# Characterization of the Chemical Structure of Novel Colored Maillard Reaction Products from Furan-2-carboxaldehyde and Amino Acids

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Colored compounds formed by Maillard-type reactions from furan-2-carboxaldehyde and primary and secondary amino acids including L-alanine and L-proline, respectively, have been identified. When furan-2-carboxaldehyde was heated with L-proline in aqueous solution at pH 7.0, an intensely yellow compound was generated, which was identified as 5(*S*)-(2-carboxy-1-pyrrolidinyl)-2-hydroxy-(*E*,*E*)-2,4-pentadienal-(*S*)-(2-carboxypyrrolidine)imine (**1**) by application of several one- and two-dimensional NMR experiments and, in addition, by MS, UV, and IR spectroscopy. Further thermal treatment of compound **1** resulted, upon a ring closure reaction, in the formation of (*E*)-4,5-bis[(*S*)-2-carboxy-1-pyrrolidinyl]-2-cyclopenten-1-one (**5**), which has been, to our knowledge, as yet not reported in the literature. To confirm the proposed structures, L-proline was substituted by pyrrolidine and piperidine, leading to analogous *N*-cyanines (**2** and **3**) and cyclopentenones (**4** and **6**). On the other hand, thermal treatment of an aqueous solution of furan-2-carboxaldehyde and L-alanine led to the formation of the novel red compounds **8a** and **8b**, which were identified as (*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-6-acetic acid and the corresponding 2-(*Z*)-(2-furyl)methylidene isomer. This is the first time that chromophoric compounds comprising four linked rings with an amino acid moiety incorporated were identified in a Maillard reaction system.

**Keywords:** *Nonenzymatic browning; Maillard reaction; furan-2-carboxaldehyde; alanine; proline; (S)-4-[(E)-1-formyl-2-(2-furyl)ethenyl]-5-(2-furyl)-2-[(E)-(2-furyl)methylidene]-2,3-dihydro-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 colors and flavors that occurs, e.g., during thermal processing of foods, such as roasting of meat, baking of bread, kiln-drying of malt, or roasting of coffee.

A lot of work has already been done relating to the key odorants produced during this reaction (Werkhoff et al., 1990; Silwar, 1992; Meynier and Mottram, 1995; Schieberle, 1995; Hofmann and Schieberle, 1995, 1997a; Schieberle and Hofmann, 1996). However, due to the complexity and multiplicity of the nonvolatile Maillard reaction products formed, surprisingly little is known about the structures of the compounds responsible for the typical brown color. To gain a more detailed insight into the structures of colorants, it is, therefore, a helpful approach to characterize such compounds in model systems containing two defined components that are potentially involved in the color formation. In the past 20 years some investigations [e.g., Ledl and Severin (1978, 1982)] have been performed to clarify the mechanisms of the so-called nonenzymatic browning; however, most model reactions have been carried out in organic solvent, rather than in aqueous solution, and, also, using amines not related to food ingredients instead of 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. The authors were, however, not able to suggest from which carbohydrate-derived intermediates the colorant was formed.

To elucidate precursors and formation pathways of colored Maillard products, it might, therefore, be more promising to identify the chromophoric structures formed by reacting certain carbohydrate degradation products under food relevant conditions. Furan-2-carboxaldehyde is known as one of the main reaction products formed from pentoses during thermal treatment (Ledl and Schleicher, 1990). Severin and Krönig (1972) reported that this aldehyde reacts easily with 4-hydroxy-5-methyl-3(2*H*)-furanone also derived from carbohydrate dehydration, giving rise to a yellow condensation product.

Although as yet not investigated, it might be possible that the reaction of furan-2-carboxaldehyde with amino acids might contribute to color development. The objectives of the following studies were, therefore, to identify colored compounds formed from furan-2-carboxaldehyde in the presence of primary as well as secondary amino acids and, furthermore, to gain insights into reaction pathways leading to these colorants.

## EXPERIMENTAL PROCEDURES

**Chemicals.** The following compounds were obtained commercially: furan-2-carboxaldehyde, (*E*)-*β*-(2-furyl)acrylaldehyde, pyrrolidine, piperidine, 3-hydroxypyridine, cyanogen

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bromide, trifluoracetic acid (TFA), and acetic acid (Aldrich, Steinheim, Germany); L-alanine and L-proline (Lancaster, Mühlheim, Germany); solvents of HPLC grade (Aldrich); DMSO-$d_6$, $CDCl_3$, and $CD_3OD$ (Isocom, Landshut, Germany).

4-Hydroxy-5-methyl-3(2*H*)-furanone was prepared as described recently (Hofmann and Schieberle, 1997b).

**Isolation of 5(*S*)-(2-Carboxy-1-pyrrolidinyl)-2-hydroxy-(*E*,*E*)-2,4-pentadienal-(*S*)-(2-carboxypyrrolidine) Imine (1) from the Reaction of L-Proline and Furan-2-carboxaldehyde.** A solution of L-proline (50 mmol) and furan-2-carboxaldehyde (50 mmol) in water (15 mL) was heated for 15 min at 50 °C. After the unreacted furan-2-carboxaldehyde was removed by extraction with diethyl ether (5 × 15 mL), the aqueous layer was freeze-dried, the residue was taken up in methanol (3 mL), and aliquots were then fractionated by column chromatography using silica gel. After application of an aliquot (1 mL) of the raw material onto the column (40 × 2 cm), which was filled with a slurry of silica gel (silica gel 60, Merck, Darmstadt, Germany) in diethyl ether, chromatography was performed using ethyl acetate (200 mL), followed by several ethyl acetate/methanol mixtures (80:20, 60:40, 50:50 v/v; 100 mL each). Elution with ethyl acetate/methanol (40:60, v/v; 300 mL) affords a fraction containing a deep yellow colorant, which was freed from solvent in vacuo and dissolved in aqueous TFA (0.1% TFA in water; 3 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 aqueous TFA (0.1% TFA in water). After flushing with the same solvent (200 mL), the colorant was eluted with a mixture (30:70 v/v; 200 mL) of methanol and aqueous TFA (0.1% TFA in water). The collected fraction was freed from methanol in vacuo and then freeze-dried, affording **1** as an intensely yellow solid (yield = ∼5.3%, 2.65 mmol). For spectral measurements the pure colorant was then isolated by preparative RP-HPLC monitoring the effluent at $\lambda$ = 446 nm. Starting with a 15:85 (v/v) mixture of acetonitrile and aqueous TFA (0.1% TFA in water), the acetonitrile content was increased to 100% within 50 min. The colorant eluting between 10.5 and 11.5 min was collected, freeze-dried, and taken up in $CD_3OD$: $^1$H- and $^{13}$C NMR data are given in Tables 1 and 2; MS (ESI) 309 (87; $[M + 1]^+$), 194 (100; $[M - \text{proline}]^+$), 196 (15), 152 (14), 150 (10), 277 (6), 265 (4; $[M - CO_2]^+$); IR (KBr) 3432, 1540, 1384, 1160, 1120 cm$^{-1}$; UV $\lambda_{max}$ = 446 nm; $\epsilon = 5.55 \times 10^4$ L mol$^{-1}$ cm$^{-1}$ (pH 7.0; water).

