# Identification of Novel Colored Compounds Containing Pyrrole and Pyrrolinone Structures Formed by Maillard Reactions of Pentoses and Primary Amino Acids

Thomas Hofmann†

Deutsche Forschungsanstalt für Lebensmittelchemie, Lichtenbergstrasse 4, D-85748 Garching, Germany

Heating of pentoses with alanine in a ratio of 10:1 in aqueous solution at pH 7.0 generated the yellow 2-[(2-furyl)methylidene]-4-hydroxy-5-methyl-2*H*-furan-3-one (**1**), being well in line with data reported in the literature. Decreasing the relative concentrations of the pentose produced further colored nitrogen-containing compounds, among which (*S*)-4-hydroxy-5-methyl-2-[*N*-(1′-carboxyethyl)-pyrrolyl-2-methylidene]-2*H*-furan-3-one (**2**) could be identified by spectroscopic and synthetic experiments. On the other hand, thermal treatment of an aqueous solution of pentose and L-alanine in the presence of furan-2-carboxaldehyde led to the formation of the novel red (2*R*)-4-oxo-3,5-bis-[(2-furyl)methylidene] tetrahydropyrrolo[1,2-*c*]-5(*S*)-(2-furyl)oxazolidine and its 5(*R*)-(2-furyl)oxazolidine diasteromer (**3a/3b**), which were characterized by several 1D- and 2D-NMR techniques, LC/MS, and UV−vis spectroscopy as well as by synthesis of the chromophoric substructure. In addition, the red compounds (*S*)-4-[(*E*)-1-formyl-2-(2-furyl)ethenyl]-5-(2-furyl)-2-[(*E*)-(2-furyl)methylidene]-2,3-dihydo-α-amino-3-oxo-1*H*-pyrrole-1-acetic acid (**4b**) and the corresponding 2-[(*Z*)-(2-furyl)-methylidene] isomer (**4b**) were identified in this Maillard mixture. Quantitative studies on the formation of these colorants clearly demonstrates the key role of 3-deoxypentos-2-ulose as an intermediate in the formation of **4a**/**4b**. Reaction pathways leading to the colorants **2**, **3a**/**3b**, and **4a**/**4b** from pentoses and alanine are discussed.

**Keywords:** *Maillard reaction; colored compounds; (S)-4-hydroxy-5-methyl-2-[N-(1′-carboxyethyl)-pyrrolyl-2-methylidene]-2H-furan-3-one; (2R)-4-oxo-3,5-bis[(2-furyl)methylidene]tetrahydropyrrolo-[1,2-c]-5(S)-(2-furyl)oxazolidine; (S)-4-[(E)-1-formyl-2-(2-furyl)ethenyl]-5-(2-furyl)-2-[(E)-(2-furyl)-methylidene]-2,3-dihydo-α-amino-3-oxo-1H-pyrrole-1-acetic acid*

## INTRODUCTION

The Maillard reaction between reducing carbohydrates and compounds bearing an amino group is chiefly responsible for the development of desirable colors and flavors occurring, for example, during thermal processing of foods, such as roasting of meat or coffee, baking of bread, or kiln-drying of malt. However, due to the complexity and multiplicity of the nonvolatile Maillard reaction products formed, surprisingly few studies have been aimed at identifying the structures of the compounds responsible for the typical brown color.

Some model studies [e.g., Ledl and Severin (1978, 1982)] have been performed to clarify the mechanisms of this so-called nonenzymatic browning; however, most reactions have been carried out with synthetically related amines instead of food-related amino acids.

In a very recent work, Arnoldi et al. (1997) isolated a yellow colorant with a three-ring carbocyclic structure from a xylose/lysine Maillard system. However, although a high amount of lysine was used in the model experiment, surprisingly, no nitrogen-containing colored compound was identified by the authors.

However, it is a well-known fact that color development in carbohydrate/amino acid mixtures runs in parallel with the amino acid content. For example, Narziss and Stippler (1976) found that the color of a beer product was related to the nitrogen content of the malt used, indicating that amino acids are involved as catalysts and/or reaction partners in the formation of browning products during kilning of malt or wort boiling in beer production.

To gain a more detailed insight into the role of the amino acid as reactant in the nonenzymatic browning, we have recently studied Maillard reactions leading to nitrogen-containing colorants. In an aqueous reaction mixture of L-proline and furan-2-carboxaldehyde, a major dehydration product from pentoses, we identified the previously unknown intense yellow 5-(*S*)-(2-carboxy-1-pyrrolidinyl)-2-hydroxy-(*E*,*E*)-2,4-pentadienal-(*S*)-(2-carboxypyrrolidine)imine as the colored main compound formed even at low temperatures (Hofmann, 1997a, 1998a). In the presence of the primary amino acid alanine, the novel red compound (*S*)-4-[(*E*)-1-formyl-2-(2-furyl)ethenyl]-5-(2-furyl)-2-[(*E*)-(2-furyl)methylidene]-2,3-dihydo-α-amino-3-oxo-1*H*-pyrrole-1-acetic acid and the corresponding 2-[(*Z*)-(2-furyl)methylidene] isomer (PYRED) were formed from furan-2-carboxaldehyde upon thermal treatment (Hofmann, 1997b, 1998a). This was the first time that chromophoric compounds comprising four linked rings with an amino acid moiety incorporated were identified in a Maillard reaction system. Additional studies with proteins and furan-2-carboxaldehyde revealed that the ϵ-amino function of lysine residues acts as an anchor to form the corresponding lysine derivative of PYRED, linked to a protein (Hofmann, 1997a, 1998b).

† Telephone +49-89-289 14181; fax +49-89-289 14183; e-mail hofmann@dfa.leb.chemie.tu-muenchen.de

10.1021/jf980477i CCC: $15.00 
Published on Web 09/05/1998

To gain a more detailed insight into the role of the amino acid in the network of browning reactions, the present investigation was aimed at characterizing additional nitrogen-containing colored compounds in Maillard reactions of pentoses and primary amino acids.

## EXPERIMENTAL PROCEDURES

**Chemicals.** The following compounds were obtained commercially: D-xylose, L-alanine, furan-2-carboxaldehyde, thallium(I) ethylate, 2-formylpyrrole, ethyl 2-bromopropionate, potassium iodide, 3-hydroxypyrrolidine, 2-*tert*-butoxycarbonyloximino-2-phenylacetonitrile, triethylamine, 1,4-dioxane, pyridinium chlorochromate, piperidine, and acetic acid (Aldrich, Steinheim, Germany). Furan-2-carboxaldehyde was distilled at 30 °C in a high vacuum prior to use. Solvents were of HPLC grade (Aldrich). DMSO-$d_6$ and $CD_3OD$ were obtained from Isocom (Landshut, Germany).

The following compounds were synthesized following closely the methods recently described in the literature given in parentheses: 4-hydroxy-5-methyl-2*H*-furan-3-one (Hofmann and Schieberle, 1998a), 3-deoxypentos-2-ulose (Hofmann and Schieberle, 1998a), *N*-(1-deoxy-D-xylulos-1-yl)-L-alanine (Hofmann, 1998c), (*S*)-4[(*E*)-1-formyl-2-(2-furyl)ethenyl)]-5-(2-furyl)-2-[(*E*)-(2-furyl)methylidene]-2,3-dihydro-α-methyl-3-oxo-1*H*-pyrrole-1-acetic acid (**4a**; Hofmann, 1998a) and its 2-[(*Z*)-(2-furyl)methylene] isomer (**4b**; Hofmann, 1998a).

**Isolation of 2-[(2-Furyl)methylidene]-4-hydroxy-5-methyl-2*H*-furan-3-one (1) from a Heated Xylose/Alanine Solution.** A solution of xylose (60 mmol) and alanine (6 mol) in phosphate buffer (40 mL; 1 mmol/L, pH 6.0) was refluxed for 2.5 h. After cooling, the mixture was extracted with diethyl ether (4 × 30 mL), dried over $Na_2SO_4$, and concentrated to 2 mL. The raw material was applied onto a column (25 × 350 mm) filled with a slurry of silica gel (100 g, silica gel 60, Merck, Darmstadt, Germany) in toluene. Chromatography was performed using toluene (300 mL), followed by toluene/ethyl acetate (8:2; 300 mL) and toluene/ethyl acetate (7:3; 300 mL). Elution with toluene/ethyl acetate (6:4; 300 mL) gave an intense yellow fraction, which was further analyzed by RP-18-HPLC using a methanol/water gradient as the mobile phase. A peak corresponding to **1** (12 mg; ∼0.1% in yield) showed UV−vis spectra, LC/MS spectra, and retention time at RP-18 identical with those of the reference compound synthesized from 4-hydroxy-5-methyl-2*H*-furan-3-one and furan-2-carboxaldehyde (Hofmann, 1998d).

