Formation of Tetrahydro-β-carbolines and β-Carbolines during the Reaction of L-Tryptophan with D-Glucose

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The reaction of L-tryptophan (Trp) with D-glucose under conditions that can occur during food processing and preparation was studied by high-performance liquid chromatography with diode array detection (HPLC/DAD). Besides the well-established glucose−tryptophan Amadori product (AP), (1*R*,3*S*)-1-(D-*gluco*-1,2,3,4,5-pentahydroxypentyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (PHP-THβC) was identified as an important product of this reaction. For preparation, PHP-THβC was obtained in high yields when Trp and D-glucose were reacted under strongly acidic conditions after heating in methanol. At elevated reaction temperatures (150 °C) 1-acetyl-β-carboline (acetyl-βC), was detected in significant concentrations. The mixtures were heated under variations of reaction time and temperature, and AP, PHP-THβC, and acetyl-βC were quantified. In the presence of air oxygen or mild, food relevant oxidants, such as L-dehydroascorbic acid, PHP-THβC was readily oxidized to a product that was identified as the previously unknown 1-(D-*gluco*-1,2,3,4,5-pentahydroxypentyl)-β-carboline (PHP-βC). Formation of PHP-THβC and PHP-βC in foodstuffs would deserve particular interest because multiple physiological activity of THβC and βC derivatives has been shown previously.

Keywords: *Maillard reaction; tryptophan; tetrahydro-β-carboline; β-carboline*

INTRODUCTION

Besides its nutritional value, the essential amino acid L-tryptophan (Trp) influences several physiological processes, such as brain function, hypertension, and behavior (Friedman and Cuq, 1988). Although Trp is quite stable in neutral solution, it is readily degraded in the presence of reducing sugars, such as glucose or fructose (Leahy and Warthesen, 1983). D-Glucose reacts with the α-amino group of the free amino acid and, after rearrangement, the Amadori product (AP; for structure, see Figure 1) is formed (Lee et al., 1979; Huber et al., 1989). In the presence of reactive carbonyl compounds, Trp undergoes cyclization, the so-called Pictet−Spengler reaction, which has been used successfully to synthesize tetrahydro-β-carbolines (THβC) (Bailey et al., 1994). Several THβC derivatives, for example, 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid and 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, have been detected in foodstuffs (Gutsche and Herderich, 1997), such as fermented alcoholic beverages (Herraiz and Ough, 1993), smoked food (Papavergou and Clifford, 1992), or soy sauce (Adachi et al., 1991; Herraiz, 1996). Under harsher conditions THβC can undergo decarboxylation and oxidation and, as a consequence, β-carboline (βC) derivatives have been isolated from reaction mixtures of sugars and Trp or tryptamine (Severin and Bräutigam, 1973; Bräutigam and Severin, 1974; Wang et al., 1999). βC derivatives, which are substituted with a hydroxymethylfuranyl group in position 1, have been identified in soy sauce (Nakatsuka et al., 1986).

The formation of THβC and βC compounds in foodstuffs is of particular interest because it has been demonstrated that they are of physiological relevance. Numerous THβC and βC derivatives show binding to the benzodiazepine receptor (Cain et al., 1982). Furthermore, premutagenicity (Kinae et al., 1986), inhibitory activity on monoamine oxidase (Ho, 1972), and membrane translocation mechanisms (Kehr et al., 1978) and psychotropic effects (Naranjo, 1969) have been reported besides others.

Here we report the formation of (1*R*,3*S*)-1-(D-*gluco*-1,2,3,4,5-pentahydroxypentyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (PHP-THβC) in Maillard reaction mixtures of D-glucose and Trp. The product yields were determined by HPLC and related to those of the AP and 1-acetyl-β-carboline. Furthermore, it was revealed that oxidation of the PHP-THβC derivative by air or by L-dehydroascorbic acid gives rise to the previously unknown 1-(D-*gluco*-1,2,3,4,5-pentahydroxypentyl)-β-carboline (PHP-βC).

