectively. For pneumatically assisted electrospray ionization, the spray

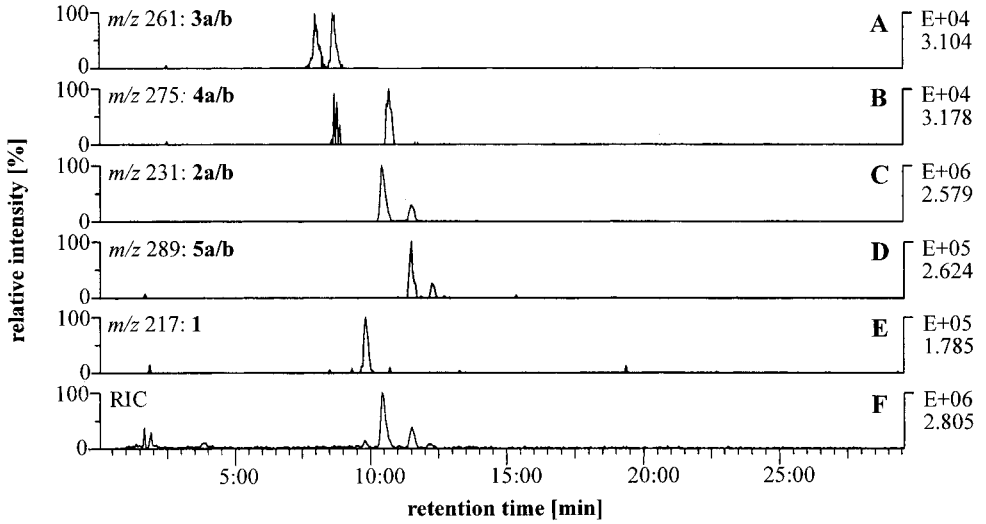


Figure 1. HPLC−MS/MS analysis of soy sauce, constant neutral loss scanning (−73 amu; 19 eV; 2.0 mTorr Ar). (A − E) Reconstructed ion chromatograms showing precursor ions $[M + H]^+$ of compounds **1**−**5**. (F) Reconstructed ion chromatogram (RIC).

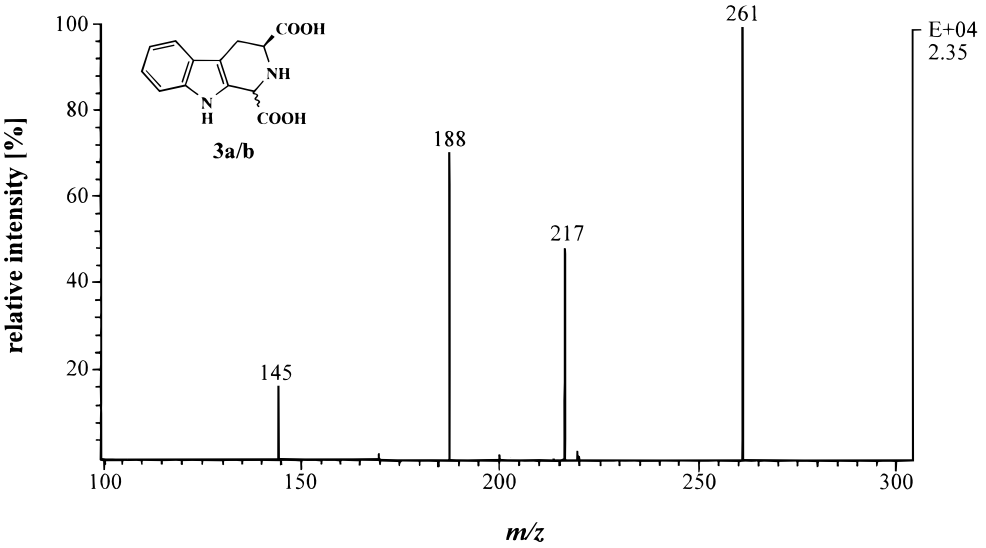


Figure 2. Product ion spectrum of 1,2,3,4-tetrahydro-β-carboline-1,3-dicarboxylic acid **3a/b**, precursor ion m/z 261 $[M + H]^+$ (15 eV, 2.0 mTorr Ar).