**Isolation of 5-(1-Pyrrolidinyl)-2-hydroxy-(*E*,*E*)-2,4-pentadienal (Pyrrolidine) Imine (2) from the Reaction of Furan-2-carboxaldehyde and Pyrrolidinium Acetate.** An aqueous solution (5 mL) of furan-2-carboxaldehyde (30 mmol), pyrrolidine (30 mmol), and acetic acid (30 mmol) was heated for 10 min at 50 °C. After extraction with diethyl ether (5 × 15 mL), the aqueous layer was freeze-dried and the residue was taken up in methanol (3 mL) and then fractionated by column chromatography using silica gel as discribed above. After application of an aliquot (1 mL) of the raw material onto the column conditioned with ethyl acetate, chromatography was performed using ethyl acetate (200 mL), followed by ethyl acetate/methanol (80:20 v/v, 200 mL). Elution with ethyl acetate/methanol (60:40 v/v, 200 mL) affords a deep yellow fraction, which was freed from the solvent in vacuo. The residue was dissolved in aqueous TFA (0.1% TFA in water; 3 mL) and then further fractionated by flash chromatography using RP-18 material as descibed above. The solution was placed onto the column (20 × 1.6 cm), which was conditioned with a 60:40 (v/v) mixture of methanol and aqueous TFA (0.1% TFA in water). Chromatography with the latter solvent (1.5 mL/min) afforded the colorant after 100−150 mL. The collected eluate was freeze-dried, and the intensely yellow residue was taken up in $CD_3OD$ (yield = ∼11.6%; 3.5 mmol): $^1$H NMR (360 MHz; $CD_3OD$, COSY, TOCSY; arbitrary numbering of the carbon atoms refers to formula **2** in Figure 1): $\delta$ 1.93−1.98 [m, 8H, H−C(7,7′,8,8′)], 3.20−3.25 [m, 8H, H−C(6,6′,9,9′)], 5.73 [dd, 1H, $^3J_{4,3}$ = 12.8 Hz, $^3J_{4,5}$ = 11.9 Hz, H−C(4)], 6.84 [d, 1H, $^3J_{3,4}$ = 12.8 Hz, H−C(3)], 7.19 [bs, 1H, H−C(1)], 7.63 [d, 1H, $^3J_{5,4}$=11.9 Hz, H−C(5)]; MS (ESI) 221 (100; $[M + 1]^+$), 150 (58; $[M - \text{pyrrolidine}]^+$); IR (KBr) 3417, 1555 cm$^{-1}$; UV $\lambda_{max}$ = 446 nm; $\epsilon = 4.90 \times 10^4$ L mol$^{-1}$ cm$^{-1}$ (pH 7.0).

**Synthesis of 5-(1-Pyrrolidinyl)-2-hydroxy-(*E*,*E*)-2,4-pentadienal (Pyrrolidine) Imine (Figure 2).** Cyanogen bromide (1.0 mmol) was added slowly to a stirred solution of 3-hydroxypyridine (1.0 mmol) in ethanol (4 mL). After 10 min of heating at 70 °C, pyrrolidine (2.0 mmol) was added and the mixture was then stirred for 20 min at room temperature. The solvent was evaporated in vacuo, the residue was taken up in methanol (1 mL) and the target compound (yield = 35%, 77 mg) was isolated as described above. MS and NMR data of (*E*,*E*)-5-(1-pyrrolidinyl)-2-hydroxy-2,4-pentadienal (pyrrolidine) imine were identical with those obtained for **2** isolated from the reaction mixture of furan-2-aldehyde and pyrrolidinium acetate.

**Isolation of 5-(1-Piperidinyl)-2-hydroxy-(*E*,*E*)-2,4-pentadienal (Piperidine) Imine (3) from the Reaction of Furan-2-carboxaldehyde and Piperidinium Acetate.** Preparation and isolation of compound **3** was performed following the procedure described above for colorant **2** (yield = ∼10.5%; 3.2 mmol): $^1$H NMR (360 MHz, $CD_3OD$, COSY, TOCSY; arbitrary numbering of the carbon atoms refers to formula **3** in Figure 1) $\delta$ 1.66−1.75 [m, 4H, H−C(8,8′)], 1.75−1.93 [m, 8H, H−C(7,7′,9,9′)], 3.15−3.23 [m, 8H, H−C(6,6′,10,10′)], 5.73 [dd, 1H, $^3J_{4,3}$ = 12.8 Hz, $^3J_{4,5}$ = 11.9 Hz, H−C(4)], 6.83 [d, 1H, $^3J_{3,4}$ = 12.8 Hz, H−C(3)], 7.17 [bs, 1H, H−C(1)], 7.62 [d, 1H, $^3J_{5,4}$ = 11.9 Hz, H−C(5)]; MS (ESI) 249 (100; $[M + 1]^+$), 164 (61; $[M - \text{piperidine}]^+$); IR (KBr) 3419, 1553 cm$^{-1}$; UV $\Lambda_{max}$ = 446 nm; $\epsilon = 4.85 \times 10^4$ L mol$^{-1}$ cm$^{-1}$ (pH 7.0).

**Preparation of (*E*)-4,5-Bis(1-piperidinyl)-2-cyclopenten-1-one (4).** Compound **3** (0.5 mmol) was heated for 45 min at 70 °C in methanol (10 mL). A compound generated during heating was purified by preparative HPLC on RP-18 using a gradient starting with a 70:30 mixture of acetonitrile (containing 0.1% triethylamine) and increasing the acetonitrile content to 100% within 30 min. The effluent between 5.5 and 6.5 min was collected, diluted with water, and extracted with $CHCl_3$. After drying over $Na_2SO_4$, the solvent was evaporated in vacuo and the residue (yield = 15%, 18 mg) was taken up in $CDCl_3$: $^1$H NMR (360 MHz, $CDCl_3$, COSY, TOCSY; arbitrary numbering of the carbon atoms refers to formular **4** in Figure 4): $\delta$ 1.45 [m, 4H, H−C(8,8′)], 1.54 [m, 4H, H−C(7,9)], 1.59 [m, 4H, H−C(7′,9′)], 2.54 [m, 6H, H−C(10,6′,10′)], 2.74 [m, 2H, H−C(6)], 3.27 [d, 1H, $^3J_{5,4}$ = 3.1 Hz, H−C(5)], 3.79 [ddd, 1H, $^3J_{4,5}$ = 3.1 Hz, $^3J_{4,3}$ = 1.77 Hz, $^4J_{4,2}$ = 1.77 Hz, H−C(4)], 6.16 [dd, 1H, $^3J_{2,3}$ = 6.19 Hz, $^4J_{2,4}$ = 1.77 Hz, H−C(2)], 7.58 [dd, 1H, $^3J_{3,2}$ = 6.19 Hz, $^3J_{3,4}$ = 1.77 Hz, H−C(3)]; $^{13}$C NMR ($CDCl_3$, 135° DEPT, HMQC, HMBC; the numbering of the carbon atoms refers to formula **4** in Figure 4) $\delta$ 27.6 [$CH_2$, C(8)], 27.7 [$CH_2$, C(8′)], 29.6 [$CH_2$, C(7,9)], 29.8 [$CH_2$, C(7′,9′)], 53.9 [$CH_2$, C(6,-10)], 54.3 [$CH_2$, C(6′,10′)], 70.9 [CH, C(5)], 71.6 [CH, C(4)], 138.1 [CH, C(2)], 165.3 [CH, C(3)], 211.1 [C, C(1)]; MS(ESI) 249 (100; $[M + 1]^+$), 164 (25; $[M - \text{piperidine}]^+$); UV $\lambda_{max}$ = 220 nm; IR ($CHCl_3$) 2937, 1706, 1607 cm$^{-1}$.

**Preparation of (*E*)-4,5-Bis(*S*)-(2-carboxy-1-pyrrolidinyl)-2-cyclopenten-1-one (5).** Compound **1** (0.5 mmol) was heated in methanol (10 mL) for 45 min at 70 °C. After concentration of the mixture, isolation of the compound generated was achieved by preparative HPLC monitoring at $\lambda$ = 220 nm. Starting with a 15:85 mixture of acetonitrile and aqueous TFA acid (0.1% in water), the acetonitrile content was increased to 100% within 40 min. The colorant eluting between 9.0 and 10.0 min was collected, freeze-dried, and taken up in $CD_3OD$ (yield = 11%, 17 mg): $^1$H NMR ($CD_3OD$, COSY, TOCSY; the numbering of the carbon atoms refers to formula **5** in Figure 4) $\delta$ 1.90−2.10 [m, 4H, H−C(8,8′)], 2.06−2.15 [m, 2H, H−C(7a,7′a)], 2.17−2.28 [m, 2H, H−C(7b,7′b)], 3.43−3.51 [m, 2H, H−C(9a,9′a)], 3.49 (m, 1H, $^3J_{5,4}$ = 3.1 Hz, H−C(5)], 3.54−3.61 [m, 2H, H−C(9b, 9′b)], 3.89 [dd, 2H, $^3J_{6,7a}$ = $^3J_{6',7'a}$ = 8.4 Hz, $^3J_{6,7b}$ = $^3J_{6',7'b}$ = 3.5 Hz, H−C(6,6′)], 4.04 [ddd, 1H, $^3J_{4,5}$ = 3.1 Hz, $^3J_{4,3}$ = 1.77 Hz, $^4J_{4,2}$ = 1.77 Hz, H−C(4)], 6.23 [dd, 1H, $^3J_{2,3}$ = 6.19 Hz, $^4J_{2,4}$ = 1.77 Hz, H−C(2)], 7.89 [dd, 1H, $^3J_{3,2}$ = 6.19 Hz, $^3J_{3,4}$ = 1.77 Hz, H−C(3)]; MS (ESI) 309 (100; $[M + 1]^+$), 194 (38; $[M - \text{proline}]^+$.

