Reaction of Tryptophan with Carbohydrates: Mechanistic Studies on the Formation of Carbohydrate-Derived β-Carbolines

Stefanie Diem and Markus Herderich*

Institut für Pharmazie und Lebensmittelchemie, Universität Würzburg, Am Hubland, 97074 Würzburg, Germany

Numerous carbohydrate-derived β-carbolines have been identified for the first time in model reactions of tryptophan with glucose and ribose, as well as in food samples. Extending these structural studies, we performed detailed investigations to elucidate the corresponding intermediates and formation pathway of these alkaloids. Degradation experiments with purified tryptophan glycoconjugates established that only *glyco*-tetrahydro-β-carboline-3-carboxylic acids, and not the N-glycosides nor the C-glycoconjugates represented the direct precursors of carbohydrate-derived β-carbolines. In addition, the significance of the oxidative decarboxylation reaction as the initial step for formation of 1-substituted β-carbolines was proven. Finally, the stereochemistry of the carbohydrate-derived side chain was studied by means of CD spectroscopy and HPLC-CD experiments. These detailed stereochemical analyses yielded experimental evidence for the racemization steps required for formation of the carbohydrate-derived harman alkaloids and confirmed the proposed reaction pathway.

Keywords: *L-Tryptophan; β-carbolines; Maillard reaction; stereochemistry; HPLC-CD*

INTRODUCTION

Tetrahydro-β-carboline-3-carboxylic acids are easily formed by Pictet–Spengler condensation of tryptophan with aldehydes or α-keto acids (*1*). Oxidative decarboxylation of tetrahydro-β-carboline-3-carboxylic acids results in formation of the respective β-carboline alkaloids (*2*). Both tetrahydro-β-carboline-3-carboxylic acids and β-carbolines have been detected in food samples such as smoked meat, soy sauce, cheese or yogurt, and in alcoholic fermentation products such as beer, wine, or sake (*3–8*). Interestingly, these alkaloids also occur in biological tissues and fluids where they might act as neuromodulators (*9*, *10*). Pharmacologically relevant effects of these indole alkaloids include the interaction with benzodiazepine receptors (*11*, *12*) and the inhibition of monoamine oxidase A and monoamine uptake (*13*). In addition, their bioactivation by *N*-methylation can give rise to endogenous neurotoxins that are of interest in the etiology of Parkinson's disease (*14*, *15*). Furthermore, the β-carbolines harman and norharman have been demonstrated to have particular comutagenic and genotoxic properties (*16*).

With regard to the reaction of aldohexoses with tryptophan, the formation of various carbohydrate-derived tetrahydro-β-carboline-3-carboxylic acids and β-carbolines has been observed. Thus, we detected novel *glyco*-tetrahydro-β-carboline-3-carboxylic acids in model reactions of tryptophan with hexoses and pentoses, as well as in food samples (*17*, *18*). Rönner and co-workers (*19*) investigated the oxidative decarboxylation of 1-(D-*gluco*-1,2,3,4,5-pentahydroxypentyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid which resulted in formation of 1-(D-*gluco*-1,2,3,4,5-pentahydroxypentyl)-β-carboline. Wang and co-workers (*20*) identified 1-(1,3,4-trihydroxybutyl)-β-carboline and 1-(1,4-dihydroxybutyl)-β-carboline in model reactions of tryptophan with xylose. Furthermore, we recently identified numerous hexose- and pentose-derived β-carbolines in food samples (*18*, *21*). In this paper, we report on detailed studies we performed to elucidate the formation pathway of these novel alkaloids.

MATERIALS AND METHODS

Apparatus. An Applied Biosystems (BAI, Bensheim, Germany) 140b pump was used for C_{18}-HPLC-MS. For chiral HPLC-MS, a Knauer (Berlin, Germany) HPLC 64 pump with an analytical pump head was used. HPLC-DAD analysis, ESI-MS, MS/MS, and HRMS experiments were performed as described previously (*21*). CD spectra were recorded on a Jasco (Gross-Umstadt, Germany) J-600 spectropolarimeter. For HPLC-CD analyses, a Jasco PU-1580 intelligent HPLC pump and a Jasco gradient device LG-980–02S was used. The detector was a Jasco J-715 spectropolarimeter, equipped with the standard flow-probe detection cell from Jasco. UV–Vis and fluorescence spectra were recorded on a Shimadzu (Duisburg, Germany) UV-2101PC spectrophotometer and a Shimadzu RF-540 spectrofluorophotometer, respectively. NMR spectra were acquired with a Bruker (Rheinstetten, Germany) DMX 600 spectrometer; chemical shifts are reported relative to the solvent signal (CD_3OD: 3.31 ppm for ^1H NMR, 49.0 ppm for ^{13}C NMR) as reference.

Reagents. Water of HPLC gradient grade, L-tryptophan, D-glucose, TLC plates (SiO_2 60) and Lichroprep C_{18} (particle size 43–60 μm) were from Merck (Darmstadt, Germany). Silica gel (particle size 32–63 μm) was from ICN (Eschwege, Germany). Trifluoroacetic acid (TFA, spectroscopic grade), tryptamine hydrochoride and D-ribose were from Fluka (Buchs, Switzerland). Acetonitrile, isohexane, and 2-propanol, all of HPLC gradient grade, were from Fisher (Loughborough, UK). All other chemicals were of analytical purity.

Isolation of β-Carboline 4 from Model Reactions. A total of 500 mg of L-tryptophan and 900 mg of D-glucose were dissolved in 8 mL of water and adjusted to pH 1 with 2 M

* Corresponding author (fax +49-931-8885484; e-mail mherderi@pzlc.uni-wuerzburg.de).

