Influence of L-Cysteine on the Formation of Bitter-Tasting Aminohexose Reductones from Glucose and L-Proline: Identification of a Novel Furo[2,3-*b*]thiazine

Thomas Hofmann†

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

Thermal treatment of a 1 + 1 mixture of glucose and L-proline led to the development of an intense bitter taste being reflected in high amounts of the bitter-tasting bispyrrolidino- (**1**) and pyrrolidinohexose reductones (**2**) formed. Heating the reaction mixture in the presence of L-cysteine drastically reduced the amounts of these aminohexose reductones and, thereby, the intensity of the bitter taste. Studies on the mechanism of the cysteine-induced reduction of the bitter taste revealed that the precursor of the aminohexose reductones, the hexose-derived acetylformoin (**3**), reacted more easily with L-cysteine to form the 7-hydroxy-4a,6-dimethyl-2*H*,3*H*,4a*H*-furo[2,3-*b*]thiazine (**4**), a previousely unknown Maillard reaction product, than with L-proline to the aminohexose reductones **1** and **2**, thereby blocking the formation of bitter-tasting compounds.

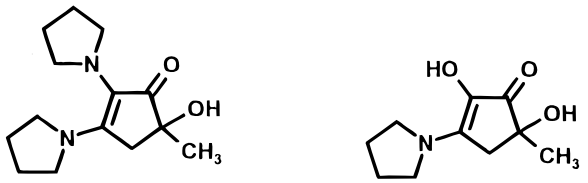
Keywords: *Acetylformoin; pyrrolidinohexose reductone; bispyrrolidinohexose reductone; 7-hydroxy-4a,6-dimethyl-2H,3H,4aH-furo[2,3-b]thiazine; Maillard reaction; bitter taste; taste dilution analysis*

INTRODUCTION

The Maillard reaction between reducing carbohydrates and amino acids is chiefly responsible for the development of aromas and colors occurring during the thermal processing of foods. Besides these desirable attributes of Maillard reaction products, bitter-tasting compounds are formed from carbohydrates and amino acids, among which L-proline was found to be most effective, especially when heated at elevated temperatures (Chen, 1979; Papst et al., 1984, 1985). Such bitter taste can be found in, for example, bread crust, roasted coffee, and grilled meat, or in beer wort when processed at too high temperatures. Also, the manufacturers of reaction flavors, prepared by heating carbohydrate/amino acid mixtures under controlled conditions, have to struggle with bitter-tasting compounds, because not only the odorous volatile fraction but also the total reaction mixture including the nonvolatile bitter-tasting compounds are applied to convenience foods and snack products. Because the consumer does not accept such a bitter taste in these products, it is an important task to inhibit the formation of bitter-tasting compounds from carbohydrates and amino acids.

Investigating the structure of bitter-tasting compounds in Maillard mixtures, Papst et al. (1984) isolated the bispyrrolidinylhexose reductone (**1** in Figure 1) in a dry-heated 1 + 3 mixture of saccharose and L-proline. Very recently, Hofmann (1998) successfully identified the bispyrrolidinylhexose reductone (**1** in Figure 1) and, in addition, the corresponding pyrrolidinylhexose reductone (**2** in Figure 1) in a heated 1 + 1 mixture of L-proline and glucose.

However, as yet no studies have been performed aimed at reducing the undesired bitter taste of such carbohydrate/amino acid mixtures. Chen (1979) reported that heated carbohydrate/L-cysteine mixtures exhibit only weak bitter taste. It might be, therefore, a promising approach to study whether addition of L-cysteine also reduces the bitter taste of carbohydrate/L-proline mixtures, for example, by reacting with certain bitter compound precursors.



1 **2**

Figure 1. Structures of the bitter-tasting bispyrrolidinohexose reductone (**1**) and pyrrolidinohexose reductone (**2**) identified in heated carbohydrate/L-proline mixtures.

The purpose of the present paper was, therefore, to study the influence of L-cysteine on the bitter taste of glucose/L-proline mixtures and to investigate its influence on the formation of the bitter-tasting aminohexose reductones **1** and **2**.

EXPERIMENTAL PROCEDURES

Chemicals. The following compounds were obtained commercially: D-glucose, L-proline, L-cysteine, dimethylformamide, and methanol (Aldrich, Steinheim, Germany); cysteamine (Fluka, Deisenhofen, Germany). Deuterated solvents were obtained from Isocom (Landshut, Germany).