**Preparation of (*E*)-4,5-Bis(1-pyrrolidinyl)-2-cyclopenten-1-one (6).** The compound generated upon thermal treatment of *N*-cyanine **2** (0.5 mmol) was islolated following the procedure described for cyclopentenone **4** (yield = 11%, 13 mg): $^1$H NMR (360 MHz, $CDCl_3$, COSY, TOCSY; the numbering of the carbon atoms refers to formula **6** in Figure 4) $\delta$ 1.92−1.98 [m, 8H, H−C(7,8,7′,8′)], 2.98−3.06 [m, 8H, H−C(6,9,6′,9′)], 3.36 [d, 1H, $^3J_{5,4}$ = 3.1 Hz, H−C(5)], 3.96 [ddd, 1H, $^3J_{4,5}$ = 3.1 Hz, $^3J_{4,3}$ = 1.77 Hz, $^4J_{4,2}$ = 1.77 Hz, H−C(4)], 6.25 (d, 1H, $^3J_{2,3}$ = 6.19 Hz, $^4J_{2,4}$ = 1.77 Hz, H−C(2)], 7.66 [dd, 1H, $^3J_{3,2}$ = 6.19 Hz, $^3J_{3,4}$ = 1.77 Hz, H−C(3)]; MS(ESI) 221 (100; $[M + 1]^+$), 150 (29; $[M -$ pyrrolidine$]^+$).

**Identification of the (*E*)-4,5-Diamino-2-cyclopenten-1-one 4, 5, or 6 in Heated Aqueous Solutions of the Corresponding *N*-Cyanine 3, 1, or 2.** *N*-Cyanine **1**, **2**, or **3** (0.5 mmol each) was heated in water (5 mL) for 20 min at 70 °C. The peaks eluting at the same time during RP-HPLC and exhibiting the UV and MS spectra identical with those of the prepared compounds (see above) were identified as the 4,5-diamino-2-cyclopenten-1-ones **4**, **5**, or **6**.

**Isolation 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-6-acetic Acid (8a) and Its 2-[(*Z*)-(2-furyl)methylidene] Isomer (8b) from the Reaction of Furan-2-carboxaldehyde and L-Alanine.** A solution of furan-2-carboxaldehyde (250 mmol) and L-alanine (250 mmol) in phosphate buffer (0.2 mol/L; pH 7.0; 150 mL) was heated for 1 h at 70 °C. The mixture was cooled to room temperature and extracted with ethyl acetate (10 × 100 mL); the organic layer was dried over $Na_2SO_4$ and then concentrated at 30 °C under vacuum (100 mbar) to ∼100 mL. The solution was then distilled in high vacuum (0.04 mbar) at 40 °C, and the dark red residue was taken up in ethyl acetate (10 mL). Aliquots of the solution were then subfractionated by column chromatography (40 × 2 cm) using silica gel (silica gel 60, Merck). After application of an aliquot of the raw material (5 mL) onto the column conditioned with diethyl ether, chromatography was performed using diethyl ether (150 mL), followed by ethyl acetate (150 mL). Elution with ethyl acetate/methanol (80:20, v/v; 150 mL) affords a deep red fraction, which was further subfractionated by preparative thin-layer chromatography on silica gel (20 × 20 cm; 0.5 mm; Merck) using ethyl acetate/methanol (75:25, v/v) as the eluent. The dark red band with $R_f$ = 0.27 was scraped off and suspended in methanol. After filtration and concentration in vacuo to ∼2 mL, this fraction was separated by anion exchange chromatography. The solution was placed onto a column (15 × 2 cm) filled with a slurry of (diethylaminoethyl)cellulose (DE−52, Whatman Ltd., Maidstone, U.K.) in methanol/water (80:20, v/v). Neutral compounds were first eluted with methanol/water (80:20, v/v; 200 mL). The acidic components were then isolated by elution with a mixture (80:20, v/v; 200 mL) of methanol and aqueous NaCl solution (0.01 mol/L). This fraction was concentrated in vacuo to ∼50 mL, water (200 mL) was added, and the solution was then extracted with ethyl acetate (5 × 20 mL). The solvent was distilled off in vacuo, and the residue was taken up in methanol (2 mL). For spectral measurements the colorant was then isolated by preparative RP-HPLC. Starting with a mixture of methanol (20%) and water (80%), the methanol content was increased to 100% within 45 min. The effluent was collected between 14.5 and 15.5 min, diluted with water, and extracted with ethyl acetate. The solvent was then distilled off in vacuo, affording the pure colorant as a deep red solid (yield = ∼0.4%, 416 mg). NMR studies revealed an equilibrium between a major isomer with *E*/*E* configuration (**8a**) and a minor isomer with *Z*/*E* configuration (**8b**), both consisting of a mixture of two atropisomers. The chemical shifts of both *E*/*E*-configured atropisomers **8a**, separated by dashs, were as follows: $^1$H NMR (600 MHz, DMSO-$d_6$, COSY, TOCSY; the numbering of the carbon atoms refers to formula **8a** in Figure 7) $\delta$ 1.60/1.43 [each d, 3H, $^3J_{22,21}$ = 7.3 Hz, H−C(22)], 4.64/4.75 [each q, 1H, $^3J_{21,22}$ = 7.3 Hz, H−C(21)], 6.55/6.58 [each dd, 1H, $^3J_{19,18}$ = 3.5 Hz, $^3J_{19,20}$ = 1.4 Hz, H−C(19)], 6.61−6.63/6.61−6.63 [m, each 2H, $^3J_{8,7}$ = 3.5 Hz, $^3J_{8,9}$ = 1.4 Hz, $^3J_{12,11}$ = 3.5 Hz, $^3J_{12,13}$ = 1.4 Hz, H−C(8,12)], 6.83/7.02 [each d, 1H, $^3J_{11,12}$ = 3.5 Hz, H−C(11)], 6.87/6.69 [each d, 1H, $^3J_{18,19}$ = 3.5 Hz, H−C(18)], 6.90/7.10 [each s, 1H, H−C(5)], 7.55/7.49 [each s, 1H, H−C(16)], 7.84/7.85 [each d, 1H, $^3J_{9,8}$ = 1.4 Hz, H−C(9)], 7.85/7.81 [each d, 1H, $^3J_{20,19}$ = 1.4 Hz, H−C(20)], 7.87/7.82 [each d, 1H, $^3J_{13,12}$ = 1.4 Hz, H−C(13)], 8.21/8.23 [each d, 1H, $^3J_{7,8}$ = 3.5 Hz, H−C(7)], 9.48/9.48 [s, each 1H, H−C(15)]; $^{13}$C NMR (360 MHz, DMSO-$d_6$, DEPT-135, HMQC, HMBC; the numbering of the carbon atoms refers to formular **8a** in Figure 7) 17.8/17.1 [$CH_3$, C(22)], 57.4/57.2 [CH, C(21)], 105.7/104.9 [C, C(2)], 109.7/110.6 [CH, C(5)], 112.5/112.4 [CH, C(12)], 113.2/113.2 [CH, C(8)}, 113.5/113.5 [CH, C(19)], 115.9/116.0 [CH, C(7)], 116.4/116.5 [CH, C(18)], 130.3/130.1 [C, C(14))] 133.8/132.7 [C, C(4)], 137.7/136.2 [CH, C(16)], 144.1/144.1 [CH, C(13)], 145.5/145.6 [CH, C(9)], 146.2/146.1 [CH, C(20)], 150.7/150.7 [C, C(6)], 151.1/151.1 [C, C(17)], 151.2/151.2 [C, C(10)], 153.1/153.1 [C, C(1)], 171.8/172.3 [C, C(23)], 180.3/180.5 [C, C(3)], 193.1/193.1 [CH, C(15)]; MS(ESI) 420 (100, $[M + 1]^+$), 442 (24, $[M + Na]^+$), 392 (20), 319 (13); UV ($H_2O$, pH 7.0) $\lambda_{max1}$ = 330 nm, $\lambda_{max2}$ = 414 nm ($\epsilon = 2.9 \times 10^4$ L mol$^{-1}$ cm$^{-1}$), $\lambda_{max3}$ = 480 nm; IR ($CHCl_3$) 3016, 2975, 1653, 1469, 1046 cm$^{-1}$. The atomic assignment of the NMR data of **8a** and the spectral data of the minor isomer **8b** will be published elsewhere in more detail (Hofmann, 1997).