10.1021/jf010379o CCC: $20.00 © 2001 American Chemical Society
Published on Web 10/19/2001

HCl. The reaction mixtures were kept at 80 °C for 12 days. Model reactions were filtered, adjusted to pH 8 with 4 M NaOH, and extracted with ethyl acetate. Completeness of extraction was established by TLC on silica gel plates using acetone−chloroform−25% aqueous NH_4OH (6:2:1, v/v/v, use of organic phase) as eluent and detection of fluorescent spots with excitation at 366 nm. The combined ethyl acetate extracts were dried with anhydrous sodium sulfate, and the solvent was removed under vacuum at 40 °C. The extract was applied to a silica gel column (77 × 2.1 cm) and eluted with acetone−chloroform−25% aqueous NH_4OH (6:2:1, v/v/v, use of the organic phase). Fractions (10 mL) were collected and checked by TLC as described above as well as by HPLC-DAD (200−600 nm) with an Eurospher 100 C_{18} ODS column (250 × 4 mm i.d., particle size 5 μm; Knauer) using a linear binary gradient. Solvent A was 0.05% TFA in water (v/v) and solvent B was acetonitrile. HPLC was programmed as follows: 0 min, 5% B; 30 min, 50% B; 31 min, 100% B; 35 min, 100% B. The flow rate was 1 mL/min, and the injection volume was 20 μL for all analyses. Fractions containing **4** were pooled and concentrated by evaporation of the solvent under vacuum at 40 °C. The concentrated fractions were adjusted to pH 7 and further purified by column chromatography with Lichroprep C_{18} material. The column (15 × 1.6 cm) was equilibrated with water/TFA (100:0.05, v/v) and then eluted with 100 mL water/acetonitrile/TFA (95:5:0.05, v/v/v), 100 mL water/acetonitrile/TFA (90:10:0.05, v/v/v), and 100 mL water/acetonitrile/TFA (85:15:0.05, v/v/v). Fluorescent fractions were analyzed by HPLC-DAD as descibed above. Fractions containing only **4** were pooled and freeze-dried and yielded **4** as pale yellow amorphous powder.

4: ESI-MS, $[M+H]^+$ *m/z* 253; ESI-MS/MS (precursor ion *m/z* 253, 20 eV, 267 mPa Ar), *m/z* 253, 235, 233, 220, 206, 198, 197; EI−HRMS, calculated for $C_{16}H_{16}N_2O$ (M^+): 252.1263, found: 252.1264; UV λ_{max} (H_2O), 351 nm, 289 nm, 241 nm, 208 nm; fluorescence (H_2O/acetonitrile/TFA 75:25:0.05, v/v/v), excitation λ_{max} 256, 302, or 372 nm, emission λ_{max} 448 nm; R_f (acetone−chloroform−25% aqueous NH_4OH 6:2:1, v/v/v, use of the organic phase), 0.82; ^{1}H NMR (600 MHz, CD_3OD), δ in ppm: 8.57 (d, 1H, H-4), 8.41 (d, 1H, H-5), 8.34 (d, 1H, H-3), 7.83−7.77 (m, 2H, H-7 and H-8), 7.47 (dd, 1H, H-6), 5.59 (m, 1H, H-1′), 5.53 (m, 1H, H-3′), 5.35 (dd, 1H, H-4′), 2.79 (m, 1H, H-2′a), 2.70 (m, 1H, H-2′b), 1.52 (d, 3H, H-5′a, H-5′b and H-5′c); $J_{5,6}$ = 8.1 Hz, $J_{6,7}$ = 6.4 Hz, $J_{3,4}$ = 6.2 Hz, $J_{3',4'}$ = 15.3, $J_{4',5'a}$ = $J_{4',5'b}$ = $J_{4',5'c}$ = 6.4; ^{13}C NMR (150 MHz, CD_3OD), δ in ppm: 145.5 (C-8a), 144.4 (C-1), 135.5 (C-4a), 133.2 (C-7), 132.9 (C-9a), 131.1 (C-4′), 129.3 (C-3), 125.6 (C-3′), 124.1 (C-5), 123.1 (C-6), 121.2 (C-4b), 117.1 (C-4), 113.9 (C-8), 69.6 (C-1′), 40.9 (C-2′), 18.0 (C-5′). Signal assignment was confirmed by DEPT, HH-COSY, HMQC, and HMBC experiments.

Isolation of β-Carbolines 7a and 7b from Model Reactions. A total of 250 mg of l-tryptophan and 375 mg of D-ribose were dissolved in 8 mL of water, adjusted to pH 1 with concentrated HCl, and kept at 80 °C for 31 days. For isolation of β-carbolines, model reactions were filtered, adjusted to pH 8 with 4 M NaOH, and extracted with ethyl acetate. Completeness of extraction was proved by TLC as described above. The combined ethyl acetate extracts were dried with anhydrous sodium sulfate, and the solvent was removed under vacuum at 40 °C. The extract was applied to a silica gel column (68 × 2.1 cm) and eluted with acetone−chloroform−25% aqueous NH_4OH (6:2:1, v/v/v, use of the organic phase). Fractions were collected and checked by TLC and HPLC-DAD as described above. Fractions containing **7a/b** were pooled and concentrated by evaporation of the solvent under vacuum at 40 °C. The concentrated fractions were adjusted to pH 7 and further purified by column chromatography with Lichroprep C_{18} material. The column (33 × 3.3 cm) was equilibrated with water/acetonitrile/TFA (90:10:0.05, v/v/v) and then eluted with 3.5 L of water/acetonitrile/TFA (90:10:0.05, v/v/v), 500 mL of water/acetonitrile/TFA (85:15:0.05, v/v/v) and 500 mL of water/acetonitrile/TFA (75:25:0.05, v/v/v). Fluorescent fractions were analyzed by HPLC-DAD as described above. Fractions containing only **7a** or **7b** were pooled, freeze-dried, and yielded the β-carbolines as pale yellow amorphous powders:

7a: ESI-MS, $[M+H]^+$ *m/z* 257; ESI-MS/MS (precursor ion *m/z* 257, 20 eV, 267 mPa Ar), *m/z* 257, 239, 221, 195; EI-HRMS, calculated for $C_{15}H_{16}N_2O_2$ (M^+): 256.1212, found: 256.1208; UV λ_{max} (H_2O), 349 nm, 289 nm, 241 nm, 208 nm; fluorescence (H_2O/TFA 100:0.05, v/v), excitation λ_{max} 257, 302, or 370 nm, emission λ_{max} 451 nm; R_f (acetone−chloroform−25% aqueous NH_4OH 6:2:1, v/v/v, use of the organic phase), 0.56; ^{1}H NMR (600 MHz, CD_3OD), δ in ppm: 8.56 (d, 1H, H-4), 8.40 (d, 1H, H-5), 8.34 (d, 1H, H-3), 7.82−7.77 (m, 2H, H-7 and H-8), 7.47 (dd, 1H, H-6), 5.73 (m, 1H, H-1′), 4.09 (m, 1H, H-3′), 2.19−2.14 (m, 2H, H-2′a and H-2′b), 1.25 (d, 3H, H-4′a, H-4′b and H-4′c); $J_{5,6}$ = 7.9 Hz, $J_{6,7}$ = 5.2 Hz, $J_{3,4}$ = 6.3 Hz, $J_{3',4'a}$ = $J_{3',4'b}$ = $J_{3',4'c}$ = 6.3; ^{13}C NMR (150 MHz, CD_3OD), δ in ppm: 145.4 (C-8a), 144.7 (C-1), 135.4 (C-4a), 133.1 (C-7), 132.9 (C-9a), 129.3 (C-3), 124.0 (C-5), 123.0 (C-6), 121.3 (C-4b), 117.0 (C-4), 113.9 (C-8), 68.3 (C-1′), 65.8 (C-3′), 45.4 (C-2′), 24.0 (C-4′). Signal assignment was confirmed by DEPT, HH-COSY, HMQC, and HMBC experiments.

7b: ESI-MS, $[M+H]^+$ *m/z* 257; ESI-MS/MS (precursor ion *m/z* 257, 20 eV, 267 mPa Ar), *m/z* 257, 239, 221, 195; EI-HRMS, calculated for $C_{15}H_{16}N_2O_2$ (M^+): 256.1212, found: 256.1211; UV λ_{max} (H_2O), 349 nm, 289 nm, 241 nm, 208 nm; fluorescence (H_2O/TFA 100:0.05, v/v), excitation λ_{max} 257, 302, or 370 nm, emission λ_{max} 450 nm; R_f (acetone−chloroform−25% aqueous NH_4OH 6:2:1, v/v/v, use of the organic phase), 0.53; ^{1}H NMR (600 MHz, CD_3OD), δ in ppm: 8.58 (d, 1H, H-4), 8.41 (d, 1H, H-5), 8.34 (d, 1H, H-3), 7.83−7.76 (m, 2H, H-7 and H-8), 7.47 (dd, 1H, H-6), 5.72 (dd, 1H, H-1′), 4.27 (m, 1H, H-3′), 2.04−1.94 (m, 2H, H-2′a and H-2′b), 1.26 (d, 3H, H-4′a, H-4′b and H-4′c); $J_{5,6}$ = 8.0 Hz, $J_{6,7}$ = 6.8 Hz, $J_{3,4}$ = 6.2 Hz, $J_{1',2'a}/J_{1',2'b}$ = 3.5/9.3 Hz (assignment exchangeable), $J_{3',4'a}$ = $J_{3',4'b}$ = $J_{3',4'c}$ = 6.3; ^{13}C NMR (150 MHz, CD_3OD), δ in ppm: 145.5 (C-8a), 145.3 (C-1), 135.6 (C-4a), 133.2 (C-7), 132.6 (C-9a), 129.3 (C-3), 124.0 (C-5), 123.1 (C-6), 121.2 (C-4b), 117.1 (C-4), 113.9 (C-8), 66.9 (C-1′), 65.0 (C-3′), 46.1 (C-2′), 24.3 (C-4′). Signal assignment was confirmed by DEPT, HH-COSY, HMQC, and HMBC experiments.

