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# The role of methylglyoxal in the non-enzymatic conversion of tryptophan, its methyl ester and tryptamine to 1-acetyl-β-carbolines

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**Abstract**—Non-enzymatic modification of L-tryptophan (**1**) and its metabolites and derivatives with aldehydes, via the Pictet–Spengler reaction, affords β-carbolines. Here we demonstrate that methylglyoxal (**2**) generates 1-acetyl-β-carbolines from tryptophan (**1**), from its methyl ester (**6**) and from tryptamine (**4**); however, **2** did not generate 1-(1-hydroxyethyl)-β-carboline derivates. HPLC analysis of model reaction systems showed formation of 1-acetyl-β-carboline (**3**) and 1-acetyl-β-carboline-3-carboxylic acid (**5**) during incubation of **1** and **2**, at pH 5.7 and 7.4, at 100 °C, and only **5** at 37 °C, at the same pH values, with limited access of oxygen. Aerobic conditions caused higher formation of **3** at 37 °C at both pH values, while, at higher temperature, the same effect was only observed at pH 5.7. Lack of oxygen did not much influence the formation of **3** or **5** at both pH and temperature values, in comparison with the formation at limited access of oxygen. Incubation of **2** and **6** generated methyl-1-acetyl-β-carboline-3-carboxylate (**7**) together with **3** and **5** as a result of hydrolysis of **6** into **1** and, partially, **7** into **5**, while in incubation mixtures of **2** and **4** only unstable 1-acetyl-3,4-dihydro-β-carboline (**8**) was observed. Incubation of **1** with D-glucose as well as incubation of tryptophan with Amadori product **18** under similar conditions did not generate carbolines **3** or **5**. For the first time, we were able to demonstrate the presence of 1-acetyl-β-carboline-3-carboxylic acid (**5**) in some commercially available ketchups and in previously heated tomato concentrate.


## 1. Introduction

Tryptophan, an essential nutrition ingredient, is a building block of proteins and the precursor, not only of a variety of biologically active compounds (serotonin, melatonin, tryptamine, quinolinic, and kynurenic acids), but also of the coenzymes, NAD and NADP.[1] Under physiological conditions, and during thermal food processing or storing, tryptophan, like other amino acids, undergoes non-enzymatic modifications with reducing carbohydrates (non-enzymatic glycation) via the Maillard and/or the Pictet–Spengler reactions (Scheme 1). The Maillard reaction is initiated by nucleophilic attack of the amino group of an amino acid at a sugar carbonyl group, leading to a reversible Schiff base which spontaneously rearranges to form more stable, covalently bound, Amadori rearrangement products. The latter undergo further rearrangements to form irreversibly linked adducts called advanced glycation end products (AGEs).[2] In the Pictet–Spengler reaction the Schiff base undergoes ring closure resulting in the formation of tetrahydro-β-carbolines and β-carbolines,[3] a group of compounds with a large array of pharmacological activities.[4] These compounds have recently been detected in a variety of food and biological matrices.[5]

We recently showed that methylglyoxal, a physiological metabolite and a key intermediate in the Maillard reaction,[6–9] and about 20,000 times more reactive than glucose in glycation reactions,[10,11] could spontaneously modify tryptophan, its methyl ester and tryptamine via the Pictet–Spengler reaction to yield the corresponding β-carbolines.[12]

To gain more insight into the possible formation of these β-carbolines during food processing and storage, as well as under physiological temperature and pH

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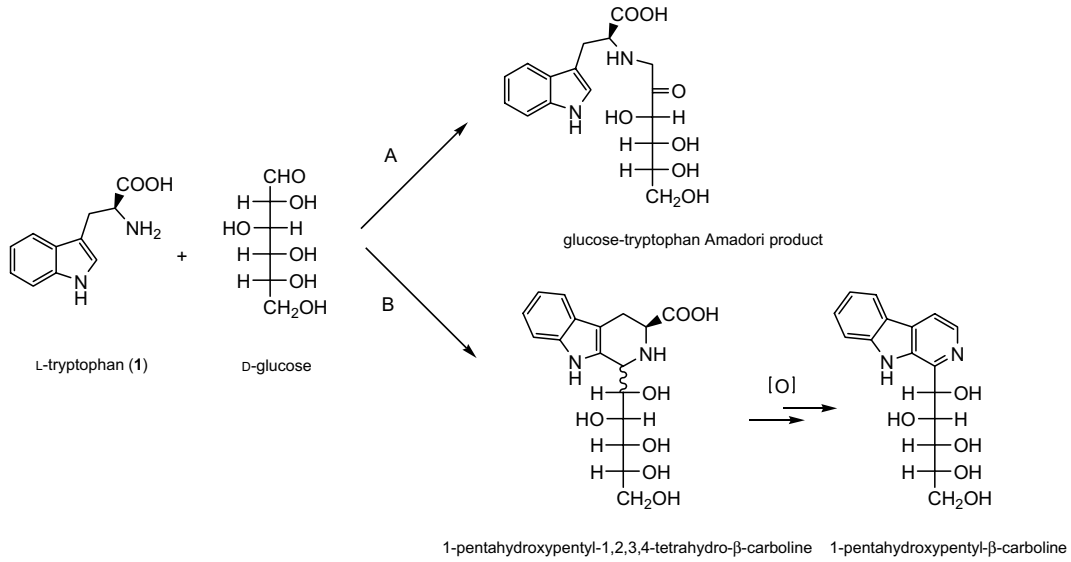


**Scheme 1.** Tryptophan in the Maillard (Path A) and Pictet–Spengler (Path B) reaction.

value, here we describe methods for the laboratory synthesis of β-carbolines derived from tryptophan (**1**), tryptamine (**4**) and tryptophan methyl ester (**6**) with methylglyoxal (**2**), together with a detailed study of their generation in different model systems and identification of 1-acetyl-β-carboline-3-carboxylic acid (**5**) in a ketchup and a tomato concentrate after thermal treatment.

## 2. Results

### 2.1. Standards synthesis

Figure 1 summarizes the syntheses performed reacting methylglyoxal (**1**) with L-tryptophan (**2**), tryptamine (**4**) or L-tryptophan methyl ester (**6**) under conditions characteristic for the Pictet–Spengler reaction (acidic aqueous medium), along with a number of alternative procedures used to prepare the desired β-carbolines.

As we recently communicated, acid-catalyzed condensation of methylglyoxal (**2**) with L-tryptophan (**1**) afforded a mixture of 1-acetyl-β-carboline (**3**) and 1-acetyl-β-carboline-3-carboxylic acid (**5**). Replacing **1** by its methyl ester (**6**) led to the formation of methyl-1-acetyl-β-carboline-3-carboxylate (**7**). Replacement by tryptamine (**4**) afforded partially saturated 1-acetyl-3,4-dihydro-β-carboline (**8**), which required exposure to alkaline conditions to convert to the fully aromatized product **3**.[12]

As it is well known that all the above β-carbolines arise via tetrahydro-β-carbolines as intermediates, we postulated that the latter should be present in the described reaction mixtures. However, addition of potassium dichromate, as a known initiator of tetrahydro-β-carboline oxidation into the corresponding β-carbolines,[13] to reaction mixtures containing L-tryptophan (**1**) and methylglyoxal (**2**) did not increase the yield of 1-acetyl-β-carboline (**3**), likely because 1-acetyl-1,2,3,4-tetrahydro-β-carboline underwent spontaneous oxidative aromatization immediately following the ring closure. This concept is corroborated by experiments on the reduction of tryptamine-derived β-carboline **8** with sodium cyanoborohydride. TLC of the crude reaction mixture confirmed complete consumption of **8**, accompanied by the formation of a new compound. However, after removal of the sodium cyanoborohydride, only starting compound **8** was isolated in almost quantitative yield.

In addition to the formation of 1-acetyl-β-carbolines, in the above reactions, the corresponding 1-(1-hydroxyethyl)-β-carbolines were expected as by-products, as it was already shown that direct condensation of phenylglyoxal or methylglyoxal with L-tryptophan affords 1-(1-hydroxybenzyl)- or 1-(1-hydroxyethyl)-β-carbolines, respectively.[14,15] Considering the complexity of the previously described reaction mixtures, we attempted to synthesize the hydroxyethyl derivates using the following alternative approaches. First we resorted to the Bischler–Napieralski reaction. Amides **10** and **12** required for this purpose were prepared by *N*,*N*-dicyclohexylcarbodiimide (DCC) mediated condensation of lactic acid (**9**) with tryptamine (**4**) or L-tryptophan methyl ester (**6**), in yields of 34 and 36.4%. Subsequent acetylation of the hydroxyl groups in **10** and **12** afforded **11** and **13** in quantitative yield. Amides **10**, **11**, and **13** were subjected to Bischler–Napieralski cyclization. However, refluxing amide **11** in $POCl_3$,[16] afforded 1-acetyl-3,4-dihydro-β-carboline (**8**) ($\eta$ = 13.4%), instead of the expected 1-hydroxyethyl analogue. Refluxing amide **10** in TFA,[17] gave the same (**8**) product, but in much lower yield ($\eta$ = 2%). Even though the reaction mixtures were treated with saturated aqueous $NaHCO_3$, the formation of **8** was most likely the result of deacetylation and subsequent oxidation of the deprotected hydroxyl group induced by acid and heating during the reaction of $POCl_3$ or TFA with water. Submission of amide **13** to the Bischler–Napieralski reaction in $POCl_3$ resulted in reductive dehydration and formation of methyl-1-ethyl-β-carboline-3-carboxylate (**14**) ($\eta$ = 33%). Using $PCl_5$[18] in the same reaction scheme starting with