**Isolation of (*S*)-4-Hydroxy-5-methyl-2-[(*E*)-*N*-(1′-carboxyethyl)pyrrolyl-2-methylidene]-2*H*-furan-3-one (2) from a Heated Aqueous Solution of Xylose and Alanine.** A solution of xylose (60 mmol) and alanine (60 mol) in phosphate buffer (40 mL; 1 mmol/L, pH 6.0) was heated under reflux for 2.5 h. After cooling, the pH of the mixture was adjusted to 3.0 and then extracted with ethyl acetate (6 × 30 mL). The organic layer was dried over $Na_2SO_4$, then concentrated to ∼3 mL, and, finally, fractionated by column chromatography (35 × 400 mm) on silica gel (150 g, silica gel 60, Merck). After application of the raw material onto the column conditioned with ethyl acetate, chromatography was performed using ethyl acetate (300 mL), followed by ethyl acetate/methanol (80:20; 300 mL). Elution with ethyl acetate/methanol (50:50; 500 mL) affords a fraction that was subfractionated by preparative thin-layer chromatography on silica gel (20 × 20 cm; 0.5 mm; Merck) using ethyl acetate/methanol (50:50, v/v) as the eluent. The yellow band with $R_f = 0.7-0.8$ was scraped off and suspended in hot methanol. After filtration and concentration, the colorant was purified by flash chromatography on RP-18 material (15,0 g; Lichroprep 25−40 μm, Merk) using methanol/water (60:40, v/v) as the mobile phase. After application of the raw material, chromatography with the same eluent afforded the target compound in the effluent >75 mL. The combined eluates were freeze-dried, yielding the colored compound as a yellow powder (5 mg, ∼0.04% in yield); LC/MS 264, $[M + 1]^+$; $^1$H NMR (360 MHz, MeOD-$d_3$; the arbitrary numbering of the carbon atoms refers to Figure 2) $\delta$ 1.73 [d, 3H, $^3J_{7,6} = 7.08$ Hz, H−C(7)], 2.31 [s, 3H, H−C(13)], 5.02 [q, 1H, $^3J_{6,7} = 7.08$ Hz, H−C(6)], 6.31 [dd, 1H, $^3J_{4,5} = 2.66$ Hz, $^3J_{4,3} = 3.98$ Hz, H−C(4)], 6.86 (s, 1H, H at C-1), 7.08 [dd, 1H, $^3J_{3,4} = 3.98$ Hz, $^4J_{3,5} = 1.33$ Hz, H−C(3)], 7.18 [dd, 1H, $^3J_{5,4} = 2.66$ Hz, $^4J_{5,3} = 1.33$ Hz, H−C(5)]; UV $\lambda_{max} = 405$ nm, $\epsilon = 0.7$ ($10^4$ L mol$^{-1}$ cm$^{-1}$).

**Synthesis of *N*-(1′-Carboxyethyl)-2-formylpyrrole.** *Ethyl 2-(2′-Formylpyrrol-1-yl)propanoate.* Thallium(I) ethylate (30 mmol) was added to a solution of 2-formylpyrrole (20 mmol) in anhydrous acetonitrile (10 mL) under an atmosphere of argon. Over a period of 60 min, ethyl 2-iodpropionate (20 mmol), freshly prepared from ethyl 2-brompropionate and potassium iodide in boiling acetone, was added dropwise to the ice-cooled mixture. After stirring for 12 h at room temperature, the suspension was applied onto a slurry of silica gel (25 × 200 mm, 30 g, silica gel 60, Merck) in methanol/ethyl acetate (20:80, v/v). Elution with the same solvent mixture, followed by evaporation of the solvent, afforded the ethyl 2-(2′-formylpyrrol-1-yl)propanoate (14.3 mmol; 72% in yield) as a colorless oil: GC/MS(EI) 122 (100), 94 (64), 195-(31), 149(26), 167(22), 93(15), 121(13), 104(10).

*N-(1′-Carboxyethyl)-2-formylpyrrole.* Ethyl 2-(2′-formylpyrrol-1-yl)propanoate (14.0 mmol) was refluxed for 15 min in a mixture (30 mL; 50:50, v/v) of methanol and aqueous sodium hydroxide (3 mol/L NaOH). After evaporation of the solvent, the aqueous phase was extracted with methylene chloride (3 × 10 mL) and then adjusted to pH 3 using hydrochloric acid (5 mol/L). The target compound was then extracted with methylene chloride (5 × 20 mL). Evaporation of the solvent and recrystallization from ethyl acetate yielded the *N*-(1′-carboxyethyl)-2-formylpyrrole (7.8 mmol; 56% in yield) as colorless crystals: LC/MS 168 100, $[M + 1]^+$; IR (KBr) 3109, 2400−3200, 1732, 1614 cm$^{-1}$; UV $\lambda_{max1} = 290$ nm; $^1$H NMR (360 MHz, MeOD-$d_3$; the arbitrary numbering of the carbon atoms refers to Figure 3) $\delta$ 1.73 [d, 3H, $^3J_{7,6} = 7.07$ Hz, H−C(7)], 5.72 [quart., 1H, $^3J_{6,7} = 7.07$ Hz, H−C(6)], 6.30 [dd, 1H, $^3J_{4,5} = 2.66$ Hz, $^3J_{4,3} = 3.98$ Hz, H−C(4)], 7.06 [dd, 1H, $^3J_{3,4} = 3.98$ Hz, $^4J_{3,5} = 1.33$ Hz, H−C(3)], 7.24 [dd, 1H, $^3J_{5,4} = 2.66$ Hz, $^4J_{5,3} = 1.33$ Hz, H−C(5)], 9.42 [d, 1H, $J = 0.89$ Hz, H−C(1)]; $^{13}$C and $^{13}$C/DEPT NMR (360 MHz, MeOD-$d_3$; the arbitrary numbering of the carbon atoms refers to Figure 3) $\delta$ 17.8 [$CH_3$, C(7)], 56.8 [CH, C(6)], 110.9 [CH, C(4)], 126.9 [CH, C(3)], 131.1 [CH, C(5)], 132.8 [CH, C(2)], 174.1 [COOH, C(8)], 180.9 [CHO, C(1)].

**Formation of 4-Hydroxy-5-methyl-2-[(*E*)-*N*-(1′-carboxyethyl)pyrrolyl-2-methylidene]-2*H*-furan-3-one from an Aqueous Mixture of 1-(1′-Carboxyethyl)-2-formylpyrrole and 4-Hydroxy-5-methyl-2*H*-furan-3-one.** A mixture of 1-(1′-carboxyethyl)-2-formylpyrrole (5 mmol) and 4-hydroxy-5-methyl-2*H*-furan-3-one (5 mmol) in phosphate buffer (pH 7.0; 0.2 mol/L; 10 mL) was heated at 90 °C for 60 min, with stirring. After freeze-drying of the reaction mixture, the residue was fractionated by flash chromatography on RP-18 material (15.0 g; Lichroprep 25−40 μm, Merck) using methanol/water (60:40, v/v) as the mobile phase. After application of the crude material and chromatography with the same eluent, the target compound was isolated in the effluent >75 mL. Final purification was performed by RP-HPLC using the following solvent gradient: starting with a mixture (30:70, v/v) of acetonitrile and aqueous TFA (0.1% TFA in water), the acetonitrile content was increased to 100% within 65 min. Monitoring the effluent at 405 nm gave a peak at 11 min, which was collected in several runs. The combined eluates were freeze-dried, yielding the colored compound (0.3 mmol; 6% in yield). The spectroscopic data were very well in line with those obtained from **2** isolated from the pentose/alanine mixture.

**Isolation of (2*R*)-4-Oxo-3,5-bis[(2-furyl)methylidene]-tetrahydropyrrolo[1,2-*c*]-5(*S*)-(2-furyl)oxazolidine and Its 5(*R*)-(2-Furyl)oxazolidine Diastereomer (3a/b) from a Xylose/Alanine Mixture Heated in the Presence of Furan-2-carboxaldehyde.** A solution of xylose (1.34 mol) and alanine (0.32 mol) dissolved in a mixture of phosphate buffer (1800 mL; 1 mmol/L, pH 7.0) and methanol (500 mL) was refluxed for 15 min, furan-2-carboxaldehyde (2 mol) was

**Table 1. Assignment of $^1$H-NMR Signals (350 MHz, $CD_3OD$) of (2*R*)-4-Oxo-3-[(*E*)-(2-furyl)methylidene]-5-[(*Z*)-(2-furyl)methylidene]tetrahydropyrrolo[1,2-*c*]-5(*S*)-(2-furyl)oxazolidine and Its 5(*R*)-(2-Furyl)oxazolidine Diastereomer (3a/3b)**

| H at relevant C atom[a] | $\delta^b$ (ppm) 3a | 3b | I[c] | M[c] | J[c] (Hz) | COSY[d] | TOCSY[d] |
|---|---|---|---|---|---|---|---|
| H−C(5a) | 3.84 | 3.83 | 1 | t | 8.4 | H−C(4), H−C(5b) | H−C(4), H−C(5b), H−C(16) |
| H−C(5b) | 4.31 | 4.30 | 1 | t | 8.4 | H−C(4), H−C(5a) | H−C(4), H−C(5a), H−C(16) |
| H−C(4) | 5.25 | 5.25 | 1 | m | 8.4 | H−C(5a), H−C(5b) | H−C(5a), H−C(5b), H−C(16) |
| H−C(6) | 6.15 | 6.14 | 1 | s | | | |
| H−C(14) | 6.46 | 6.46 | 1 | dd | 3.5, 1.4 | H−C(13), H−C(15) | H−C(13), H−C(15) |
| H−C(9) | 6.51 | 6.51 | 1 | dd | 3.5, 1.4 | H−C(8), H−C(10) | H−C(8), H−C(10) |
| H−C(11) | 6.56 | 6.55 | 1 | s | | | |
| H−C(13) | 6.62 | 6.61 | 1 | d | 3.5 | H−C(14) | H−C(14), H−C(15) |
| H−C(19) | 6.66 | 6.66 | 1 | dd | 3.5, 1.4 | H−C(18), H−C(20) | H−C(18), H−C(20) |
| H−C(8) | 6.68 | 6.67 | 1 | d | 3.5 | H−C(9) | H−C(9), H−C(10) |
| H−C(18) | 7.02 | 7.01 | 1 | d | 3.5 | H−C(19) | H−C(19), H−C(20) |
| H−C(16) | 7.36 | 7.36 | 1 | s | | | |
| H−C(15) | 7.38 | 7.38 | 1 | d | 1.4 | H−C(14) | H−C(13), H−C(14) |
| H−C(10) | 7.56 | 7.55 | 1 | d | 1.4 | H−C(9) | H−C(8), H−C(9) |
| H−C(20) | 7.81 | 7.80 | 1 | d | 1.4 | H−C(19) | H−C(18), H−C(19) |