Degradation Studies with Reference Compounds. Aqueous solutions (2 mg/mL, adjusted to pH 1.2 with 2 M HCl) of *N*-glycoside 1-(β-D-glucosyl)-L-tryptophan, *C*-glycoconjugate 2-(β-D-galactosyl)-L-tryptophan, *trans-galacto*-, *cis-galacto*-tetrahydro-β-carboline-3-carboxylic acids, and a mixture of *trans*- and *cis-gluco*-tetrahydro-β-carboline-3-carboxylic acids were stored at 80 °C. Aliquots were sampled after 0, 6, 11, and 13 d, were diluted, and were directly analyzed by HPLC-MS. An aqueous solution (1 mg/mL, adjusted to pH 1.2 with 2 M HCl) of 1-(D-*gluco*-1,2,3,4,5-pentahydroxypentyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (obtained from Prof. Pischetsrieder, University of Erlangen) was treated in the same way. Here, aliquots were sampled after 0, 5, 12, 18, 27, 35, and 62 d, were diluted and directly analyzed by HPLC-MS. Chromatographic separation was performed on a Symmetry C_{18} ODS column (150 × 2.1 mm i.d., particle size 5 μm; Waters, Milford, MA) using a binary gradient. Solvent A was 0.05% TFA in water (v/v), and solvent B was acetonitrile. HPLC was programmed as follows: pressurizing with 50% B, equilibration time 10 min at 5% B and linear gradient elution (0 min, 5% B; 30 min, 30% B; 31 min, 100% B; 35 min, 100% B). The flow rate was 200 μL/min, and the injection volume was 5 μL for all analyses. For pneumatically assisted ESI, the spray voltage was set to 3.5 kV; the temperature of the heated capillary was 220 °C. Nitrogen served both as sheath (60 psi) and auxiliary gas (10 units). Product ion scanning was performed at a collision gas pressure of 267 mPa Ar and a collision energy of 20 or 22 eV with a total scan duration of 1.0 s for a single spectrum.

Model Reactions of Tryptamine with Glucose. A total of 246 mg of tryptamine hydrochloride and 450 mg of D-glucose were dissolved in 8 mL of water, adjusted to pH 1 with concentrated HCl and kept at 80 °C. Aliquots were taken after 8 and 26 d, were diluted with water (1/100 v/v), and were directly analyzed by HPLC-MS as described above.

Chiral HPLC-MS Analysis of Carbohydrate-Derived β-Carbolines. Chromatographic separation was performed on a Chiralcel OD-H column (250 × 4.6 mm i.d., 5 μm; Daicel, Tokyo, Japan) with isocratic elution (isohexane/2-propanol 80/

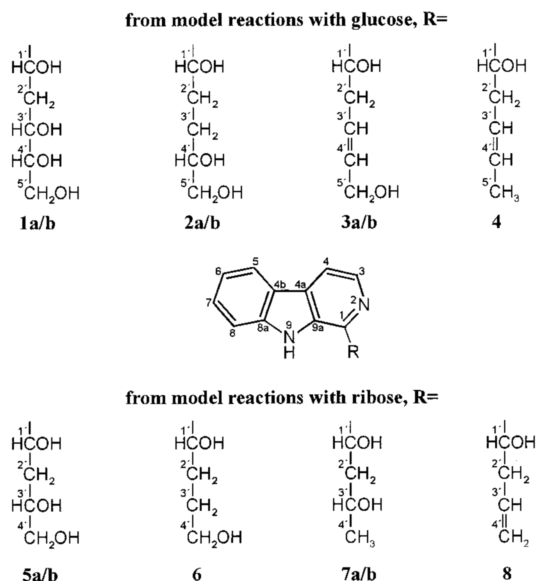

Figure 1. Carbohydrate-derived β-carbolines from model reactions of tryptophan with glucose or ribose (**1a/b**: 1-(1,3,4,5-tetrahydroxypent-1-yl)-β-carbolines; **2a/b**: 1-(1,4,5-trihydroxypent-1-yl)-β-carbolines; **3a/b**: (*Z*)-/(*E*)-1-(1,5-dihydroxypent-3-en-1-yl)-β-carboline; **4**: (*E*)-1-(1-hydroxypent-3-en-1-yl)-β-carboline; **5a/b**: 1-(1,3,4-trihydroxybut-1-yl)-β-carbolines; **6**: 1-(1,4-dihydroxybut-1-yl)-β-carboline; **7a/b**: 1-(1,3-dihydroxybut-1-yl)-β-carbolines; **8**: 1-(1-hydroxybut-3-en-1-yl)-β-carboline).

20). The flow rate was 500 μL/min, and the injection volume was 5 or 10 μL. Using a postcolumn T-splitter 225 μL/min were directed into the mass spectrometer via the ESI interface. For pneumatically assisted ESI, nitrogen served both as sheath (70 psi) and auxiliary gas (10 units). All other ESI-MS/MS parameters were as described above.

Chiral HPLC-CD Analysis of Carbohydrate-Derived β-Carbolines. Chromatographic separation was performed on a Chiralcel OD-H column (250 × 4.6 mm i.d., 5 μm; Daicel) with isocratic elution (isohexane/2-propanol 80/20). The flow rate was 500 μL/min, and the injection volume was 20 μL.

RESULTS AND DISCUSSION

Structures and Occurrence of Carbohydrate-Derived β-Carbolines. Our studies on the reaction of tryptophan with sugars have revealed the formation of various carbohydrate-derived β-carbolines (Figure 1). In addition to the recently published compounds **1a/b**, **2a/b**, **3a/b**, **5a/b**, **6**, and **8** (*18*, *21*), we detected the β-carbolines **4** and **7a/b** in model reactions of tryptophan with glucose and ribose. Isolation and subsequent characterization by tandem mass spectrometry and NMR spectroscopy led to identification of **4** as (*E*)-1-(1-hydroxypent-3-en-1-yl)-β-carboline, and **7a/b** as diastereomeric 1-(1,3-dihydroxybut-1-yl)-β-carbolines, respectively. Significant formation of **7a/b** besides the isomeric 1-(1,4-dihydroxybut-1-yl)-β-carboline **6** was only observed under strongly acidic conditions below pH 2. Furthermore, analysis of food samples proved the occurrence of these carbohydrate-derived β-carbolines in various nutritional sources. With the help of HPLC-MS/MS, **1a/b**, **2a/b**, **3a/b**, **5a/b**, **6**, and **8** were identified in products such as soy sauce, fish sauce, or ketchup, and in alcoholic beverages such as beer, wine, or sherry (*18*, *21*). Quantitative determinations of **1a/b** and **2a/b** revealed contents ranging from a few micrograms per liter up to several milligrams per liter (*21*).

