4-Alkylidene-2-imino-5-[4-alkylidene-5-oxo-1,3-imidazol-2-inyl]azamethylidene-1,3-imidazolidine—A Novel Colored Substructure in Melanoidins Formed by Maillard Reactions of Bound Arginine with Glyoxal and Furan-2-carboxaldehyde

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When N^{α}-acetyl-L-arginine was heated with glyoxal in aqueous solution at pH 7.0 in the presence of furan-2-carboxaldehyde, an intense red-brown color developed. The compound mainly evoking this color was identified as (*S*,*S*)-1-[4-(acetylamino)-4-carboxy-1-butyl]-2-imino-4-[(*Z*)-(2-furyl)-methylidene]-5-{2-[1-[4-(acetylamino)-4-carboxy-1-butyl]-4-[(*E*)-(2-furyl)methylidene]-5-oxo-1,3-imidazol-2-inyl]}azamethylidene-1,3-imidazolidine (**1**, BISARG) by application of several one- and two-dimensional NMR experiments and, in addition, by LC/MS^n measurements and UV−vis spectroscopy. This is the first time that a chromophoric compound comprising four linked rings with two arginine moieties incorporated was identified in a Maillard reaction system. This novel type of chromophore indicates the possibility that food melanoidins might be generated by protein oligomerization via colored cross-linking structures, for example, between two arginine residues.

Keywords: *Arginine; furan-2-carboxaldehyde; glyoxal; Maillard reaction; colored compounds; cross-link amino acid; melanoidins*

INTRODUCTION

The development of the typical brown color during the thermal treatment of foods, such as coffee, roasted beef, or bread crust, mainly originates from interactions between reducing carbohydrates and amino compounds, known as the Maillard reaction. In addition to low molecular weight colored compounds, especially high molecular weight browning products with molecular weights up to 100000 Da, the so-called melanoidins, are proposed to be formed during food processing. However, due to the complexity and multiplicity of the nonvolatile Maillard reaction products (Nursten and O'Reilly, 1986; Ames, 1992; Ledl and Schleicher, 1992), it is a helpful approach to characterize such compounds in suitable model systems containing Maillard reaction intermediates, which are undoubtedly involved in color formation.

Despite extensive studies in the past 20 years, the chemical nature of melanoidins is as yet not clear. One theory is that high molecular weight colored structures are formed by polymerization of low molecular weight Maillard reaction intermediates such as pyrrole derivatives (Tressl et al., 1998a,b). On the other hand, it was recently found that heating an aqueous solution of glucose in the presence of amino acids produces colored compounds exhibiting exclusively molecular weights <3000 Da (Hofmann, 1998a). The fact that polymers with molecular weights >3000 Da were not detected indicates that the formation of melanoidins by polymerization of low molecular weight intermediates is not very likely to occur in a food system (Hofmann, 1998a).

Because Aurich et al. (1967) were able to identify several amino acids in a hydrolysate of coffee melanoidins, we assumed that melanoidins might be generated by a cross-linking reaction between low molecular weight colored Maillard reaction products and high molecular weight noncolored proteins. This hypothesis was recently confirmed by an experiment in which an aqueous solution of glucose was heated in the presence of β-casein (Hofmann, 1997a, 1998b). Fractionation of the brown solution revealed that the predominant part of the colored compounds consists of colored casein pentamers or even higher oligomers exhibiting molecular weights >100000 Da. These colored protein-oligomerization products showed characteristics typical for food melanoidins (Hofmann, 1998a).

The carbohydrate degradation product furan-2-carboxaldehyde has been identified in various thermally treated foods, for example, wheat and rye bread crusts (Schieberle and Grosch, 1987) as well as roasted coffee, barley, and chicory (Kanjahn et al., 1996). We therefore studied the formation of melanoidins from the reaction of furan-2-carboxaldehyde with casein (Hofmann, 1997a, 1998b). Brown-orange melanoproteins showing characteristics typical for food melanoidins were successfully characterized as chromophores linked to the protein skeleton via the ϵ-amino group of a lysine residue (Hofmann, 1997a, 1998b). These findings for the first time verified the proposal that in food melanoidins low molecular weight chromophores are covalently bound to a noncolored biopolymer. Further investigations (Hofmann, 1998b) showed that N^{α}-protected lysine can be used as a suitable model compound to monitor reactions between lysine residues of proteins and carbohydrate degradation products, for example, furan-2-carboxaldehyde. Such model studies, carried out under conditions typical for food processing, are very helpful to gain insight into the formation of food melanoidins.