**Isolation of (*E*,*E*)-2-[3-(2-Furyl)-2-propen-1-ylidene]-4-hydroxy-5-methyl-3(2*H*)-furanone (9) from the Reaction of (*E*)-β-(2-Furyl)acrylaldehyde and 4-Hydroxy-5-methyl-3(2*H*)-furanone.** A solution of 4-hydroxy-5-methyl-3(2*H*)-furanone (10 mmol) and (*E*)-β-(2-furyl)acrylaldehyde (10 mmol) in phosphate buffer (30 mL; 0.5 mol/L; pH 7.0) was heated for 1 h at 70 °C. The reaction mixture was adjusted to pH 5.0 with aqueous hydrochloric acid (0.1 mol/L) and then extracted with ethyl acetate (5 × 10 mL). After drying over $Na_2SO_4$, the organic layer was concentrated in vacuo to ∼1 mL. Storage at −30 °C afforded component **9** as purple-red crystals (yield = ∼14%; 305 mg): $^1$H and $^{13}$C NMR data are given in Tables 3 and 4; LC/MS(ESI) 219 (100; $[M + 1]^+$); UV (water, pH 7.0) $\lambda_{max}$ = 391 nm.

**High-Performance Liquid Chromatography (HPLC).** HPLC was performed with a gradient system consisting of two pumps type 422 (Kontron, Eching, Germany), a gradient mixer M800 (Kontron), a Rheodyne injector (100 μL loop), and a diode array detector DAD type 440 (Kontron). A column packed with RP-18 material (ODS-Hypersil, 5 μm, 10 nm, Shandon, Frankfurt, Germany) was used for analytical (250 × 4.6 mm, flow rate = 0.8 mL/min) chromatography. The column was protected with a guard cartridge (25 × 4.6 mm) packed with the same material. The preparative column (250 × 10 mm) was also an ODS-Hypersil (5 μm, 10 nm, Shandon) used with a flow rate of 1.8 mL/min.

**Liquid Chromatography/Mass Spectrometry (LC/MS).** Analytical HPLC (Nucleosil 100-5C18, Macherey and Nagel, Dürren, Germany) was coupled with an LCQ-MS (Finnigan MAT GmbH, Bremen, Germany) using either electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI). After injection of the sample (2.0 μL), analysis was performed using a gradient starting with a mixture (10:90, v/v) of acetonitrile and 0.1% aqueous formic acid and increasing the acetonitrile content to 100% within 15 min.

**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, Mainz, Germany) coupled with an MD-800 mass spectrometer (Fisons Instruments); sample application (0.5 μL) was done by on-column injection at 40 °C.

**UV−Visible Spectroscopy (UV−Vis).** UV−visible spectra were measured over a range of 220−600 nm using a U-2000 spectrometer (Colora Messtechnik Gmbh, Lorch, Germany).

**Infrared Spectroscopy (IR).** IR spectra were obtained by means of a 288 B spectrometer (Perkin-Elmer, Überlingen, Germany).

**Nuclear Magnetic Resonance Spectroscopy (NMR).** $^1$H, $^{13}$C, DEPT-135, DQF−COSY, TOCSY, HMQC, and HMBC experiments were performed on Bruker AC-200, a Bruker AM-360, and a Bruker AMX-600 spectrometers using the acquisi-

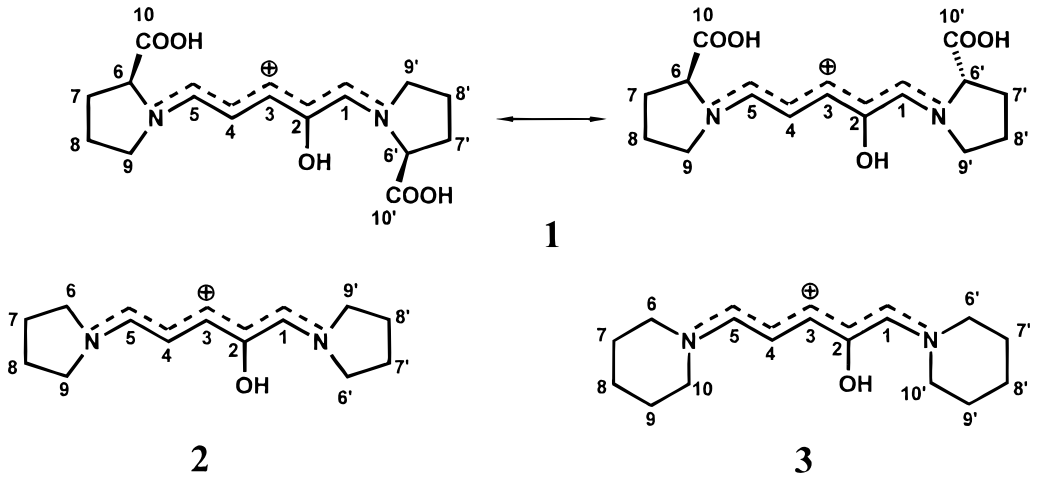


**Figure 1.** Structures of the yellow *N*-cyanines **1**−**3**: 5(*S*)-(2-carboxy-1-pyrrolidinyl)-2-hydroxy-(*E*,*E*)-2,4-pentadienal-(*S*)-(2-carboxypyrrolidine) imine (**1**); 5-(1-pyrrolidinyl)-2-hydroxy-(*E*,*E*)-2,4-pentadienal (pyrrolidine) imine (**2**); and 5-(1-piperidinyl)-2-hydroxy-(*E*,*E*)-2,4-pentadienal (piperidine) imine (**3**).

tion parameters described recently by Hofmann (1997). Samples were dissolved in $CDCl_3$, DMSO-$d_6$, or $CD_3OD$; chemical shifts expressed in parts per million were measured from tetramethylsilane (TMS) as the internal standard or from residual $CHCl_3$ (7.24 ppm) or DMSO-$d_5$ (2.49 ppm) in the proton dimension and with the carbon signal of $CHCl_3$ (78.0 ppm) or DMSO-$d_6$ (39.5 ppm) in the carbon dimension. Evaluation of the experiments was done with 1D- and 2D-WIN-NMR software.

## RESULTS AND DISCUSSION

Thermal treatment of aqueous solutions of furan-2-carboxaldehyde in the presence of several amino compounds led to a rapid colorization of the reaction mixtures. The colorants generated were registered after separation of the reaction mixtures by RP-HPLC using either a diode array detector (DAD) monitoring in the wavelength range between 220 and 500 nm or an LC/MS. The structures of colored compounds generated from the reaction of furan-2-carboxaldehyde with either L-proline, pyrrolidine, piperidine, or L-alanine were investigated.

**Reaction of Furan-2-carboxaldehyde with Proline and Cyclic Secondary Amines.** When furan-2-carboxaldehyde and L-proline were heated in aqueous solution, the color of the reaction mixture rapidly turned deep yellow. An intensely yellow reaction product was characterized by DAD exhibiting an absorption maximum at 446 nm.