[a] Numbering of carbon atoms refers to structures **3a** and **3b** in Figure 5. [b] The $^1$H chemical shifts are given in relation to $CD_3OD$. [c] Determined from 1D spectrum. [d] Observed homonuclear $^1$H,$^1$H connectivities by TOCSY and DQF−COSY.

added, and heating was continued for another 80 min. After cooling to room temperature, the solution was extracted with diethyl ether (10 × 300 mL), and the organic layer was dried over $Na_2SO_4$ and concentrated to ∼150 mL at 25 °C in vacuo (100 mbar). To remove unreacted furan-2-carboxaldehyde, the extract was then distilled in high vacuo (0.04 mbar) at 35 °C. The intensely colored residue was dissolved in a 1:1 mixture (10 mL) of toluene and ethyl acetate, and aliquots were then fractionated by column chromatography (35 × 450 mm) on silica gel (200 g, silica gel 60, Merck). After application of an aliquot of the crude material (5 mL) onto the column conditioned with toluene, chromatography was performed using toluene (300 mL) and toluene/ethyl acetate (9:1, v/v; 300 mL). The fraction, obtained by using toluene/ethyl acetate (8:2, v/v; 300 mL) as the mobile phase, was further subfractionated by a further column chromatography (20 × 400 mm) on silica gel (50 g, silica gel 60, Merck). The fraction was concentrated and applied onto water-cooled silica gel, which was conditioned with *n*-pentane. After flushing with pentane (200 mL), followed by pentane/diethyl ether (9:1, v/v; 200 mL), elution with pentane/diethyl ether (8:2, v/v; 200 mL) afforded a fraction containing a deep red compound, which was freed from solvent in vacuo and dissolved in methanol (1 mL). The colored compound was further purified by flash chromatography using an RP-18 stationary phase (15.0 g; Lichroprep 25−40 $\mu$m, Merck). The solution was placed onto the column (20 × 1.6 cm), which was conditioned with methanol/water (8:2, v/v). Flushing with the same solvent mixture (120 mL) afforded a fraction containing the red colorant. After the solvent had been removed, the aqueous phase was extracted with ethyl acetate (3 × 20 mL) and, after drying over $Na_2SO_4$, the red colorant was isolated in 98% purity by preparative thin-layer chromatography on silica gel (20 × 20 cm; 0.5 mm; Merck) using *n*-pentane/toluene/ethyl acetate (70:15:15, v/v/v) as the eluent. A red band at $R_f = 0.62$ was scraped off and dissolved in methanol (20 mL). After filtration, the solvent was evaporated to dryness, affording **1** as an intense red solid (1.3 mmol; ∼0.1% in yield): LC/MS *m*/*z* 350 (100, $[M + 1]^+$); UV $\lambda_{max1} =$ 456 nm ($\epsilon = 1.0 \times 10^4$ L mol$^{-1}$ cm$^{-1}$), $\lambda_{max2} = 349$ nm ($\epsilon = 1.2 \times 10^4$ L mol$^{-1}$ cm$^{-1}$); $^1$H and $^{13}$C-NMR data are listed in Tables 1 and 2.

**Synthesis of the 2,4-Bis[(2-furyl)methylidene]pyrrolidine-3-one Chromophore.** *N-(tert-Butoxycarbonyl)-3-hydroxypyrrolidine.* 3-Hydroxypyrrolidine (15 mmol), 2-*tert*-butoxycarbonyloximino-2-phenylacetonitrile (16.5 mmol), and triethylamine (22.5 mmol) were stirred for 2 h at room temperature in 1,4-dioxane/water (20 mL; 1:1, v/v). The organic solvent was then distilled off in vacuo, and, after addition of water (50 mL), the oily phase was fractionated by flash chromatography on a diol phase as described recently (Hofmann and Schieberle, 1998b). The target compound (11.9 mmol, 79% in yield) was characterized by HRGC/MS: MS(EI) 57 (100), 56 (48), 114 (33), 87 (28), 132 (22), 131 (17), 86-(6), 130 (5), 187 (4); MS(CI, isobutane) 188 (100, $[M + 1]^+$).

**Table 2. Assignment of $^{13}$C-NMR Signals (360 MHz, $CD_3OD$) of the Major Diastereomer of 4-Oxo-3-[(*E*)-(2-furyl)methylidene]-5-[(*Z*)-(2-furyl)methylidene]-tetrahydropyrrolo[1,2-*c*]-5-(2-furyl)oxazolidine (3a)**

| H at relevant C atom[a] | $\delta^b$ (ppm) | DEPT[c] | heteronuclear $^1$H,$^{13}$C multiple−quantum coherence[d] via $^1J$(C,H) | via $^{2,3}J$(C,H) |
|---|---|---|---|---|
| C(4) | 62.2 | CH | H−C(4) | H−C(5), H−C(6), H−C(16) |
| C(5) | 70.1 | $CH_2$ | H−C(5) | H−C(4), H−C(6) |
| C(6) | 90.1 | CH | H−C(6) | H−C(4), H−C(5) |
| C(11) | 99.8 | CH | H−C(11) | H−C(13) |
| C(19) | 108.4 | CH | H−C(19) | H−C(18), H−C(20) |
| C(9) | 109.6 | CH | H−C(9) | H−C(8), H−C(10) |
| C(14) | 111.9 | CH | H−C(14) | H−C(13), H−C(15) |
| C(8) | 112.7 | CH | H−C(8) | H−C(6), H−C(9), H−C(10) |
| C(13) | 112.8 | CH | H−C(13) | H−C(11), H−C(14), H−C(15) |
| C(18) | 119.3 | CH | H−C(18) | H−C(16), H−C(19), H−C(20) |
| C(16) | 119.7 | CH | H−C(16) | H−C(4), H−C(18) |
| C(3) | 131.6 | C | | H−C(4), H−C(5), H−C(16) |
| C(1) | 141.0 | C | | H−C(6), H−C(11) |
| C(10) | 142.5 | CH | H−C(10) | H−C(8), H−C(9) |
| C(15) | 143.5 | CH | H−C(15) | H−C(13), H−C(14) |
| C(20) | 147.1 | CH | H−C(20) | H−C(18), H−C(19) |
| C(12) | 150.2 | C | | H−C(14), H−C(15) |
| C(17) | 150.6 | C | | H−C(19), H−C(20) |
| C(7) | 151.4 | C | | H−C(9), H−C(10) |
| C(2) | 189.9 | C | | H−C(4), H−C(11), H−C(16) |

[a] Numbering of carbon atoms refers to structure **3a** in Figure 5. [b] The $^{13}$C chemical shifts are given in relation to $CD_3OD$. [c] DEPT-135 spectroscopy. [d] Assignments based on HMQC ($^1J$) and HMBC ($^{2,3}J$) experiments.

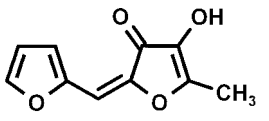
*N-(tert-Butoxycarbonyl)pyrrolidine-3-one.* A mixture of *N*-(*tert*-butoxycarbonyl)-3-hydroxypyrrolidine (10 mmol) and pyridinium chlorochromate (15 mmol) in dichloromethane (25 mL) was stirred for 4 h at room temperature. After addition of diethyl ether (40 mL), the organic phase was separated and dried over $Na_2SO_4$ and the solvent was distilled off in vacuo. The residue was purified by flash chromatography as described recently (Hofmann and Schieberle, 1998b), affording *N*-(*tert*-butoxycarbonyl)pyrrolidine-3-one (3.5 mmol, 35% in yield) as a colorless oil: MS(EI) 57 (100), 56 (74), 85 (39), 112 (21), 130 (19), 105 (13), 103 (11), 129 (6), 185 (4); MS(CI, isobutane) 186 (100, $[M + 1]^+$).

