

Two Novel β -Carboline Compounds from the Maillard Reaction between Xylose and Tryptophan

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Two nonvolatile β -carboline alkaloids were isolated from the Maillard reaction between xylose and tryptophan by solvent extraction and flash silica gel column chromatography. Their structures were elucidated by spectral methods (UV, MS, and NMR) as 1-(1,4-dihydroxybutyl)- β -carboline and 1-(1,3,4-trihydroxybutyl)- β -carboline.

Keywords: *Maillard reaction; β -carboline alkaloid*

INTRODUCTION

The Maillard reaction, also known as nonenzymatic browning reaction, involves the reaction of carbonyl compounds, especially reducing sugars, with compounds possessing a free amino group. Maillard reaction is an important chemical reaction in foods. Apart from the generation of flavor and the formation of color in many foods, it is also responsible for the formation of anti-mutagenic compounds (Yen, 1992). Some Maillard reaction products also show antioxidant activity in tested food models (Lingnert and Waller, 1983; Yen and Hsieh, 1995; Bedinghaus and Ockerman, 1995). However, due to their complexity and multiplicity, the vast majority of Maillard reaction products, particularly the nonvolatile products, still remain uncharacterized. Recently, some attempts have been made to study nonvolatile Maillard reaction products. One study compared reverse-phase HPLC, ion-exchange HPLC, isoelectric focusing, and capillary zone electrophoresis for the separation of methanol-extractable nonvolatile Maillard products of a model extrusion-cooked cereal product (Ames et al., 1997). Other researchers have dealt with isolation and structural elucidation of nonvolatile compounds from model systems such as xylose and lysine, xylose and glycine, furan-2-carboxaldehyde and proline, and 3-deoxy-D-hexos-2-ulose and arginine systems; a few novel compounds have been characterized from those studies (Hofmann, 1998; Arnoldi et al., 1997; Hayase, 1997).

To our knowledge, nonvolatile Maillard reaction products from tryptophan and xylose models have not been studied. In this paper, we report the isolation and structural elucidation of two novel nonvolatile β -carboline alkaloids from this model system.

MATERIALS AND METHODS

General Procedures. ^1H NMR and ^{13}C NMR spectra were obtained on a Varian Gemini-200 instrument at 200 and 50 MHz, respectively. $\text{CH}_3\text{OH}-d_4$ was used as a solvent, and chemical shifts were expressed in parts per million (δ). ^{13}C NMR multiplicity was determined by APT experiment. Desorption chemical ionization mass spectra were measured on a Finnigan MAT-90 spectrometer, using CH_4 as a reactant gas.

UV spectra were obtained over the range of 200–400 nm using a Hitachi U-3110 spectrophotometer. Thin-layer chromatography was performed on Sigma-Aldrich TLC plates (250 μm thickness, 2–25 μm particle size), with compounds visualized by spraying with 5% (v/v) H_2SO_4 in ethanol solution. Silica gel (130–270 mesh) was purchased from Aldrich Chemical Co. (Milwaukee, WI) and used for column chromatography. All solvents used for chromatography were of analytical grade quality and purchased from Fisher Scientific (Springfield, NJ). D-(+)-Xylose and tryptophan were purchased from the Aldrich Chemical Co. (Milwaukee, WI).

Preparation of the Model System. Samples containing 0.1 mol of xylose and 0.1 mol of tryptophan were added to 100 mL of distilled water. The mixture was refluxed at 100 $^\circ\text{C}$ for 20 h in a 250-mL flask coupled to a reflux condenser unit; 100 mL of distilled water was added to the resulting brown mixture, which was partitioned with methylene chloride (3 \times 200 mL), and then the water layer was extracted with ethyl acetate (3 \times 200 mL). The combined ethyl acetate extracts were dried with anhydrous sodium sulfate, and the solvent was removed under vacuum at 50 $^\circ\text{C}$.