Studies by Okitani et al. (1984) had revealed that incubation of lysozyme with glucose resulted in polym-

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Table 1. Assignment of ^{1}H NMR Signals (360 MHz, DMSO-d_6) of (*S,S*)-1-[4-(Acetylamino)-4-carboxy-1-butyl]-2-imino-4-[(*Z*)-(2-furyl)methylidene]-5-{2-[1-[4-(acetylamino)-4-carboxy-1-butyl]-4-[(*E*)-(2-furyl)methylidene]-5-oxo-1,3-imidazol-2-inyl]}azamethylidene-1,3-imidazolidine (1)

H at relevant C atom[a]	δ[b]	I[c]	M[c]	J[c] (Hz)	connectivity[d] with
H−C(3′)	1.60−1.80	2	m		H−C(2′), H−C(4′)
H−C(3)	1.60−1.80	2	m		H−C(2), H−C(4)
H−C(4′)	1.60−1.80	2	m		H−C(3′), H−C(5′)
H−C(4)	1.60−1.80	2	m		H−C(3), H−C(5)
H−C(15′)	1.81	3	s		
H−C(15)	1.85	3	s		
H−C(5′)	3.78−3.82	2	m		H−C(4′)
H−C(5)	3.78−3.82	2	m		H−C(4)
H−C(2′)	4.19	2	m		H−C(3′), HN−C(2′)
H−C(2)	4.36	1	m		H−C(3), HN−C(2)
H−C(12)	6.83	1	dd	3.5, 1.8	H−C(11), H−C(13)
H−C(12′)	6.83	1	dd	3.5, 1.8	H−C(11), H−C(13)
H−C(9)	7.07	1	s		
H−C(11)	7.16	1	d	3.5	H−C(12)
H−C(11′)	7.26	1	d	3.5	H−C(12′)
H−C(9′)	7.32	1	s		
H−C(13)	7.92	1	d	1.8	H−C(12)
H−C(13′)	8.01	1	d	1.8	H−C(12′)
HN−C(2′)	8.16	1	d	7.96	H−C(2′)
HN−C(2)	8.19	1	d	7.96	H−C(2)
HOOC−C(1/1′)	9.25	bs			
H_2N−C(6)	10.01	2	bs		
HN−C(6)	11.52	1	bs		

[a] Numbering of carbon atoms refers to formula **1** in Figure 1. [b] The ^{1}H chemical shifts are given in relation to DMSO-d_6. [c] Determined from 1D spectrum. [d] Observed homonuclear ^{1}H,^{1}H connectivities by DQF−COSY.

erization of the protein as well as browning of the reaction mixture. Comparing the amino acid composition of the protein before and after the reaction with glucose revealed an impairment of the amino acid residues, among which arginine, lysine, and tryptophan showed the most significant losses.

The following investigation was, therefore, undertaken to study the role of bound arginine residues in color formation with carbohydrate degradation products.

EXPERIMENTAL PROCEDURES

Chemicals. The following compounds were obtained commercially: N^{α}-acetyl-L-arginine, glyoxal (30% solution in water), furan-2-carboxaldehyde, formic acid, and trifluoracetic acid (TFA) were from Aldrich (Steinheim, Germany). Furan-2-carboxaldehyde was freshly distilled prior to use. Solvents were of HPLC grade (Aldrich, Steinheim, Germany). DMSO-d_6 was obtained from Isocom (Landshut, Germany).