Bispyrrolidinohexose reductone (**1**), pyrrolidinohexose reductone (**2**), and acetylformoin (**3**) were synthesized following procedures recently described by Hofmann (1998).

Syntheses. *N-(1-Deoxy-*D*-fructos-1-yl)proline.* A mixture of powdered anhydrous D-glucose (300 mmol) and L-proline (400 mmol) was refluxed in anhydrous methanol (400 mL) with stirring. After 40 min of heating, malonic acid (90 mmol) was added and the solution was refluxed for an additional 180 min. The mixture was cooled to room temperature and concentrated

† Telephone +49-89-289 14170; fax +49−89−289 14183; e-mail thomas.hofmann@lrz.tum.de

10.1021/jf9903152 CCC: $18.00
Published on Web 10/09/1999

to ~100 mL under vacuum and then stored at −20 °C. Dropwise addition of acetone yielded the Amadori rearrangement product as a white hydroscopic solid. Concentration of the mother liquor, recooling, and addition of acetone yielded additional raw product. Both crops were combined. Recrystallization from a mimimum amount of methanol and acetone yielded *N*-(1-deoxy-D-fructos-1-yl)-L-proline as a white powder with a purity of ~95% (100 mmol; yield = 30%): LC/MS 222 (100, $[M + 1]^+$), 204 (25, $[M + 1 - H_2O]^+$); ^{1}H NMR (360 MHz in D_2O; DQF-COSY) δ 1.90−2.15 (m, 4H, 2 × $-CH_2-$), 2.30−2.50 (m, 2H, $-CH_2-$), 3.22 (m, 1H, ^2J = 13.0 Hz, $-CH_aH_bN$), 3.30 (m, 1H, ^2J = 13.0 Hz, $-CH_aH_bN$), 3.32−3.35 [m, 1H, $-CH$(COOH)N], 3.62 (dd, 1H, ^2J = 12.8 Hz, ^3J = 1.5 Hz, $-CH_aH_bO$), 3.72 (d, 1H, ^3J = 9.8 Hz, $-CHOH-$), 3.86 (dd, 1H, ^2J = 9.8 Hz, ^3J = 3.5 Hz, $-CHOH-$), 3.97 (m, 1H, ^3J = 1.5 Hz, ^3J = 1.0 Hz, ^3J = 3.5 Hz, $-CHOH-$), 4.01 (dd, 1H, ^2J = 12.8 Hz, ^3J = 1.0 Hz, $-CH_aH_bO$); ^{13}C NMR (360 MHz; D_2O, DEPT, HMQC, HMBC) δ 24.2 ($-CH_2-$), 29.3 ($-CH_2-$), 57.8 ($-CH_2N$), 61.1 ($-CH_2-$), 61.5 ($-CH_2-$), 64.2 [$-CH$(COOH)N], 69.8 ($-CHOH-$), 70.0 ($-CHOH-$), 70.4 ($-CHOH-$), 96.4 [N*C*(OH)], 174.1 ($-COOH$).

7-Hydroxy-4a,6-dimethyl-2H,3H,4aH-furo[2,3-b]thiazine. A solution of acetylformoin (10 mmol) and cysteamine (10 mmol) in dimethylformamide (5 mL) was stirred for 2 h at 80 °C. After cooling, the mixture was applied onto the top of a column designed for flash chromatography (20 cm × 2 cm i.d.; J. T. Baker BV, Deventen, The Netherlands, no. 7022-01) and equipped with a solvent reservoir (250 mL) and a pressure regulator. The column was then filled with a slurry of Bakerbond-Diol gel (50 g; J. T. Baker BV, Deventen, The Netherlands) in *n*-pentane. Chromatography was performed under nitrogen pressure maintaining a constant flow rate of 5 mL/min and by using the following solvents: *n*-pentane (100 mL, fraction A), *n*-pentane/diethyl ether (80:20, v/v; 100 mL, fraction B), *n*-pentane/diethyl ether (70:30, v/v; 100 mL, fraction C), *n*-pentane/diethyl ether (60:40, v/v; 100 mL, fraction D), *n*-pentane/diethyl ether (50:50, v/v; 100 mL, fraction E) followed by *n*-pentane/diethyl ether (30:70, v/v; 100 mL, fraction F). Fractions D and E were collected, and the volatiles were removed by high-vacuum distillation, affording the target compound as a residual white powder with a purity of ~96% (4.9 mmol; 49% in yield): GC/MS (CI, methane) 186 (100, $[M + 1]^+$); MS(EI) spectrum is displayed in Figure 3; ^{1}H and ^{13}C NMR data as well as signal assignments are given in Tables 3 and 4.