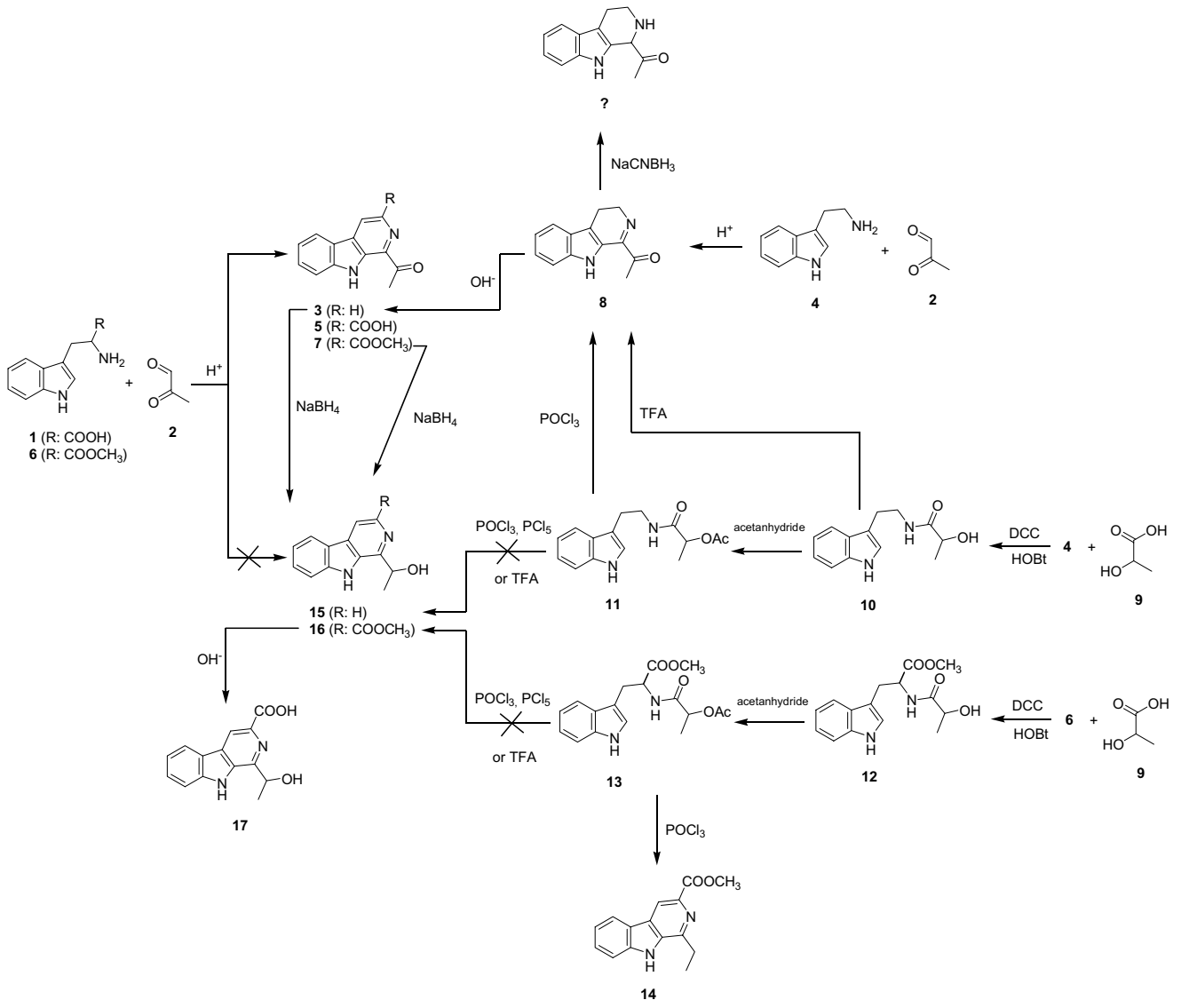


**Figure 1.** Synthesis of 1-acetyl- and 1-(1-hydroxyethyl)-β-carbolines.

amides **11** or **13** did not result in β-carboline formation (Fig. 1).

Because the Bischler–Napieralski reaction, under the previously described conditions, failed for 1-(1-hydroxyethyl)-β-carbolines **15**–**17**, compounds **15** and **16** were prepared by reduction of the corresponding acetyl derivates **3** and **7** with sodium borohydride, in yields of 45 and 79%, respectively, while **17** was obtained by alkaline hydrolysis of ester **16**. However, HPLC and TLC analysis of the respective incubation mixtures showed that 1-(1-hydroxyethyl)-β-carbolines cannot be generated by direct condensation of L-tryptophan (**1**), tryptamine (**4**), and L-tryptophan methyl ester (**6**) with methylglyoxal (**2**) (Fig. 1).

### 2.2. Formation of methylglyoxal (2) derived 1-acetyl-β-carbolines in model systems under physiological and food storage or processing temperatures and pH values

As shown in Section 2.1, 1-acetyl-β-carbolines can be formed in spontaneous non-enzymatic reactions of L-tryptophan (**1**), its methyl ester **6** or its metabolite **4** with methylglyoxal (**2**) under acidic conditions. Thus amines **1**, **4**, and **6** were incubated with methylglyoxal (**2**) in 50 mM phosphate buffer, at pH 5.7 and 7.4, at 37 and 100 °C. In addition, with the aim to examine the role of oxidative processes in the formation of β-carbolines **3** and **5** and their distribution following interaction of **1** and **2**, incubations were also conducted under conditions with different availability of oxygen.

When L-tryptophan (**1**) and methylglyoxal (**2**) were reacted in airtight closed vessels (limited oxygen availability; Fig. 2), at 37 °C, 1-acetyl-β-carboline-3-carboxylic acid (**5**) was detected at both examined pH values, while 1-acetyl-β-carboline (**3**) was found only at pH 5.7, after 3 days (Fig. 2A and B). After the first day of incubation, the same amount of β-carboline **5** was obtained at both pH values; further accumulation of **5** was only observed at pH 7.4. At 100 °C, **3** and **5** were formed at both pH values, (Fig. 2C and D), but while the amount of **5** was the same, in both cases, the formation of β-carboline **3** was enhanced at the higher pH (Fig. 2D).

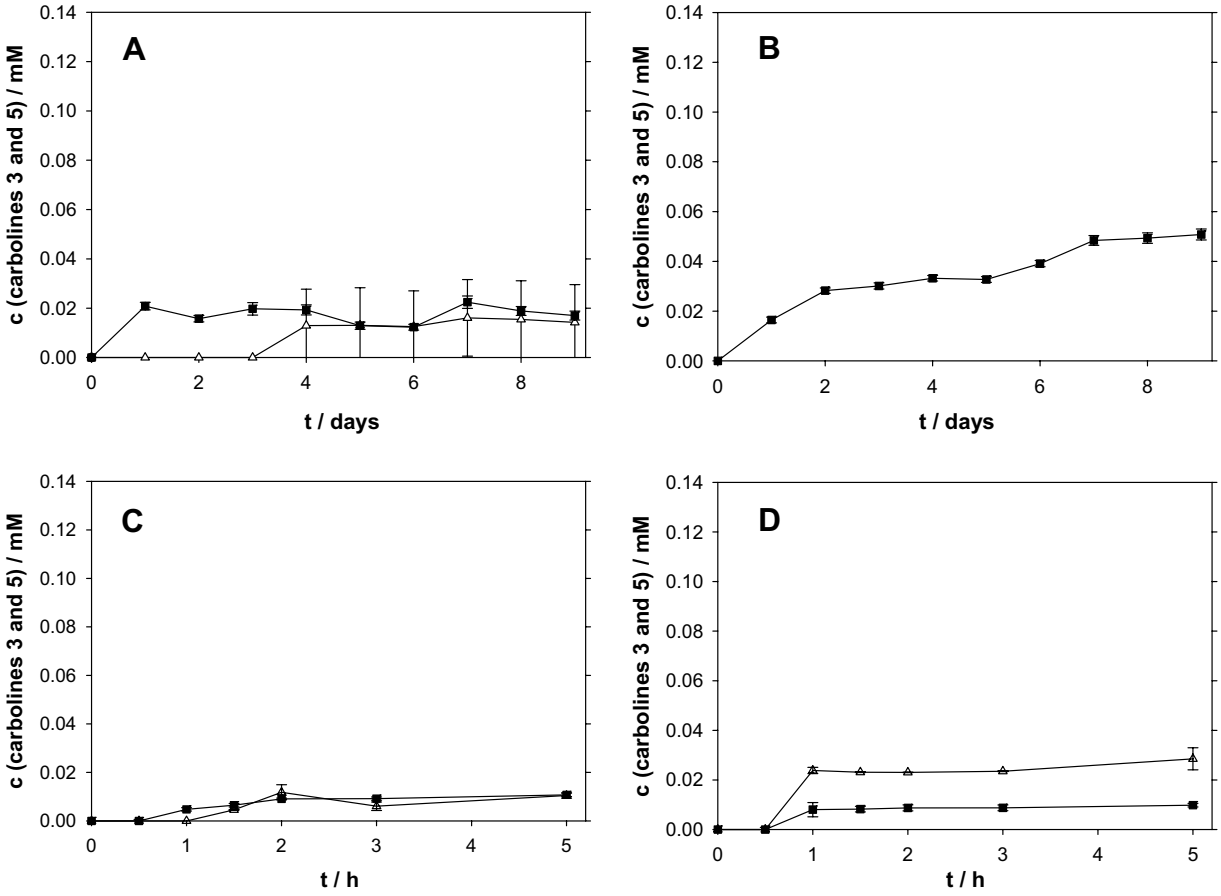


**Figure 2.** Formation of 1-acetyl-β-carboline (**3**; △) and 1-acetyl-β-carboline-3-carboxylic acid (**5**; ■) during incubation of L-tryptophan (**1**) with methylglyoxal (**2**) in phosphate buffer at pH 5.7 (A and C) and 7.4 (B and D) at 37 °C (A and B) and 100 °C (C and D), under limited availability of oxygen.

When the incubations were carried out under aerobic conditions, at 37 °C, (Fig. 3A and B) 1-acetyl-β-carboline (**3**) was formed at both pH values, but an approximately two times higher amount accumulated at pH 5.7 (Fig. 3A) than at pH 7.4 (Fig. 3B). On the other hand the trend of 1-acetyl-β-carboline-3-carboxylic acid (**5**) formation was almost identical to that with the limited oxygen availability (Fig. 2A and B). A similar trend in the distribution of **3** in spite of oxygen availability was also observed at 100 °C and pH 7.4 (Figs. 2D and 3D). Additional oxygen (Fig. 3 vs Fig. 2) also increased the formation of the examined β-carbolines, at both temperatures, but considerably only at pH 5.7 in which case the amounts of **5** were three times and those of **3** even ten times higher (Fig. 3A and C vs Fig. 2A and C), while at pH 7.4 the amounts of **3** and **5** were similar to those obtained with limited amounts of oxygen (Fig. 3B and D vs Fig. 2B and D).