Isolation of (*S,S*)-1-[4-(Acetylamino)-4-carboxy-1-butyl]-2-imino-4-[(*Z*)-(2-furyl) methylidene]-5-{2-[1-[4-(acetylamino)-4-carboxy-1-butyl)-4-[(*E*)-(2-furyl)methylidene]-5-oxo-1,3-imidazol-2-inyl]}azamethylidene-1,3-imidazolidine (1, BISARG) from a Heated Aqueous Solution of N^{α}-Acetyl-L-arginine in the Presence of Glyoxal and Furan-2-carboxaldehyde. A solution of N^{α}-acetyl-L-arginine (50 mmol) and glyoxal (50 mmol) in phosphate buffer (50 mL, 0.5 mol/L, pH 7.0) was heated for 90 min at 80 °C. Furan-2-carboxaldehyde (50 mmol) was then added and heating continued for an additional 60 min. After cooling to room temperature, the mixture was extracted with ethyl acetate (4 × 10 mL), the organic layer was discarded, and the aqueous phase was freeze-dried. The residue was taken up in a mixture (5 mL, 60:40, v/v) of methanol and aqueous TFA (0.1% TFA in water) and was then fractionated by flash chromatography using RP-18 material (20.0 g; Lichroprep 25−40 μm, Merck, Darmstadt, Germany) as the stationary phase. The material was applied onto the column (16 × 200 mm), which was conditioned with a mixture (5 mL, 60:40, v/v) of methanol and aqueous TFA (0.1% TFA in water). A deep red fraction was collected, freed from methanol in vacuo, and freeze-dried. The residue was, then, rechromatographed by semipreparative HPLC on RP-18 material. Starting with a 10:90 (v/v) mixture of acetonitrile and aqueous TFA (0.1% TFA in water), the acetonitrile content was increased to 100% within 40 min. A red peak, which was eluted between 20.5 and 22.0 min, was collected, and the solvent was removed in vacuo. Freeze-drying of the collected red fraction affords the colorant (∼162 mg; yield = 1% of the theory) as deep red needles (purity = 98%): UV−vis (water) λ_{max1} = 314 nm ($\epsilon = 0.8 \times 10^4$ L mol^{-1} cm^{-1}), λ_{max2} = 472 nm ($\epsilon = 1.6 \times 10^4$ L mol^{-1} cm^{-1}); LC/MS^n spectra are displayed in Figure 2; ^{1}H and ^{13}C NMR data are summarized in Tables 1 and 2.

Table 2. Assignment of ^{13}C NMR Signals (360 MHz, DMSO-d_6) of (*S,S*)-1-[4-(Acetylamino)-4-carboxy-1-butyl]-2-imino-4-[(*Z*)-(2-furyl)methylidene]-5-{2-[1-[4-(acetylamino)-4-carboxy-1-butyl]-4-[(*E*)-(2-furyl)methylidene]-5-oxo-1,3-imidazol-2-inyl]}azamethylidene-1,3-imidazolidine (1)

H at relevant C atom[a]	δ[b]	DEPT[c]	heteronuclear ^{1}H, ^{13}C coherence[d] via ^1J(C,H)	via $^{2,3,4}J$(C,H)
C(15′)	22.2	CH_3	CH_3(15′)	H−C(14′)
C(15)	22.3	CH_3	CH_3(15)	H−C(14)
C(4′)	24.0	CH_2	H−C(4′)	H−C(2′), H−C(3′), H−C(5′)
C(4)	25.5	CH_2	H−C(4)	H−C(2), H−C(3), H−C(5)
C(3′)	27.9	CH_2	H−C(3′)	H−C(2′), H−C(4′), H−C(5′)
C(3)	28.1	CH_2	H−C(3)	H−C(2), H−C(4), H−C(5)
C(5′)	42.6	CH_2	H−C(5′)	H−C(3′), H−C(4′)
C(5)	45.6	CH_2	H−C(5)	H−C(3), H−C(4)
C(2′)	51.0	CH	H−C(2′)	HN−C(2′), H−C(3′), H−C(4′)
C(2)	51.4	CH	H−C(2)	HN−C(2), H−C(3), H−C(4)
C(9)	104.0	CH	H−C(9)	H−C(11)
C(9′)	104.9	CH	H−C(9′)	H−C(11′)
C(12)	113.8	CH	H−C(12)	H−C(11), H−C(13)
C(12′)	114.1	CH	H−C(12′)	H−C(11′), H−C(13′)
C(11)	118.7	CH	H−C(11)	H−C(12), H−C(13)
C(11′)	118.9	CH	H−C(11′)	H−C(12′), H−C(13′)
C(7)	122.2	C		H−C(9)
C(7′)	130.8	C		H−C(9′)
C(13)	147.0	CH	H−C(13)	H−C(11), H−C(12)
C(13′)	147.4	CH	H−C(13′)	H−C(11′), H−C(12′)
C(10)	149.2	C		H−C(12), H−C(13)
C(10′)	149.6	C		H−C(12′), H−C(13′)
C(6′)	154.8	C		
C(6)	158.5	C		H−C(5)
C(8)	162.2	C		H−C(5), H−C(9)
C(14′)	169.3	C		H−C(15′), HN−C(2′)
C(14)	169.4	C		H−C(15), HN−C(2)
C(1′)	173.2	C		H−C(2′)
C(1)	173.2	C		H−C(2)
C(8′)	173.5	C		H−C(5′), H−C(9′)

[a] Arbitrary numbering of carbon atoms refers to formula **1** in Figure 1. [b] The ^{13}C chemical shifts are given in relation to DMSO-d_6. [c] DEPT-135 spectroscopy. [d] Assignments based on HMQC (^1J) and HMBC ($^{2,3}J$) experiments.