Roasting of Glucose/L-Proline in the Absence or Presence of L-Cysteine, Respectively. Glucose (1 mmol) and L-proline (1 mmol) were powdered in a mortar without or with addition of L-cysteine (0.2, 0.5, or 1.0 mmol), respectively, mixed with silica gel (0.2 g), and then heated in an alumina block for 15 min at 180 °C.

Quantification of Bispyrrolidino- (1) and Pyrrolidinohexose Reductone (2) in Thermally Treated Maillard Mixtures. The heated reaction mixture was dissolved in water and made up to 5 mL. An aliquot (2 mL) was fractionated by ultracentrifugation with a cutoff of 1000 Da (Centriplus, Amicon, Witten, Germany) using a centrifuge with a swinging-bucket rotor adapter at 3000*g* until filtration was complete. The filtrate was then directly used for RP-HPLC analysis. Identification of the aminohexose reductones **1** and **2** was performed by comparison of the LC/MS and the UV−vis spectra as well as the retention times (RP-HPLC; **2**, 16.0 min; **1**, 37.6 min) with those obtained for the synthetic reference compounds. Quantification of the aminohexose reductones **1** and **2** was performed by comparing the peak areas obtained at λ = 300 nm with those of defined standard solutions of each reference compound in methanol.

Identification of 7-Hydroxy-4a,6-dimethyl-2*H*,3*H*,4a*H*-furo[2,3-*b*]thiazine (4) in Mixtures of Glucose/L-Proline, *N*-(1-Deoxy-D-fructos-1-yl)-L-proline, or Acetylformoin/L-Proline Heated in the Presence of L-Cysteine or Cysteamine, Respectively. Glucose (5 mmol)/L-proline (5 mmol) or *N*-(1-deoxy-D-fructos-1-yl)-L-proline (5 mmol) was mixed with L-cysteine (5 mmol) or cysteamine (5 mmol), respectively, ground with silica gel (1.0 g), and heated in an alumina block for 15 min at 180 °C. Acetylformoin (1 mmol) and L-cysteine (1 mmol) or cysteamine (1 mmol) were mixed with silica gel (0.2 g) and heated in an alumina block at 180 °C for 15 or 10 min, respectively. After cooling, each reacted mixture was filled in a chromatography column (20 cm × 1 cm i.d.) containing silica gel (3.0 g) as the stationary phase. After chromatography with ethyl acetate (50 mL), the column was flushed with methanol/ethyl acetate (40:60, v/v; 50 mL), and the eluate was collected and concentrated in vacuo. GC/MS analysis revealed a compound showing the same chromatographical and mass spectroscopical data as the synthetic 7-hydroxy-4a,6-dimethyl-2*H*,3*H*,4a*H*-furo[2,3-*b*]thiazine.

Dry-Heating of 7-Hydroxy-4a,6-dimethyl-2*H*,3*H*,4a*H*-furo[2,3-*b*]thiazine (4) in the Presence of L-Proline. 7-Hydroxy-4a,6-dimethyl-2*H*,3*H*,4a*H*-furo[2,3-*b*]thiazine (0.5 mmol) and L-proline (0.5 mmol) were intimately mixed with silica gel (0.3 g) and then heated for 10 min at 180 °C. The heated reaction mixture was suspended in water (2 mL) and filtered. The filtrate was then directly used for RP-HPLC analysis, demonstrating that the aminohexose reductones **1** and **2** were absent.

Determination of Taste Dilution (TD) Factors of the Maillard Mixtures. The reacted Maillard mixtures were dissolved in water (50 mL). These solutions were then stepwise diluted with water until a difference in bitter taste between an aliquot (10 mL) and two blanks (tap water; 10 mL) could just be sensorially detected using a triangle test. Using this procedure, a *t*aste *d*ilution (TD) factor could be defined for each reaction mixture.