The results presented in Figure 4 showed that β-carbolines **3** and **5** can be formed even under oxygen deficiency.

During the incubation of L-tryptophan methyl ester (**6**) with methylglyoxal (**2**) at 37 °C and 100 °C (Fig. 5), part of the ester (**6**) was hydrolyzed and the free L-tryptophan (**1**) formed reacted with methylglyoxal (**2**) as already described. The hydrolysis occurred faster at higher pH and temperature (Fig. 5B and D). At the same time **2** and **6** also reacted directly. Thus, at 37 °C (Fig. 5A and B) 1-acetyl-β-carboline-3-carboxylic acid (**5**) and methyl-1-acetyl-β-carboline-3-carboxylate (**7**) were formed at both pH values while 1-acetyl-β-carboline (**3**) was not detected in any incubation mixture. At pH 7.4 and 37 °C β-carboline **7** reached its maximum after the first day of incubation and then rapidly disappeared (Fig. 5B). As the concentration of β-carboline **5** constantly increased during the incubation period, but its amounts were the same as those detected when L-tryptophan (**1**) and methylglyoxal (**2**) were incubated (Fig. 2B), it may be concluded that hydrolysis of **7** into **5** had non-considerable influence on the final concentration of **5**. At pH 5.7 (Fig. 5A), the amount of β-carboline **5** formed was lower than during the incubation of **1** and **2** under similar conditions (Fig. 2A) which is probably the result of slower hydrolysis of ester **6**, that is, smaller concentration of **1**. β-Carboline **7** reached its maximum after 24 h of incubation at acidic pH, at both temperatures (Fig. 5A and C). However, although **7** was degraded during incubation it was constantly formed as a result of slower ester **6** hydrolysis in an acidic medium. As no parallel increase of β-carboline **5** was observed, it was assumed that β-carboline **7** was consumed in other processes which were beyond the

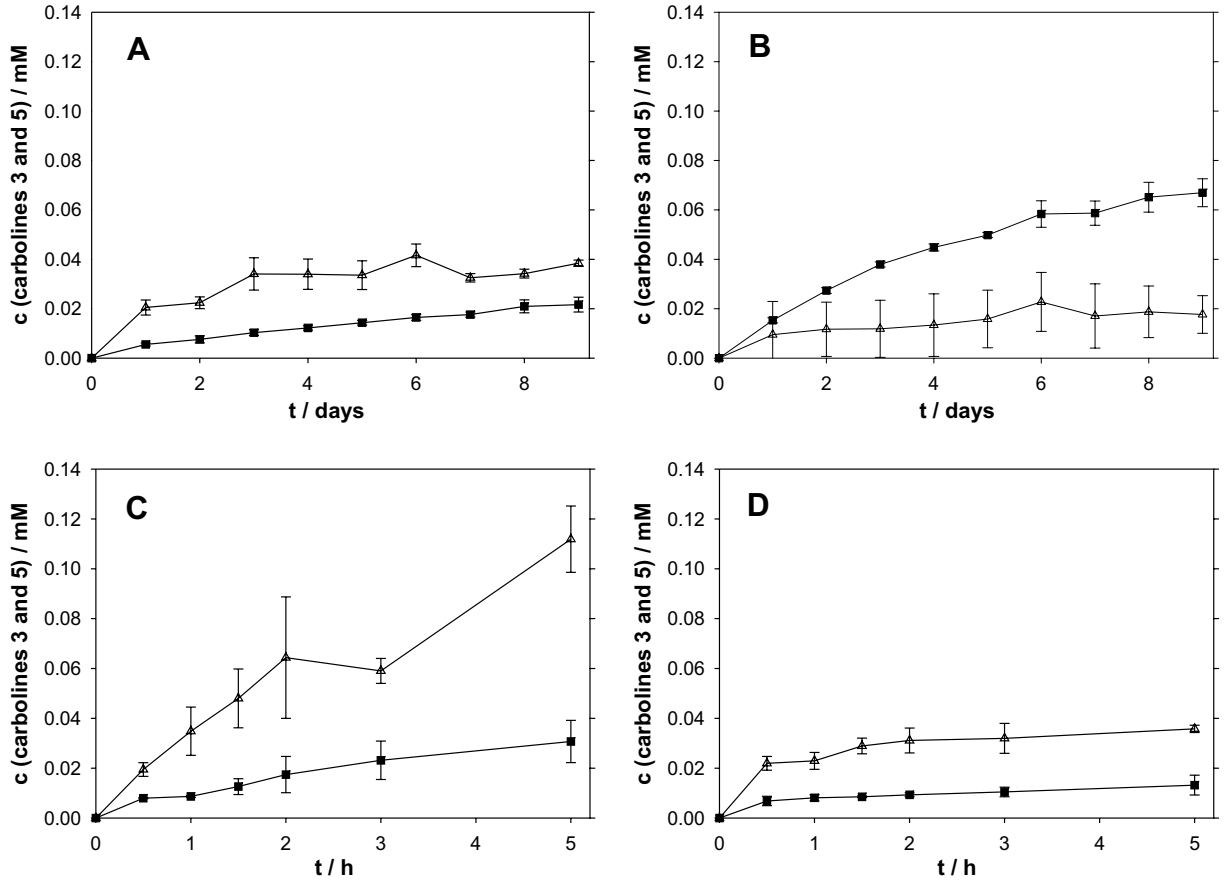


**Figure 3.** Formation of 1-acetyl-β-carboline (**3**; △) and 1-acetyl-β-carboline-3-carboxylic acid (**5**; ■) during incubation of L-tryptophan (**1**) with methylglyoxal (**2**) in phosphate buffer at pH 5.7 (A and C) and 7.4 (B and D) at 37 °C (A and B) and 100 °C (C and D), under aerobic conditions.

scope of this study (Fig. 5A). Similar observations were made at 100 °C (Fig. 5C and D), although, under these conditions, 1-acetyl-β-carboline (**3**) was also detected. In addition, comparison of the amounts of β-carboline **5** formed from **6** in alkaline medium (Fig. 5D), with those observed in incubations of **1** and **2** under similar conditions (Fig. 2D), suggested that **5** may also arise by hydrolysis of **7**. During the incubations of tryptamine (**4**) and methylglyoxal (**2**) (data not shown), 1-acetyl-3,4-dihydro-β-carboline (**8**) was formed under all examined conditions (pH 5.7 and 7.4, at 37 and 100 °C), and during all incubation periods, excluding pH 7.4 at 100 °C, in which case **8** disappeared after 2 h of incubation. 1-Acetyl-β-carboline (**3**) was not detected in any of those incubations presumably because the pH was not alkaline enough and/or the degradation rate was faster than complete aromatization.

The possible formation of 1-acetyl-β-carbolines **3** and **5** from D-glucose and L-tryptophan (**1**) under the above conditions were examined by incubation of D-glucose and L-tryptophan (**1**) in different molar ratios (10:1 and 20:1), as well as by incubation of previously synthesized *N*-(1-deoxy-D-fructos-1-yl)-L-tryptophan (**18**), the Amadori product of L-tryptophan (**1**), with and without the presence of L-tryptophan (**1**) in phosphate buffer at pH 5.7 and 7.4, at 37 °C, for 21 days, and at 100 °C, for 5 h. Under these conditions, RP HPLC analysis of the incubation mixtures did not show formation of 1-acetyl-β-carbolines **3** or **5**. 1-Acetyl-β-carboline-3-carboxylic acid (**5**) was, however, detected in some commercially available ketchups and in a tomato concentrate, after heating at 100 °C, for 1 h, while no **5** was found before heating. Detection, in this case, was by a highly sensitive and specific LC–MS/MS method (Fig. 6). Unlike **5**, 1-acetyl-β-carboline (**3**) was not detected in any of the analyzed samples of food or beverage.

## 3. Discussion

β-Carbolines represent a large group of alkaloids widely distributed in nature, occurring in plants, marine organisms, insects and, most importantly, in foods and in human tissues and body fluids. The compounds are of great interest due to their diverse biological activities, such as inhibition of topoisomerase and monoamine oxidase, binding to benzodiazepine and serotonin receptors and intercalating into DNA. In addition, these alkaloids also exhibit a broad spectrum of pharmacological activities including sedative, anxiolytic, hypnotic, anticovulsant, antitumor, and antimicrobial effects.[4]

In the current study, it was demonstrated that the endogenous metabolite and intermediate in the Maillard reaction, methylglyoxal (**2**),[6–9] reacts with L-tryptophan