High-Performance Liquid Chromatography (HPLC). The HPLC apparatus (Kontron, Eching, Germany) consisted of two type 422 pumps , an M800 gradient mixer, a Rheodyne injector (100 μL loop), and a type 440 diode array detector (DAD) monitoring the effluent in a wavelength range between 220 and 600 nm. Separations were performed on a stainless steel column packed with RP-18 (ODS-Hypersil, 5 μm, Shandon, Frankfurt, Germany) in a semipreparative 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 aqueous formic acid (0.1% formic acid in water); the acetonitrile content was increased to 100% within 15 min. For LC/MS^n measurements, the sample (10 μg/mL) was dissolved in methanol and was then

applied to the MS by using the loop injection technique (injection speed = 10 μL/min). For each further fragmentation step, one ion was separated by means of an ion trap, and, after exitation, fragmentation was induced by collision with molecules of reactant gas (He).

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 by Hofmann (1997b). Tetramethylsilane (TMS) was used as the internal standard.

RESULTS AND DISCUSSION

Recent studies (Hofmann, 1997a, 1998b) revealed that the reaction of lysine residues of proteins with furan-2-carboxaldehyde led to melanoidin formation. We therefore studied in the present investigation whether, in addition to the reaction with lysine residues, furan-2-carboxaldehyde also reacts with arginine moieties upon color formation. Thermal treatment of an aqueous solution of N^{α}-acetyl-L-arginine and furan-2-carboxaldehyde did, however, not result in a significant browning of the reaction mixture.

We were, therefore, interested in whether arginine residues might generally be not involved in melanoidin formation or whether arginine moieties lead to colored compounds only in the presence of certain other carbohydrate degradation products. It might be possible that only after "activation" of the guanidyl function of arginine by the reaction with a dicarbonyl compound, furan-2-carboxaldehyde might react to colored compounds. A common dicarbonyl compound is glyoxal. This compound was detected as a degradation product of carbohydrates (Nedvidek et al., 1992) and is reported as a product of lipid autoxidation in edible oil (Hirayama et al., 1984).

To study this hypotheses in more detail, we heated an aqueous solution of N^{α}-acetyl-L-arginine and glyoxal in the presence of furan-2-carboxaldehyde. In comparison to the mixture without glyoxal, an orange-brown colorization of the reaction mixture occurred very rapidly. After separation of the reaction products by RP-HPLC, the colorants generated were registered using either a DAD monitoring the wavelength range between 220 and 600 nm or an LC/MS. A red compound was detected exhibiting absorption maxima at 314 and 472 nm.

After extraction with solvents, the residual aqueous layer was freeze-dried and the intense red main colored reaction product was isolated by flash chromatography using RP-18 material as the stationary phase. After final purification by RP-HPLC, the determination of its chemical structure was performed by several one- and two-dimensional NMR techniques and, in addition, by LC/MS^n and UV−vis spectroscopy.

The spectroscopic data were consistent with structure **1** displayed in Figure 1. For spectral measurements the compound was isolated in the protonated form. LC/MS measurements (Figure 2A) gave an intense molecular ion at *m*/*z* 651 (100%), confirming the proposed ionic structure **1** with an even number of nitrogen atoms (Figure 1). Selection of this molecular ion, excitation, and fragmentation revealed the pattern of the daughter ions displayed in Figure 2B (LC/MS^2). The loss of 18