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). An aliquot of the sample (0.5 μL) was applied by the cold on-column injection technique at 40 °C. After 2 min, the temperature of the oven was raised by 40 °C/min to 60 °C, held 1 min isothermally, raised by 8 °C/min to 240 °C, and held for 20 min.

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 540) 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 an analytical scale (4.6 × 250 mm, flow rate = 0.8 mL/min). For quantification of aminohexose reductones, the following solvent gradient was used: starting with a mixture (20:80, v/v) of methanol and aqueous triethylamine formate buffer (20 mmol/L; pH 8.5), the methanol content was increased to 100% within 50 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 mixture (20:80, v/v) of methanol and aqueous ammonium formate (20 mmol/L; pH 8.5) and increasing the methanol content to 100% within 40 min.

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

RESULTS AND DISCUSSION

Sensorial evaluation of a dry-heated, equimolar mixture of glucose and L-proline revealed a strong bitter taste being well in line with data reported by Chen (1979) and Papst et al. (1984, 1985).

Table 1. Influence of L-Cysteine on the Bitter Taste of Heated Glucose/L-Proline (1:1) Mixtures[a]

cysteine content (mmol)	TD factor[b]	cysteine content (mmol)	TD factor[b]
0.0	2048	0.5	64
0.2	128	1.0	16

[a] Mixtures of glucose (1 mmol) and L-proline (1 mmol) were reacted in the presence of various amounts of L-cysteine at 180 °C for 15 min. [b] The taste dilution (TD) factor is defined as the dilution of the reacted sample in water at which the bitter taste is just detectable sensorially in a triangle test using water as the blank. The data are the means of triplicates.

To study whether the intensity of the bitter taste developed in this Maillard mixture can be reduced by L-cysteine, glucose and L-proline were thermally treated in the presence of various amounts of L-cysteine. After these compounds had been dissolved in water, the bitter intensity of these solutions was evaluated by diluting them step by step with tap water and comparing the bitter-taste intensity of each dilution with two blanks of tap water using the triangle test. The dilution, at which a difference in bitter taste between the sample and the blanks could just be sensorially detected, was defined as the *t*aste *d*ilution (TD) factor. Because the TD factor, by definition, corresponds to the threshold of the Maillard mixture in water, a solution exhibiting no bitter taste has a TD factor of <1. This taste dilution analysis, therefore, ranks the single reaction mixtures on the basis of their relative intensities in bitter taste. As given in Table 1, by far the highest TD factor of 2048 was evaluated for the glucose/L-proline mixture heated in the absence of L-cysteine. Addition of L-cysteine prior to thermal treatment led to a drastic decrease of the intensity of the bitter taste; for example, addition of 0.2, 0.5, or 1.0 mmol of cysteine led to a reduction of the bitter-taste intensity by factors of 16, 32, or 128, respectively (cf. Table 1). These data indicated that the formation of bitter-tasting Maillard compounds in glucose/L-proline mixtures can be significantely counteracted by L-cysteine, also when added in lower than equimolar amounts.

To gain more detailed insights as to whether the cysteine-induced decrease in bitterness is reflected in a decrease of the amounts of bitter-tasting aminohexose reductones, bispyrrolidinohexose reductone (**1**) and pyrrolidinohexose reductone (**2**) were quantified in glucose/L-proline mixtures heated in the absence or in the presence of various amounts of cysteine. The reaction mixtures were, therefore, fractionated by ultracentrifugation, the low molecular fraction was separated by RP-HPLC, and the amounts of bitter-tasting compounds **1** and **2** were determined by means of DAD using the synthetic reference compounds as external standards. The data, given in Table 2, showed that these aminohexose reductones were generated in by far the highest concentrations in the glucose/L-proline mixture when heated in the absence of cysteine, for example, 5.96 and 3.20 mg/mmol of glucose of **1** and **2**, respectively, were formed. Addition of already 0.2 mmol L-cysteine to the glucose/L-proline mixture lowered the amounts of the aminohexose reductones **1** and **2** by factors of >3. Heating a mixture containing equimolar ratios of both amino acids generated compounds **1** and **2** in amounts of only 0.62 and 0.18 mg/kg. These data indicate that the cysteine-induced reduction of the bitter-taste intensity in glucose/L-proline mixtures is reflected in a drastic decrease of the aminohexose reductones **1** and **2** evoking strong bitter taste.