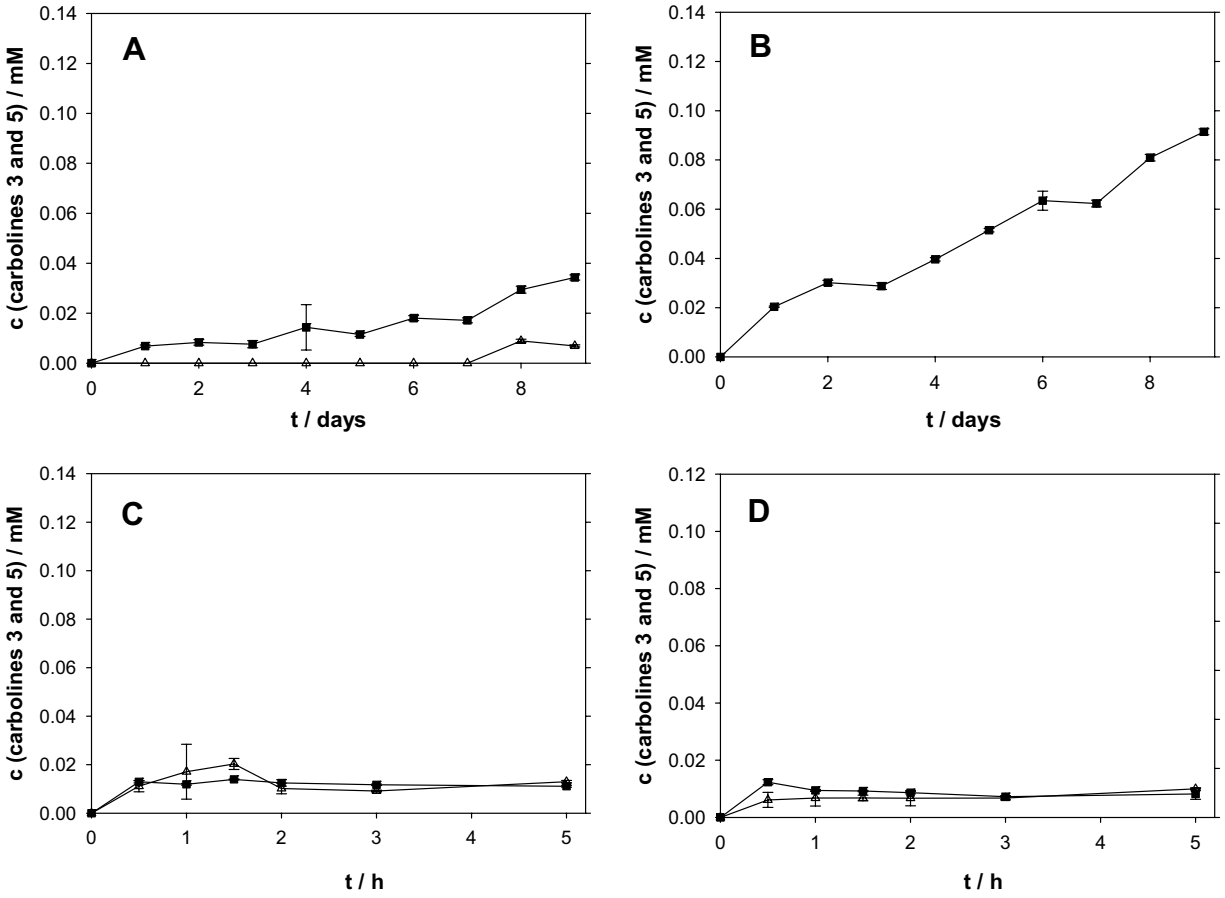


**Figure 4.** Formation of 1-acetyl-β-carboline (**3**; △) and 1-acetyl-β-carboline-3-carboxylic acid (**5**; ■) during incubation of L-tryptophan (**1**) with methylglyoxal (**2**) in phosphate buffer, pH 5.7 (A and C) and 7.4 (B and D) at 37 °C (A and B) and 100 °C (C and D), under nitrogen.

(**1**), its metabolite **4** and derivate **6**, under physiological and during food storing or processing temperatures and pH values, to yield 1-acetyl-β-carbolines **3**, **5**, **7**, and **8**. Some of them were previously isolated from a number of plants[19–21] and microorganisms,[22] and their high affinity for the brain benzodiazepine recognition site received particular attention.[23]

While some dicarbonyl compounds were previously shown to condense with L-tryptophan (**1**) to yield 1-hydroxylcarbolines via the Pictet–Spengler reaction,[14,15] we failed to observe formation of 1-(1-hydoxyethyl)-derivates **15**–**17** in mixtures of methylglyoxal (**2**) and **1**, **4**, or **6**, under the conditions adopted in this work.

The systematic study outlined above showed that, at 100 °C, methylglyoxal (**2**) and L-tryptophan (**1**) generated 1-acetyl-β-carboline (**3**) at both pH 5.7 and 7.4, regardless of oxygen availability. At 37 °C, this happened, at a comparable rate, only under slightly acidic conditions, else only after prolonged incubation or in the presence of oxygen. Lower temperatures favored conservation of the carboxyl group at the newly formed ring, why 1-acetyl-β-carboline-3-carboxylic acid (**5**), on the other hand, was detected in the incubation mixtures of **1** and **2** under all examined conditions with non-considerable influence of oxygen on its formation. These results clearly demonstrate that methylglyoxal-derived β-carboline **5** can also be formed under physiological pH and temperature (pH 7.4, 37 °C), in contrast to the restricted reactivity of some other physiological aldehydes, such as glucose, which can generate corresponding β-carbolines from L-tryptophan (**1**) (regardless of the temperature) only in acidic solution.[4] The different distribution of products at different pH values, together with beneficial influence of oxygen on the decarboxylation step, in particular under acidic conditions, suggests that oxidation of the respective intermediate 1,2,3,4-tetrahydro-β-carbolines does not involve only one, but likely a combination of several mechanisms. L-Tryptophan methyl ester (**6**) and tryptamine (**4**) formed β-carbolines **7** and **8**, under the examined model conditions. Under certain conditions (100 °C, pH 7.4), incubation of **6** and **2** also afforded carbolines derived from L-tryptophan (**1**), due to partial hydrolysis of **6** into **1** and **7** into **5**. The overall reactivity of the amino group toward methylglyoxal (**2**) decreased in the order **6** > **1** > **4** (details not shown). This can be rationalized by comparison of the p$K_a$ values of the three bases which decrease in the same order. The tryptophan methyl ester imine intermediate (Schiff base), formed before the attack of electrons from the indole moiety and ring closure,[3] appears to be more electrophilic than the imine formed from tryptamine (**4**).

The presence of 1-acetyl-β-carboline-3-carboxylic acid (**5**) in tomato concentrate after heating at 100 °C, for

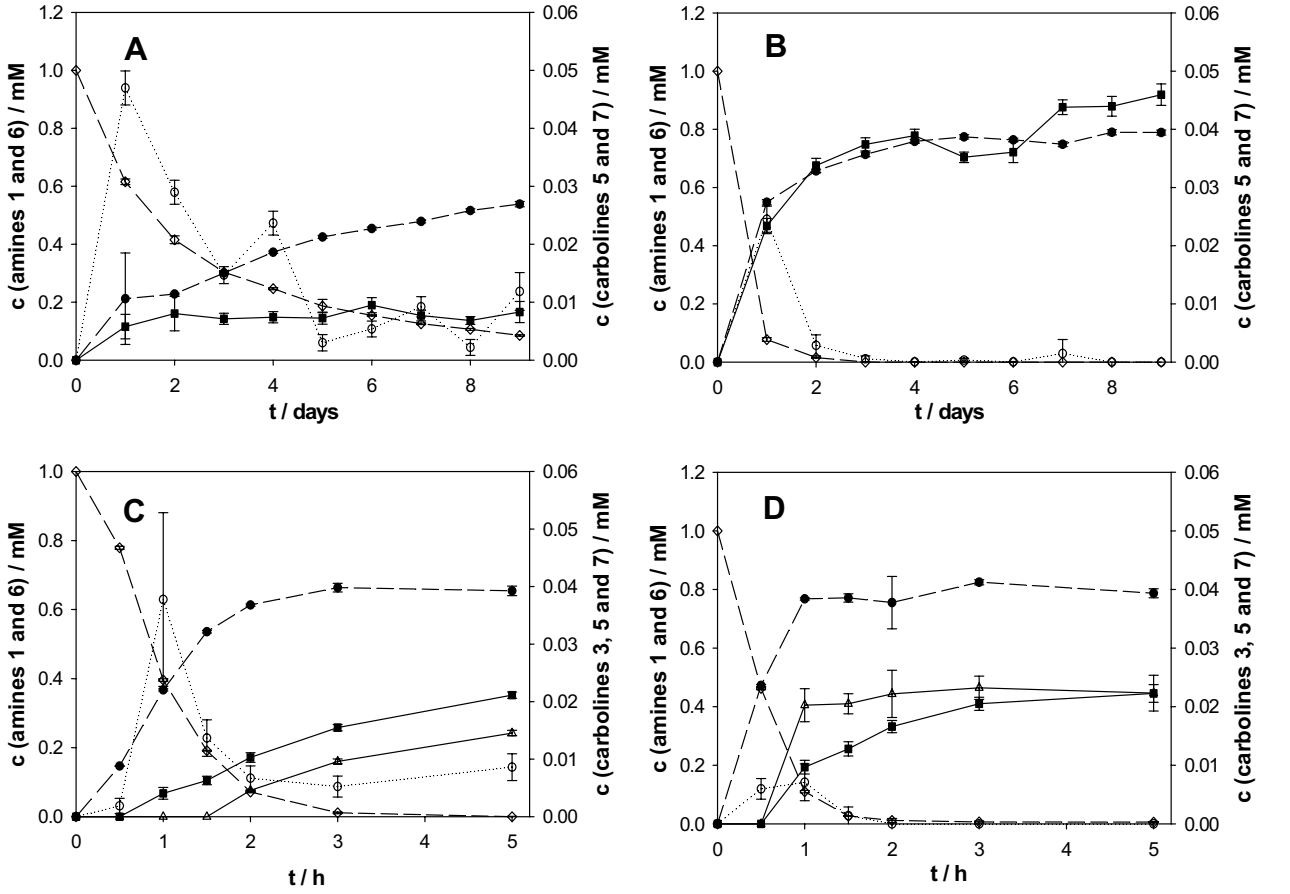


**Figure 5.** Formation of 1-acetyl-β-carboline (**3**; △; solid line), 1-acetyl-β-carboline-3-carboxylic acid (**5**; ■; solid line), methyl-1-acetyl-β-carboline-3-carboxylate (**7**; ○; dotted line) and L-tryptophan (**1**; •; dashed line) during incubation of L-tryptophan methyl ester (**6**; ◇; dashed line) with methylglyoxal (**2**) in phosphate buffer at pH 5.7 (A and C) and 7.4 (B and D) at 37 °C (A and B) and 100 °C (C and D).