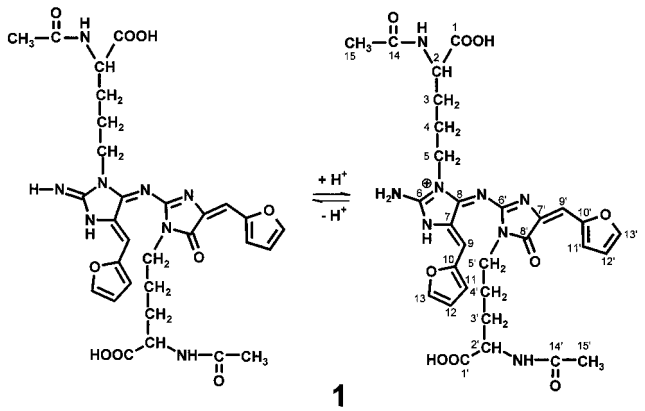

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Figure 1. Structures of the deprotonated and protonated forms of the red (*S*,*S*)-1-[4-(acetylamino)-4-carboxy-1-butyl]-2-imino-4-[(*Z*)-(2-furyl)methylidene]-5-{2-[1-[4-(acetylamino)-4-carboxy-1-butyl]-4-[(*E*)-(2-furyl)methylidene]-5-oxo-1,3-imidazol-2-inyl]}azamethylidene-1,3-imidazolidine (**1**, BISARG).

to *m*/*z* 633 and, in addition, the loss of 44 to *m*/*z* 607 correspond most likely to the elimination of H_2O and CO_2, respectively, corroborating the proposed structure of the carboxylic acid. The loss of 42 to *m*/*z* 609 demonstrates the cleavage of a molecule of ketene most likely originating from the *N*-acetyl groups. As confirmed by LC/MS^3 (Figure 2C), the ion with *m*/*z* 546 results from its mother ion *m*/*z* 633 by the loss of CO, followed by a McLafferty-type elimination of acetamide. Further fragmentation of the base ion *m*/*z* 609 outlined in Figure 2D revealed the ion *m*/*z* 494 most likely corresponding to the cleavage of an 2-aminopentenoic acid moiety and, in addition, the ion *m*/*z* 334 reflecting the heterocyclic core after cleavage of both arginine residues from the proposed structure **1**.

The ^{1}H NMR spectrum of compound **1** showed 23 resonance signals (Table 1). Double-quantum-filtered δ,δ correlation spectroscopy (DQF−COSY) as well as total correlated spectroscopy (TOCSY) revealed several strongly coupled ^{1}H spin systems. H,H shift correlations between the hydrogens HN−C(2)/H−C(2), H−C(2)/H−C(3), H−C(3)/H−C(4), and H−C(4)/H−C(5) as well as HN−C(2′)/H−C(2′), H−C(2′)/H−C(3′), H−C(3′)/H−C(4′), and H−C(4′)/H−C(5′) and, in addition, the two singlets at 1.81 and 1.85 ppm, each corresponding to an acetyl group, confirmed the presence of two N^{α}-acetylarginine moieties in compound **1**. The homonuclear correlations with J = 3.5 and 1.8 Hz observed for the ^{1}H spin systems H−C(11)/H−C(12)/H−C(13) and H−C(11′)/H−C(12′)/H−C(13′) corresponded to the pattern expected for furan rings substituted in the 2-position. Two furan rings are, therefore, proposed to be incorporated in structure **1**. In addition, two singlets were recorded at 7.07 and 7.32 ppm resonating in the chemical shift range expected for olefinic hydrogens. The proton of the carboxylic acids and the protons connected with two nitrogen atoms, one incorporated in the heterocyclic core and one as an exocyclic amino function, were deduced from the broad signals at 9.25, 10.01, and 11.52 ppm.

The ^{13}C NMR spectrum of compound **1** showed 30 signals among which 12 quarternary carbon atoms were elucidated by comparing the ^{13}C NMR data with the results of the DEPT-135 experiment (Table 2). Unequivocal assignment of the quarternary carbon atoms could then be successfully achieved by means of heteronuclear multiple-bond/multiquantum coherence experiments optimized for ^2J(C,H) and ^3J(C,H) coupling