After its isolation by column chromatography and preparative RP-HPLC, the determination of its chemical structure was performed by several one- and two-dimensional NMR techniques and, in addition, by MS, UV, and IR spectroscopy. The spectroscopic data were consistent with structure **1** existing in two isomers (Figure 1). For spectral measurements the compound was isolated in the protonated form. LC/MS measurements gave an intense molecular ion at *m*/*z* 309 (87%), being well in line with the ionic structure proposed for **1**. In addition, the base peak at *m*/*z* 194 (100%) demonstrates a loss of 115 most likely corresponding to a proline moiety. The $^1$H NMR spectrum measured in $CD_3OD$ showed two sets of 10 resonance signals each in a ratio of 4:1, indicating the existence of two isomers. Further NMR data, fitting well with the assignment of structure **1**, are given in Table 1. The chemical shifts of the signals at 7.67, 7.30, 6.97, and 5.83 ppm for the major isomer were in the expected range of olefinic hydrogen atoms, whereas the five multiplets in the range of 1.9−3.9 ppm were assigned as the hydrogens of two proline moieties. Double-quantum-filtered $\delta/\delta$-correlation spectroscopy (COSY) as well as total correlated spectroscopy (TOCSY) revealed several strongly coupled $^1$H-spin systems, confirming the existence of two 2-carboxypyrrolidine groups in **1** and demonstrating the three hydrogens H−C(3), H−C(4), and H−C(5) incorporated in a polyene system. The coupling constants of 11.9 and 12.9 Hz for the olefinic hydrogens indicated similar C−C distances in the olefinic system and are very well in line with data measured for all-trans-configured *N*-cyanines, e.g., 11.8 and 12.7 Hz for 5-(1-pyrrolidinyl)-heptatrienylidene pyrrolidinium salts (Scheibe et al., 1966). The broad singlet resonating at 7.30 ppm showed no homonuclear couplings with other hydrogens; however, heteronuclear coupling (Table 2) was observed with the carbon atoms C(6′) and C(9′) of a proline moiety and, in addition, with C(2) and C(3). These data confirm that the amino function of proline is directly linked to the iminium carbon atom C(1). A comparison of the $^{13}$C NMR spectrum, in which 10 signals appeared, with the results of the DEPT experiment indicated two signals corresponding to quarternary carbon atoms (Table 2). Unequivocal assignment of these quarternary carbon atoms could be successfully achieved by means of heteronuclear multiple-bond (HMBC)/multiquantum (HMQC) coherence experiments optimized for $^2J$(C,H) and $^3J$(C,H) coupling constants (Table 2). These experiments led to the assignment of the signal at 179.4 ppm as the carboxy function of two equivalent proline moieties and the signal at 142.7 as the olefinic carbon atom C(2). In addition to the correlation of H−C(5) with C(3) and C(4), H−C(5) showed a cross peak with H−C(6) and H−C(9) of a proline moiety. These data demonstrate the direct link of the proline moiety at carbon atom C(5). In summary, the obtained spectroscopical data are consistent with the proposed structure of **1** as 5(*S*)-(2-carboxy-1-pyrrolidinyl)-2-hydroxy-(*E*,*E*)-2,4-pentadienal-(*S*)-(2-carboxypyrrolidine) imine (Figure 1). To our knowledge, this intensely colored compound **1**, showing a high extinction coefficient of $5.55 \times 10^4$ L mol$^{-1}$ cm$^{-1}$ (in water, pH 7.0), has not been previously described in the literature.

**Table 1. Assignment of $^1$H NMR Signals (360 MHz, $CD_3OD$) of 5(*S*)-(2-Carboxy-1-pyrrolidinyl)-2-hydroxy-(*E*,*E*)-2,4-pentadienal (*S*)-(2-Carboxypyrrolidine) Imine (1)**

| H at relevant C atom[b] | $\delta^a$ (ppm) 1a | $\delta^a$ (ppm) 1b | $I^a$ | $M^a$ | $J^a$ (Hz) | connectivity[c] with |
|---|---|---|---|---|---|---|
| H−C(5) | 7.67 | 7.70 | 1 | d | $^3J_{5,4} = 11.9$ | H−C(4) |
| H−C(1) | 7.30 | 7.30 | 1 | bs | | |
| H−C(3) | 6.97 | 6.94 | 1 | d | $^3J_{3,4} = 12.8$ | H−C(4) |
| H−C(4) | 5.83 | 5.67 | 1 | dd | $^3J_{4,3} = 12.8$<br>$^3J_{4,5} = 11.9$ | H−C(3), H−C(5) |
| H−C(6)<br>H−C(6′) | 4.57 | 4.41 | 1 | dd | $^3J_{6,7a} = 8.4$<br>$^3J_{6,7b} = 3.5$<br>$^3J_{6',7a'} = 8.4$<br>$^3J_{6',7b'} = 3.5$ | H−C(7a), H−C(7b)<br>H−C(7a′), H−C(7b′) |
| H−C(9a)<br>H−C(9a′) | 3.69−3.92 | 3.69−3.92 | 2 | m | | H−C(8)<br>H−C(8′) |
| H−C(9b)<br>H−C(9b′) | 3.45−3.64 | 3.45−3.64 | 2 | m | | H−C(8)<br>H−C(8′) |
| H−C(7a),<br>H−C(7a′) | 2.30−2.49 | 2.30−2.49 | 2 | m | | H−C(6)<br>H−C(6′) |
| H−C(7b)<br>H−C(7b′) | 2.14−2.29 | 2.14−2.29 | 2 | m | | H−C(6)<br>H−C(6′) |
| H−C(8)<br>H−C(8′) | 1.90−2.13 | 1.90−2.13 | 4 | m | | H−C(9a), H−C(9b)<br>H−C(9a′), H−C(9b′) |

[a]Determined from 1D spectrum. [b]Arbitrary numbering of carbon atoms refers to **1** in Figure 1. [c]Homonuclear $^1$H,$^1$H connectivities determined by TOCSY and DQF−COSY.

**Table 2. Assignment of $^{13}$C NMR Signals (360 MHz, $CD_3OD$) of 5(*S*)-(2-Carboxy-1-pyrrolidinyl)-2-hydroxy-(*E*,*E*)-2,4-pentadienal (*S*)-(2-Carboxypyrrolidine) Imine (1)**

| | | | heteronuclear $^1$H,$^{13}$C multiple-quantum coherence[c] | |
|---|---|---|---|---|
| C atom[a] | $\delta$ (ppm) | DEPT[b] | via $^1J$(C,H) | via $^{2,3}J$(C,H) |
| C(10)<br>C(10′) | 179.4 | C | | H−C(6), H−C(7a), H−C(7b)<br>H−C(6′), H−C(7a′), H−C(7b′) |
| C(5) | 162.5 | CH | H−C(5) | H−C(3), H−C(4), H−C(6), H−C(7) |
| C(3) | 156.8 | CH | H−C(3) | H−C(1), H−C(4), H−C(5) |
| C(1) | 154.8 | CH | H−C(1) | H−C(2), H−C(3), H−C(6′), H−C(7′) |
| C(2) | 142.7 | C | | H−C(1), H−C(3), H−C(4) |
| C(4) | 106.1 | CH | H−C(4) | H−C(3), H−C(5) |
| C(6)<br>C(6′) | 71.7 | $CH_2$ | H−C(6)<br>H−C(6′) | H−C(5), H−C(7)<br>H−C(1), H−C(7′) |
| C(9)<br>C(9′) | 54.2 | $CH_2$ | H−C(9)<br>H−C(9′) | H−C(5), H−C(8)<br>H−C(1), H−C(8′) |
| C(7)<br>C(7′) | 36.1 | $CH_2$ | H−C(7)<br>H−C(7′) | H−C(6), H−C(8)<br>H−C(6′), H−C(8′) |
| C(8)<br>C(8′) | 29.9 | $CH_2$ | H−C(8)<br>H−C(8′) | H−C(7a), H−C(7b), H−C(9a), H−C(9b)<br>H−C(7a′), H−C(7b′), H−C(9a′), H−C(9b′) |

[a]Arbitrary numbering of carbon atoms refers to **1** in Figure 1. [b]DEPT-135 spectroscopy. [c]Assignments based on HMQC ($^1J$) and HMBC ($^{2,3}J$) experiments.