MATERIALS AND METHODS

Apparatus. ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz), H,H-COSY, C,H-COSY (correlated spectroscopy), and attached proton transfer (APT) spectra were recorded with a JEOL 400 GSX spectrometer with $(CH_3)_4Si$ as internal standard. NOE difference experiments were performed on a Bruker AM 360 FT-NMR spectrometer at 360.13 MHz. Chemical shifts are reported in parts per million. Mass spectral analyses were obtained with an HP 5989 A MS engine and positive and

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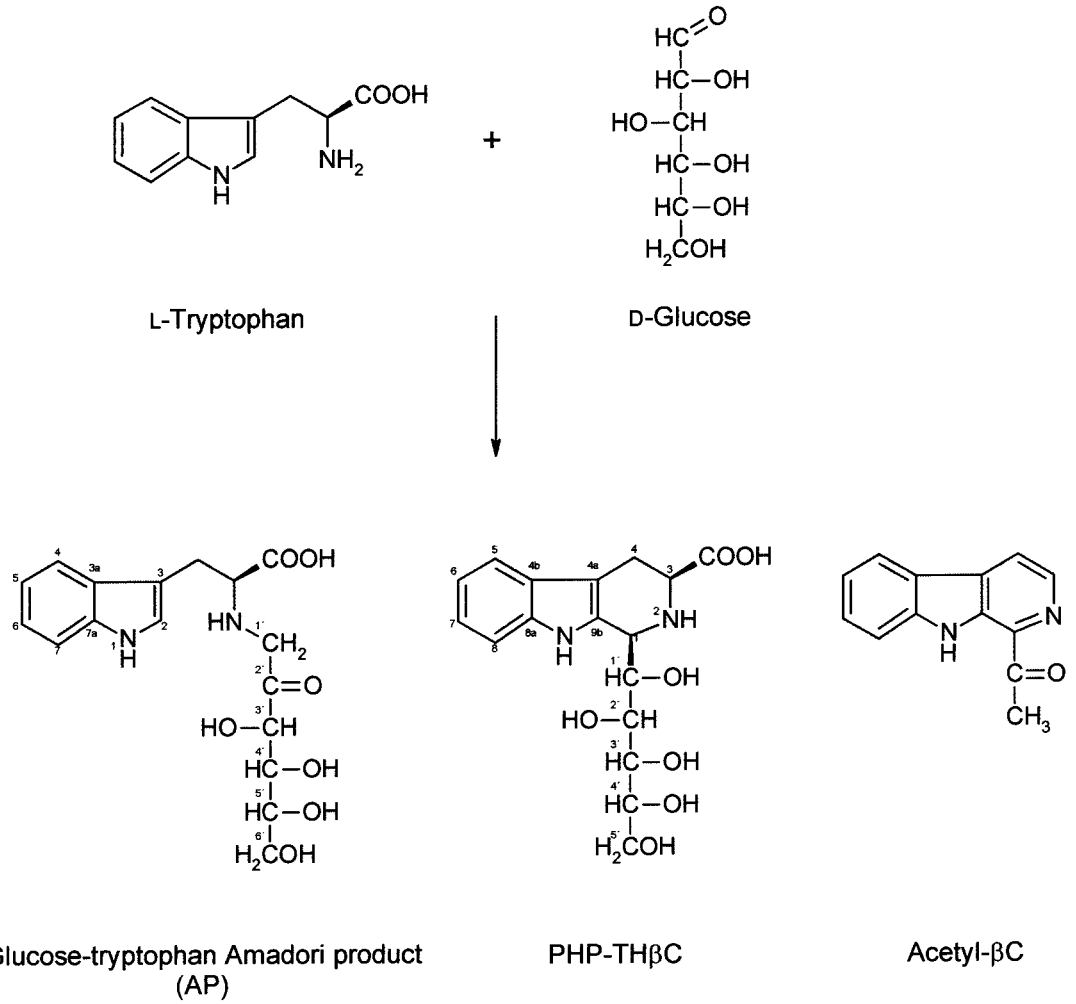

Figure 1. Formation of AP, PHP-THβC, and acetyl-βC during the reaction of Trp with D-glucose.

negative FABMS data with a Kratos MS 80 RFA spectrometer. Elemental analyses were obtained with a Heraeus Rapid instrument. UV spectra were taken from DAD.

Reagents. Methanol Lichrosolv, chromatography grade, was purchased from Merck (Darmstadt, Germany). Deionized water was distilled before use for HPLC. Amberlite 200 (strongly acidic cation exchanger) and palladium (10%) on activated charcoal were obtained from Fluka (Deisenhofen, Germany), and *N*-acetyl-L-tryptophan was from Bachem (Heidelberg, Germany).