capillary voltage was set to 3.5 kV and the temperature of the heated inlet capillary was 220 °C. Nitrogen served both as sheath (50 psi) and auxiliary gas (10 units). Positive ions were detected with a total scan duration of 1.0 s for a single full spectrum. MS/MS experiments, such as product ion scanning (mass range 20−350 amu) and neutral loss experiments (scanning from 200 to 350 amu), were performed at a collision gas pressure of 2.0 mTorr Ar with a total scan duration of 3.0 s for a single spectrum. Quantitative evaluations were carried out using standard solutions (0, 100, 1000, and 10000 ng/mL) of reference compound **3b** in H_2O for external calibration. Values were calculated from signal intensities of the respective $[M + H]^+$ ions as obtained by HPLC−ESI−MS analysis.

RESULTS AND DISCUSSION

Initial experiments had revealed that the electrospray process could effectively ionize tetrahydro-β-carboline-3-carboxylic acids such as **1** and **2a/b**; in the positive mode, exclusively protonated molecules $[M + H]^+$ were obtained. In addition, low-energy CID of protonated molecules had been demonstrated to yield characteristic product ion spectra for tetrahydro-β-carbolines, i.e., the most abundant product ions were formed by neutral loss of the iminoacetic acid moiety $C_2H_3NO_2$ (−73 amu) due to retro-Diels−Alder fragmentation (Gutsche et al., 1996).

In order to extend our knowledge to the occurrence and relevance of tetrahydro-β-carboline derivatives other than **1** and **2a/b** we analyzed numerous food samples by means of HPLC coupled to tandem mass spectrometry. The application of constant neutral loss scanning in combination with subsequent product ion experiments directly led to the substructure specific identification ("profiling") of various Pictet−Spengler condensation products in complex matrices such as fermented beverages, seasoning sauces, yeast extract, and fruit products.

Detection limits for HPLC−ESI−MS/MS analysis of THCCs by means of neutral loss scanning were established at 100 ng mL^{-1} (ca. 2.5 pmol on column) using standard solutions of reference compounds. As a representative example the profile of THCCs from soy sauce analysis is outlined in Figure 1. In addition to protonated molecular ions $[M + H]^+$ of well-documented 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid **1** (m/z 217) and 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-car-

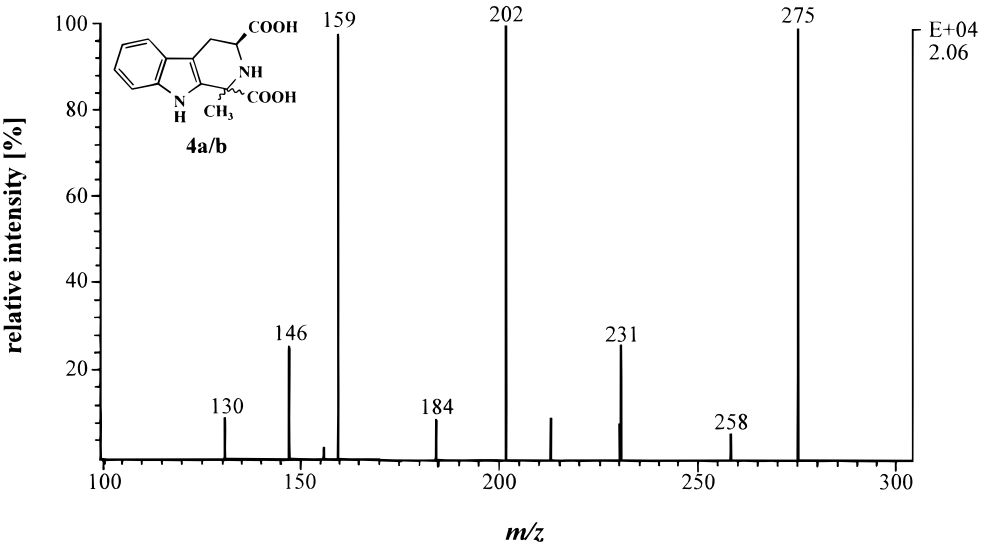


Figure 3. Product ion spectrum of 1-methyl-1,2,3,4-tetrahydro-β-carboline-1,3-dicarboxylic acid **4a/b**, precursor ion *m/z* 275 $[M + H]^+$ (15 eV, 2.0 mTorr Ar).