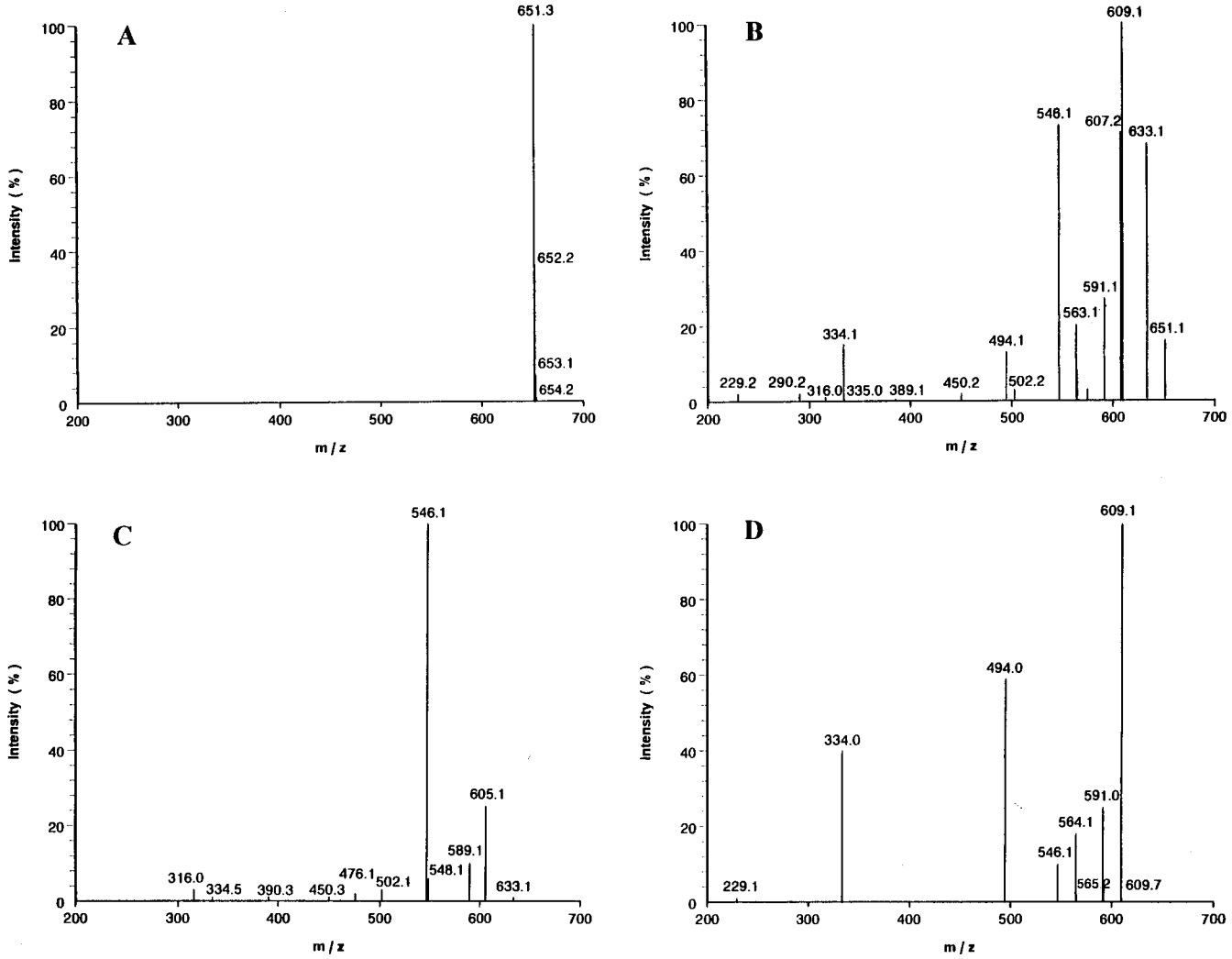

Figure 2. (A) LC/MS of the isolated red compound **1**; (B) fragmentation of the isolated ion *m/z* 651 (LC/MS^2); (C) fragmentation of the isolated ion *m/z* 633 (LC/MS^3); (D) fragmentation of the isolated ion *m/z* 609 (LC/MS^3).

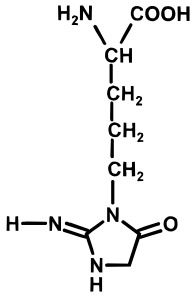

Figure 3. Structure of 1-(4-amino-4-carboxybutyl)-2-imino-5-oxoimidazolidine formed from arginine and glyoxal (Schwarzenbolz et al., 1997).