High-Performance Liquid Chromatography (HPLC). Analytical HPLC was performed with a Merck L-7100 gradient pump and a Merck L-7450 photodiode array detector including Merck-Hitachi model D-7000 Chromatography Data Station software. A column packed with Nucleosil 100-5 (C-18 HD, 250 × 4 i.d., guard cartridge, 8 × 4 mm) from Macherey and Nagel (Düren, Germany) was used. For elution a gradient was used of 100% A from 0 to 5 min, 0−75% B from 5 to 35 min, and 100% B from 40 to 50 min at a flow rate of 0.8 mL/min (solvent A, 50 mM triethylammonium acetate, pH 5.8; solvent B, methanol). The substances were detected by a diode array detector at a wavelength range from 200 to 400 nm.

For preparative HPLC, a Merck L-6250 pump, a Merck L-4000 UV detector, and a Merck D-2500 chromatointegrator were used. Preparative HPLC was performed on a Supelcosil 250-21,2 column (Supelco) packed with LC-18-DB, 5 μm particle size. System A: As eluent 60% A from 0 to 23 min and 40% A from 23 to 55 min at a flow rate of 10 mL/min (solvent A, 5 mM ammonium formate, pH 4.5; solvent B, methanol) was used. The detection wavelength was 275 nm. System B: The eluent was 85% A from 0 to 29 min and 70% A from 29 to 51 min at a flow rate of 10 mL/min. The detection wavelength was 250 nm. System C: The eluent was 85% A at a flow rate of 10 mL/min. The detection wavelength was 220 nm.

Synthesis of *N*-(1-Deoxy-D-fructos-1-yl)tryptophan (AP). *N*-(1-Deoxy-D-fructos-1-yl)tryptophan was prepared according to the literature (Heyns and Noack, 1964). The AP was dried over P_2O_5 in high vacuum (3×10^{-2} Torr) and additionally for several hours at 80 °C. The product was obtained predominantly as the cyclic semiacetal with the following composition. Anal. Calcd for $C_{17}H_{22}N_2O_7$: C, 55.73; H, 6.05; N, 7.65. Found: C, 55.54; H, 6.41; N, 7.47. The spectral data were in accordance with the literature (Röper et al., 1983). UV λ_{max} 279 nm.

Synthesis of (1*R*,3*S*)-1-(D-*gluco*-1,2,3,4,5-Pentahydroxypentyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic Acid (PHP-THβC). The synthesis of the compound was achieved by a Pictet−Spengler type reaction. [For general procedures of Pictet−Spengler reactions, see, for example, Bailey et al. (1993).] L-Tryptophan (510 mg, 2.5 mmol) and NaOH (100 mg, 2.5 mmol) were dissolved in 5 mL of methanol. After evaporation of the solvent, the L-tryptophan sodium salt was dried under high vacuum, dissolved together with D-glucose (2.25 g, 12.5 mmol) in absolute methanol (60 mL), and heated under reflux for 2.5 h. The solution was concentrated under reduced pressure to a volume of 10 mL and, after cooling, added dropwise to methanolic 2 N HCl (10 mL). The mixture was stirred for 1 h at room temperature. After filtration (to remove precipitated NaCl), the solvent was evaporated. The residue was suspended in deionized water (50 mL) and filtered. Strongly acidic cation exchanger (50 g, Amberlite 200, Na^+-form, Sigma) was added without further conditioning. The mixture was stirred for 30 min at room temperature. To the

excess of sugar and amine-free reaction products, the cation exchanger was washed with 1000 mL of deionized water. The reaction product was eluted with 0.2 M NH_3. After removal of NH_3 under reduced pressure, the solution was lyophilized and purified by preparative HPLC (system C). (1*R*,3*S*)-1-(D-*gluco*-1,2,3,4,5-Pentahydroxypentyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid was obtained as a colorless solid with the following composition. Anal. Calcd for $C_{17}H_{22}N_2O_7$: C, 55.73; H, 6.05; N, 7.65. Found: C, 55.10; H, 6.73; N, 7.59.