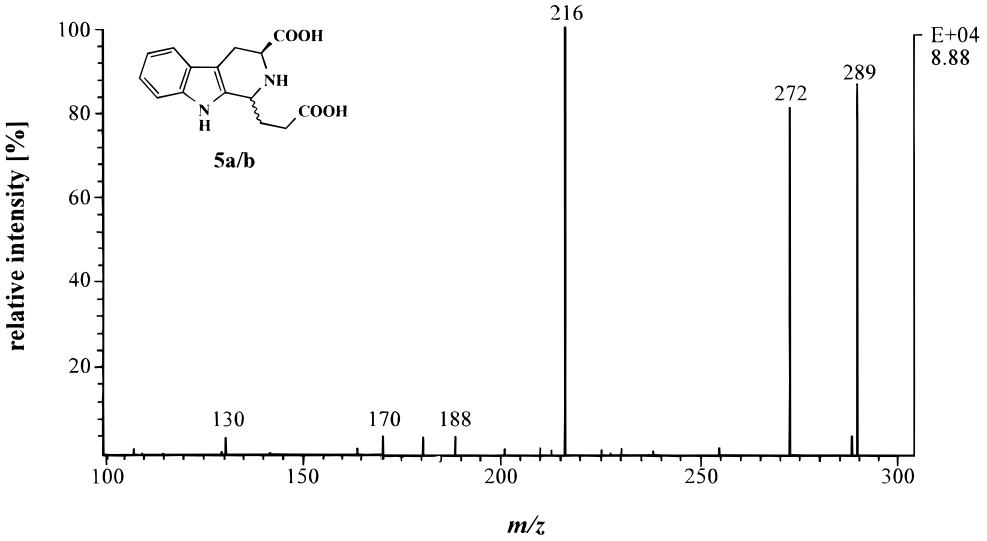


Figure 4. Product ion spectrum of 1-(2′-carboxyethyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid **5a/b**, precursor ion *m/z* 289 $[M + H]^+$ (15 eV, 2.0 mTorr Ar).

boxylic acid **2a/b** (*m/z* 231) (traces C and E, Figure 1), precursor ions *m/z* 261, *m/z* 275, and *m/z* 289 revealed for the first time the presence of additional THCC derivatives (traces A, B, and D, Figure 1). Product ion spectra of these putative tetrahydro-β-carbolines as obtained by low-energy CID showed characteristic fragmentation patterns dominated by the loss of 73 amu (Figures 2−4). In addition, product ions *m/z* 217 (Figure 2; **3a/b**) and *m/z* 231 (Figure 3; **4a/b**) apparently resulted from the loss of CO_2 (−44 amu) characteristic for carboxylic acids; product ions *m/z* 145 ($[M + H - 116]^+$) of **3a/b** (Figure 2) and *m/z* 159 ($[M + H - 116]^+$) of **4a/b** (Figure 3) were indicative of the loss of the indole moiety.

Under chromatographic conditions selected in the reconstructed ion chromatogram RIC (Figure 1, trace F) as well as in the fluorescence chromatogram (excitation 290 nm, emission 360 nm, data not shown) diastereomers of 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid **2a/b** were separated neither from the second eluting diastereomer of **4** nor from the first eluting diastereomer of **5**. Additionally, the first eluting diastereomer of **4** and the second eluting diastereomer of **3** could not be differentiated by chromatography. Therefore, only application of tandem mass spectrometry provided an approach for the reliable assignment of numerous structurally related THCC derivatives in complex food samples as coeluting tetrahydro-β-carboline derivatives were identified unequivocally by HPLC−ESI−MS/MS.