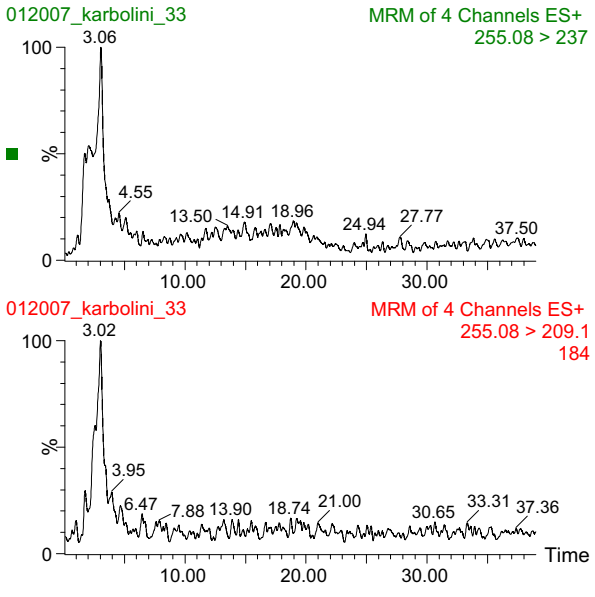


**Figure 6.** Detection of 1-acetyl-β-carboline-3-carboxylic acid (*m/z* 255.08) by LC–ESI/MS/MS analysis in a tomato concentrate previously heated 1 h at 100 °C. Multiple reaction monitoring (MRM) with *m/z* 237.0 and 209.1 as characteristic fragment ions.

1 h, while no **5** was detected prior the thermal treatment and to the best of our knowledge, represents the first demonstration of **5** as a food contaminant. Its possible origin could be explained in accord with the model systems outlined above.

Occurrence of β-carbolines, primarily β-carboline (norharman) and 1-methyl-β-carboline (harman), was demonstrated in different foodstuffs and biological fluids and tissues.[24] Recently Herraiz and Galisteo demonstrated the presence of sugar-derived tetrahydro-β-carbolines in fruit juices and jams,[5] which during heating could be rearranged into 1-acetyl-β-carbolines. Because of that and because of the possible formation of methylglyoxal (**2**) during the Maillard reaction of sugars (glucose) with amino acids, formation of 1-acetyl-β-carboline **3** should be expected in systems containing free L-tryptophan (**1**). Moreover, Rönner et al. showed that heating of D-glucose and L-tryptophan (**1**) (5:1 molar ratio) in 0.1 M phosphate buffer, at pH 5, generated 1-acetyl-β-carboline (**3**).[25] In the model systems described above compounds **3** and/or **5** were detected. The β-carboline **3** formed from D-glucose and **1** was observed only at higher temperatures, that is, as Rönner et al. demonstrated above 120 °C.[25] Also, the methylglyoxal formed in the reaction systems may be consumed in additional possible pathways in which the formation of compounds similar to the already isolated and characterized methylglyoxal amine derivates, such as carboxyethyl lysine (CEL)[26] or

methylglyoxal-derived lysine dimer (MOLD),[27] can be expected.

## 4. Conclusion

The described results and interpretations show that methylglyoxal (**2**) and L-tryptophan (**1**) as well as its metabolite **4** and derivate **6** generate 1-acetyl-β-carbolines in model systems mimicking in vivo and/or food processing and storage pH values and temperatures. These results, together with the detection of one of the described β-carbolines in some foods, corroborate the proposed chemical pathways based on the Maillard reaction.

Further studies focusing on the quantification of the described β-carbolines and their biological activity are required to demonstrate the distribution of these β-carbolines and their physiological impact.

## 5. Experimental

### 5.1. General methods

Melting points were determined in open capillaries on a Töttoli (Büchi) apparatus and are uncorrected. NMR spectra were recorded on a Bruker AV 600 spectrometer operating at 150.91 MHz for $^{13}C$ and 600.13 MHz for $^{1}H$. The spectra were measured in $D_2O$ and DMSO-$d_6$ solution at 25 °C. Chemical shifts in parts per million (ppm) were referenced to TMS in DMSO-$d_6$ and to dioxane in $D_2O$. Spectra were assigned based on 1D ($^{1}H$ and APT) an 2D homonuclear (COSY) and heteronuclear (HMQC, HMBC) experiments. Preparative HPLC was performed on a Varian ProStar HPLC system equipped with a Eurospher 100 C-18 semipreparative column (5 μm; 8 × 250 mm) (flow rate: 1.0 mL/min), while analytical HPLC was performed on a HP 1090 system equipped with a diode-array detector using an Eurospher 100C-18 analytical column (5 μm; 4 × 250 mm) (flow rate: 0.5 mL/min). The solvents used for HPLC were 40% aqueous methanol containing 0.1% TFA (solvent A); 75% aqueous methanol containing 0.1% TFA (solvent B); 72% aqueous methanol containing 0.1% TFA (solvent C) and 35% aqueous methanol containing 0.1% TFA (solvent D). Elution was programmed as follows: *Gradient 1*: isocratic A for 10 min; 100% A to 100% B in 10 min; isocratic B for 10 min; 100% B to 100% A in 5 min, and isocratic A for 5 min to return to the initial conditions; *Gradient 2*: isocratic D for 10 min; 100% D to 100% B in 10 min; isocratic B for 10 min; 100% B to 100% D in 5 min, and isocratic D for 5 min to return to the initial conditions. Thin layer chromatography was carried out with TLC glass plates precoated with silica gel 60 $F_{254}$ (Merck, Darmstadt, Germany). Visualization was accomplished using ninhydrin (2% in ethanol), the chlorine–iodine reagent, or by heating with $H_2SO_4$ (10% in water). Flash column chromatography was performed on silica gel (0.040–0.063 mm; Merck, Darmstadt, Germany). The following solvents were used: ethyl acetate (E); ethyl acetate/methanol (1:1, v/v) (F), and ethyl acetate/ethanol/water/acetic acid (7:1:1:1, v/v/v/v) (G). The identification of β-carbolines in biological samples was accomplished by LC–MS/MS using a Waters 2695 Separation Module with a Micromass Quatromicro triple quadropole mass spectrometry detector (Waters-Micromass, Manchester, UK). For the chromatographic separation, an Atlantis dC-18 column (3 μm, 2.1 × 50 mm; Waters, Milford, MA, USA) (flow rate: 0.2 mL/min) was used. Ammonium formate (26 mM, pH 3.8) (solvent H) and 90% aqueous acetonitrile (solvent I) were used in *gradient 3* according to the following program: 60% H and 40% I to 40% H and 60% I in 10 min; 40% H and 60% I to 10% H and 90% I in 2 min; isocratic 10% H and 90% I for 7 min; 10% H and 90% I to 60% H and 40% I in 3 min; and isocratic 60% H and 40% I for 9 min to return to the initial conditions.

### 5.2. Chemicals

Reagents of the highest quality available were obtained from Sigma (St. Louis, MO, USA); only ammonium formate and acetonitrile were from Burdick & Jackson (Muskegon, MI, USA). Aqueous solutions of methylglyoxal were prepared as previously described.[28] Aqueous solutions of glyoxal, methylglyoxal, and butan-2,3-dione were standardized using Friedmann's volumetric method.[29] 1-Acetyl-β-carboline (**3**), 1-acetyl-β-carboline-3-carboxylic acid (**5**), and methyl-1-acetyl-β-carboline-3-carboxylate (**7**) were prepared as previously described.[12]

**5.2.1. Synthesis of 1-acetyl-β-carboline (3).** The title compound was prepared in three different ways, of which the following two approaches were previously described[12]: from L-tryptophan (**1**) and methylglyoxal (**2**) (Method A) or from 1-acetyl-3,4-dihydro-β-carboline (**8**) (Method B).

*Method C.* L-Tryptophan (**1**) (200 mg; 0.98 mmol) dissolved in aqueous HCl (0.1 M; 5 mL) was stirred with methylglyoxal (**2**) (288.5 mM; 6.8 mL; 1.96 mmol) at 80 °C, for 2 h. An aqueous solution of potassium dichromate (10%, 6 mL) was then added. The precipitate formed was filtered off and purified by RP HPLC (solvent B, $t_R$ = 14.1 min) to yield 1-acetyl-β-carboline (**3**) (2 mg; $\eta$ = 2%).

**5.2.2. Synthesis of 1-acetyl-3,4-dihydro-β-carboline (8).** The title compound was prepared in four different ways, of which the condensation of tryptamine hydrochloride (**4**) with methylglyoxal (**2**) (Method A) was previously described.[12]

*Method B.* To a flask containing tryptamine hydrochloride (**4**) (200 mg; 1.02 mmol) dissolved in a mixture of aqueous HCl (0.1 M; 2.5 mL) and methanol (4 mL), an aqueous solution of methylglyoxal (**2**) (482 mM; 4.2 mL; 2.04 mmol) was added. The mixture was stirred at 40 °C overnight, neutralized with saturated aqueous $NaHCO_3$, and extracted with chloroform (3× 20 mL). The combined organic layers were washed with water, dried over anhydrous $Na_2SO_4$, and evaporated. Purifica-

tion by flash column chromatography on silica gel (solvent E; $R_f = 0.92$) afforded 1-acetyl-3,4-dihydro-β-carboline (**8**) (17 mg; $\eta = 7.9\%$).

*Method C.* *N*-Lactoyltryptamine (**10**) (50 mg; 0.22 mmol) was dissolved in concd TFA (3 mL) and heated under reflux for 45 min. After cooling, the mixture was neutralized with saturated aqueous $NaHCO_3$ and extracted with chloroform (3× 20 mL). The combined organic layers were washed with water, dried over anhydrous $Na_2SO_4$, and evaporated. Purification by flash column chromatography on silica gel (solvent E; $R_f = 0.92$) afforded 1-acetyl-3,4-dihydro-β-carboline (**8**) (0.9 mg; $\eta = 2\%$).

*Method D.* *N*-(*O*-Acetyl-lactoyl)tryptamine (**11**) (160 mg; 0.58 mmol) was dissolved in $POCl_3$ (4 mL) and heated under reflux for 2 h. After cooling the mixture was added dropwise to ice-cooled, saturated aqueous $NaHCO_3$ (40 mL). Final neutralization was done by adding solid $NaHCO_3$. The resulting mixture was extracted with chloroform (3× 50 mL). The combined organic layers were washed with water, dried over anhydrous $Na_2SO_4$, and evaporated. The crude product was purified by flash column chromatography on silica gel (solvent E; $R_f = 0.92$) to yield 1-acetyl-3,4-dihydro-β-carboline (**8**) (16.6 mg; $\eta = 13.4\%$).