Purification of Nonvolatile Compounds. The ethyl acetate extract (1.0 g) was subjected to column chromatography with silica gel and eluted with chloroform–methanol (10:1, 8:1, 6:1, each 500 mL) and yielded two fractions. Fraction 1 was further purified with the silica gel column being eluted with chloroform–methanol (10:1) to obtain 15 mg of **1**. Fraction 2 was rechromatographed on silica gel column and eluted with chloroform–methanol–water (6:1:0.1) to give 10 mg of **2**.

1 is a pale yellow powder, UV $\lambda_{\text{max}}^{\text{EtOH}}$ 222, 262, 295, 360. DCI-MS (m/z): 257 [$\text{M} + 1$] $^+$, 239, 198, 169. ^1H NMR (200 MHz, in CD_3OD): 8.21 (1H, d, $J = 5.4$ Hz, H-3), 8.16 (1H, d, $J = 8.0$ Hz, H-5), 7.90 (1H, d, $J = 5.4$ Hz, H-4), 7.64 (1H, d, $J = 8.0$ Hz, H-8), 7.55 (1H, td, $J = 8.0, 1.2$ Hz, H-7), 7.28 (1H, td, $J = 8.0, 1.2$ Hz, H-6), 5.19 (1H, t, $J = 6.3$ Hz, H-1'), 3.59 (2H, t, $J = 6.5$ Hz, H-4'), 2.03 (2H, m, H-2'), 1.72 (2H, m, H-3').

2 is a pale yellow powder, UV $\lambda_{\text{max}}^{\text{EtOH}}$ 223, 263, 295, 360. DCI-MS (m/z): 273 [$\text{M} + 1$] $^+$, 257, 198, 169, 103, 91, 75, 69. ^1H NMR (200 MHz, in CD_3OD): 8.22 (1H, d, $J = 5.4$ Hz, H-3), 8.17 (1H, d, $J = 8.0$ Hz, H-5), 7.99 (1H, d, $J = 5.4$ Hz, H-4), 7.64 (1H, d, $J = 8.0$ Hz, H-8), 7.57 (1H, t, $J = 8.0$ Hz, H-7), 7.25 (1H, d, $J = 8.0$ Hz, H-6), 5.40 (1H, t, $J = 6.3$ Hz, H-1'), 4.62 (1H, m, H-3'), 3.59 (2H, d, $J = 5.6$ Hz, H-4'), 2.14 (2H, m, H-2')

RESULTS AND DISCUSSION

Two major nonvolatile compounds were isolated from the Maillard reaction between xylose and tryptophan. **1** was isolated as a pale yellow powder, and the