On the basis of structural information as implied by tandem mass spectrometry of putative THCC derivatives, authentic reference compounds were synthesized and characterized spectroscopically. Upon reaction with glyoxylic acid or pyruvic acid, L-tryptophan yielded diastereomers of 1,2,3,4-tetrahydro-β-carboline-1,3-dicarboxylic acid **3a/b** and 1-methyl-1,2,3,4-tetrahydro-β-carboline-1,3-dicarboxylic acid **4a/b**, respectively (Table 1). In contrast, reaction of L-tryptophan and α-oxoglutaric acid led to a complex mixture of several unstable conjugates. 1-(2′-Carboxyethyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid **5a/b** was identified as the major product by HPLC−ESI−MS/MS analysis. Unfortunately, **5a/b** rapidly decomposed during the isolation procedure. Thus, **5a/b** was synthesized directly by reaction of L-tryptophan and succinic semialdehyde. Identity of purified 1,2,3,4-tetrahydro-β-carbolines **3a/b**, **4a/b**, and **5a/b** was established by DEPT−,

Table 2. Novel Tetrahydro-β-carbolines in Food[a]

food sample	3a/b	4a/b	5a/b
seasoning sauce	+++	+++	+++
soy sauce	+++	+++	+++
worcestershire sauce	+	+	+
yeast extract	++	++	+
white wine	+	+	nd
red wine	+	+	nd
vinegar 1	+	+	nd
vinegar 2	+	+	nd
sherry	+	+	nd
beer	+	+	nd
juice prepared from dried plums	+	nd	nd
fruit syrup	+	+	nd
caramel color	nd	nd	nd

[a] Symbols: +++, >10 μg/mL; ++ >500 ng/mL; +, >10 ng/mL; nd; not detectable.

CH−COSY−, and HMBC−NMR experiments. Subsequently, analytical data such as retention times, protonated molecular ions, and characteristic product ion spectra of synthesized reference compounds confirmed identification of novel THCC derivatives in food samples under study. Any artifactual formation of THCCs during sample preparation could be excluded because no d_4-labeled THCC derivatives were detectable after addition of L-tryptophan-d_5 prior to preconcentration (Gutsche et al., 1996).

In order to clarify the relevance of the newly identified THCCs numerous food samples including seasoning sauce, alcoholic beverages, vinegar, and yeast extract were analyzed. Results are summarized in Table 2. Notably, most samples under study contained dicarboxylic acids **3a/b** and **4a/b** together with established THCCs **1** and **2a/b**. **5a/b** was identified in soy sauce, worcestershire sauce, seasoning sauce, and yeast extract.

CONCLUSION

Tetrahydro-β-carboline-1,3-dicarboxylic acids **3a/b** and **4a/b** as well as 1-(2′-carboxyethyl) derivatives **5a/b** were identified for the first time in numerous food samples and underlined the relevance of Pictet−Spengler condensation for the formation of tryptophan-derived degradation products from α-oxo acids. In addition, HPLC−MS/MS was demonstrated to efficiently facilitate studies examining the structural variety of tryptophan-derived alkaloids. In combination with research on nutritional biochemistry and toxicology of THCC derivatives **3a/b**, **4a/b**, and **5a/b**, HPLC−MS/MS will provide a reliable analytical basis for elucidating the relevance of individual β-carbolines in food.

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LITERATURE CITED

Adachi, J.; Mizoi, Y.; Naito, T.; Ogawa, Y.; Uetani, Y.; Ninomiya, I. Identification of tetrahydro-β-carboline-3-carboxylic acid in foodstuffs, human urine and human milk. *J. Nutr.* **1991**, *121*, 646−652.

Arai, S.; Abe, M.; Yamashita, M.; Kato, H.; Fujimaki, M. Applying proteolytic enzymes on soybean. *Agric. Biol. Chem.* **1971**, *35*, 552−559.

Argoudelis, C. J. In vitro reaction of L-tryptophan and vitamin B_6. Synthesis of the corresponding β-tetrahydrocarbolines. *J. Agric. Food Chem.* **1994**, *42*, 2372−2375.