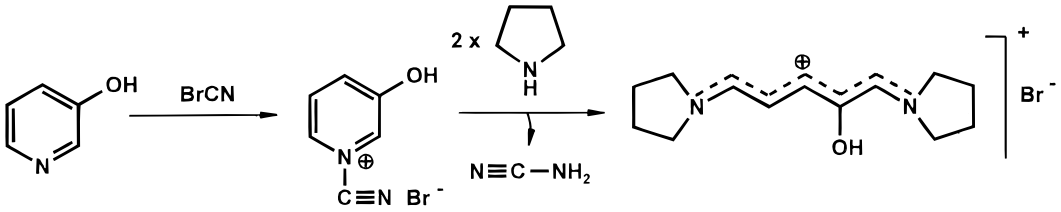



**Figure 2.** Synthesis of 5-(1-pyrrolidinyl)-2-hydroxy-(*E*,*E*)-2,4-pentadienal (pyrrolidine) imine.

To confirm that the existence of two spectrum sets is due to a diastereomeric splitting and is not the result of, e.g., an *E*/*Z* equilibrium, we substituted the L-proline moiety in the reaction with furan-2-carboxaldehyde by the achiral cyclic amines pyrrolidine and piperidine. We were successful in isolating 5-(1-pyrrolidinyl)-2-hydroxy-(*E*,*E*)-2,4-pentadienal (pyrrolidine) imine (**2**; Figure 1) and 5-(1-piperidinyl)-2-hydroxy-(*E*,*E*)-2,4-pentadienal (piperidine) imine (**3**; Figure 1) as well. Both *N*-cyanines showed only a sole $^1$H NMR signal set, establishing that the spectrum splitting of **1** is caused by the existence of two diastereomeres as outlined in Figure 1.

For further confirmation of the proposed *N*-cyanines, 5-(1-pyrrolidinyl)-2-hydroxy-(*E*,*E*)-2,4-pentadienal (pyrrolidine) imine was synthesized by a Koenigs cleavage of 3-hydroxypyridine as outlined in Figure 2. *N*-Cyano-3-hydroxypyridinium bromide, obtained by treatment of 3-hydroxypyridine with cyanogen bromide, was reacted with pyrrolidine, resulting in 5-(1-pyrrolidinyl)-2-hydroxy-(*E*,*E*)-2,4-pentadienal (pyrrolidine) imine. The spectroscopical data obtained were identical with those of **2** isolated from the reaction mixture of furan-2-aldehyde and pyrrolidinium acetate.

The formation of *N*-cyanines has been described earlier in the literature from the reaction of furan-2-

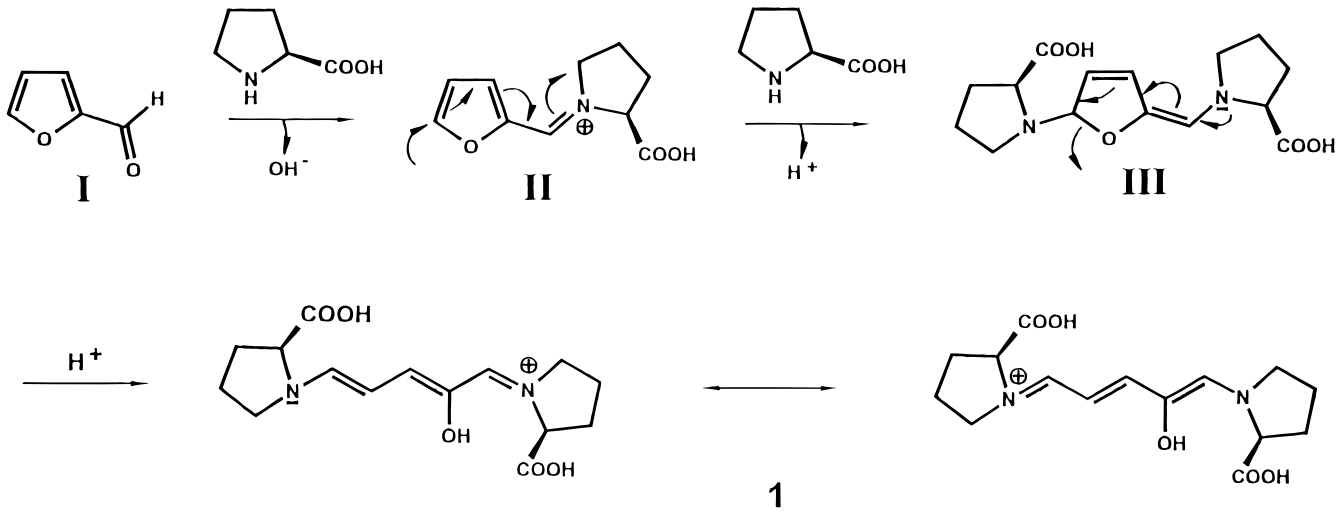



**Figure 3.** Reaction mechanism leading from furan-2-carboxaldehyde and L-proline to 5(*S*)-(2-carboxy-1-pyrrolidinyl)-2-hydroxy-(*E*,*E*)-2,4-pentadienal-(*S*)-(2-carboxypyrrolidine) imine (**1**).

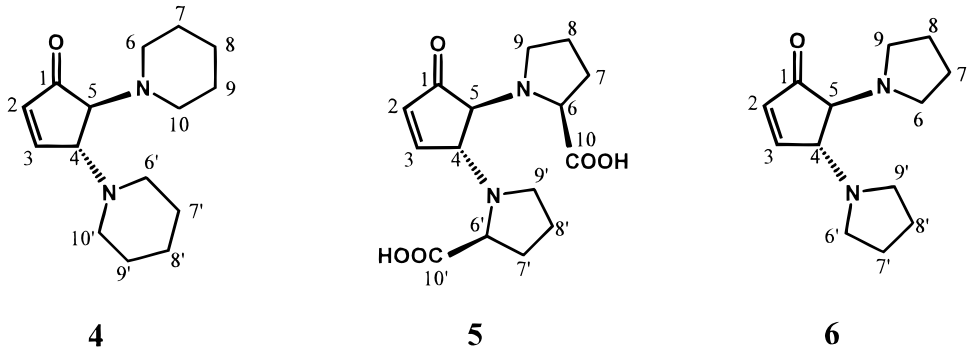



**Figure 4.** Structures of compounds **4**−**6**: (*E*)-4,5-bis(1-piperidinyl)-2-cyclopenten-1-one (**4**); (*E*)-4,5-bis[(*S*)-2-carboxypyrrolidinyl]-2-cyclopenten-1-one (**5**); and (*E*)-4,5-bis(1-pyrrolidinyl)-2-cyclopenten-1-one (**6**).

aldehyde with primary aromatic amines, e.g., aniline (Zinke and Mulhausen, 1905), and is known in organic chemistry as the so-called Stenhouse reaction.

A reaction pathway for the formation of **1** is proposed in Figure 3. Reaction of proline with the aldehyde function of furan-2-aldehyde (**I**) affords a furfurylimmonium ion (**II**). Due to the strong electron acceptor properties at the 4-position of the furan ring, nucleophilic attack of a second molecule of L-proline results in the formation of intermediate **III**, which upon ring opening of the vinylogue cyclic hemiaminal gives rise to 5(*S*)-(2-carboxy-1-pyrrolidinyl)-2-hydroxy-(*E*,*E*)-2,4-pentadienal-(*S*)-(2-carboxypyrrolidine) imine (**1**).

To study the stability of these *N*-cyanines, an aqueous solution of **3** was heated and the reaction mixture analyzed by RP-HPLC. A colorless reaction product was detected showing an absorption maximum at 220 nm. Because this compound was generated in higher amounts when the *N*-cyanine was heated in methanol, it was isolated from a methanolic reaction mixture. The assignment of its structure is based on spectroscopical data. LC/MS-APCI measurements revealed a molecule peak at *m*/*z* 249, indicating a molecular mass of 248 amu and a loss of 84 to *m*/*z* 164, most likely corresponding to the elimination of a piperidine moiety.