*N-(tert-Butoxycarbonyl)-2,4-bis[(2-furyl)methylidene]pyrrolidine-3-one.* A mixture of *N*-(*tert*-butoxycarbonyl)pyrrolidine-3-one (2 mmol), furan-2-carboxaldehyde (5 mmol), piperidine (100 $\mu$L), and acetic acid (100 $\mu$L) in ethanol/water (1:1, v/v;

25 mL) was heated for 1 h at 60 °C. The organic solvent was then evaporated in vacuo (30 mbar) at 35 °C, and the reaction mixture was adjusted to pH 6.0 with aqueous hydrochloric acid (0.1 mol/L). After extraction with ethyl acetate (4 × 10 mL) and drying over $Na_2SO_4$, the organic layer was concentrated in vacuo to ~1 mL and the target compound was isolated by semipreparative thin-layer chromatography using silica gel (20 × 20 cm; 0.5 mm; Merck) as the stationary phase and a mixture of pentane/toluene/ethyl acetate (75:20:5, v/v/v) as the mobile phase. The band with $R_f = 0.22$ was scraped off and suspended in methanol. Filtration and evaporation of the solvent gives *N*-(*tert*-butoxycarbonyl)-2,4-bis[(2-furyl)methylene]-3-oxopyrrolidine (0.6 mmol, 30% in yield) as deep red crystals: LC/MS 342 (100, $[M + 1]^+$), 286 (94, $[M + 1 -$ isobutene$]^+$), 242 (16, $[M + 1 - BOC]^+$), 364 (5, $[M + Na]^+$); $^1$H NMR (360 MHz; DMSO-$d_6$; DQF−COSY; the arbitrary numbering of the carbon atoms refers to Figure 6) 1.32 [s, 9H, H−C(17−19)], 4.80 [s, 2H, H−C(4)], 6.64 [dd, 1H, $^3J_{13,12} = 3.54$ Hz, $^3J_{13,14} = 1.77$ Hz, H−C(13)], 6.67 [s, 1H, H−C(10)], 6.74 [s, 1H, H−C(5)], 6.77 [dd, 1H, $^3J_{8,7} = 3.54$ Hz, $^3J_{8,9} = 1.77$ Hz, H−C(8)], 7.12 [d, 1H, $^3J_{7,8} = 3.54$ Hz, H−C(7)]. 7.33 [d, 1H, $^3J_{12,13} = 3.54$ Hz, H−C(12)], 7.86 [d, 1H, $^3J_{9,8} = 1.77$ Hz, H−C(9)], 8.08 [d, 1H, $^3J_{14,13} = 1.77$ Hz, H−C(14)]; $^{13}$C NMR (360 MHz; DMSO-$d_6$; DEPT, HMQC, HMBC; the arbitrary numbering of the carbon atoms refers to Figure 6) $\delta$ 27.2 [$CH_3$, C(17−19)], 49.7 [$CH_2$, C(4)], 80.9 [C, C(16)], 104.5 [CH, C(5)], 112.4 [CH, C(8)], 113.4 [CH, C(13)], 115.9 [CH, C(10)], 118.9 [CH, C(12)], 119.2 [CH, C(7)], 126.6 [C, C(3)], 131.3 [C, C(1)], 144.6 [CH, C(9)], 147.6 [CH, C(14)], 150.2 [C, C(6)], 150.7 [C, C(11)], 151.4 [C, C(15)], 187.7 [C, C(2)]; UV $\lambda_{max1} = 461$ nm ($\epsilon = 1.0 \times 10^4$ L mol$^{-1}$ cm$^{-1}$), $\lambda_{max2} = 377$ nm ($\epsilon = 1.1 \times 10^4$ L mol$^{-1}$ cm$^{-1}$).

**Identification and Quantification of (*S*)-4[(*E*)-1-Formyl-2-(2-furyl)ethenyl)]-5-(2-furyl)-2-[(*E*)-(2-furyl)methylidene]-2,3-dihydro-α-methyl-3-oxo-1*H*-pyrrole-1-acetic Acid (4a) and Its 2-[(*Z*)-(2-Furyl)methylene] Isomer (4b) in Heated Aqueous Solutions of Xylose/Alanine, *N*-(1-Deoxy-D-xylulos-1-yl)-L-alanine, and 3-Deoxypentosulose/Alanine, Respectively, in the Presence of Furan-2-carboxaldehyde.** Solutions of either alanine (30 mmol) and xylose (30 mmol), *N*-(1-deoxy-D-xylulos-1-yl)-L-alanine (30 mmol) or alanine (30 mmol) and 3-deoxypentos-2-ulose (30 mmol), each in phosphate buffer (90 mL; 1 mmol/L, pH 7.0), were refluxed for 15 min. After addition of furan-2-carboxaldehyde (90 mol), heating was continued for another 80 min. The mixture was cooled to room temperature and extracted with ethyl acetate (5 × 30 mL), and the organic layer was dried over $Na_2SO_4$ and concentrated at 25 °C under vacuum (100 mbar) to ~80 mL. To remove volatile compounds, the extract was then distilled in high vacuo (0.04 mbar) at 35 °C. The residue was dissolved in ethyl acetate and fractionated by column chromatography (35 × 450 mm) on silica gel (200 g, silica gel 60, Merck). After application of the crude material onto the column conditioned with diethyl ether, chromatography was performed using diethyl ether (500 mL), followed by ethyl acetate (500 mL). Elution with ethyl acetate/methanol (8:2, v/v; 300 mL) affords a colored fraction, which was freed from solvent in vacuo. The residue was taken up in methanol (2 mL) and was then analyzed by analytical HPLC. Starting with a mixture of methanol (20%) and water (80%), the methanol content was increased to 100% within 45 min. Two peaks eluting after 14.0 and 16.5 min showed UV−vis spectra, MS spectra, and retention time at RP-18 material identical with those of the reference compounds of **4a** and **4b** (Hofmann, 1997b, 1998a). Using the same HPLC conditions, the colorants **4a**/**4b** were quantified by comparing the peak areas of **4a** and **4b** monitored at 414 nm with those of the pure reference compounds used as external standards.

**Gas Chromatography/Mass Spectroscopy (GC/MS).** HRGC was performed with a type 5160 gas chromatograph (Fisons Instruments, Mainz, Germany) using an SE-54 capillary (30 m × 0.32 mm, 0.25 μm; J&W Scientific, Fisons Instruments) coupled with an MD-800 mass spectrometer (Fisons Instruments); sample application (0.5 μL) was done by on-column injection at 40 °C.




**1**

**Figure 1.** Structure of 2-[(2-furyl)methylidene]-4-hydroxy-5-methyl-2*H*-furan-3-one (**1**).

**High-Performance Liquid Chromatography (HPLC).** The HPLC apparatus (Kontron, Eching, Germany) consisted of two pumps (type 422), a gradient mixer (M 800), a Rheodyne injector (100 μL loop), and a diode array detector (DAD, type 440) monitoring the effluent in a wavelength range between 220 and 500 nm. Separations were performed on a stainless steel column packed with RP-18 (ODS-Hypersil, 5 μm, 10 nm, Shandon, Frankfurt, Germany) in either an analytical (4.6 × 250 mm, flow rate = 0.8 mL/min) or a preparative scale (10 × 250 mm, flow rate = 1.8 mL/min).

**Liquid Chromatography/Mass Spectrometry (LC/MS).** An analytical HPLC column (Nucleosil 100-5C18, Macherey and Nagel, Dürren, Germany) was coupled to an LCQ-MS (Finnigan MAT GmbH, Bremen, Germany) using electrospray ionization (ESI). After injection of the sample (2.0 μL), analysis was performed using a gradient starting with a 10:90 (v/v) mixture of acetonitrile and water and increasing the acetonitrile content to 100% within 15 min.

**UV−Vis Spectrocopy**. UV−vis spectra were obtained using a U-2000 spectrometer (Colora Messtechnik GmbH, Lorch, Germany).

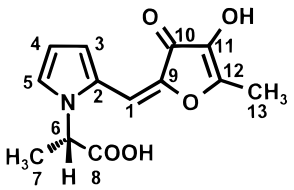
**Nuclear Magnetic Resonance Spectroscopy (NMR).** $^1$H, $^{13}$C, DEPT-135, DQF−COSY, TOCSY, HMQC, and HMBC experiments were performed on a Bruker AC-200 and a Bruker AM-360 spectrometer (Bruker, Rheinstetten, Germany) using the acquisition parameters described recently (Hofmann, 1997b). Tetramethylsilane (TMS) was used as the internal standard.

## RESULTS AND DISCUSSION

Heating of aqueous solutions of xylose and L-alanine in ratios of 10:1 or 1:1 led to intense browning of both reaction mixtures; however, the colorization occurred much more rapidly in the latter mixture. This is well in line with the catalytic effect of the amino acid on the carbohydrate degradation. In the solvent extract of the 10:1 mixture, 2-[(2-furyl)methylidene]-4-hydroxy-5-methyl-2*H*-furan-3-one (**1** in Figure 1) could be identified as one of the main colored reaction products by comparing the data of the UV−vis and the LC/MS spectra as well the retention time (RP-18-HPLC) with those obtained for the synthesized reference compound (Hofmann, 1998d). This result is well in line with data reported earlier by Severin and Krönig (1972) as well as Nursten and O'Reilly (1986), who isolated compound **1** from xylose heated in the presence of isopropyl ammoniumacetate and glycine, respectively.

Heating of xylose and L-alanine in a 1:1 mixture, however, gave, besides **1**, another colorant, which was monitored after separation of the solvent extract by RP-HPLC using either a DAD operating at wavelengths between 220 and 500 nm or an LC/MS. The intensely yellow product was characterized by DAD to have an absorption maximum at 405 nm.

After chromatographic isolation, the determination of its chemical structure was performed by $^1$H-NMR, LC/MS, and UV−vis spectroscopy. LC/MS measurements gave an intense $[M + 1]^+$ ion at *m*/*z* 264 (100%), fitting well with the proposed structure for **2** (Figure 2). The $^1$H-NMR spectrum measured in $CD_3OD$ showed seven resonance signals. The singlets at 2.31 and 6.86 ppm



**2**

**Figure 2.** Structure of (*S*)-4-hydroxy-5-methyl-2-[*N*-(1′-carboxyethyl)pyrrolyl-2-methylidene]-2*H*-furan-3-one (**2**).