^{1}H NMR (CD_3OD/CF_3COOD, H,H-COSY) δ 3.22 [ddd, 1H, ^2J (4*y*, 4*x*) = 16 Hz, ^3J (4*y*, 3) = 12 Hz, *J* (4*y*, 1) = 2.5, *H-4y*], 3.43 [ddd, 1H, ^2J (4*x*, 4*y*) = 16 Hz, ^3J (3, 4*x*) = 5.2 Hz, *J* (4*x*, 1) = 1.3, *H-4x*], 3.70−3.84 (m, 3H, *H-4′*, *H-5′a*, *H-5′b*), 3.93 [dd, 1H, ^3J (3′, 2′) = 1.9 Hz, ^3J (3′, 4′) = 7.5 Hz, *H-3′*], 4.28 [dd, 1H, ^3J (2′, 1′) = 4 Hz, ^3J (2′, 3′) = 1.9 Hz, *H-2′*], 4.42 [dd, 1H, ^3J (3, 4*x*) = 5.2 Hz, ^3J (3, 4*y*) = 12 Hz, *H-3*], 4.63 [dd, 1H, ^3J (1′, 1) = 1.8 Hz, ^3J (1′, 2′) = 4 Hz, *H-1′*], 5.08 (m, 1H, *H-1*), 6.96 ('t', 1H, *J* ∼ 8 Hz, *H-6*)*, 7.06 ('t', 1H, *J* ∼ 8 Hz, *H-7*)*, 7.28 ('d', 1H, *J* ∼ 8 Hz, *H-8*)*, 7.38 ('d', 1H, *J* ∼ 8 Hz, *H-5*)*. *(Signal assignment in single quotes indicates the apparent signal which represent obviously a signal of higher order.)

^{13}C NMR (CD_3OD/CF_3COOD, APT, C,H-COSY) δ 21.6 (*C-4*), 55.7 (*CH-3*), 56.5 (*CH-1*), 63.3 (CH_2-*5′*), 70.1 (*CH-1′*), 70.9 (*CH-2′*), 71.1 (*CH-3′*), 71.7 (*CH-4′*), 107.4 (*C-4a*), 111.2 (*CH-8*), 117.6 (*CH-5*), 119.4 (*CH-6*), 122.3 (*CH-7*), 126.1 (*C-4b*), 127.3 (*C-9a*), 136.4 (*C-8a*), 169.8 (*C*OOH).

FAB-MS (Cs, 20 kV, glycerin), *m*/*z* 367 $[M + H]^+$, 365 $[M - H]^+$. UV λ_{max} 273 nm.

Synthesis of the Diastereomers (3*S*)-1-(1,2-Dihydroxyethyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic Acid. According to the literature (Severin and Heidenhain, 1966) the diastereomers A and B of 1-(1,2-dihydroxyethyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid were prepared using DL-glyceraldehyde and L-tryptophan. The diastereomers were found in a ratio of A:B = 5:3.

^{1}H NMR (D_2O/D_3CCOOD) A: δ 3.05−3.22, 3.4−3.51 (m, 2H, *H-2a′*, *H-2b′*), 3.93−4.07 (m, 2H, *J* ∼ 12, 5 Hz, *H-3*, *H-4y*), 4.24−4.28 [dd, 1H, ^2J (4*x*, 4*y*) = 12.08 Hz, ^3J (4*x*, 3) = 4.76 Hz, *H-4x*], 4.54−4.56 (dt, 1H, *J* = 2.6, 4.3 Hz, *H-1′*), 5.08 (d, 1H, *H-1*), 7.12 (dd, 1H, ^3J = 6.96, 8.06 Hz, *H-6*), 7.21 (dd, 1H, ^3J = 6.96, 8.06 Hz, *H-7*), 7.43 (d, 1H, ^3J = 8.06 Hz, *H-8*), 7.53, 7.54 (d, 1H, ^3J = 7.69 Hz, *H-5*). B: 3.05−3.22, 3.4−3.51 (m, 2H, *H-2a′*, *H-2b′*), 3.76−3.94 (m, 2H, *J* ∼ 12, 5 Hz, *H-3*, *H-4y*), 4.12−4.19 [dd, 1H, ^2J (4*x*, 4*y*) = 12.08 Hz, ^3J (4*x*, 3) = 5.13 Hz, *H-4x*], 4.46−4.47 (dt, 1H, *J* = 3.4, 5.2 Hz, *H-1′*), 5.03 [d, 1H, ^3J (1, 1′) = 4.76 Hz, *H-1*]; signals H-5−H-8 are identical to those of B.

^{13}C NMR (D_2O/D_3CCOOD, APT) δ 21.0, 21.5 (CH_2-*4*), 56.1, 56.2, 56.3 (*CH-1*, *CH-3*), 62.2, 62.8 (CH_2-*2′*), 67.9, 68.4 (*CH-1′*), 106.5, 107.3 (*C-4a*), 110.7 (*CH-8*), 117.3 (*CH-5*), 119.0 (*CH-6*), 121.9 (*CH-7*), 124.6, 124.9, 125.2, 136.1 (*C-4b*, *C-8a*, *C-9a*), 171.4, 175.1 (*C*OOH); UV λ_{max} 270 nm.