Table 2. Influence of L-Cysteine on the Amounts of Bispyrrolidino- (1) and Pyrrolidinohexose Reductone (2) Generated upon Heating of a Glucose/L-Proline (1:1) Mixture[a]

cysteine content (mmol)	amount (mg/mmol of glucose) of	
	1	**2**
0.0	3.20	5.96
0.2	0.96	1.89
0.5	0.23	0.83
1.0	0.18	0.62

[a] Mixtures of glucose (1 mmol) and L-proline (1 mmol) were reacted in the presence of various amounts of L-cysteine at 180 °C for 15 min.

Table 3. Assignment of ^{1}H NMR Signals (360 MHz, $CDCl_3$) of 7-Hydroxy-4a,6-dimethyl-2*H*,3*H*,4a*H*-furo[2,3-*b*]thiazine (4)

H at relevant C atom[a]	δ[b]	I[c]	M[c]	J[c] (Hz)	connectivity[d] with
H−C(8)	1.61	3	s		
H−C(9)	2.11	3	s		
H_a−C(3)	2.78	1	ddd	11.5, 5.3, 0.9	H_b−C(3), H_a−C(2), H_b−C(2)
H_b−C(3)	3.03	1	ddd	11.5, 3.1, 1.3	H_a−C(3), H_a−C(2), H_b−C(2)
H_a−C(2)	3.29	1	ddd	12.8, 5.3, 3.1	H_b−C(2), H_a−C(3), H_b−C(3)
H_b−C(2)	3.80	1	ddd	12.8, 3.1, 0.9	H_a−C(2), H_a−C(3), H_b−C(3)

[a] Numbering of carbon atoms refers to formula **4** in Figure 4. [b] The ^{1}H chemical shifts are given in relation to $CDCl_3$. [c] Determined from 1D spectrum. [d] Observed homonuclear ^{1}H,^{1}H connectivities by DQF-COSY.

In a very recent investigation (Hofmann, 1998), the C_6-reductone acetylformoin (**3**) formed upon thermal degradation of hexoses was identified as an effective progenitor of aminohexose reductones **1** and **2** when heated in the presence of L-proline or pyrrolidine (Figure 2). To study whether L-cysteine blocks the formation of bitter-tasting aminohexose reductones upon reaction with the intermediate acetylformoin, this C_6-reductone was heated in mixture with L-proline in the absence as well as in the presence of L-cysteine. Sensorial evaluation revealed an intense bitter taste of the thermally treated acetylformoin/L-proline mixture but a lack of bitterness when the mixture was heated in the presence of L-cysteine (data not shown). Obviously, the sulfur-containing amino acid might inhibit the formation of bitter-tasting aminohexose reductones from acetylformoin.

For further confirmation of this assumption, the methanol-soluble reaction products of the heated cysteine-containing mixture were analyzed by GC/MS. A compound was detected with a retention index >2700 showing the mass spectrum displayed in Figure 3. MS using chemical ionization confirmed the molecular mass as 185 Da. The same compound could be detected in lower yields in the glucose/L-proline mixture or *N*-(1-deoxy-D-fructos-1-yl)-L-proline, respectively, when heated in the presence of L-cysteine. To gain more insights into the chemical structure of this compound, its elementary composition was analyzed by high-resolution MS, revealing a sum formula of $C_8H_{11}NO_2S$ in the molecular ion (*m*/*z* 185). Taking these data into account and considering four double-bond equivalents, which were calculated from the ratio of carbon to hydrogen atoms, a bicyclic condensation product of acetylformoin and cysteamine, the Strecker degradation product of L-cysteine, was assumed to be the unknown compound.

For further confirmation, synthetic acetylformoin was heated with cysteamine, and the reaction mixture was then analyzed by GC/MS. Because the reaction of