The $^1$H NMR data display nine signals including five signals according to two piperidine moieties. The chemical shifts of the two double doublets at 6.16 and 7.58 ppm integrating for one proton each were in the expected range of olefinic hydrogen atoms, whereas the signals at 3.27 and 3.79 ppm were assigned as the α-hydrogen of an alkylamino function. In the $^{13}$C NMR spectrum 11 signals could be distinguished, of which the signal at 211.1 ppm was assigned by DEPT-NMR to a quarternary carbon atom. The links between protons and carbons were determined by DQF−COSY, TOCSY, HMQC, and HMBC experiments. The IR spectra showed an absorption band at 1706 cm$^{-1}$ ($\nu$CO). The relatively intense absorption at 1646 cm$^{-1}$ ($\nu$C=C) characteristic for enamines was lacking; however, weak absorption at 1607 cm$^{-1}$ ($\nu$C=C) was observed. These data confirmed the NMR data indicating that the amino functions are not connected to the double bond but are linked with the 4- and 5-positions of a 2-cyclopenten-1-one ring system. The homonuclear coupling constants $J_{2,3}$ and $J_{2,4}$ of 6.19 and 1.77 Hz are consistent with values for similar 2-cyclopenten-1-ones (Matsumoto et al., 1968), while $J_{3,4}$ and $J_{4,5}$ of 1.77 and 3.10 Hz can be rationalized by assuming a distorted *E* relationship of the two bulky piperidine moieties. The preference of the *E* configuration was also reported for 4,5-(diarylamino)-2-cyclopenten-1-ones formed in heated ethanolic solutions of Stenhouse salts of arylamines by Lewis and Mulquiney (1971). On the basis of these data, the reaction product generated during thermal treatment of the *N*-cyanine **3** was identified as (*E*)-4,5-bis(1-piperidinyl)-2-cyclopenten-1-one (**4** in Figure 4).

The same model reaction was performed in aqueous solution with the L-proline-derived cyanine **1** as well as the pyrrolidine-derived cyanine **2**. On the basis of spectroscopical data the compounds displayed in Figure 4 were identified as the novel Maillard reaction product

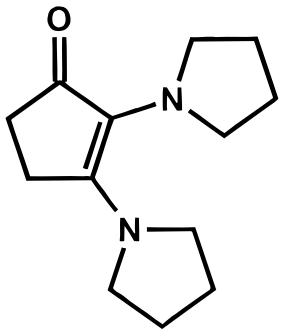


**Figure 5.** 2,3-Bis(1-pyrrolidinyl)-2-cyclopenten-1-one (**7**) isolated from a heated mixture of L-proline and sucrose (Papst et al., 1985).

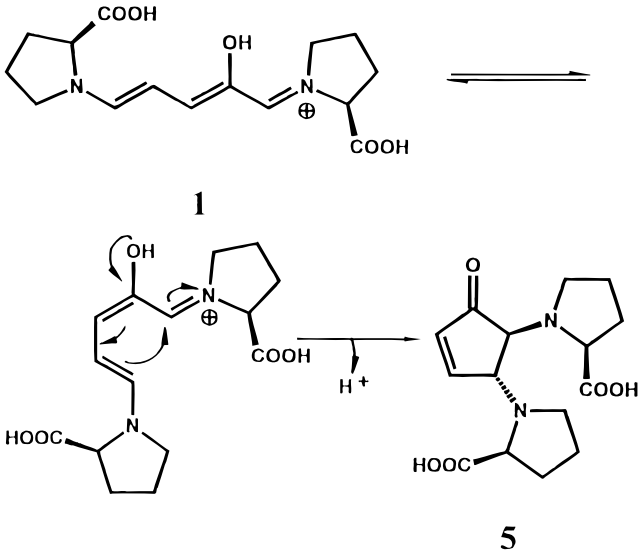
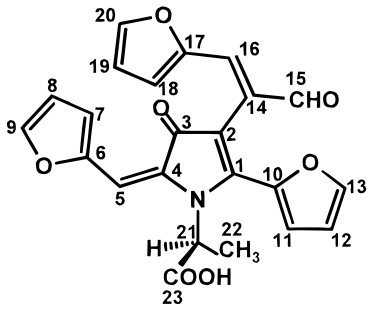


**Figure 6.** Reaction mechanism leading from *N*-cyanine **1** to (*E*)-4,5-bis[(*S*)-2-carboxypyrrolidinyl]-2-cyclopenten-1-one (**5**).

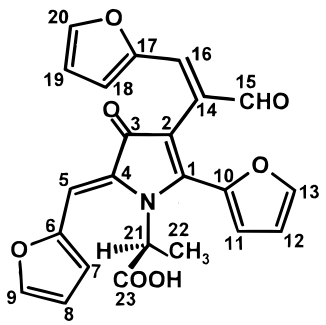
(*E*)-4,5-bis[(*S*)-2-carboxy-1-pyrrolidinyl]-2-cyclopenten-1-one (**5**) and (*E*)-4,5-bis(1-pyrrolidinyl)-2-cyclopenten-1-one (**6**), respectively. It is interesting to note that the isomer 2,3-bis(1-pyrrolidinyl)-2-cyclopenten-1-one (**7**) outlined in Figure 5 has previously been identified as a bitter-tasting component isolated from a dry-heated mixture of L-proline and sucrose (Papst et al., 1985).

A reaction pathway for the formation of (*E*)-4,5-bis-[(*S*)-2-carboxy-1-pyrrolidinyl]-2-cyclopenten-1-one (**5**) is proposed in Figure 6. Upon thermal treatment, (*S*,*S*)-5-(2-carboxy-1-pyrrolidinyl)-2-hydroxy-(*E*,*E*)-2,4-pentadienal (2-carboxypyrrolidine) imine (**1**) might tautomerize, upon thermal treatment, into the *Z*,*E* isomer, which, upon 1,5-cyclization, gives rise to (*E*)-4,5-bis[(*S*)-2-carboxy-1-pyrrolidinyl]-2-cyclopenten-1-one (**5**). It was surprising that, although the formation of diastereomers of the (*E*)-4,5-diamino-2-cyclopenten-1-ones was expected, only one spectrum set was detected by the NMR measurements. This observation indicates a stereospecific ring closure.

**Reaction of Furan-2-carboxaldehyde with L-Alanine and Primary Amines.** When furan-2-carboxaldehyde was reacted with L-alanine, two red reaction products characterized by absorption maxima at 330, 414, and 480 nm were detected by HPLC. For their isolation, the reaction mixture was extracted with ethyl acetate and unreacted furan-2-carboxaldehyde was evaporated at room temperature in high vacuum. The dark red residue was then fractionated by column



**8a**



**8b**

**Figure 7.** Structures of the red compounds **8a** and **8b**: (*S*)-*N*-(1-carboxyethyl)-2(*E*)-(2-furylmethylene)- and (*S*)-*N*-(1-carboxyethyl)-2(*Z*)-(2-furylmethylene)-4(*E*)-(1-formyl-2-furyl-1-ethenyl)-5-(2-furyl)-3(2*H*)-pyrrolinone.

chromatography followed by semipreparative thin-layer chromatography using silica gel. The colorants were further purified by anion exchange chromatography followed by preparative RP-HPLC.

Several one- and two-dimensional NMR techniques and MS, UV, and IR spectroscopy as well as synthetic experiments led to the structures of the two novel, colored Maillard reaction products (*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-6-acetic acid (**8a**) and the corresponding 2-[(*Z*)-(2-furyl)methylidene] isomer (**8b**). The details of the structure determination of **8a** and **8b** appearing in a ratio of 15:1 are published elsewhere (Hofmann, 1997).

The structure of **8a/8b** (Figure 7) makes it possible that, in analogy to the reaction with L-proline, the backbone of the colorant might be formed by ring opening of one molecule of furan-2-carboxaldehyde. In a second step, this intermediate might then condense with three molecules of furan-2-carboxaldehyde, leading to **8a**/**8b**. An unequivocal clarification of the reaction route leading to colored 1*H*-pyrrol-3(2*H*)-ones was very recently performed by application of $^{13}$C-labeling experiments in combination with $^{13}$C NMR spectroscopy (Hofmann, 1998).