Preparation of Acetyl-βC. According to the modified method of Mahboobi et al. (1999), a suspension of the diastereomers 1-(1,2-dihydroxyethyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (905 mg, 2.7 mmol) and 10% Pd/C (540 mg) in dry xylene (90 mL) were refluxed for 2 h. The catalyst was filtered off through a glass filter funnel from Schott (Mainz, Germany), pore size 4, the filter was washed with warm EtOH, and the solvent was evaporated. The residue containing educt and various reaction products was purified by preparative HPLC (system A) by collecting the fractions between 40 and 50 min. After evaporation of the solvent, the fractions were lyophilized to obtain acetyl-βC as a yellowish solid. Spectral data of the product were in accordance with the literature (Bracher and Hildebrand, 1993). UV λ_{max} 285, 309, 381 nm. Retention time for the analytical HPLC system described before was 40.8 min.

Reaction of L-Tryptophan with D-Glucose in Phophate Buffer at Different Temperatures. L-Tryptophan (1.021 g, 5 mmol) and D-glucose anhydrous (4.5 g, 25 mmol) were dissolved in 120 mL of 0.1 M phosphate buffer (pH 5) and heated under reflux. Samples were taken after 0.5, 1, 2, 4, 6, 7, 8, and 9 h.

Additionally, L-tryptophan (51 mg, 0.25 mmol) and D-glucose anhydrous (225 mg, 1.25 mmol) were dissolved in 6 mL of 0.1 M phosphate buffer (pH 5) and incubated in closed vessels for 7 h at 120 or 150 °C, respectively.

Quantification. The contents of *N*-(1-deoxy-D-fructos-1-yl)-tryptophan, (1*R*,3*S*)-1-(D-*gluco*-1,2,3,4,5-pentahydroxypentyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, and 1-acetyl-β-carboline in the reaction mixtures of L-tryptophan and D-glucose were determined. Identification of the products in the reaction mixtures was achieved by comparison of the retention times and UV spectra with those of the synthesized or isolated reference compounds. *N*-Acetyltryptophan was used as an internal standard. Response factors were calculated from injections of solutions of 0.77 mg/mL *N*-(1-deoxy-D-fructos-1-yl)tryptophan, 0.25 mg/mL (1*R*,3*S*)-1-(D-*gluco*-1,2,3,4,5-pentahydroxypentyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, 0.080 mg/mL 1-acetyl-β-carboline, and 0.11 mg/mL N^{α}-acetyltryptophan. Prior to injection into the HPLC, 100 μL of an N^{α}-acetyltryptophan standard solution (1.1 mg/mL bidistilled water) was added to 900 μL of the filtered samples. Quantification was achieved using a DAD-System-Manager Software D-7000 Chromatography Data Station (Merck-Hitachi) with manual baseline correction.

The yields are given as a percentage relative to the starting concentration of L-tryptophan and are means of triplicates.

Reaction of (1*R*,3*S*)-1-(D-*gluco*-1,2,3,4,5-Pentahydroxypentyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic Acid with L-Dehydroascorbic Acid or in the Presence of Air. Various amounts of PHP-THβC and L-dehydroascorbic acid were dissolved in 0.1 M phosphate buffer (pH 5) and incubated in closed vessels at 100 °C for 1−20 h. In other experiments the reaction was carried out without L-dehydroascorbic acid but in the presence of air. After incubation, the reaction mixtures were filtered and analyzed by HPLC/DAD.

Isolation of 1-(D-*gluco*-1,2,3,4,5-Pentahydroxypentyl)-β-carboline (PHP-βC). PHP-THβC (0.25 mmol) and L-dehydroascorbic acid (0.25 mmol) were dissolved in 10 mL of 0.1 M phosphate buffer (pH 5) and incubated in closed vessels at 100 °C for 90 h. The mixture was subsequently separated by preparative HPLC (system B). To isolate the main reaction product, the fractions between 44 and 49.5 min were collected. After evaporation of the solvent, the fractions were lyophilized to obtain 1-(D-*gluco*-1,2,3,4,5-pentahydroxypentyl)-β-carboline as a yellowish solid. ^{1}H NMR (DMSO-d_6, H,H-COSY) δ 3.29−3.33 (m, 2H, *H-3′*, *H-5a′*), 3.48−3.56 (m, 2H, *H-4′*, *H-5b′*), 4.17 [dd, 1H, ^3J (2′, 1′) = 5.49 Hz, ^3J (2′, 3′) = 1.7 Hz, *H-2′*], 5.16 [d, 1H, ^3J (1′, 2′) = 5.49 Hz, *H-1′*], 7.18 (dd, 1H, ^3J = 6.96, 7.69 Hz, *H-6*), 7.48 (dd, 1H, ^3J = 6.96, 8.06 Hz, *H-7*), 7.65 (d, 1H, ^3J = 8.06 Hz, *H-8*), 7.97 (d, 1H, ^3J = 5.49 Hz, *H-4*), 8.17 (d, 1H, ^3J = 7.69 Hz, *H-5*), 8.20 (d, 1H, ^3J = 5.49 Hz, *H-3*).