Table 4. Assignment of ^{13}C NMR Signals (360 MHz, $CDCl_3$) of 7-Hydroxy-4a,6-dimethyl-2*H*,3*H*,4a*H*-furo[2,3-*b*]thiazine (4)

relevant C atom[a]	δ[b]	DEPT[c]	heteronuclear $^1H,^{13}C$ multiple-quantum coherence[d] via ^1J(C,H)	via $^{2,3,4}J$(C,H)
C(9)	10.87	CH_3	H−C(9)	
C(8)	23.04	CH_3	H−C(8)	
C(3)	34.07	CH_2	H_a−C(3), H_b−C(3)	H_a−C(2), H_b−C(2)
C(2)	48.82	CH_2	H_a−C(2), H_b−C(2)	H_a−C(3), H_b−C(3)
C(4a)	75.00	C		H_b−C(2), H_b−C(3), H−C(8)
C(6)	133.96	C		H−C(9)
C(7)	156.70	C		H_a−C(2), H_b−C(2), H−C(9)
C(7a)	196.16	C		H_b−C(2), H−C(8)

[a] Numbering of carbon atoms refers to formula **4** in Figure 4. [b] The ^{13}C chemical shifts are given in relation to $CDCl_3$. [c] DEPT-135 spectroscopy. [d] Assignments based on HMQC (^1J) and HMBC (^2J, ^3J, ^4J) experiments.

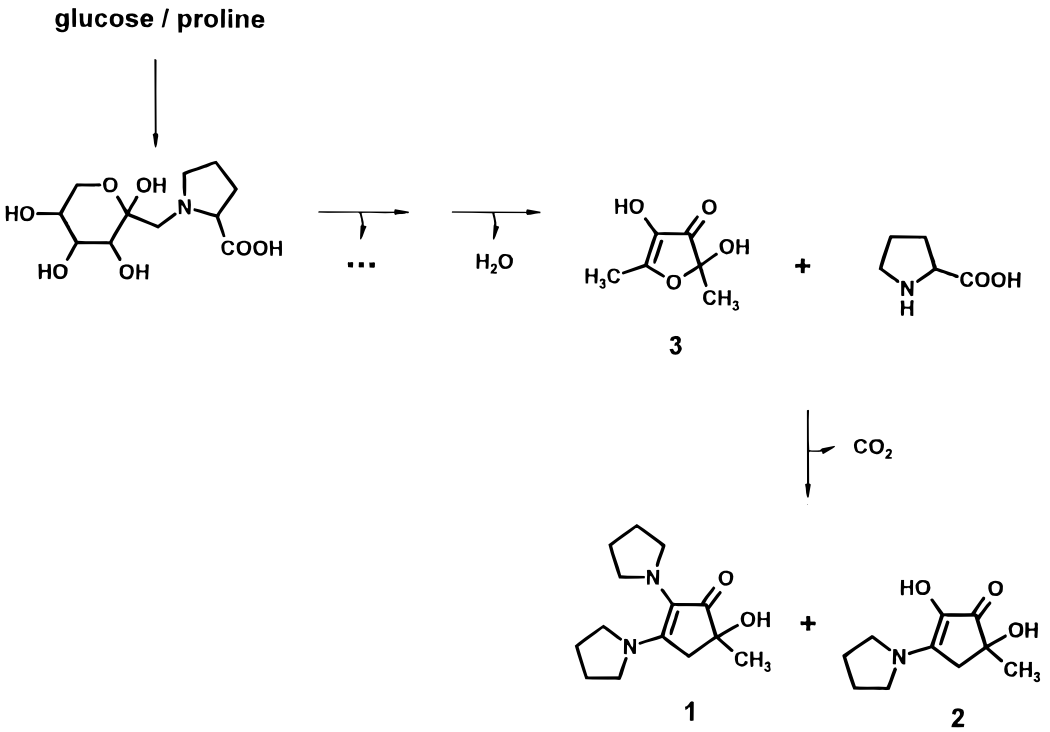


Figure 2. Formation of aminohexose reductones **1** and **2** from glucose and L-proline via acetylformoin (**3**) as the key intermediate.

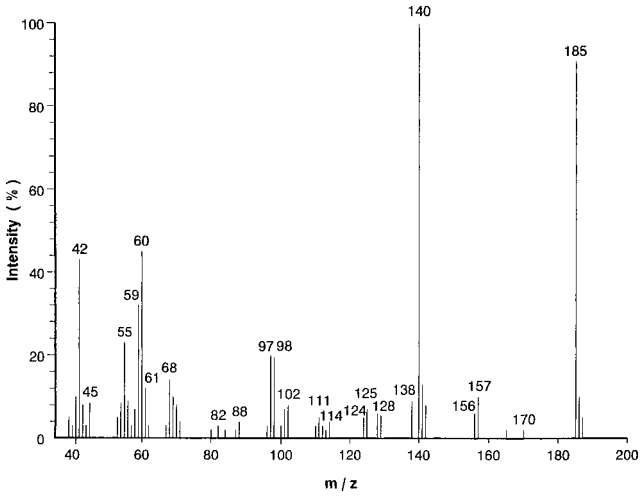
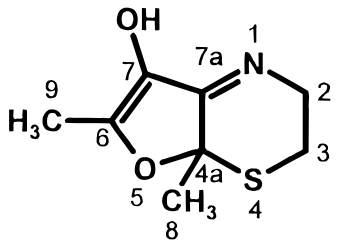