We studied, in addition, whether the chromophoric system of the colorants **8a/8b** might be extended by further reactions with other Maillard reaction intermediates. **8a** bears a α,β-unsaturated aldehyde group, which might act as a bridge extending the chromophoric system upon condensation with methylene active compounds formed in the Maillard reaction. To study this in more detail, the α,β-unsaturated (*E*)-β-(2-furyl)-acrylaldehyde was heated with 4-hydroxy-5-methyl-3(2*H*)-furanone, a major pentose dehydration product,

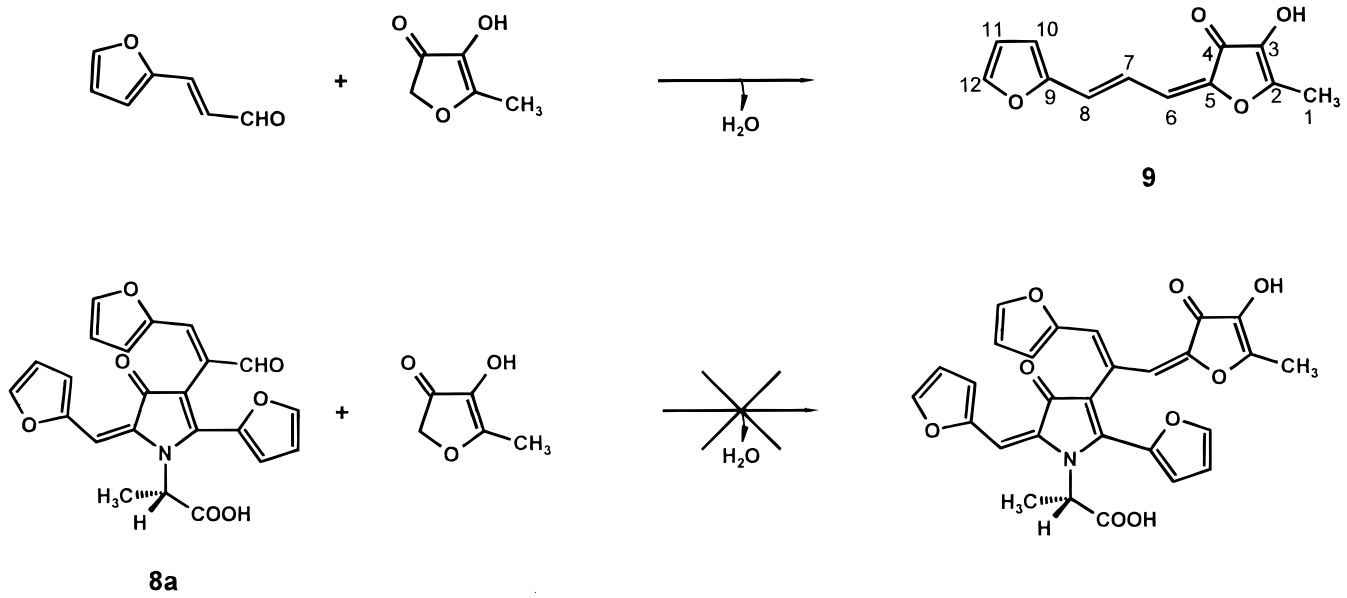



**Figure 8.** Reaction of 4-hydroxy-5-methyl-3(2*H*)-furanone with (*E*)-*β*-(2-furyl)acrylaldehyde and colorant **8a**, respectively.

**Table 3. Assignment of $^1$H NMR Signals (360 MHz, $CDCl_3$) of (*E*,*E*)-2-[3-(2-Furyl)-2-propen-1-ylidene]-4-hydroxy-5-methyl-3(2*H*)-furanone (9)**

| H at relevant C atom[a] | $\delta^b$ (ppm) | $I^b$ | $M^b$ | $J^b$ (Hz) | connectivity[c] with |
|---|---|---|---|---|---|
| H−C(1) | 2.36 | 3 | s | | |
| H−C(11) | 6.48 | 1 | dd | $^3J_{11,10} = 3.5$, $^3J_{11,12} = 1.7$ | H−C(10), H−C(12) |
| H−C(10) | 6.55 | 1 | dd | $^3J_{10,11} = 3.5$ | H−C(11) |
| H−C(6) | 6.64 | 1 | d | $^3J_{6,7} = 11.8$ | H−C(7) |
| H−C(8) | 6.78 | 1 | d | $^3J_{8,7} = 15.3$ | H−C(7) |
| H−C(7) | 7.10 | 1 | dd | $^3J_{7,8} = 15.3$, $^3J_{7,6} = 11.8$ | H−C(8), H−C(6) |
| H−C(12) | 7.49 | 1 | d | $^3J_{12,11} = 1.7$ | H−C(11) |

[a]Arbitrary numbering of carbon atoms refers to **9** in Figure 8. [b]Determined from 1D spectrum. [c]Homonuclear $^1$H,$^1$H connectivities determined by DQF−COSY.

**Table 4. Assignment of $^{13}$C NMR Signals (360 MHz, $CD_3OD$) of (*E*,*E*)-2-[3-(2-Furyl)-2-propen-1-ylidene]-4-hydroxy-5-methyl-3(2*H*)-furanone (9)**

| | | | heteronuclear $^1$H,$^{13}$C multiple-quantum coherence[c] | |
|---|---|---|---|---|
| C atom[a] | $\delta$ (ppm) | DEPT[b] | via $^1J$(C,H) | via $^{2,3}J$(C,H) |
| C(1) | 12.3 | $CH_3$ | H−C(1) | |
| C(11) | 112.4 | CH | H−C(11) | H−C(10), H−C(12) |
| C(10) | 112.9 | CH | H−C(10) | H−C(8), H−C(11), H−C(12) |
| C(6) | 115.1 | CH | H−C(6) | H−C(7), H−C(8) |
| C(7) | 118.4 | CH | H−C(7) | H−C(6), H−C(8) |
| C(8) | 128.2 | CH | H−C(8) | H−C(6), H−C(7), H−C(10) |
| C(2) | 136.5 | C | | H−C(1) |
| C(5) | 144.2 | C | | H−C(6) |
| C(12) | 145.2 | CH | H−C(12) | H−C(10), H−C(11) |
| C(9) | 152.5 | C | | H−C(7), H−C(8), H−C(10), H−C(11) |
| C(3) | 159.3 | C | | H−C(1) |
| C(4) | 181.2 | C | | H−C(6) |

[a]Arbitrary numbering of carbon atoms refers to **9** in Figure 8. [b]DEPT-135 spectroscopy. [c]Assignments based on HMQC ($^1J$) and HMBC ($^{2,3}J$) experiments.

in aqueous solution at pH 7.0. The color of the reaction mixture rapidly turned deep orange. An intensely orange reaction product was characterized by DAD, exhibiting an absorption maximum at 391 nm. After extraction of the reaction mixture with ethyl acetate and crystallization of the colorant, its chemical structure was determined by several one- and two-dimensional NMR techniques and, in addition, by MS and UV spectroscopy. The singlet at 2.36 ppm in the $^1$H NMR spectrum and the signal pattern in the $^1$H and $^{13}$C NMR spectra obtained for the furan ring indicated that a condensation reaction has occurred between the aldehyde function of (*E*)-*β*-(2-furyl)acrylaldehyde and the methylene active group of the 3(2*H*)-furanone. The DQF−COSY experiment and, in addition, the coupling constants of 11.8 and 15.3 Hz between hydrogens resonating in the olefinic region of the $^1$H NMR spectrum demonstrate the existence of an *E*,*E*-configured diene system. In summary, the NMR data given in Tables 3 and 4 were consistent with structure **9** identified as the previously unknown (*E*,*E*)-2-[3-(2-furyl)-2-propen-1-ylidene]-4-hydroxy-5-methyl-3(2*H*)-furanone (**9**, Figure 8).

To study whether also the aldehyde group in the (*E*)-*β*-(2-furyl)acrylaldehyde moiety of **8a** might extend the chromophoric system by condensation with methylene active compounds, **8a** was heated in aqueous solution with 4-hydroxy-5-methyl-3(2*H*)-furanone. Monitoring the product spectrum by TLC and HPLC revealed no condensation product was formed. This fits well the steric hindered environment at the aldehyde function in **8a**, which was recently established by performing conformation analysis using 2D-NOESY and 2D-ROESY NMR spectroscopy (Hofmann, 1997).

These results imply that the prolongation of a mesomeric system by condensation reactions with methylene active compounds might generally be counteracted by the steric hindrance of bulky substituents. The existence of huge chromophoric systems should, therefore, be very unlikely in the Maillard reaction. Investigations to confirm this hypotheses by additional experiments are now in progress.

## CONCLUSIONS

The identification of colored *N*-cyanines and 1*H*-pyrrol-3(2*H*)-ones formed by reacting the food volatile furan-2-carboxaldehyde with primary and secondary amino acids, respectively, provides 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.

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