^{13}C NMR (DMSO-d_6, APT, C,H-COSY) δ 63.4 (CH_2-*5′*), 71.4 (*CH-4′*), 71.8 (*CH-2′*), 72.1 (*CH-3′*), 75.7 (*CH-1′*), 112.3 (*CH-8*), 113.5 (*CH-4*), 118.9 (*CH-6*), 120.4 (*C-4b*), 121.4 (*CH-5*), 127.8 (*CH-7*), 128.2, 133.7, 140.6 (*C-4a*, *C-8a*, *C-9a*), 136.4 (*CH-3*), 146.3 (*C-1*).

FAB-MS (Cs, 20 kV, m-NBA), *m*/*z* 319 $[M + H]^+$, 317 $[M - H]^+$. UV λ_{max} 215, 237, 286, 337 nm.

Retention time for the analytical HPLC system described before: 28.7 min.

Molecular Modeling Methods. Force field calculations were performed with the MM3 program package [Allinger, N., et al., MM3(96) software, Tripos and Associates, St. Louis, MO], and semiempirical calculations were performed with the MOPAC6 program package (MOPAC6, Seiler, F., U.S. Air Force Academy, Colorado Springs, CO) within the SYBYL program package (Sybyl version 6.6, Tripos and Associates, 1999) on a Silicon Graphics Indigo2 R10000 workstation. Geometrics were first minimized with MM3 as full optimizations with default parameters and additional pi-calculations for aromatic systems. Starting with these MM3 geometries AM1 calculations were performed as full optimizations using the keywords *precise xyz geo-ok*. Closed shell systems were calculated as singlet states; in charged systems net charges were set with the keyword *charge* according to their formal charges.

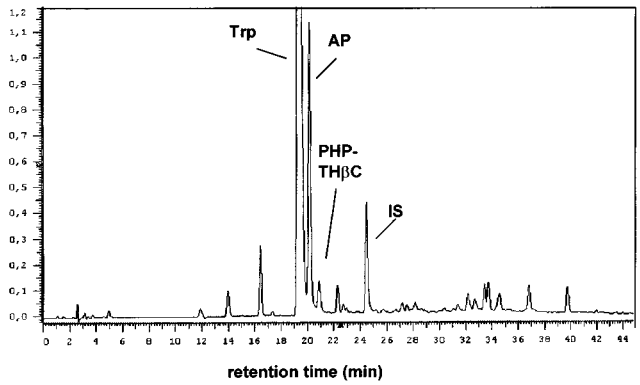

Figure 2. HPLC chromatogram of a mixture of D-glucose and L-tryptophan that was refluxed for 7 h in phosphate buffer (pH 5) (UV detection at 276 nm). IS, internal standard *N*-acetyl-L-tryptophan. Under these conditions acetyl-βC, PHP-βC, and the *trans* isomer of PHP-THβC were not detected. For chromatographic conditions and retention times of the other products see Materials and Methods.

RESULTS

D-Glucose was reacted with Trp in phosphate buffer, and the reaction mixtures were analyzed by HPLC/DAD (Figure 2). As main product the glucose−tryptophan AP was formed, which was identified by comparison of retention time and UV spectrum with those of the synthesized reference compound. During prolonged heating the formation of a new product could be observed. The compound was obtained in high yields, when D-glucose and sodium tryptophan were allowed to react in strongly acidic solution after heating in methanol. The product was isolated from the latter reaction mixture and identified by spectral data as PHP-THβC (Figure 1). The unambiguous assignment of the structure as the *cis*-1,3 isomer is difficult, because both compounds, the *cis*-1,3 and the *trans*-1,3 isomers, are expected to display very similar proton and carbon shifts in NMR. Therefore, semiempirical AM1 calculations and force field calculations were performed to determine the distance between H-1 and H-3. For the distance in the *cis*-1,3 isomer (both groups in equatorial position) a distance of 2.49 Å was calculated, whereas for the *cis*-1,3 isomer (both groups in axial position) and for the *trans*-1,3 isomer distances of 3.99 and 3.91 Å, respectively, were determined. In NOE difference experiments only protons at a distance of <3 Å show an effect. Therefore, NOE difference spectra were recorded, where a strong effect between H-1 and H-3 was observed. These results indicate that the product is the *cis*-1,3 isomer.