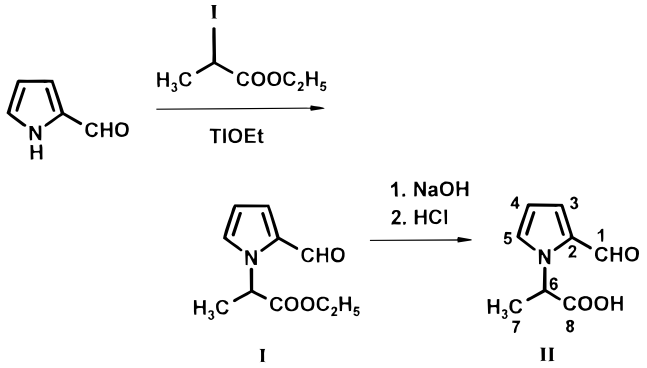


**Figure 3.** Synthetic sequence for the preparation of *N*-(1′-carboxyethyl)-2-formylpyrrole.

were in the range of the chemical shifts of the methyl group and the methylidene proton, respectively, found for the colored 2*H*-furan-3-one **1** (Hofmann, 1998c). Double-quantum-filtered homonuclear $\delta,\delta$ correlation spectroscopy (DQF−COSY) revealed two strongly coupled $^1$H spin systems, confirming the presence of a pyrrole ring system and an alanine moiety in **2**. Due to the chemical shift of the α-hydrogen of the alanine moiety, the amino acid must be incorporated in the pyrrole ring. Due to the low amounts that could be isolated from the Maillard mixture, an unequivocal assignment of the $^{13}$C-NMR data could, however, not be achieved.

In analogy to the formation of **1** from furan-2-carboxaldehyde and 4-hydroxy-5-methyl-2*H*-furan-3-one (Ledl and Severin, 1978; Hofmann, 1998d), **2** might be assumed to be formed from 4-hydroxy-5-methyl-2*H*-furan-3-one and *N*-(1′-carboxyethyl)-2-formylpyrrole. These intermediates were, therefore, used for the synthesis as displayed in Figure 3. 2-Formylpyrrole was N-alkylated with ethyl 2-iodopropionate, yielding the ethyl 2-(2′-formylpyrrol-1-yl)propanoate (**I**), which after hydrolysis of the carboxylic ester gives rise to *N*-(1′-carboxyethyl)-2-formylpyrrole (**II**). Heating an aqueous solution of the synthetic aldehyde with 4-hydroxy-5-methyl-2*H*-furan-3-one gave a main colored reaction product, which showed identical $^1$H-NMR, UV−vis, and LC/MS spectra as well as the same retention times at RP-18 as compound **2** isolated from the xylose/alanine mixture.

In summary, the spectroscopic and synthetic data obtained are consistent with the proposed structure of **2** as (*S*)-4-hydroxy-5-methyl-2-[(*E*)-*N*-(1′-carboxyethyl)-pyrrolyl-2-methylidene]-2*H*-furan-3-one (Figure 4). The predominance of the (*E*) configuration of the methylidene double bond was evidenced by molecular mechanics calculations using an MM3 force field. To our knowledge, this yellow compound **2**, showing an extinction coefficient of $0.7 \times 10^4$ L mol$^{-1}$ cm$^{-1}$ (in water, pH 7.0), has previously not been described in the literature.

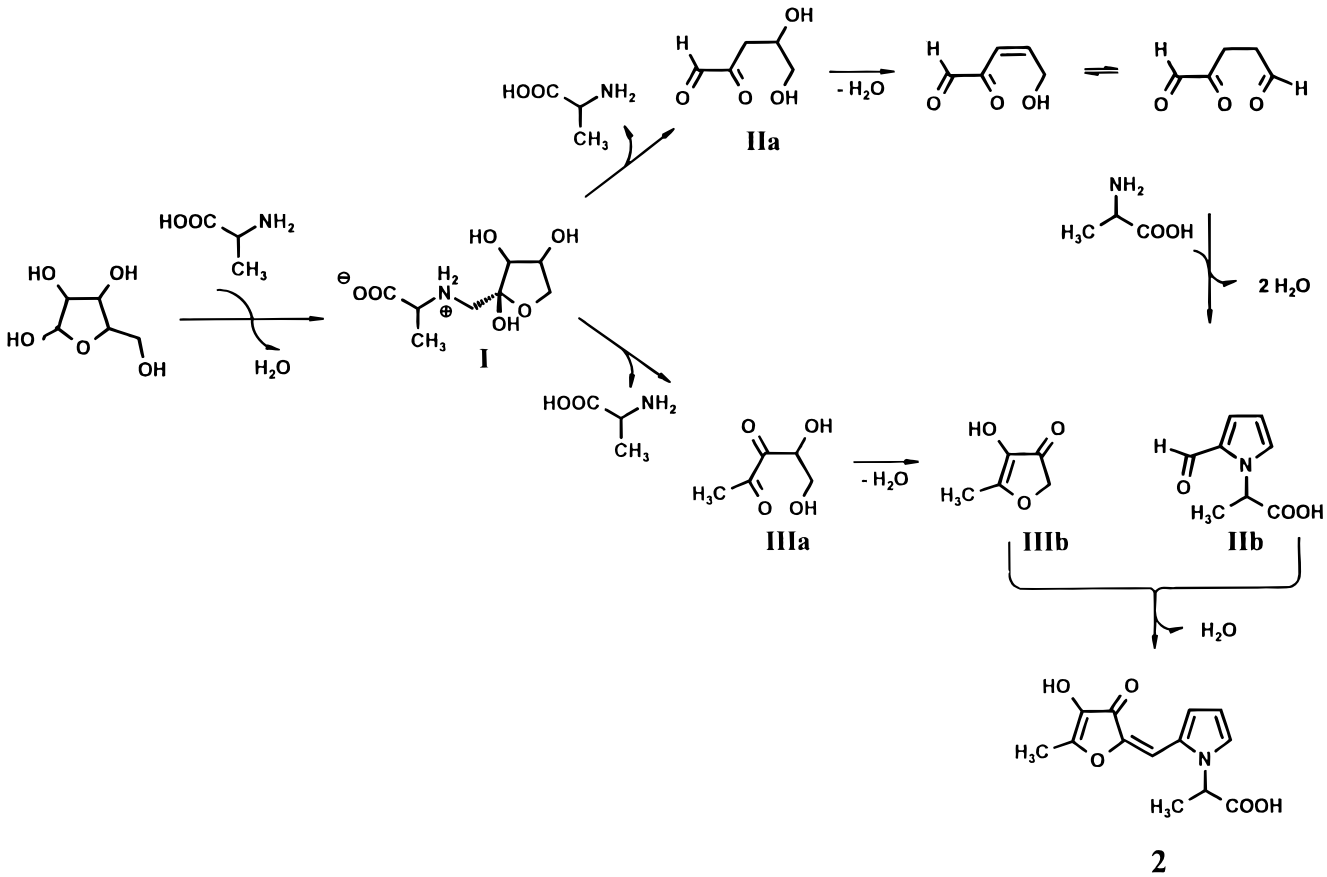


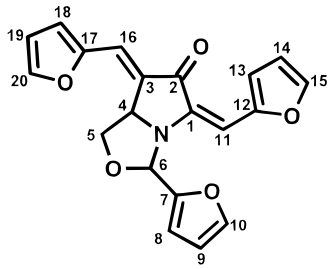
**Figure 4.** Formation pathway leading to (*S*)-4-hydroxy-5-methyl-2-[(*E*)-*N*-(1′-carboxyethyl)-pyrrolyl-2-methylidene]-2*H*-furan-3-one (**2**) from pentoses and L-alanine.

A homologuous colorant derived from methylamine as well as from glycine was reported earlier by Ledl and Severin (1978), indicating that the formation of this nitrogen-containing type of chromophore runs independently from the amino moiety present.

On the basis of the data obtained, the reaction pathway displayed in Figure 4 was proposed for the formation of colorant **2**. Dehydration of *N*-(1-deoxypentulos-1-yl)-L-alanine (**I**), the Amadori rearrangement product formed from pentoses and L-alanine, at the 3-position gives the 3-deoxypentosone (**IIa**) after hydrolysis. Further dehydration of **IIa** and cyclization with one molecule of alanine leads to *N*-(1′-carboxyethyl)-2-formylpyrrole (**IIb**). Deamination at the 1-position of the Amadori rearrangement product (**I**) yields 1-deoxypentosulose (**IIIa**), which, upon cyclization and dehydration, leads to the formation of 4-hydroxy-5-methyl-2*H*-furan-3-one (**IIIb**). As confirmed by synthetic experiments, the condensation reaction between the methylene-active intermediate **IIIb** and the aldehyde **IIb** then results in the colored (*S*)-4-hydroxy-5-methyl-2-[(*E*)-*N*-(1′-carboxyethyl)-pyrrolyl-2-methylidene]-2*H*-furan-3-one (**2**).

The formation of **2** from *N*-(1′-carboxyethyl)-2-formylpyrrole and 4-hydroxy-5-methyl-2*H*-furan-3-one confirms the hypothesis of Ledl and Severin (1978, 1982) that the condensation reaction between methylene-active compounds and carbonyl compounds is an important general reaction leading to color development during the Maillard reaction. The amino acid assisted conversion of carbohydrates, however, results in a tremendous variety of reactive carbonyls such as furan-2-carboxaldehyde, *N*-(1′-carboxyethyl)-2-formylpyrrole, or 1- and 3-deoxyosones, as well as methylene-active compounds such as 4-hydroxy-5-methyl-2*H*-furan-3-one. This large variety of reactants leads, therefore, to the multiplicity and the low yields of the colored Maillard reaction products, making the identification of such browning products a big challenge. To propose possibilities on how to clarify general reaction types involved in Maillard-type browning, Hofmann (1998d) recently reacted aqueous solutions of reducing carbohydrates and amino acids in the presence of a surplus amount of one carbonyl compound. Because furan-2-carboxaldehyde is known as one of the main dehydration products from pentoses (Ledl and Schleicher, 1990; Hofmann and Schieberle, 1998a), it was chosen for these experiments. Using this strategy, all methylene-active color precursors are forced to react with the same aldehyde, offering the possibility to characterize the key types of chromophores.