**5.2.3. Synthesis of *N*-lactoyltryptamine (10).** Tryptamine hydrochloride (**4**) (300 mg; 1.53 mmol) was dissolved in THF/water (6 mL; 3:2, v/v) and neutralized with NEM (*N*-ethyl morpholine) (196 μL; 1.53 mmol). The solution was added dropwise to a solution of lactic acid (**9**) (228 μL; 3.05 mmol), 1-hydroxybenzotriazole (HOBt) (412 mg; 3.05 mmol) and DCC (686 mg; 3.33 mmol) in THF (10 mL), at −15 °C. The mixture was stirred overnight at room temperature. Dicyclohexylurea was filtered off, and the filtrate was evaporated. The residue was dissolved in ethyl acetate (40 mL) and extracted with saturated aqueous $NaHCO_3$ (2× 40 mL), 10% aqueous citric acid (2× 40 mL), and saturated brine (2× 40 mL). After drying over anhydrous $Na_2SO_4$, the organic phase was evaporated and the residue purified by flash column chromatography on silica gel (solvent E; $R_f = 0.45$) to yield *N*-lactoyltryptamine (**10**) (122.7 mg; $\eta = 34\%$). Mp (ethyl acetate/*n*-hexane): 94–97 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ (ppm): 1.20 (d, $J = 6.7$ Hz, 3H, C$H_3$); 2.83 (t, $J = 7.5$ Hz, 2H, 4-H); 3.15 (m, 1H, C*H*OH); 3.33 (m, 2H, 3-H); 5.49 (d, $J = 4.6$ Hz, 1H, CHO*H*); 6.97 (m, 1H, 7-H); 7.06 (m, 1H, 6-H); 7.15 (s, 1H, 2-H); 7.33 (d, $J = 8.0$ Hz, 1H, 5-H); 7.56 (d, $J = 7.9$ Hz, 1H, 8-H); 7.77 (t, $J = 5.6$ Hz, 1H, N*H*CO); 10.81 (s, 1H, N*H*). $^{13}$C NMR (DMSO-$d_6$) $\delta$ (ppm): 21.03 (*C*H$_3$); 25.23 (C-4); 38.90 (C-3); 67.26 (*C*HOH); 111.31 (C-5); 111.73 (C-4a); 118.17 (C-7); 118.31 (C-8), 120.89 (C-6); 122.54 (C-2); 127.19 (C-4b); 136.24 (C-8a); 174.25 (NH*C*O). Anal. Calcd for $C_{13}H_{16}N_2O_2$: C, 67.22; H, 6.94; N, 12.06. Found: C, 67.14; H, 6.78; N, 11.88.

**5.2.4. Synthesis of *N*-(*O*-acetyl-lactoyl)tryptamine (11).** To *N*-lactoyltryptamine (**10**) (100 mg; 0.43 mmol), dissolved in abs pyridine (4 mL), acetanhydride (2 mL) was added. The mixture was stirred at room temperature, for 2 h, poured into ice cold water (40 mL) and then stirred for an additional 2 h, at room temperature. The mixture was evaporated as an azeotropic mixture with toluene. *N*-(*O*-Acetyl-lactoyl)tryptamine (**11**) was obtained as a colorless transparent oil (118 mg; $\eta = 100\%$; $R_f$ (E) = 0.93). $^1$H NMR (DMSO-$d_6$) $\delta$ (ppm): 1.31 (*d*, $J = 6.8$ Hz, 3H, C$H_3$); 2.06 (s, 3H, COC$H_3$); 2.83 (t, $J = 7.4$ Hz, 2H, 4-H); 3.34 (m, 2H, 3-H); 4.92 (q, $J = 6.8$ Hz, 1H, C*H*O); 6.98 (m, 1H, 7-H); 7.07 (m, 1H, 6-H); 7.14 (s, 1H, 2-H); 7.34 (d, $J = 8.1$ Hz, 1H, 5-H); 7.55 (d, $J = 7.9$ Hz, 1H, 8-H); 8.09 (t, $J = 5.6$ Hz, 1H, N*H*CO); 10.83 (s, 1H, N*H*). $^{13}$C NMR (DMSO-$d_6$) $\delta$ (ppm): 17.78 (*C*H$_3$); 20.88 (CO*C*H$_3$) 25.12 (C-4); 39.64 (C-3); 69.81 (*C*HO); 111.45 (C-5); 111.75 (C-4a); 118.32 (C-7); 118.34 (C-8), 121.00 (C-6); 122.80 (C-2); 127.32 (C-4b); 136.35 (C-8a); 169.80 (O*C*O); 169.94 (NH*C*O). Anal. Calcd for $C_{15}H_{18}N_2O_3 \times 0.1H_2O$: C, 65.25; H, 6.64; N, 12.06. Found: C, 65.07; H, 6.07; N, 10.31.

**5.2.5. Synthesis of *N*-lactoyl-L-tryptophan methyl ester (12).** A solution of L-tryptophan methyl ester (**6**) (500 mg; 2.08 mmol) and NEM (262 μL; 2.08 mmol) in THF/water (10 mL, 3:2, v/v) was added dropwise to a cooled (−15 °C) solution of lactic acid (**9**) (310 μL; 4.16 mmol), HOBt (561 mg; 4.16 mmol), and DCC (1.03 g; 4.57 mmol) in THF (30 mL). The resulting mixture was stirred overnight at room temperature. The dicyclohexylurea was filtered off, and the filtrate was evaporated. The residue was dissolved in ethyl acetate (50 mL) and extracted with saturated aqueous $NaHCO_3$ (2× 50 mL), 10% aqueous citric acid (2× 50 mL) and brine (2× 50 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$ and evaporated. The crude product was purified by flash column chromatography on silica gel (solvent E; $R_f = 0.45$) to yield *N*-lactoyl-L-tryptophan methyl ester as a colorless transparent oil (**12**) (416 mg; $\eta = 36.4\%$). $^1$H NMR (DMSO-$d_6$) $\delta$ (ppm): 1.15 (*d*, $J = 6.9$ Hz, 3H, C$H_3$); 3.19 (d, 2H, 4-H); 3.61 (s, 3H, COOC$H_3$); 3.97 (m, 1H, C*H*OH); 4.59 (m, 2H, 3-H); 5.60 (br, 1H, CHO*H*); 6.99 (m, 1H, 7-H); 7.07 (m, 1H, 6-H); 7.12 (s, 1H, 2-H); 7.34 (d, $J = 8.1$ Hz, 1H, 5-H); 7.46 (d, $J = 7.9$ Hz, 1H, 8-H); 7.74 (t, $J = 7.9$ Hz, 1H, N*H*CO); 10.90 (s, 1H, N*H*). $^{13}$C NMR (DMSO-$d_6$) $\delta$ (ppm): 20.83 (*C*H$_3$); 26.96 (C-4); 51.90 (COO*C*H$_3$); 52.20 (C-3); 66.98 (*C*HOH); 109.01 (C-4a); 111.40 (C-5); 118.06 (C-8); 118.41 (C-7), 120.98 (C-6); 123.67 (C-2); 127.16 (C-4b); 136.07 (C-8a); 172.08 (*C*OOCH$_3$); 174.27 (NH*C*O). Anal. Calcd for $C_{15}H_{18}N_2O4 \times 0.2H_2O$: C, 61.30; H, 6.31; N, 9.53. Found: C, 61.32; H, 6.21; N, 9.45.

**5.2.6. Synthesis of *N*-(*O*-acetyl-lactoyl)-L-tryptophan methyl ester (13).** To *N*-lactoyl-L-tryptophan methyl ester (**12**) (200 mg; 0.69 mmol), dissolved in abs pyridine (5 mL), acetanhydride (2 mL) was added. The reaction mixture was stirred at room temperature for 2 h, poured into ice cold water (40 mL), and then stirred at room temperature, for an additional 2 h. The mixture was evaporated as an azeotropic mixture with toluene. *N*-(*O*-Acetyl-lactoyl)-L-tryptophan methyl ester (**13**) was obtained as a colorless transparent oil (118 mg;

$\eta$ = 100%; $R_f$ (E) = 0.82). $^1$H NMR (DMSO-$d_6$) $\delta$ (ppm): 1.26 (d, $J$ = 6.9 Hz, 3H, C$H_3$); 2.03 (s, 3H, COC$H_3$); 3.14 (m, 2H, 4-H and 4′-H); 3.58 (s, 3H, COOC$H_3$); 4.52 (m, 2H, 3-H); 4.98 (q, $J$ = 6.9 Hz, 1H, C$H$O); 6.99 (m, 1H, 7-H); 7.08 (m, 1H, 6-H); 7.16 (s, 1H, 2-H); 7.33 (d, $J$ = 8.0 Hz, 1H, 5-H); 7.49 (d, $J$ = 7.9 Hz, 1H, 8-H); 8.39 (d, $J$ = 7.5 Hz, 1H, N$H$CO); 10.90 (s, 1H, N$H$). $^{13}$C NMR (DMSO-$d_6$) $\delta$ (ppm): 17.31 (*C*H$_3$); 20.64 (CO*C*H$_3$); 26.73 (C-4); 51.83 (COO*C*H$_3$); 52.84 (C-3); 69.23 (*C*HO); 109.24 (C-4a); 111.36 (C-5); 117.97 (C-8); 118.38 (C-7), 120.93 (C-6); 123.67 (C-2); 127.09 (C-4b); 136.04 (C-8a); 169.58 (O*C*O); 170.04 (NH*C*O); 171.96 (*C*OOCH$_3$). Anal. Calcd for $C_{17}H_{20}N_2O_5 \times 0.2H_2O$: C, 60.78; H, 6.12; N, 8.34. Found: C, 60.74; H, 6.01; N, 8.46.