Further evidence for the *cis*-1,3 configuration was provided by the fact that acetylation of PHP-THβC resulted in the formation of a peracetylated lactone in which the ester bond was formed between the carboxylate group at position 3 and the hydroxyl group at C-1'. Lactonization, however, should only be possible if the groups are in *cis*-1,3 configuration (see also Discussion).

During synthesis of PHP-THβC the formation of a minor compound was detected. Preliminary analyses suggested that the product was the 1,3-*trans* isomer of PHP-THβC: Both products display the identical UV spectra, and a ^{1}H NMR that was recorded of the mixture of both compounds showed a second set of signals which is very similar to the signals of PHP-THβC. However, because the product was not detected in the samples where the maximum yield of the *cis*-1,3 isomer was <3%, further efforts to accomplish its structural assignment were not undertaken.

Under the applied conditions, at temperatures >100 °C in addition to AP and PHP-THβC a compound was detected that showed a UV spectrum different from the other products. The compound was identified as acetyl-βC by synthesis of the reference compound. Acetyl-βC was previously isolated from tryptamine and D-glucose that had been reacted under roasting conditions (Severin and Bräutigam, 1973).

After identification of the main products, Trp was heated with D-glucose under variations of reaction time and temperature, and AP, PHP-THβC, and acetyl-βC were quantified by HPLC. The results are summarized in Figure 3: When the mixture was heated under reflux, AP could be detected immediately after <30 min with the maximum yield of almost 9% (related to Trp) reached after 4 h of reaction. However, AP is not a stable end product, so a decrease of product yield was observed during prolonged heating. In contrast, the amount of PHP-THβC gradually increases during the entire reaction up to 1%. When the reaction temperature is raised to 120 °C, AP and PHP-THβC are formed in similar concentrations (2−2.5%). Additionally, the formation of acetyl-βC can be observed (2.6% yield). At an elevated temperature of 150 °C, neither AP nor PHP-THβC formation was detected; acetyl-βC was the only reaction product identified (4%).

β-Carbolines, which are oxidation products of THβC, deserve particular attention due to their physiological activity (Bracher and Hildebrand, 1992; Cain et al., 1982, and literature cited therein). Therefore, we have investigated the reaction conditions under which PHP-THβC is converted to the analogous βC derivative. For preparative purposes oxidation can be achieved, for example, by palladium-catalyzed dehydration of the analogous THβC derivative (Mahboobi et al., 1999). We have applied this method to THβCs with a polyhydroxyalkyl side chain, for example, to 1-(1,2-dihydroxyethyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, which is formed by the condensation of Trp with glyceraldehyde. Under these conditions, besides decarboxylation, elimination of water in the side chain took place and acetyl-βC was obtained. On the other hand, when PHP-THβC was reacted with mild oxidants, such as L-dehydroascorbic acid, the formation of a main product was detected by HPLC. The compound was isolated and identified as 1-(D-*gluco*-1,2,3,4,5-pentahydroxypentyl)-β-carboline (PHP-βC; Figure 4). PHP-βC was also detected in similar yields by oxidation with air oxygen. Therefore, it can be assumed that under the conditions of food processing and storage, PHP-THβC can be converted into the corresponding decarboxylated β-carboline, PHP-βC. Investigations to confirm this assumption by analyses of food products are currently in progress.