Concerning the formation of compounds **5a/b** and **6** from tryptophan and xylose, Wang and co-workers proposed a mechanism including Pictet–Spengler condensation, oxidative decarboxylation, tautomerization, and dehydrogenation steps (*20*). According to this pathway, racemization of the sugar-derived side chain was an essential reaction step. However, the authors did not demonstrate the occurrence of diastereomeric β-carbolines and gave no experimental evidence to further substantiate their proposed pathway. Consequently, we aimed to clarify the reactions involved in the formation of β-carbolines **1-8** and performed various mechanistic studies including detailed investigations on the stereochemistry of the carbohydrate-derived substituents at C-1.

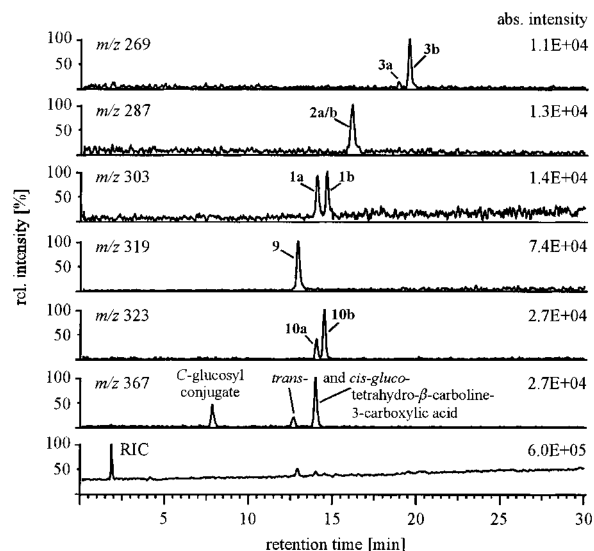

Figure 2. HPLC-MS analysis of the degradation products obtained from *gluco*-tetrahydro-β-carboline-3-carboxylic acids.

Precursors of Carbohydrate-Derived β-Carbolines. Presumably the β-carbolines **1**–**8** represented degradation products of primary tryptophan glycoconjugates such as *C*-glycosyl derivatives, *N*-glycosides, or *glyco*-tetrahydro-β-carboline-3-carboxylic acids (*17*, *18*). To identify the direct precursors of these carbohydrate-derived alkaloids, degradation studies with purified tryptophan glycoconjugates were performed. These initial model experiments demonstrated the fate of tryptophan-*C*-glycosyl and -*N*-glycoside derivatives, and of various *glyco*-tetrahydro-β-carboline-3-carboxylic acids. Following incubations at acidic pH and elevated temperatures, HPLC-MS analyses revealed formation of the β-carbolines **1a/b**, **2a/b**, and **3a/b** from all tetrahydro-β-carboline-3-carboxylic acid samples in significant amounts. In contrast, only trace amounts of these alkaloids occurred in the model experiments with the *C*-glycosyl derivative and the *N*-glycoside, respectively. Figure 2 shows the HPLC-MS chromatograms obtained from the *gluco*-tetrahydro-β-carboline-3-carboxylic acids after incubation for 13 days. In addition to the *gluco*-tetrahydro-β-carboline-3-carboxylic acids and the β-carbolines **1a/b**, **2a/b**, and **3a/b**, we detected newly formed substances in the mass chromatograms with the molecular ions $[M+H]^+$ *m/z* 367, 323, and 319. The additional peak with *m/z* 367 was identified as the corresponding tryptophan-*C*-glucosyl conjugate with the help of its characteristic product ion spectrum and retention time (*17*).

The compound having the molecular ion $[M+H]^+$ *m/z* 319 was tentatively identified as 1-(D-*gluco*-1,2,3,4,5-pentahydroxypentyl)-β-carboline **9** from the product ion

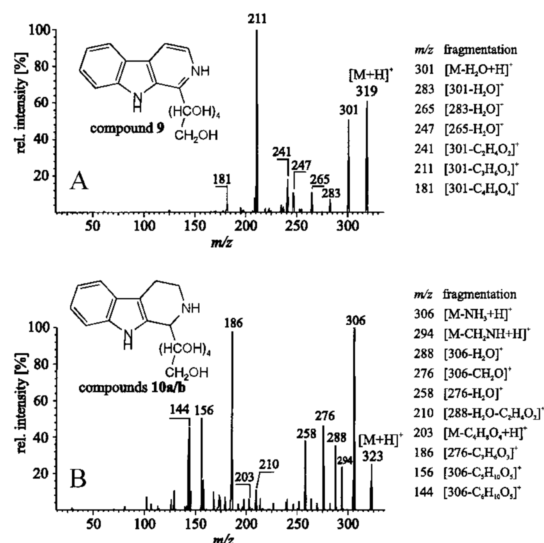

Figure 3. Product ion spectra (22 eV) of 1-(D-*gluco*-1,2,3,4,5-pentahydroxypentyl)-β-carboline **9** (A) and *gluco*-tetrahydro-β-carboline **10b** (B).

spectrum (Figure 3A), which showed the repeated neutral loss of H_2O and characteristic *retro*-aldol fragmentations. This structural assignment was confirmed with the help of an authentic reference substance. As reported by Rönner and co-workers, this polyol-β-carboline **9** is formed by oxidative decarboxylation of the *gluco*-tetrahydro-β-carboline-3-carboxylic acids. However, **9** represented no intermediate during formation of the alkaloids **1a/b**, **2a/b**, and **3a/b**. As demonstrated by further degradation studies, no formation of β-carbolines **1a/b**, **2a/b**, and **3a/b** could be observed by HPLC-MS analysis following storage of 1-(D-*gluco*-1,2,3,4,5-pentahydroxypentyl)-β-carboline **9** for 62 days at acidic pH and elevated temperatures.