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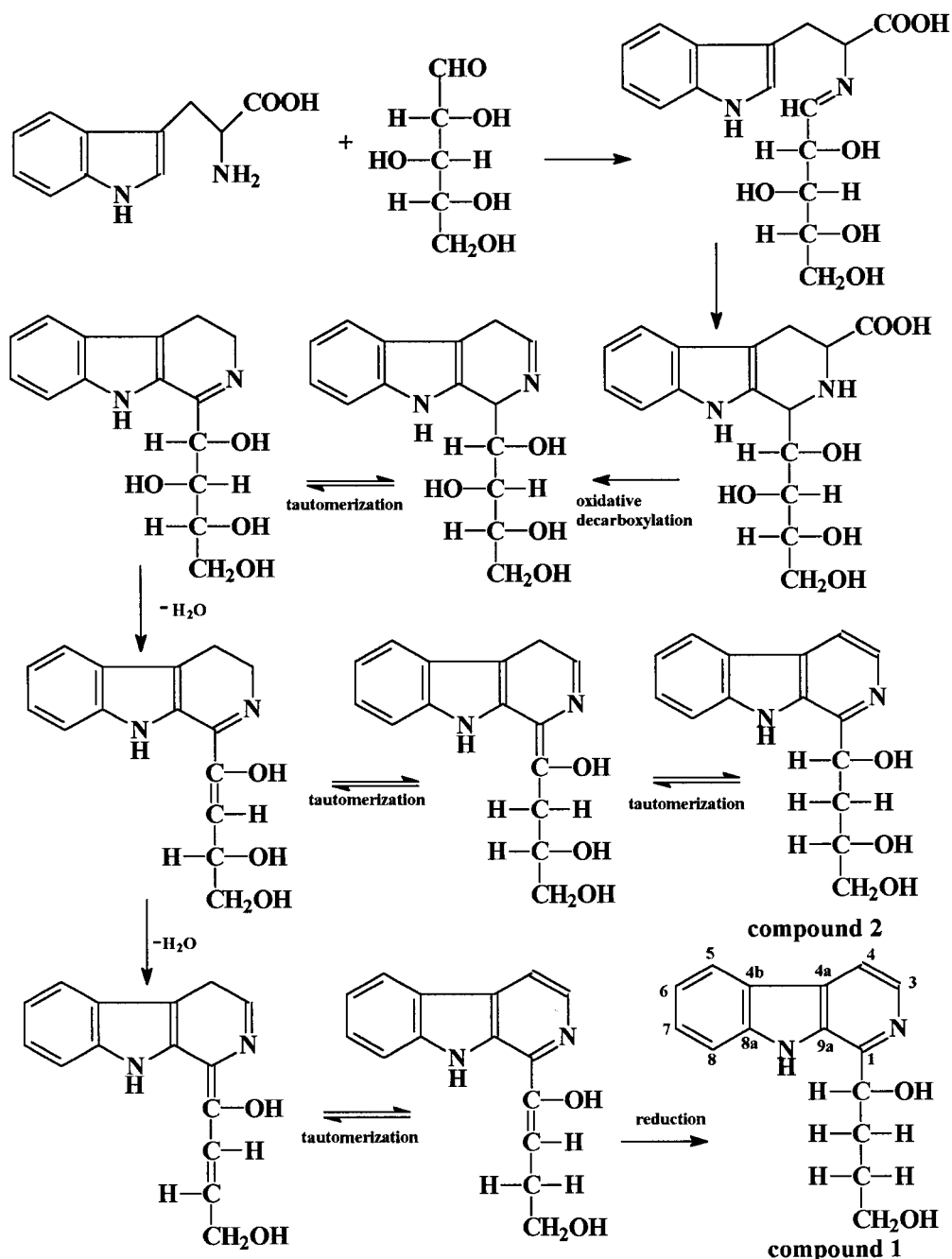


Figure 1. Proposed pathway for the formation of **1** and **2**.

Table 1. ^{13}C NMR Data for Isolated Compounds^a

position	1	2	position	1	2
1	148.9, s	148.3, s	8	113.3, d	113.4, d
3	137.6, d	137.7, d	8a	134.7, s	134.7, s
4	115.2, d	115.4, d	9a	142.8, s	142.9, s
4a	122.3, s	122.4, s	1'	75.4, d	74.2, d
4b	131.5, s	131.7, s	2'	34.9, t	41.1, t
5	122.7, d	122.7, d	3'	30.1, t	72.1, d
6	121.0, d	121.0, d	4'	63.2, t	67.4, t
7	129.9, d	129.9, d			

^a s = singlet, d = doublet, t = triplet, q = quartet.

molecular formula $\text{C}_{15}\text{H}_{16}\text{N}_2\text{O}_2$ was deduced from desorption chemical ionization–mass spectrometry (DCI–MS), which showed a $[\text{M} + 1]^+$ at 257 and ^{13}C NMR spectrum that accounted for a total of 15 carbons. The UV spectrum showed maxima at 223, 263, 295, and 360, the typical absorption of a β -carboline alkaloid (Gozler