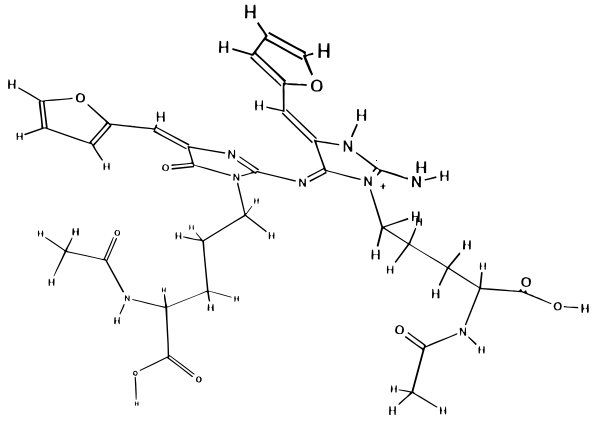

Figure 4. Conformation of **1** evidenced by molecular mechanics calculations.

constants (Table 2). The carbon atoms of the furan rings C(11) and C(11′) resonated at 118.7 and 118.9 ppm. Heteronuclear correlations of C(11) and C(11′) with the olefinic protons H−C(9) and H−C(9′), respectively, were consistent with the (2-furyl)methylidene substructures proposed for compound **1**. The olefinic proton H−C(9) showed further coupling with two quarternary carbon atoms resonating at 122.2 ppm [C(7)] and 162.5 ppm [C(8)]. In analogy, a correlation between H−C(9′) and the signal at 130.8 ppm [C(7′)] and 173.5 ppm [C(8′)] was found. In addition, the signals at 162.5 and 173.5 ppm showed a heteronuclear coupling with H−C(5) and H−C(5′) of the arginine moieties, respectively. Furthermore, the arginine protons H−C(5) and H−C(5′) showed a cross-correlation with the signals at 158.5 and 154.8 ppm, respectively, and were, therefore, assigned as the quarternary carbon atoms C(6) and C(6′). These data confirm that the guanidino functions of both arginine moieties are incorporated in two imidazolidine ring systems proposed for **1**. These findings are well in line with data reported very recently by Schwarzenbolz et al. (1997), who identified 1-(4-amino-4-carboxybutyl)-2-imino-5-oxoimidazoline as the major reaction

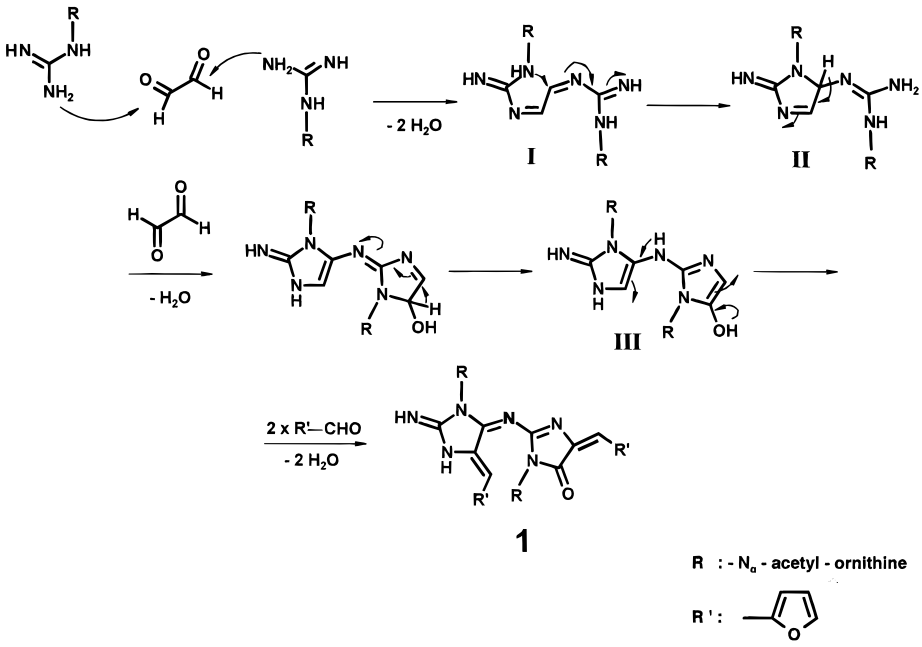

Figure 5. Proposed reaction sequence leading to the chromophoric cross-link amino acid **1** from two arginine residues and glyoxal in the presence of furan-2-carboxaldehyde.