**5.2.7. Synthesis of methyl-1-ethyl-β-carboline-3-carboxylate (14).** *N*-(*O*-Acetyl-lactoyl)-L-tryptophan methyl ester (**13**) (100 mg; 0.30 mmol) dissolved in $POCl_3$ (4 mL) was refluxed for 2 h and then added dropwise to stirred and cooled (°C) aqueous $NaHCO_3$ (40 mL). Final neutralization was done by adding solid $NaHCO_3$. The reaction mixture was extracted with chloroform (3× 50 mL). The combined organic layers were washed with water, dried over anhydrous $Na_2SO_4$, and evaporated. The crude product was purified by flash column chromatography on silica gel (solvent E; $R_f$ = 0.65) to yield unstable methyl-1-ethyl-β-carboline-3-carboxylate (**14**) (25 mg; $\eta$ = 33%). $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 1.29 (t, $J$ = 7.6 Hz, 3H, C$H_3$); 3.11 (q, $J$ = 7.6 Hz, 2H, C$H_2$); 4.00 (s, 3H, COOC$H_3$); 7.33 (m, 1H, 7-H); 7.50 (m, 2H, 5-H and 6-H); 8.15 (d, $J$ = 7.9 Hz, 1H, 8-H); 8.78 (s, 1H, 4-H); 9.82 (s, 1H, N$H$). $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 12.94 (*C*H$_3$); 27.45 (*C*H$_2$); 52.65 (COO*C*H$_3$); 112.06 (C-5); 116.51 (C-4); 120.71 (C-7); 121.50 (C-8); 121.94 (C-4a); 128.37 (C-1); 128.60 (C-6); 135.68 (C-3); 135.74 (C-4a); 140.61 (C-9a); 147.29 (C-8a); 167.00 (*C*OOCH$_3$).

**5.2.8. Synthesis of 1-(1-hydroxyethyl)-β-carboline (15).** $NaBH_4$ (18 mg; 0.48 mmol) was added to a methanol solution (25 mL) of 1-acetyl-β-carboline (**3**) (100 mg; 0.48) and stirred at room temperature for 1.5 h. The reaction mixture was poured into distilled water (50 mL) and extracted with ethyl acetate (3× 50 mL). The combined organic layers were washed with water, dried over $Na_2SO_4$, and evaporated. The crude product was purified by flash column chromatography on silica gel (solvent F; $R_f$ = 0.72) to yield 1-(1-hydroxyethyl)-β-carboline (**15**) (45 mg; $\eta$ = 45%). Mp (methanol/ethyl acetate (18:2, v/v)): 194–195 °C (decompg) (lit.: 197 °C[30]); $^1$H NMR (DMSO-$d_6$) $\delta$ (ppm): 1.56 (d, $J$ = 6.5 Hz, 3H, C$H_3$); 5.22 (q, $J$ = 6.5 Hz, 1H, C$H$OH); 5.73 (s, 1H, CHO$H$); 7.21 (m, 1H, 7-H); 7.51 (m, 1H, 6-H); 7.70 (d, $J$ = 8.2 Hz, 1H, 5-H); 7.99 (d, $J$ = 5.2, 1H, 4-H); 8.19 (d, $J$ = 7.7 Hz, 1H, 8-H); 8.23 (d, $J$ = 5.2 Hz, 1H, 3-H); 11.26 (s, 1H, N$H$). $^{13}$C NMR (DMSO-$d_6$) $\delta$ (ppm): 22.87 (*C*H$_3$); 69.28 (*C*HOH); 112.35 (C-4); 113.43 (C-5); 118.93 (C-8); 120.44 (C-4a); 121.31 (C-7); 127.75 (C-6); 128.19 (C-1); 132.26 (C-9a); 136.59 (C-3); 140.49 (C-4b); 148.65 (C-8a). Anal. ($C_{13}H_{12}N_2O \times 0.2$ $CH_3OH$) C, H, N; Calcd for C, 72.51; H, 5.90; N, 12.81. Found: C, 72.66; H, 5.84; N, 12.31.

**5.2.9. Synthesis of methyl-1-(1-hydroxyethyl)-β-carboline-3-carboxylate (16).** $NaBH_4$ (20 mg; 0.52 mmol) was added to a methanol suspension (20 mL) of methyl-1-acetyl-β-carboline-3-carboxylate (**7**) (140 mg; 0.52) and stirred at room temperature for 2 h. The reaction mixture was poured into distilled water (50 mL) and extracted with ethyl acetate (3× 50 mL). The combined organic layers were washed with water, dried over anhydrous $Na_2SO_4$, and evaporated. The crude product was purified by flash column chromatography on silica gel (solvent F; $R_f$ = 0.19) to yield methyl-1-(1-hydroxyethyl)-β-carboline-3-carboxylate (**16**) (120 mg; $\eta$ = 79%). Mp ($CHCl_3$/*n*-hexane): 136–137 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ (ppm): 1.56 (d, $J$ = 6.7 Hz, 3H, C$H_3$); 3.91 (s, 3H, COOC$H_3$); 5.24 (q, $J$ = 6.7 Hz, 1H, C$H$OH); 5.90 (br, 1H, CHO$H$); 7.28 (m, 1H, 7-H); 7.57 (m, 1H, 6-H); 7.77 (d, $J$ = 8.2 Hz, 1H, 5-H); 8.36 (d, $J$ = 7.9 Hz, 1H, 8-H); 8.83 (s, 1H, 4-H); 11.69 (s, 1H, N$H$). $^{13}$C NMR (DMSO-$d_6$) $\delta$ (ppm): 22.61 (*C*H$_3$); 51.58 (COO*C*H$_3$); 69.73 (*C*HOH); 112.92 (C-5); 116.58 (C-4); 119.96 (C-8); 120.10 (C-4a); 121.67 (C-7); 128.28 (C-1); 128.31 (C-6); 133.91 (C-9a); 135.08 (C-3); 140.95 (C-4b); 148.72 (C-8a); 166.05 (*C*OOCH$_3$). Anal. Calcd for $C_{15}H_{14}N_2O_3 \times 0.8H_2O$: C, 63.28; H, 5.52; N, 9.84. Found: C, 63.74; H, 5.14; N, 10.15.

**5.2.10. Synthesis of 1-(1-hydroxyethyl)-β-carboline-3-carboxylic acid (17).** To a solution of methyl-1-(1-hydroxyethyl)-β-carboline-3-carboxylate (**16**) (40 mg; 0.15 mmol) in dioxane (3 mL) aqueous NaOH (33%; 1 mL) and several drops of water were added. The mixture was stirred at room temperature, overnight, and then acidified with aqueous HCl (6 M). The dioxane was evaporated and the resulting aqueous solution of 1-(1-hydroxyethyl)-β-carboline-3-carboxylic acid (**17**) was allowed to stand for several days at +4 °C. The precipitate was filtered off and dried in a desiccator (14.5 mg; $\eta$ = 38%; $R_f$ (G) = 0.39). Mp: 298–300 °C (decompg); $^1$H NMR (DMSO-$d_6$) $\delta$ (ppm): 1.63 (d, $J$ = 6.6 Hz, 3H, C$H_3$); 5.73 (q, $J$ = 6.6 Hz, 1H, C$H$OH); 7.43 (m, 1H, 7-H); 7.74 (m, 1H, 6-H); 7.88 (d, $J$ = 8.2 Hz, 1H, 5-H); 8.58 (d, $J$ = 7.9 Hz, 1H, 8-H); 9.20 (s, 1H, 4-H); 12.99 (s, 1H, N$H$). $^{13}$C NMR (DMSO-$d_6$) $\delta$ (ppm): 22.81 (*C*H$_3$); 65.94 (*C*HOH); 113.40 (C-5); 117.54 (C-4); 120.24 (C-4a); 121.55 (C-8); 123.08 (C-7); 129.95 (C-1); 130.86 (C-6); 131.42 (C-9a); 132.59 (C-3); 143.14 (C-4b); 147.04 (C-8a); 163.19 (*C*OOH). Anal. Calcd for: $C_{14}H_{12}N_2O_3 \times HCl \times 0.8H_2O$: C, 55.73; H, 4.68; N, 9.28. Found: C, 55.94; H, 4.99; N, 9.68.

**5.2.11. Synthesis of *N*-(1-deoxy-D-fructos-1-yl)-L-tryptophan (18).** A suspension of L-tryptophan (**1**) (1 g; 4.90 mmol) and D-glucose (9 g; 49 mmol) in abs methanol (150 mL) was refluxed for 5 h. The mixture was evaporated, dissolved in redistilled water (50 mL), and applied to a column of Dowex 50W-X8 $H^+$ (10 × 2 cm). The excess of glucose was removed with water (200 mL), and the remaining product was then eluted with aqueous ammonia (1 M; 150 mL). The eluate was evaporated and the obtained crude product was purified by RP HPLC (solvent D; $t_R$ = 11.6 min). The fractions containing the pure *N*-(1-deoxy-D-fructos-1-yl)-L-tryptophan (**19**) were pooled and evaporated.

The resulting colorless transparent oil was dissolved in methanol and precipitated with diisopropyl ether (130 mg; $\eta = 7\%$). Mp: 135–137 °C (decompg) (lit.: 143 °C (dec)[31]); $^{13}$C NMR ($D_2O$) $\delta$ (ppm): 25.69 (Trp *C*H$_2$; C-11′, β-*p*); 25.75 (Trp *C*H$_2$; C-11′, α-*f*); 25.87 (Trp *C*H$_2$; C-11′, β-f); 52.89 (Trp *C*H; C-10′, β-*p*); 50.43 (Fru C-1; β-*f*); 51.80 (Fru C-1; α-*f*); 52.87 (Fru C-1; β-*p*); 60.90 (Fru C-6; α-*f*); 61.54 (Fru C-6; β-*f*); 62.87 (Trp *C*H; C-10′, β-*f*); 62.88 (Trp *C*H; C-10′, α-*f*); 63.82 (Fru C-6; β-*p*); 68.80 (Fru C-5; β-p); 69.29 (Fru C-4; β-*p*); 70.28 (Fru C-3; β-*p*); 73.99 (Fru C-4; β-*f*); 76.17 (Fru C-4; α-*f*); 78.08 (Fru C-3; β-*f*); 80.92 (Fru C-5; β-*f*); 82.51 (Fru C-3; α-*f*); 95.10 (Fru C-2; β-*p*); 106.97 (Trp $C_q$, C-3′); 111.99 (Trp C*H*, C-5′); 118.53 (Trp *C*H, C-8′); 119.57 (Trp *C*H, C-7′); 122.24 (Trp *C*H, C-6′); 125.13 (Trp, C*H*, C-2′); 126.66 (Trp $C_q$, C-4′); 135.41 (Trp $C_q$, C-9′); 172.69 (Trp *C*OOH, C-12′).