et al., 1996; Ohmoto et al., 1981). The ^1H NMR displayed a total of six protons in aromatic range. Among them, two doublet signals at δ 8.21 and 7.90 ($J = 5.4$ Hz) clearly belong to H-3 and H-4 of the β -carboline moiety (Gozler et al., 1996), while four signals at δ 7.28 (td, $J = 8.0, 1.2$ Hz), 7.55 (td, $J = 8.0, 1.2$ Hz), 7.64 (d, $J = 8.0$), and 8.16 (d, $J = 8.0$ Hz) suggested the nonsubstituted nature of the positions of C-5, -6, -7, and -8. The ^1H NMR of **1** also gave the signals for a methine proton associated with a secondary alcohol group as a 1H triplet at δ 5.19, terminal methylene protons associated with a primary alcohol as a 2H triplet at δ 3.59, and two CH_2 signals at δ 2.03 and δ 1.72 ppm, which suggest a $\text{CH}(\text{OH})\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$ moiety in **1**. The ^{13}C NMR of **1** (Table 1) showed signals of typical carboline alkaloids and the signal for a $\text{CH}(\text{OH})\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$ moiety (δ 75.4, 63.2, 30.1, and 34.9 ppm), so compound **1** was elucidated as 1-(1,4-dihydroxybutyl)- β -carboline.

2 was isolated as a pale yellow powder with the molecular formula $C_{15}H_{16}N_2O_3$, which was indicated by its DCI-MS and ^{13}C NMR. The 1H NMR of **2** showed similar signals in the aromatic region, δ 8.22 (1H, d, $J = 5.4$ Hz, H-3), 8.17 (1H, d, $J = 8.0$ Hz, H-5), 7.99 (1H, d, $J = 5.4$ Hz, H-4), 7.64 (1H, d, $J = 8.0$ Hz, H-8), 7.57 (1H, t, $J = 8.0$ Hz, H-7), and 7.25 (1H, t, $J = 8.0$ Hz, H-6), suggesting a 1-substituted β -carboline moiety in **2**. Comparing the molecular weight of **2** with that of **1** suggests one more hydroxyl group in **2**, in the 1H NMR of **2**; the 1'-position hydrogen (δ 5.40) still remained a triplet, suggesting that this hydroxyl group can only be substituted at the 3'-position, so its structure was elucidated as 1-(1,3,4-trihydroxybutyl)- β -carboline. Its ^{13}C NMR (Table 1) were assigned according to literature (Erra-Balsells et al., 1988; Ohmoto and Koike, 1982, 1984).

Alkaloids with β -carboline structure are widely distributed in nature. Among them, norharman (β -carboline) and harman (1-methyl- β -carboline) are well-known compounds of tobacco and cigarette smoke, having been characterized in the 1960s (Poindexter and Carpenter, 1962). 1,2,3,4-Tetrahydro- β -carboline-3-carboxylic acid and 1-methyl-1,2,3,4-tetrahydro- β -carboline-3-carboxylic acid have been found in beverages, and 1-methyl-1,2,3,4-tetrahydro- β -carboline-3-carboxylic acid has been reported as a precursor of mutagenic *N*-nitroso compounds when tested in Ames tests (Herraiz et al., 1993; Wakabayashi, 1983). Many β -carboline alkaloids have also been found in plants, and one alkaloid creatine was shown to possess antibacterial activity but no antifungal activity (Mitsunaga et al., 1994; Gozler et al., 1996; Ajayeoba et al., 1995; Liu et al., 1988; Ohmoto and Koike, 1982, 1984; Ohmoto et al., 1981). Some volatile β -carboline alkaloids were found after the roasting of tryptophan with reducing sugars and sugar degradation products, and the authors proposed the mechanism as intramolecular cyclization of the resulting azomethines and subsequent dehydrogenation producing the thermally stable β -carboline structures (Knoch and Baltes, 1992).

In this study, β -carbolines were formed by reacting tryptophan with xylose in an aqueous solution. Only two major carboline alkaloids could be observed on the TLC plates in the ethyl acetate extracts. There was no experimental evidence to support any certain mechanism for the formation of these compounds; however, intramolecular cyclization, dehydrogenation, and equilibrium between different tautomeric forms, as depicted in Figure 1, seem reasonable.

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