product from the reaction of one arginine residue with glyoxal (Figure 3). The proposed structure **1** consisted of a 2-amino-5-imino-1-imidazoline ring [N−C(5)/C(6)/N−C(6)/C(7)/C(8)] connected to a 2-amino-5-oxo-2-imidazoline ring [N−C(5′)/C(6′)/N−C(6′)/C(7′)/C(8′)] via an azamethylidene bridge. In comparison to the chemical shift of C(8′), the signal of C(8) is highfield shifted. The shielding of this amidine carbon atom is due to the less electron-withdrawing effect of the nitrogen atom in comparison to the more electronegative oxygen in the carboxylic amide structure. The (*E*) configuration of the double bonds C(7)═C(9) and C(7′)═C(9′) was shown by molecular dynamics calculations using an MM3 force field. The energy-minimized structure of **1** is displayed in Figure 4.

In summary, the spectroscopic data obtained are consistent with the proposed structure of **1** as (*S*,*S*)-1-[4-(acetylamino)-4-carboxy-1-butyl]-2-imino-4-[(*Z*)-(2-furyl)methylidene]-5-{2-[1-[4-(acetylamino)-4-carboxy-1-butyl]-4-[(*E*)-(2-furyl)methylidene]-5-oxo-1,3-imidazol-2-inyl]}azamethylidene-1,3-imidazolidine (Figure 1). To our knowledge, this intensely red compound **1**, showing a high extinction coefficient of 1.6×10^4 L mol^{-1} cm^{-1} at 472 nm, has previously not been reported in the literature.

A reaction pathway for the formation of BISARG is proposed in Figure 5. Two arginine residues react with glyoxal to form a glyoxal diimine (**I**), which, upon cyclization, gives rise to a 5-guanidyl-2-imino-3-imidazoline (**II**). Reaction of the guanidyl group with an additional molecule of glyoxal followed by a vinylogous imine−enamine tautomerism leads to the methylene-active intermediate **III**, which, upon condensation with two molecules of furan-2-carboxaldehyde, finally gives rise to BISARG (**1**).

After condensation of the guanidyl group of two arginine moieties of proteins with two molecules of glyoxal, both of the methylene-active functions of the intermediate **III** (Figure 5) might also condense with other carbohydrate-derived carbonyl compounds, for example, acetaldehyde, glycerinaldehyde, 5-(hydroxymethyl)furan-2-carboxaldehyde, or 1- or 3-deoxyosones, indicating that a wide range of 4-alkylidene-2-imino-5-[4-alkylidene-5-oxo-1,3-imidazol-2-inyl]azamethylidene-1,3-imidazolidine chromophores can be assumed to be formed via the proposed reaction pathway.

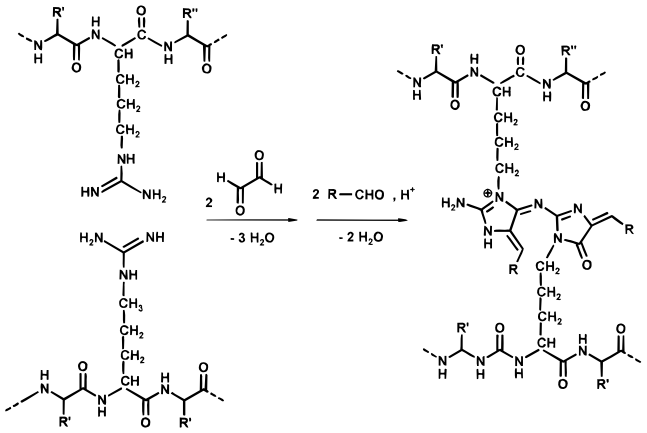

Figure 6. Formation of melanoidins by a protein-cross-linking and chromophore-generating reaction of two arginine side chains of proteins with glyoxal in the presence of a carbonyl compound.

GENERAL CONSIDERATIONS

The identification of **1** indicates that this type of compound stands for a new type of cross-link amino acid yielding cross-links as well as color. As shown in Figure 6, this type of chromophore monitors, how food melanoidins might be formed by protein oligomerization via colored cross-linking structures, for example, between two arginine residues. Owing to the ubiquitous occurrence of proteins and to the high contents of furan-2-carboxaldehyde and glyoxal in several processed foods, for example, wheat and rye bread crusts (Schieberle and Grosch, 1987) as well as roasted coffee, barley, and chicory (Kanjahn et al., 1996), this kind of chromophoric protein cross-link might also be involved in the forma-

tion of, for example, high molecular weight coffee melanoidins.

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