For the identification of further nitrogen-containing compounds from pentoses and amino acids, the model mixtures were, therefore, reacted in the presence of furan-2-carboxaldehyde. When xylose and L-alanine were reacted in aqueous solution in the presence of furan-2-carboxaldehyde, the color of the reaction mixture rapidly turned into deep red-brown. An intensely red reaction product was detected by HPLC analyses, and diode array detection gave absorption maxima at 379 and 456 nm. After isolation of this colorant, the determination of its chemical structure was performed by several one- and two-dimensional NMR techniques and, in addition, by LC/MS and UV−vis spectroscopy. The spectroscopic data were consistent with structure **3**, given in Figure 5, existing in the two diastereomers **3a** and **3b**.



**3a / 3b**

**Figure 5.** Structure of (2*R*)-4-oxo-3,5-bis[(2-furyl)methylidene]-tetrahydropyrrolo[1,2-*c*]-5(*S*)-(2-furyl)oxazolidine and its 5(*R*)-(2-furyl)oxazolidine isomer (**3a**/**3b**).

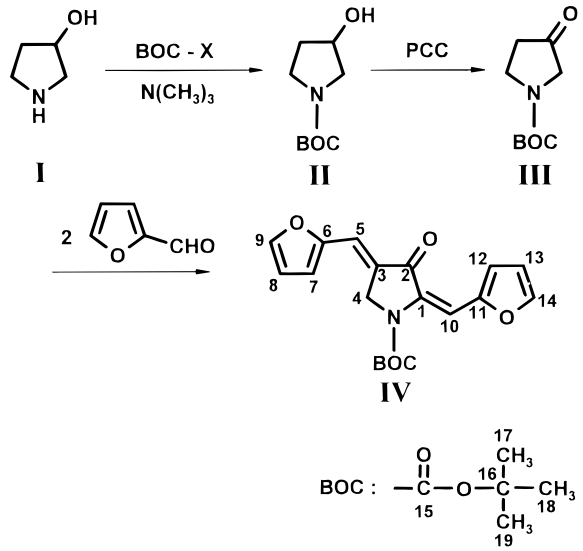



**Figure 6.** Synthesis of N-protected 2,4-bis[(2-furyl)methylidene]pyrrolidine-3-one.

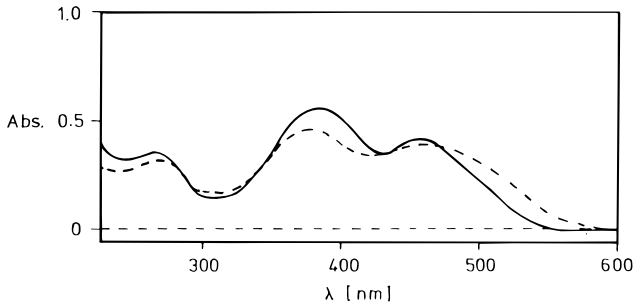



**Figure 7.** UV−vis spectrum of (—) colored Maillard product **3a**/**3b** and (- - -) synthetic 2,4-bis[(2-furyl)methylidene]pyrrolidine-3-one.

LC/MS measurements showed an intense $[M + 1]^+$ ion at *m*/*z* 350 fitting well with the structure of **3a**/**3b**. LC/MS$^2$ revealed a loss of 18 and 96, yielding *m*/*z* 332 and 254, respectively, most likely corresponding to the elimination of one molecule of water and furan-2-carboxaldehyde. These data are well in line with the proposed cyclic hemi aminal structure for **3a/3b**.

The $^1$H-NMR spectrum measured in $CD_3OD$ showed two sets of 15 resonance signals each in a ratio of about 2:1, corroborating the existence of two diastereomeric forms. Further NMR data, fitting well with the structures **3a** and **3b**, are given in Table 1. In the following, the structure determination of the predominating diastereomer **3a** is explained in more detail. A total of three furan rings, each substituted at the 2-position, were deduced from the characteristic coupling pattern

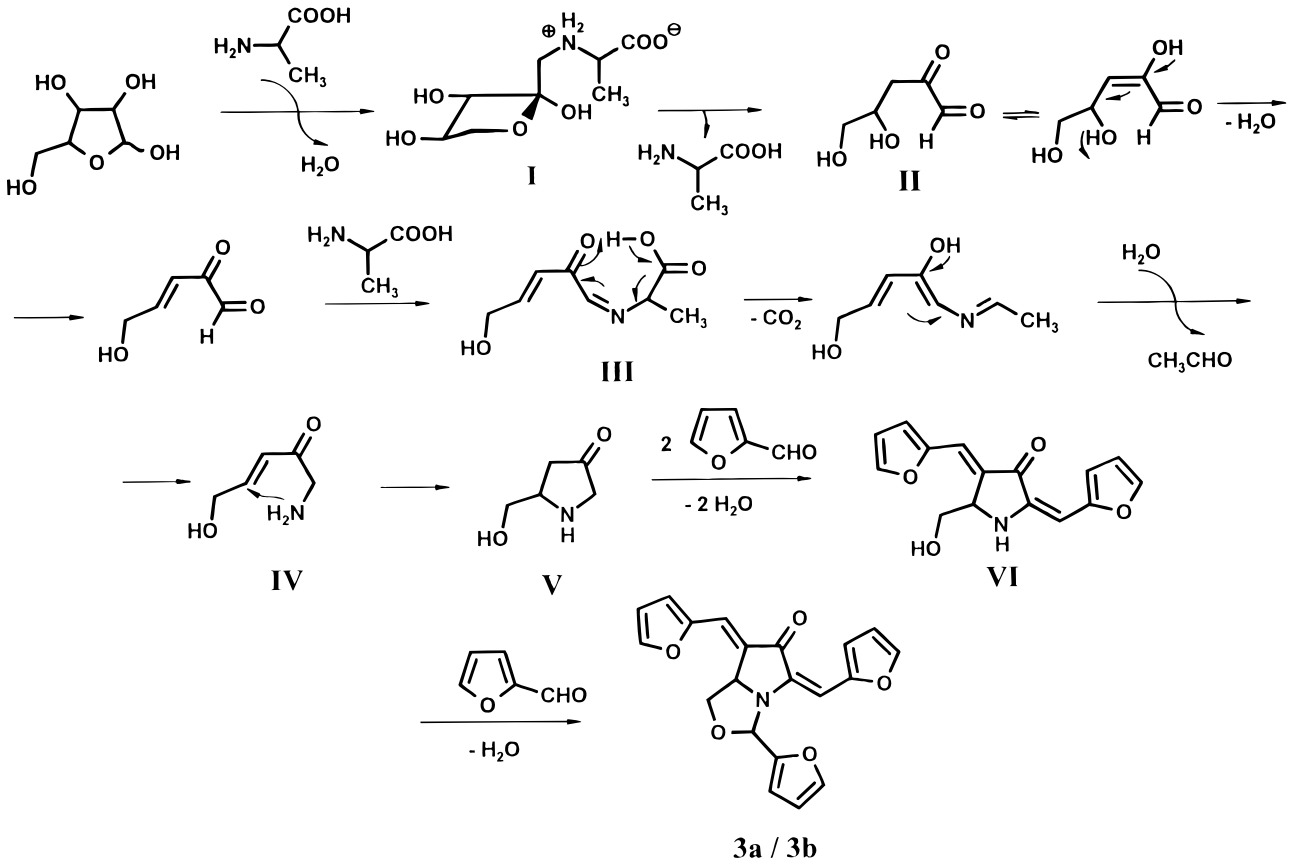



**Figure 8.** Reaction route leading to 4-oxo-3,5-bis[(2-furyl)methylidene]tetrahydropyrrolo[1,2-*c*]-5-(2-furyl)oxazolidine (**3a**/**3b**) from pentoses and alanine.

of the hydrogens H−C(8)/H−C(9)/H−C(10), H−C(13)/H−C(14)/H−C(15), and H−C(18)/H−C(19)/H−C(20). This was further confirmed by DQF−COSY as well as total correlated spectroscopy (TOCSY), indicating the expected strongly coupled $^1$H spin system in the furan rings. In addition, these homonuclear $\delta/\delta$ correlation techniques revealed a coupling of 8.4 Hz between the triplets resonating at 3.84 and 4.31 ppm and, in addition, a coupling of 8.4 Hz between these triplets and a signal resonating at 5.25 ppm. These signals were assigned as the geminal hydrogen atoms $H_a$−C(5) and $H_b$−C(5) and the vicinal methine proton H−C(4). Using molecular mechanics calculations in combination with the Karplus equation revealed dihedral angels of about 11° and 163° between H−C(4) and $H_a$−C(5) as well as between H−C(4) and $H_b$−C(5), fitting well with the measured coupling constant of 8.4 Hz and with the (*R*) configuration of this chiral center.

The chemical shifts of the signals at 6.56 and 7.36 ppm were in the range expected for olefinic hydrogen atoms, but no coupling with other hydrogens could be observed. Heteronuclear multiple-bond coherence experiments (HMBC) optimized for $^2J$(C,H) and $^3J$(C,H) coupling constants (Table 2), however, revealed a correlation between these hydrogens assigned as H−C(11) and H−C(16) with the furan carbon atoms C(13) and C(18), respectively. These data demonstrate that two furan rings are directly linked at the 2-position to a methylidene group as proposed for structure **3a**. Due to the $^3J$(C,H) coupling between the singlet at 6.15 ppm and C(8) of a furan ring, this hydrogen was evidenced as the proton H−C(6) of an amino acetal of furan-2-carboxaldehyde. The $^1$H and $^{13}$C chemical shifts of C(6) are well in line with those found for cyclic amino acetals formed from benzaldehyde and 5-(hydroxymethyl)pyrrolidine-2-one (Thottahil et al., 1986), thus confirming the proposed structure of **3a**.