The two peaks with molecular ions at *m*/*z* 323 were assigned as diastereomeric *glyco*-tetrahydro-β-carbolines **10a/b** resulting from decarboxylation of the corresponding carboxylic acids. The product ion spectra of **10a** and **10b** (Figure 3B) were identical and showed the anticipated loss of NH_3, H_2O, and CH_2O units. The tetrahydro-β-carboline structure was substantiated by the characteristic *retro*-Diels–Alder fragmentation leading to the fragment ion *m*/*z* 294 by neutral loss of the imine moiety CH_2NH.

Importance of Oxidative Decarboxylation. To evaluate the importance of the oxidative decarboxylation step for the formation of the sugar-derived β-carbolines, we studied model reactions of tryptamine with glucose. HPLC-ESI-MS analysis revealed formation of three primary tryptamine glycoconjugates with molecular ions *m*/*z* 323 $[M+H]^+$, which could be tentatively identified as the tryptamine-*N*-glucoside and the diastereomeric *gluco*-tetrahydro-β-carbolines by low-energy CID of the protonated molecule ions (data not shown). The structural assignment of the tryptamine-derived tetrahydro-β-carbolines could be further substantiated, because the retention times and product ion spectra of these diastereomeric *glyco*-tetrahydro-β-carbolines were identical when compared with the data of **10a/b** (Figure 3B). However, even after storage for 28 days, no formation of the β-carbolines **1-3** could be detected. Therefore, following formation of tricyclic *glyco*-tetrahydro-β-carboline-3-carboxylic acids by Pictet–Spengler condensation, oxidative decarboxylation of these carboxylic acids represents the essential second reaction required for the formation of the carbohydrate-derived β-carbolines. In contrast, simple loss of the carboxy moiety without oxidation results in rather stable *glyco*-tetrahydro-β-carbolines such as **10a/b**.

Racemization in the Sugar-Derived Side Chain. To obtain more information about the stereochemistry of the sugar-derived side chain, compounds **1–8** were studied by means of CD spectroscopy and HPLC-CD analysis. The CD effects, which are summarized in Table 1, resulted from differential absorption of left circularly and right circularly polarized light by chromatographically separated stereoisomeric β-carbolines.

1-(1-Hydroxybut-3-en-1-yl)-β-carboline **8** has one stereocenter at C-1′ which is enantiopure in the precursor ribose but could undergo racemization during the formation of carbohydrate-derived β-carbolines. By chiral HPLC-UV on a Chiralcel OD-H column, we achieved separation of **8** into two peaks (P1 and P2) with equal peak areas (Figure 4A). Combining chiral HPLC with on-line MS/MS-spectrometry, we revealed identical product ion spectra of P1 and P2. Thus, **8** was a racemic mixture of both enantiomers. This was further confirmed by HPLC-CD experiments. As depicted in Figure 4B, P1 and P2 have mirror-image CD spectra, characteristic of enantiomers. The observed racemization compromises the mechanism proposed by Wang and coworkers (*20*) which requires loss of the stereoinformation at C-1′. Analogous results were obtained for the β-carbolines **3a**, **3b**, **4**, and **6**.

Diastereomers **5a** and **5b** feature a 1,3,4-trihydroxybutyl moiety at C-1 with two stereocenters at C-1′ and C-3′ and could be separated on a conventional ODS column. Chromatographic analysis of either purified **5a** or **5b** on the chiral HPLC column did not reveal the presence of racemic mixtures. According to the results obtained for **8**, changes of the absolute configuration

Table 1. Results of CD and Chiral HPLC-CD Analyses

compound	CD[a]	chiral HPLC-CD[b]	interpretation
1a	mirror-image-like CD spectra	no separation	racemization at C-1′
1b		no separation	
2a/b	no CD effect	no separation	racemization at C-1′ and C-4′
3a	no CD effect	two peaks with mirror-image-like CD spectra	racemization at C-1′
3b	no CD effect	two peaks with mirror-image-like CD spectra	racemization at C-1′
4	no CD effect	two peaks with mirror-image-like CD spectra	racemization at C-1′
5a	mirror-image-like CD spectra	no separation	racemization at C-1′
5b		no separation	
6	no CD effect	two peaks with mirror-image-like CD spectra	racemization at C-1′
7a	no CD effect	no separation	racemization at C-1′ and C-3′
7b	no CD effect	no separation	racemization at C-1′ and C-3′
8	no CD effect	two peaks with mirror-image-like CD spectra	racemization at C-1′

[a] Samples can represent mixtures of stereoisomers. [b] Separation of stereoisomers.