DISCUSSION

In this paper we have studied the reaction of D-glucose with Trp. It was clearly shown that under conditions which can occur during the processing or preparation of food, THβC and βC derivatives are formed, which are substituted by the unmodified pentahydroxypentyl residue, derived from glucose. These findings lead to the conclusion that those derivatives, which might have physiological activity, can also be found in processed food products. While this paper was prepared, an article

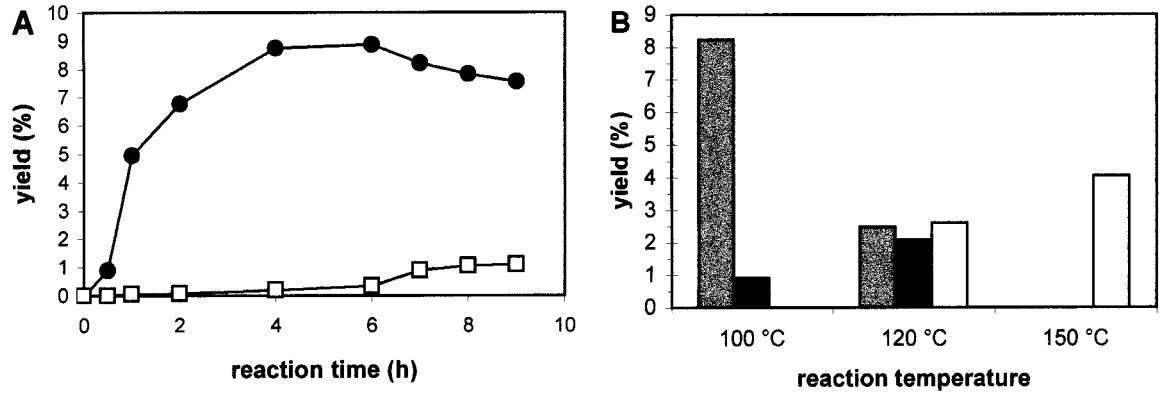

Figure 3. (A) Time-dependent formation of AP (●) and PHP-THβC (□) in a mixture of Trp and D-glucose that was refluxed at pH 5. (B) Formation of AP (gray bars), PHP-THβC (black bars), and acetyl-βC (white bars) in an analogous mixture that was heated at 100, 120, and 150 °C.

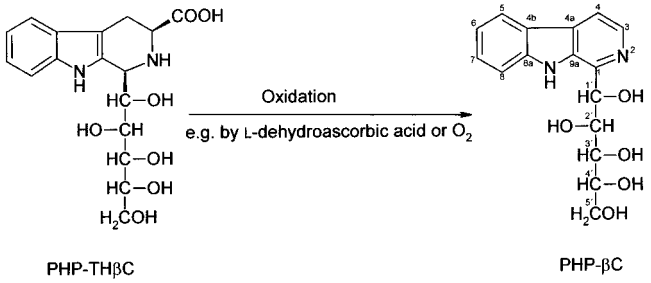

Figure 4. Oxidation of PHP-THβC by air oxygen or L-dehydroascorbic acid results in the formation of PHP-βC.

was published by Herderich and co-workers, which confirms this conclusion (Gutsche et al., 1999). In these experiments Trp was heated, for example, with glucose under acidic conditions, and the main reaction products were isolated and their structures were established by spectral data as PHP-THβC and two other Trp glycoconjugates. Subsequently, PHP-THβC, AP, and Trp-*N*-glycosides were detected in soy sauce and fruit syrup. Because Gutsche et al. were able to isolate both isomers of PHP-THβC (*cis*- and *trans*-1,3 isomers), an unambiguous assignment of the configuration could be achieved. They describe for the *cis*-1,3 isomer a coupling constant ^3J (H-1′, H-1) of 1.6 Hz, whereas the *trans* isomer shows a coupling constant ^3J (H-1′, H-1) of 8.4 Hz. These findings additionally strengthen the assumption that the compound PHP-THβC described in this paper is the *cis*-1,3 isomer because we have measured a coupling constant ^3J (H-1′, H-1) of 1.8 Hz for our compound.

Horiuchi et al. have isolated a THβC derivate from human urine, for which they have proposed the structure of PHP-THβC (Horiuchi et al., 1994). However, the recent paper of Gutsche et al. has shown that the main Trp glycoconjugate in human urine, which they suggest is the same compound isolated by Horiuchi et al., is a Trp-*C*-glycoside (Gutsche et al., 1999) and therefore does not have the PHP-THβC structure.

To our knowledge, the formation of PHP-βC in Maillard reaction mixtures has not been reported earlier.

ABBREVIATIONS USED

Acetyl-βC, 1-acetyl-β-carboline; AP, glucose−tryptophan Amadori product; DAD, diode array detection; PHP-βC, 1-(D-*gluco*-1,2,3,4,5-pentahydroxypentyl)-β-carboline; PHP-THβC, (1*R*,3*S*)-1-(D-*gluco*-1,2,3,4,5-pentahydroxypentyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid; Trp, L-tryptophan.

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