Bailey, P. D.; Hollinshead, S. P.; McLay, N. R.; Morgan, K.; Palmer, S. J.; Prince, S. N.; Reynolds, C. D.; Wood, S. D. Diastereo- and enantioselectivity in the Pictet−Spengler reaction. *J. Chem. Soc., Perkin Trans. 1* **1993**, 431−439.

Bosin, T. R.; Krogh, S.; Mais, D. Identification and quantitation of 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid and 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid in beer and wine. *J. Agric. Food Chem.* **1986**, *34*, 843−847.

Cox, E. D.; Cook, J. M. The Pictet−Spengler condensation: A new direction for an old reaction. *Chem. Rev.* **1995**, *95*, 1797−1842.

Gutsche, B.; Herderich, M. High performance liquid chromatography−electrospray ionization−tandem mass spectrometry (HPLC−ESI−MS/MS) for the analysis of 1,2,3,4-tetrahydro-β-carboline derivatives. *J. Chromatogr. A* **1997**, *767*, 101−106.

Harvey, D. G., Miller, E. J., Robsen, W. The Adamkiewicz, Hopkins and Cole, and Rosenheim tests for Tryptophan. *J. Chem. Soc.* **1941**, 153−158.

Herraiz, T. Occurrence of Tetrahydro-β-carboline-3-carboxylic acids in commercial foodstuffs. *J. Agric. Food Chem.* **1996**, *44*, 3057−3065.

Herraiz, T.; Huang, Z.; Ough, C. S. 1,2,3,4-Tetrahydro-β-carboline-3-carboxylic acid and 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid in wines. *J. Agric. Food Chem.* **1993**, *41*, 455−459.

Jacobs, W. A.; Craig, L. C. The ergot alkaloids: The synthesis of 4-carboline carbonic acids. *J. Biol. Chem.* **1936**, *113*, 759−765.

McKenna, D. J.; Towers, G. H. N. Biochemistry and pharmacology of tryptamines and β-carbolines: A Minireview. *J. Psychoact. Drugs* **1984**, *16*, 347−358.

Papavergou, E.; Clifford, M. N. Tetrahydro-β-carbolinecarboxylic acids in smoked foods. *Food Addit. Contam.* **1992**, *9*, 83−95.

Rommelspacher, H.; Damm, H.; Strauss, S.; Schmidt, G. Ethanol induces an increase of harman in the brain and urine of rats. *Naunyn−Schmiedeberg's Arch. Pharmacol.* **1984**, *327*, 107−113.

Rommelspacher, H.; May, T.; Salewski, B. Harman (1-methyl-β-carboline) is a natural inhibitor of monoamine oxidase type A in rats. *Eur. J. Pharmacol.* **1994**, *252*, 51−59.

Sen, N. P.; Seaman, S. W.; Lau, P. P.-Y.; Weber, D. Determination and occurrence of various tetrahydro-β-carboline-3-carboxylic acids and the corresponding *N*-nitroso compounds in foods and alcoholic beverages. *Food Chem.* **1995**, *54*, 327−337.

Spies, C. D.; Rommelspacher, H.; Schnapper, C.; Muller, C.; Marks, C.; Berger, G.; Conrad, C.; Blum, S.; Specht, M.; Hannemann, L.; Striebel, H. W.; Schaffartzik, W. β-Carbolines in chronic alcoholics undergoing elective tumor resection. *Alcoholism−Clin. Exp. Res.* **1995**, *19*, 969−976.

Wakabayashi, K.; Ochiai, M.; Saito, H.; Tsuda, M.; Suwa, Y.; Nagao, M.; Sugimura, T. Presence of 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, a precursor of a mutagenic nitroso compound, in soy sauce. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 2912−2916.

Yamada, S.; Akimoto, H. Reductive decyanization of α-amino nitriles with $NaBH_4$: A new synthetic approach to isoquinoline- and indole-alkaloids. *Tetrahedron Lett.* **1969**, *36*, 3105−3108.

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