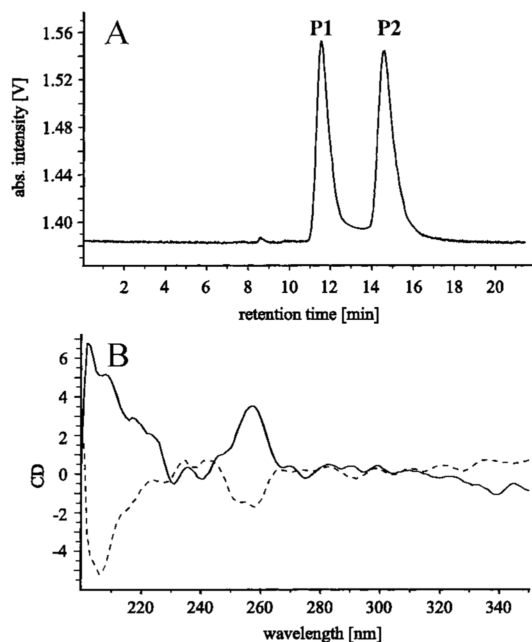

Figure 4. Separation of racemic **8** on a Chiralcel OD-H column (A) and CD spectra of enantiomers P1 (—) and P2 (- - -) (B).

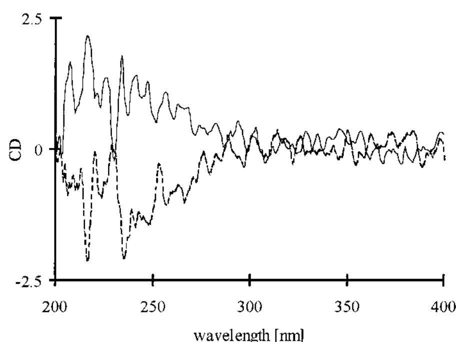

Figure 5. CD spectra of diastereomers **5a** (—) and **5b** (- - -).

were most likely to occur at C-1′. In contrast, a second racemization reaction at C-3′ should yield each of the individual diastereomers **5a** and **5b** as racemates and could be excluded. In addition, **5a** and **5b** revealed weak, but characteristic mirror-image-like CD spectra (Figure 5) which are typical for enantiopure compounds. Thus, during formation of **5a/b** racemization had taken place at C-1′, whereas C-3′ remained unchanged. Analogous results were obtained for the diastereomeric β-carbolines **1a** and **1b**.

Like **5a** and **5b**, the 1-(1,3-dihydroxybut-1-yl)-β-carbolines **7a** and **7b** feature two stereocenters at C-1′ and C-3′. The diastereomers could also be purified by conventional ODS chromatography, whereas no further separation on the chiral column was achieved. However, neither **7a** nor **7b** showed a CD effect. Thus, the diastereomeric β-carbolines **7a** and **7b** each represented racemic mixtures, and the modification had occurred at both C-1′ and C-3′. The racemization at C-3′ can be rationalized by loss of water from C-4′ and subsequent reduction of the resulting enol-structure to yield the terminal methyl group. By analogy, we assigned racemization at C-1′ and C-4′ for compounds **2a** and **2b**.

In summary, our detailed mechanistic studies on the formation of the carbohydrate-derived β-carbolines **1**−**8** confirmed and extended the proposed reaction pathway

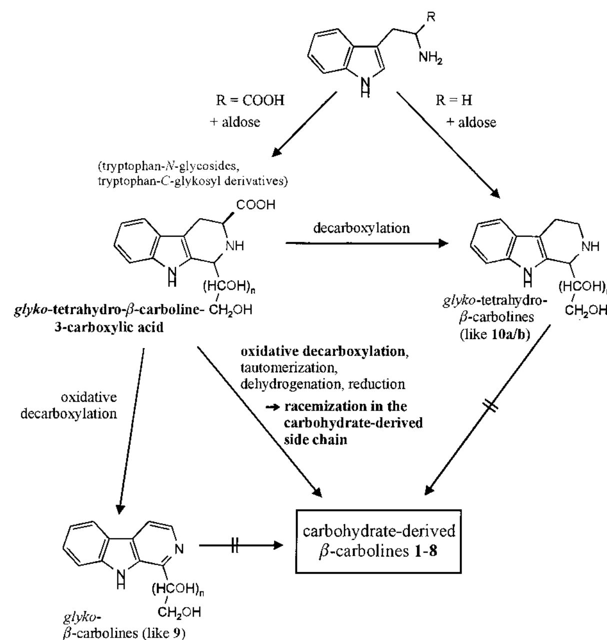

Figure 6. Precursors and relevant reactions required for the formation of carbohydrate-derived β-carbolines **1**−**8**.

(Figure 6). We demonstrated that reaction of tryptophan with reducing carbohydrates yielded tricyclic *glyco*-tetrahydro-β-carboline-3-carboxylic acids by Pictet−Spengler condensation. Only these *glyco*-tetrahydro-β-carboline-3-carboxylic acids represented suitable precursors for the β-carbolines under study. Essentially, the oxidative decarboxylation of the *glyco*-tetrahydro-β-carboline-3-carboxylic acids was the second key reaction. Finally, subsequent elimination of water from the carbohydrate-derived side chain resulted in the anticipated racemization reactions.

ABBREVIATIONS USED

CD, circular dichroism; DAD, diode array detection; ESI, electrospray ionization; HRMS, high-resolution mass spectrometry; MS/MS, tandem mass spectrometry; TFA, trifluoroacetic acid.

ACKNOWLEDGMENT

We thank M. Grüne and E. Ruckdeschel for acquisition of the NMR data, M. Wohlfahrt and K. Messer for assistance with HPLC-CD, G. Lange and F. Daderich for acquisition of the HRMS data, and Professor M. Pischetsrieder (University of Erlangen) for providing a reference sample of 1-(D-*gluco*-1,2,3,4,5-pentahydroxypentyl)-β-carboline. This study was supported by the Deutsche Forschungsgemeinschaft (DFG), Bonn (He 2599/1-5).