A comparison of the $^{13}$C-NMR spectrum of the predominant isomer **3a**, in which 20 signals appeared, with the results of the DEPT-135 experiment revealed 12 signals corresponding to 6 quarternary carbon atoms (Table 2). Unequivocal assignment of these quarternary carbon atoms could be successfully achieved by means of HMBC spectroscopy optimized for $^2J$(C,H) and $^3J$(C,H) coupling constants (Table 2). Heteronuclear correlations between the quarternary carbon atom resonating at 189.9 ppm with both of the methylidene protons H−C(11) and H−C(16) as well as with the methine proton H−C(4) led to its unequivocal assignment as the oxo group C(2).

Further correlations were observed between H−C(11) and C(1) as well as between H−C(6) and C(1), indicating that the (2-furyl)methylidene group C(11−15) is directly linked at C(1) and not at C(3) of the heterocyclic core. The fact that the (2-furyl)methylidene group is part of an exocyclic enamine structure is also well in line with the high-field shift of H−C(11) in comparison to the downfield shift of H−C(16). The CH coupling of the quarternary carbon atom resonating at 131.6 ppm with the protons H−C(4), H−C(5a), and H−C(5b) and the olefinic hydrogen H−C(16) enables a more detailed insight into the structure of **3a**, demonstrating that the second (2-furyl)methylidene group is connected with C(3) of the heterocyclic core. The oxazolidine ring in **3a** was, in addition, confirmed by heteronuclear correlations between the amino acetal proton H−C(6) and the hydrogens H−C(4) and H−C(5).

In summary, the obtained spectroscopic data are

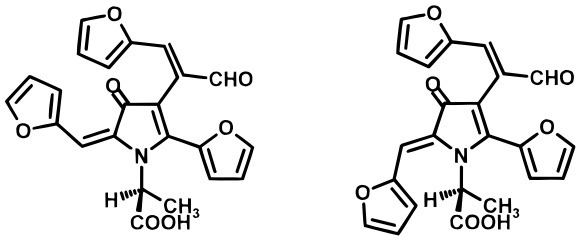


**Figure 9.** Structure of (*S*)-4-[(*E*)-1-formyl-2-(2-furyl)ethenyl]-5-(2-furyl)-2-[(*E*)-(2-furyl)methylidene]-2,3-dihydo-α-amino-3-oxo-1*H*-pyrrole-1-acetic acid (**4b**) and the corresponding 2-[(*Z*)-(2-furyl)methylidene] isomer (**4b**).

consistent with the proposed structure of **3a**/**3b** as (2*R*)-4-oxo-3,5-bis[(2-furyl)methylidene]tetrahydropyrrolo-[1,2-*c*]-5(*S*)-(2-furyl)oxazolidine and its 5(*R*)-(2-furyl)-oxazolidine diastereomer (Figure 5). To the author's knowledge, the intense red colored compound **3a/3b**, showing an extinction coefficient of $1.0 \times 10^4$ L mol$^{-1}$ cm$^{-1}$ at 456 nm (in water, pH 7.0), has previously not been described in the literature.

It is obvious from the structure **3a**/**3b** that the 2,4-bis[(2-furyl)methylidene]pyrrolidine-3-one part is responsible for the red color of this compound, whereas the furyloxazolidine moiety should not take part in the chromophoric system. To confirm the proposed chromophoric substructure in **3a**/**3b**, the 2,4-bis[(2-furyl)-methylidene]pyrrolidine-3-one substructure was synthesized following the reaction sequence outlined in Figure 6. *N*-(*tert*-Butoxycarbonyl)-3-hydroxypyrrolidine (**II**), prepared by N-protection of 3-hydroxypyrrolidine (**I**), was oxidized under mild conditions yielding the *N*-(*tert*-butoxycarbonyl)-3-hydroxypyrrolidine (**III**). Condensation with two molecules of furan-2-carboxaldehyde then yielded the N-BOC-protected 2,4-bis[(2-furyl)-methylidene]pyrrolidine-3-one (**IV**).

As outlined in Figure 7, comparison of the UV−vis spectra of **3a**/**3b** with the synthesized chromophore (**IV** in Figure 6) showed nearly identical absorption maxima, thereby confirming the 2,4-bis[(2-furyl)methylidene]-pyrrolidine-3-one substructure as the chromophore responsible for the orange-red color of **3a**/**3b**.

It is obvious from the structure of **3a**/**3b** that, besides three molecules of furan-2-carboxaldehyde, the C-5 skeleton of the pentose is incorporated. The reaction pathway outlined in Figure 8 can, therefore, be proposed for the formation of **3a/b**. Dehydration of *N*-(1-deoxy-pentulos-1-yl)-L-alanine (**I**), the Amadori rearrangement product formed from pentoses and alanine, at the 3-position gives the 3-deoxypentos-2-ulose (**II**) as a primary intermediate. After water elimination at the 4-position, this dicarbonyl compound may be reductively aminated at the aldehyde function by an oxidative decarboxylation of the amino acid. This reductive amination is well in line with the finding that **3a**/**3b** is formed independently from the amino acid moiety; for example, the colorant is formed also in the presence of valine or leucine (data not shown). Subsequent cyclization of the resulting 1-amino-5-hydroxy-3-pentene-2-one (**IV**) by an intramolecular Michael addition then gives rise to 5-hydroxymethyl-3-oxopyrrolidine (**V**). Condensation with two molecules of furan-2-carboxaldehyde forms the colored compound **VI**, which can be stabilized by reaction with a further molecule of furan-2-carboxaldehyde to give the 4-oxo-3,5-bis[(2-furyl)methylidene]-tetrahydropyrrolo[1,2-*c*]-5-(2-furyl)oxazolidine (**3a**/**3b**).

In an aqueous xylose/L-alanine solution, thermally treated in the presence of furan-2-carboxaldehyde, we

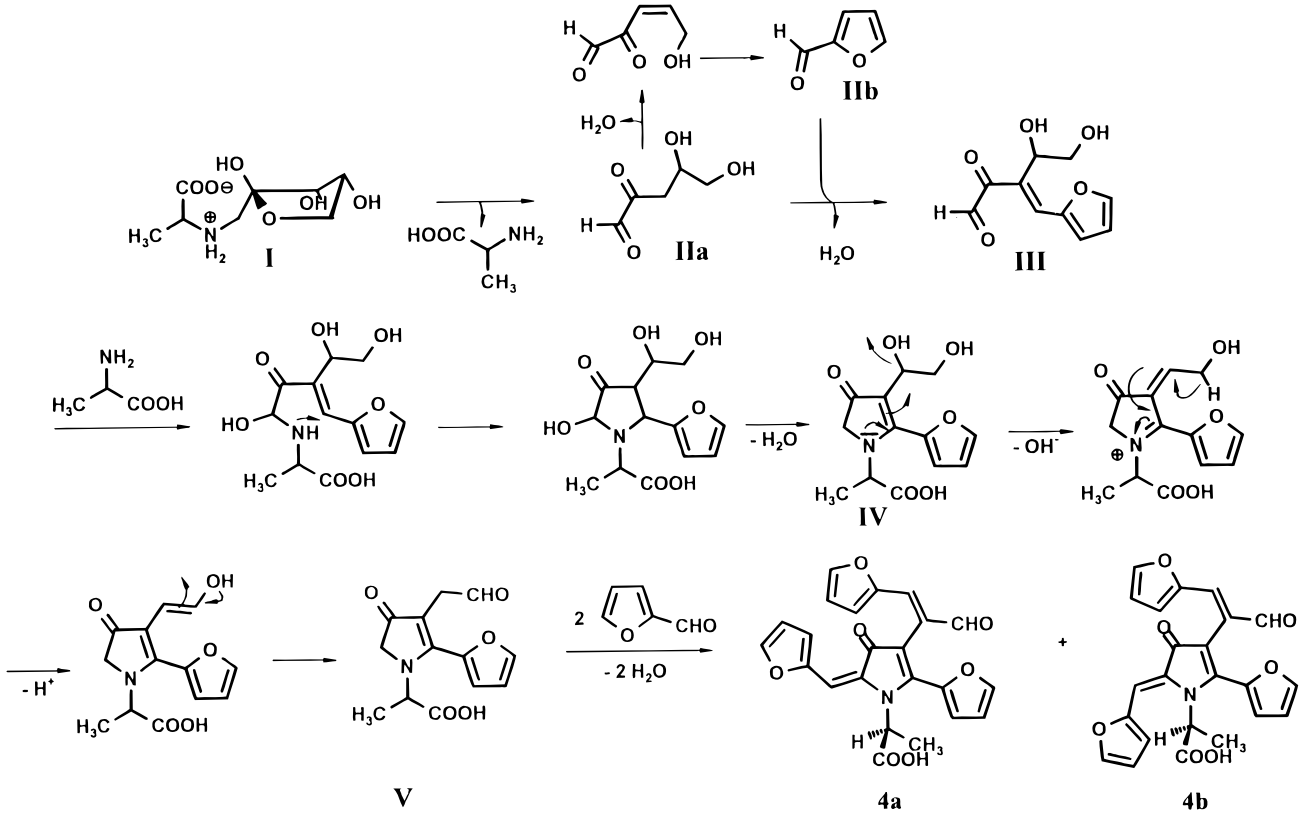


**Figure 10.** Formation pathway leading to (*S*)-4-[(*E*)-1-formyl-2-(2-furyl)ethenyl]-5-(2-furyl)-2-[(*E*)-(2-furyl)methylidene]-2,3-dihydro-α-amino-3-oxo-1*H*-pyrrole-6-acetic acid (**4a**) and its 2-[(*Z*)-(2-furyl)methylidene] isomer (**4b**) from *N*-(1-deoxy-D-xylulos-1-yl)-L-alanine.