**5.2.12. Incubation of L-tryptophan (1), its derivate (methyl ester) (6) and its metabolite (tryptamine) (4) with methylglyoxal (2).** To determine the dependence of 1-acetyl-β-carboline formation on temperature, pH, and oxygen availability during the incubation of L-tryptophan (**1**) and methylglyoxal (**2**), **1** and **2** (1.0 mM final concentrations) were incubated in phosphate buffer ($Na_2HPO_4/NaH_2PO_4$; 50 mM; pH 5.7 and 7.4). The mixtures (400 μL) were incubated in duplicate at 37 °C (individual vial for each day) and at 100 °C (individual vial for each hour) in airtight closed vessels (limited oxygen availability) and in HPLC vials under nitrogen (no oxygen availability) to avoid any influence of the oxygen during sample preparation for HPLC analysis. Unlimited oxygen availability was achieved when incubations were performed by intensive stirring of incubation mixture in open flasks with condenser. From the samples kept at 37 °C aliquots were withdrawn daily for 9 days, for samples kept at 100 °C, this was done after 0.5; 1.0; 1.5; 2.0; 3.0, and 5.0 h of incubation. All aliquots were stored at −20 °C and thawed immediately before RP HPLC analysis.

Tryptophan methyl ester (**6**) and tryptamine (**4**) were also incubated with methylglyoxal (**2**) in airtight closed vessels under conditions previously described.

Each incubation mixture was analyzed in duplicate by RP HPLC using *gradient 1*, monitoring the absorbance at 280, 254, 290 nm (for **1**, **6**, and **4**) or at 280, 254, 290, 352, and 380 nm (for **3**, **5**, and **7**). Quantification was done by determining peak areas on chromatograms, followed by comparison with the corresponding calibration curves. The latter were linear in the concentration range of 0.025–1.0 mM, for all compounds, at all examined wavelengths. Analysis of 1-acetyl-3,4-dihydro-β-carboline (**8**) was done only qualitatively due to its particular instability.

**5.2.13. Incubations of L-tryptophan (1) with D-glucose and *N*-(1-deoxy-D-fructos-1-yl)-L-tryptophan (18) with and without L-tryptophan (1).** L-Tryptophan (**1**) (1.0 mM) and D-glucose (10.0 or 20.0 mM) as well as *N*-(1-deoxy-D-fructos-1-yl)-L-tryptophan (**18**) (1 mM) were incubated in phosphate buffer ($Na_2HPO_4/NaH_2PO_4$; 50 mM; pH 5.7 and 7.4), with or without the presence of L-tryptophan (**1**) (1 mM). Samples (400 μL) were incubated in duplicate at 37 and 100 °C in airtight closed vessels. Aliquots from the samples kept at 37 °C were collected after 1, 3, 4, 7, 10, 14, 16, 18, and 21 days of the incubation period; for samples kept at 100 °C, this was done after 0.5; 1.0; 1.5; 2.0; 3.0; and 5.0 h. All aliquots were stored at −20 °C and thawed immediately before RP HPLC analysis.

Each incubation mixture was analyzed in duplicate by RP HPLC using *gradient 2*, monitoring the absorbance at 280, 254, 290 nm (for **1** and **18**) or at 280, 254, 290, 352, and 380 nm (for **3** and **5**). Quantification of each compound was done by determining peak areas on chromatograms followed by comparison with the corresponding calibration curves. The latter were linear in the concentration range of 0.025–1.0 mM, for all compounds, at all examined wavelengths.

### 5.3. LC–MS/MS analysis of β-carbolines in food samples

1-Acetyl-β-carboline-3-carboxylic acid (**5**) ($t_R$ (*gradient 3*) = 3.0 min) and 1-acetyl-β-carboline (**3**) ($t_R$ (*gradient 3*) = 4.5 min) were analyzed by electronspray positive ionization-mass spectrometric multiple reaction monitoring (ESI+ MRM). The ionization source temperature was 130 °C and the desolvation gas temperature was 400 °C. The cone gas and desolvation gas-flow rates were 850 and 150 L/h, respectively. The capillary voltage was 3.60 kV and the cone voltages for 1-acetyl-β-carboline-3-carboxylic acid (**5**) (*m*/*z* 255.08) and 1-acetyl-β-carboline (**3**) (*m*/*z* 211.13) were 20.0 and 35.0 V, respectively. The collision energies for the 1-acetyl-β-carboline-3-carboxylic acid (**5**) fragment ions at *m*/*z* 209.1 and *m*/*z* 237.0 were 20.00 and 12.00 eV, respectively. The collision energies for the 1-acetyl-β-carboline (**3**) fragment ions at *m*/*z* 169.0 and *m*/*z* 193.1 were 28.00 and 22.00 eV, respectively. Argon gas was in the collision cell. The detection limits for fragment ions *m*/*z* 209.1 and *m*/*z* 237.0 of **5** were 0.36 and 0.37 pmol, respectively, while, for fragment ions *m*/*z* 169.0 and *m*/*z* 193.1 of **3**, were 0.40 and 0.54 pmol, respectively. Programmed molecular ion and fragment ion masses were optimized to ±0.1 Da for multiple reaction monitoring detection of the analyte.

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## References and notes

1. Sainio, E.-L.; Pulkki, K.; Young, S. N. *Amino Acids* **1996**, *10*, 21.

2. Ahmed, N.; Thornalley, P. J. *Diabetes Obes. Metab.* **2007**, *9*, 233.
3. Cox, E. D.; Cook, J. M. *Chem. Rev.* **1995**, *95*, 1797.
4. Cao, R.; Peng, W.; Wang, Z.; Xu, A. *Curr. Med. Chem.* **2007**, *14*, 479.
5. Herraiz, T.; Galisteo, J. *J. Agric. Food Chem.* **2002**, *50*, 4690.
6. Nemet, I.; Turk, Z.; Duvnjak, L.; Car, N.; Varga-Defterdarović, L. *Clin. Biochem.* **2005**, *38*, 379.
7. Nemet, I.; Varga-Defterdarović, L.; Turk, Z. *Mol. Nutr. Food Res.* **2006**, *50*, 1105.
8. Thornalley, P. J. *Gen. Pharma.* **1996**, *27*, 565.
9. Kalapos, M. P. *Toxicol. Lett.* **1999**, *110*, 145.
10. Lo, T. W.; Westwood, M. E.; McLellan, A. C.; Selwood, T.; Thornalley, P. J. *J. Biol. Chem.* **1994**, *269*, 32299.
11. Zeng, J.; Davies, M. J. *Chem. Res. Toxicol.* **2006**, *19*, 1668.
12. Nemet, I.; Varga-Defterdarović, L. *Amino Acids* **2007**, *32*, 291.
13. Snyder, H. R.; Parmeter, S. M.; Katz, L. *J. Am. Chem. Soc.* **1948**, *70*, 222.
14. Kojima, E.; Kai, M.; Ohkura, Y. *Anal. Chim. Acta* **1991**, *248*, 213.
15. Yang, M.-L.; Kuo, P.-C.; Damu, A. G.; Chang, R.-J.; Chiou, W.-F.; Wu, T.-S. *Tetrahedron* **2006**, *62*, 10900.
16. Wang, X.; Tan, J.; Grozinger, K. *Tetrahedron Lett.* **1989**, *39*, 6609.
17. Previero, A.; Coletti-Previero, M. A.; Barry, L. G. *Can. J. Chem.* **1968**, *46*, 3404.
18. Ziokowski, M.; Czarnock, Z. *Tetrahedron Lett.* **2000**, *41*, 1963.
19. Fani, F.; Castillo, M.; Torres, R. *Phitochemistry* **1978**, *17*, 338.
20. Joshi, B. S.; Kamat, V. N.; Gawad, D. H. *Heterocycles* **1977**, *7*, 193.
21. Zhou, T.-S.; Ye, W.-C.; Wang, Z.-T.; Zhou, R.-H.; Xu, G.-J.; Xu, L.-S. *Phytochemistry* **1998**, *49*, 1807.
22. Proksa, B.; Uhrin, D.; Štrudíková, M.; Fuska, J. *Acta Biotechnol.* **2004**, *10*, 337.
23. Bracher, F.; Hidelbrand, D.; Häberlein, H. *Nat. Prod. Res.* **2004**, *18*, 391.
24. Pfau, W.; Skog, K. *J. Chromatogr. B* **2004**, *802*, 115.
25. Rönner, B.; Lerche, H.; Bergmüller, W.; Freilinger, C.; Severin, T.; Pischetsrieder, M. *J. Agric. Food Chem.* **2000**, *48*, 2111.
26. Ahmed, M. U.; Brinkmann Frye, E.; Degenhardt, T. P.; Thorpe, S. R.; Baynes, J. W. *Biochem. J.* **1997**, *324*, 565.
27. Brinkmann, E.; Wells-Knecht, K. J.; Thorpe, S. R.; Baynes, J. W. *J. Chem. Soc., Perkin Trans. 1* **1998**, 2817.
28. Nemet, I.; Varga-Defterdarović, L.; Turk, Z. *Clin. Biochem.* **2004**, *37*, 875.
29. Friedamann, T. E. *J. Biol. Chem.* **1927**, *73*, 331.
30. Bracher, D.; Hilderbrand, D. *Liebigs Ann. Chem.* **1993**, 837.
31. Röper, H.; Röper, S.; Heyns, K. *Carbohydr. Res.* **1983**, *116*, 184.