LITERATURE CITED

(1) Herraiz, T.; Ough, C. S. Chemical and technological factors determining tetrahydro-β-carboline-3-carboxylic acid content in fermented alcoholic beverages. *J. Agric. Food Chem.* **1993**, *41*, 959−964.

(2) Bobbitt, J. M.; Willis, J. P. Electrochemistry of natural products. 7. Oxidative decarboxylation of some tetrahydro-β-carbolinecarboxylic acids. *J. Org. Chem.* **1980**, *45*, 1978−1984.

(3) Adachi, J.; Mizoi, Y.; Naito, T.; Yamamoto, K.; Fujiwara, S.; Ninomiya, I. Determination of β-carbolines in foodstuffs by high-performance liquid chromatography and

high-performance liquid chromatography–mass spectrometry. *J. Chromatogr.* **1991**, *538*, 331–339.

(4) Sen, N. P.; Seaman, S. W.; Lau, B. P.-Y.; Weber, D.; Lewis, 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.

(5) Herraiz, T. Occurrence 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 fruit juices, purees, and jams. *J. Agric. Food Chem.* **1998**, *46*, 3484–3490.

(6) Tsuchiya, H.; Hayashi, H.; Sato, M.; Shimizu, H.; Iinuma, M. Quantitative analysis of all types of β-carboline alkaloids in medicinal plants and dried edible plants by high performance liquid chromatography with selective fluorometric detection. *Phytochem. Anal.* **1999**, *10*, 247–253.

(7) Gutsche, B.; Herderich, M. HPLC-MS/MS profiling of tryptophan-derived alkaloids in food: Identification of tetrahydro-β-carbolinedicarboxylic acids. *J. Agric. Food Chem.* **1997**, *45*, 2458–2462.

(8) Herraiz, T. Tetrahydro-β-carboline-3-carboxylic acid compounds in fish and meat: possible precursors of co-mutagenic β-carbolines norharman and harman in cooked foods. *Food Addit. Contam.* **2000**, *17*, 859–866.

(9) Rommelspacher, H.; May, T.; Susilo, R. β-Carbolines and tetrahydroisoquinolines: Detection and function in mammals. *Planta Med.* **1991**, *57*, 85–92.

(10) Musshoff, F.; Daldrup, T.; Bonte, W.; Leitner, A.; Lesch, O. M. Formaldehyde-derived tetrahydroisoquinolines and tetrahydro-β-carbolines in human urine. *J. Chromatogr. B* **1996**, *683*, 163–176.

(11) Settimj, G.; Del Giudice, M. R.; Ferretti, R.; Cotichini, V.; Bruni, G.; Romeo, M. R. β-Carbolines as inverse agonistic benzodiazepine receptor ligands. 2. Synthesis and *in vitro* and *in vivo* binding of some new 6-amino- and 6-fluoro-β-carboline-3-carboxylates. *Eur. J. Med. Chem.* **1995**, *30*, 245–251.

(12) Glennon, R. A.; Dukat, M.; Grella, B.; Hong, S. S.; Costantino, L.; Teitler, M.; Smith, C.; Egan, C.; Davis, K.; Mattson, M. V. Binding of β-carbolines and related agents at serotonin (5-HT2 and 5-HT1A), dopamine (D2) and benzodiazepine receptors. *Drug Alcohol Depend.* **2000**, *60*, 121–132.

(13) Kim, H.; Sablin, S. O.; Ramsay, R. R. Inhibition of monoamine oxidase A by β-carboline derivatives. *Arch. Biochem. Biophys.* **1997**, *337*, 137–142.

(14) Matsubara, K.; Gonda, T.; Sawada, H.; Uezono, T.; Kobayashi, Y.; Kawamura, T.; Ohtaki, K.; Kimura, K.; Akaike, A. Endogenously occurring β-carboline induces parkinsonism in nonprimate animals: A possible causative protoxin in idiopathic Parkinson's disease. *J. Neurochem.* **1998**, *70*, 727–735.

(15) Gearhart, D. A.; Collins, M. A.; Lee, J. M.; Neafsey, E. J. Increased β-carboline 9*N*-methyltransferase activity in the frontal cortex in Parkinson's disease. *Neurobiol. Dis.* **2000**, *7*, 201–211.

(16) De Meester, C. Genotoxic potential of β-carbolines: A review. *Mutat. Res.* **1995**, *339*, 139–153.

(17) Gutsche, B.; Grun, C.; Scheutzow, D.; Herderich, M. Tryptophan glycoconjugates in food and human urine. *Biochem. J.* **1999**, *343*, 11–19.

(18) Diem, S.; Albert, J.; Herderich, M. Reaction of tryptophan with carbohydrates: Identification of pentose-derived tryptophan glycoconjugates in food. *Eur. Food Res. Technol.* **2001**, in print.

(19) Rönner, B.; Lerche, H.; Bergmüller, W.; Freilinger, C.; Severin, T.; Pischetsrieder, M. Formation of tetrahydro-β-carbolines and β-carbolines during the reaction of L-tryptophan with D-glucose. *J. Agric. Food Chem.* **2000**, *48*, 2111–2116.

(20) Wang, M.; Jin, Y.; Li, J.; Ho, C.-T. Two novel β-carboline compounds from the Maillard reaction between xylose and tryptophan. *J. Agric. Food Chem.* **1999**, *47*, 48–50.

(21) Diem, S.; Herderich, M. Reaction of tryptophan with carbohydrates: Identification and quantitative determination of novel β-carboline alkaloids in food. *J. Agric. Food Chem.* **2001**, *49*, 2486-2492.

Received for review March 20, 2001. Revised manuscript received July 26, 2001. Accepted July 31, 2001.

JF010379O