**Table 3. Formation of PYRED (4a/4b) from Several C-5 Precursors**[a]

| precursors reacted in the presence of furan-2-carboxaldehyde | amount of PYRED | |
|---|---|---|
| | mg | % |
| xylose/alanine | 1.2 | 0.01 |
| *N*-(1-deoxy-D-xylulos-1-yl)-L-alanine | 1.8 | 0.02 |
| 3-deoxypentos-2-ulose/alanine | 6.8 | 0.06 |

[a] A solution of the precursors (each 30 mmol) in phosphate buffer (90 mL; 1 mmol/L, pH 7.0) was refluxed for 20 min. After addition of furan-2-carboxaldehyde (90 mmol), heating was continued for another 90 min.

additionally identified the red colorants (*S*)-4[(*E*)-1-formyl-2-(2-furyl)ethenyl)]-5-(2-furyl)-2-[(*E*)-(2-furyl)methylidene]-2,3-dihydro-α-methyl-3-oxo-1*H*-pyrrole-1-acetic acid and its 2-[(*Z*)-(2-furyl)methylene] isomer (PYRED; **4a**/**4b** in Figure 9) by comparison of the UV−vis and LC/MS spectra as well as the HPLC retention times with those obtained for the reference compounds (Hofmann, 1997b, 1998a).

In a recent investigation (Hofmann, 1997b, 1998a), PYRED was found among the main colored reaction products formed from the reaction of alanine with furan-2-carboxaldehyde. To study whether PYRED was formed in the xylose/alanine/furan-2-carboxaldehyde mixture exclusively from the reaction between furan-2-carboxaldehyde and alanine or, also, from other pentose-derived intermediates, we heated solutions of (i) xylose/alanine, (ii) the Amadori rearrangement product *N*-(1-deoxy-D-xylulos-1-yl)-L-alanine, or (iii) 3-deoxypentos-2-ulose/alanine, respectively, in the presence of furan-2-carboxaldehyde and determined the amounts of **4a/4b** generated. The results, given in Table 3, showed that the binary mixture of 3-deoxypentos-2-ulose and alanine was by far the most effective precursor system, because 5.7- and 3.8-fold more PYRED was formed compared with xylose/alanine or the *N*-(1-deoxy-D-xylulos-1-yl)-L-alanine.

On the basis of these data, it is clear that, in the presence of alanine, PYRED is not exclusively formed via furan-2-carboxaldehyde as the precursor but also from other intermediates of the 3-deoxyosone pathway. The data of the quantitative experiments revealed the 3-deoxyosone as a key intermediate in the formation of **4a**/**4b**. Because the furan rings are likely to originate from furan-2-carboxaldehyde, the following mechanism was proposed for the formation of **4a**/**4b** (Figure 10). Thermal treatment of the Amadori rearrangement product *N*-(1-deoxy-D-xylulos-1-yl)-L-alanine liberates 3-deoxypentos-2-ulose (**IIa**), which to some extent yields furan-2-carboxaldehyde (**IIb**). A condensation reaction between the C-3-methylene-active 3-deoxypentos-2-ulose and the aldehyde **IIb** leads to 3-[(2-furyl)methylidene]-3-deoxypentos-2-ulose (**III**), which, upon a cyclization reaction with alanine, forms the 4-(1,2-dihydroxyethyl)-5-(2-furyl)-2,3-dihydro-α-methyl-3-oxo-1*H*-pyrrole-1-acetic acid (**IV**). Elimination of water then gives the 4-(2-formylmethyl)-5-(2-furyl)-2,3-dihydro-α-methyl-3-oxo-1*H*-pyrrole-1-acetic acid (**V**), which, upon condensation with two molecules of furan-2-carboxaldehyde, leads to the red colorants **4a** and **4b**.

The favored formation of **4a** and **4b** from the 3-deoxyosone is well in line with the proposed reaction mechanism, because the formation of **4a**/**4b** exclusively from furan-2-carboxaldehyde needs two molecules of the amino acid (Hofmann, 1998e), whereas in its formation from the 3-deoxyosone only one amino compound is involved.

## CONCLUSION

The identification of novel nitrogen-containing colored compounds formed from pentoses and alanine clearly demonstrates that either the complete amino acid moiety can be incorporated into colored structures or only the nitrogen atom can be transferred into colorants via the Strecker reaction with deoxyosones. Such studies provide useful information to extend the knowledge on chromophores generated by Maillard-type reactions during food processing and will help to construct a route map of reactions leading to color development in heated foodstuffs.

## ACKNOWLEDGMENT

I thank the Institute of Organic Chemistry, Technical University of Munich, for the NMR measurements and the Institute of Food Chemistry, Technical University of Munich, for the LC/MS analyses. I am grateful to Mr. J. Stein for his excellent technical assistance.

## LITERATURE CITED

Arnoldi, A.; Corain, E. A.; Scaglioni, L.; Ames, J. M. New colored compounds from the Maillard Reaction between xylose and lysine. *J. Agric. Food Chem.* **1997**, *45*, 650−655.

Hofmann, T. Determination of the chemical structure of novel coloured compounds generated by Maillard-type reactions. In *Proceedings of the 6th International Symposium on the Maillard Reaction*; O'Brien, J., Nursten, H. E., Crabbe, M. J. C., Ames, J. M., Eds.; Royal Society of Chemistry, Cambridge, U.K., 1997a; in press.

Hofmann, T. Determination of the chemical structure of novel colored 1H-pyrrol-3(2H)-one derivatives formed by Maillard-type reactions. *Helv. Chim. Acta* **1997b**, *80*, 1843−1856.

Hofmann, T. Characterization of the chemical structure of novel colored Maillard reaction products from furan-2-carboxaldehyde and amino acids. *J. Agric. Food Chem.* **1998a**, *46*, 932−940.

Hofmann, T. Studies on melanoidin-type colorants generated from the Maillard reaction of protein-bound lysine and furan-2-carboxaldehyde—chemical characterisation of a red coloured domaine. *Z. Lebensm. Unters. Forsch.* **1998b**, *206*, 251−258.

Hofmann, T. Characterisation of precursors and elucidation of the reaction pathway leading to a novel coloured 2*H*,7*H*,-8a*H*-Pyrano[2, 3-*b*]pyran-3-one by quantitative studies and application of $^{13}$C-labeling experiments. *Carbohydr. Res.* **1998c**, submitted for publication.

Hofmann, T. 2*H*,7*H*,8a*H*-Pyrano[2,3-*b*]pyran-3-one—A novel type of chromophore formed from the Maillard reaction of pentoses. *Carbohydr. Res.* **1998d**, submitted for publication.

Hofmann, T. Application of site specific [$^{13}$C] enrichment and $^{13}$C NMR spectroscopy for the elucidation of the formation pathway leading to a red colored 1*H*-pyrrol-3(2*H*)-one during the Maillard reaction of furan-2-carboxaldehyde and L-alanine. *J. Agric. Food Chem.* **1998e**, *46*, 941−945.

Hofmann, T.; Schieberle, P. Quantitative model studies on the effectivity of different precursor systems in the formation of the intense food odorants 2-furfurylthiol and 2-methyl-3-furanthiol. *J. Agric. Food Chem.* **1998a**, *46*, 235−241.

Hofmann, T.; Schieberle, P. New and convenient syntheses of the important roasty, popcorn-like smelling food aroma compounds 2-acetyl-1-pyrroline and 2-acetyltetrahydropyridine from their corresponding cyclic α-amino acids. *J. Agric. Food Chem.* **1998b**, *46*, 616−619.

Ledl, F.; Schleicher, E. The Maillard reaction in foods and in the human body—new results of the chemistry, biochemistry and medicine (in German). *Angew. Chem.* **1990**, *102*, 597−734.

Ledl, F.; Severin, Th. Nonenzymatic browning reactions of pentoses and amines (in German). *Z. Lebensm. Unters. Forsch.* **1978**, *167*, 410−413.

Ledl, F.; Severin, Th. Formation of colored compounds from hexoses (in German). *Z. Lebensm. Unters. Forsch.* **1982**, *175*, 262−265.

Narziss, L.; Stippler, K. Investigation on the intermediates of color and aroma products in malt (in German). *Brauwissenschaft* **1976**, *28* (10), 332.

Nursten, H. E.; O'Reilly, R. Coloured compounds formed by the interaction of glycine and xylose. *Food Chem.* **1986**, *20*, 45−60.

Severin, Th.; Krönig, U. Structur of a colored compound from pentoses (in German). *Chem. Mikrobiol. Technol. Lebensm.* **1972**, *1*, 156−157.

Thottahil, J. K.; Przybla, C.; Malley, M.; Gougoutas, J. Z. A meso specific reaction. *Tetrahedron Lett.* **1986**, *14*, 1533−1536.

Received for review May 11, 1998. Revised manuscript received July 27, 1998. Accepted July 28, 1998.

JF980477I