Figure 3. MS(EI) spectrum of the unknown compound detected in a glucose/L-proline mixture heated in the presence of L-cysteine.

acetylformoin with cysteamine was effective in generating the unknown Maillard reaction product, the compound was isolated from this mixture by flash chromatography using silica-diol as the stationary phase. After additional purification by high-vacuum distillation, a



4

Figure 4. Structure of 7-hydroxy-4a,6-dimethyl-2*H*,3*H*,4a*H*-furo[2,3-*b*]thiazine (**4**).

white tasteless solid was obtained, which was then analyzed by 1D and 2D NMR spectroscopy. The ^1H NMR spectrum of the compound measured in $CDCl_3$ showed six resonance signals fitting well with the assignment of the structure **4** given in Figure 4. The singlets at 1.61 and 2.11 ppm, each integrating to three protons, corroborated with two methyl groups being consistent with structure **4**. In addition, four multiplets were observed resonating at 2.78, 3.03, 3.29, and 3.80 ppm. The corresponding splitting constants as well as a DQF-COSY experiment demonstrated two sets of diastereotopic protons taking part in an AA′XX′ spin system,

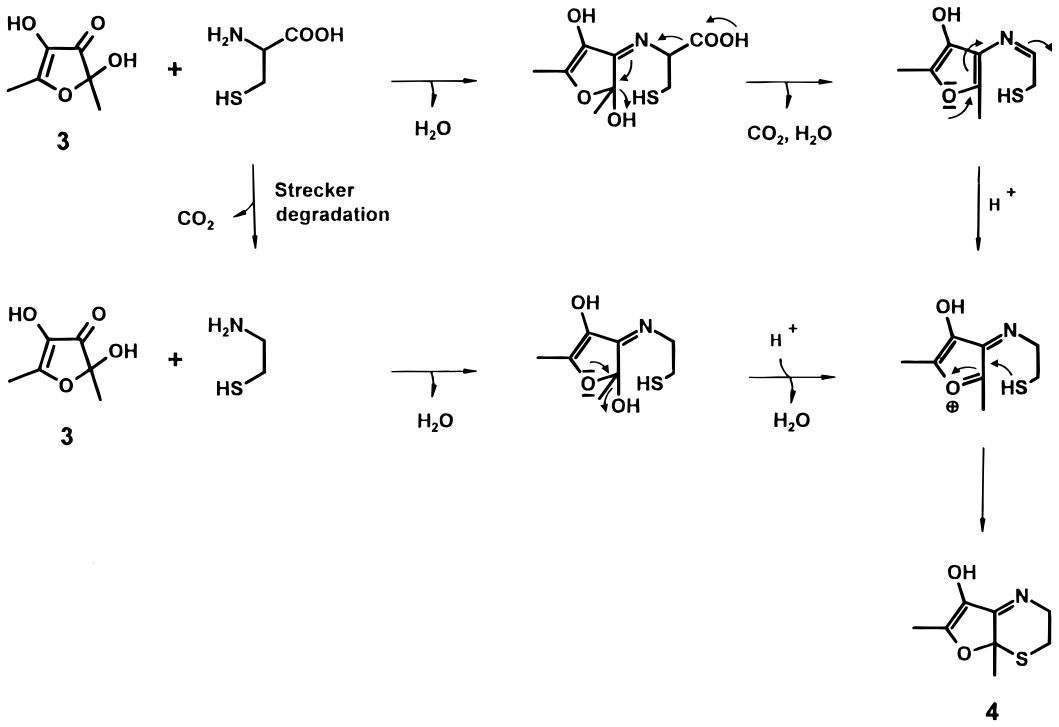


Figure 5. Formation of 7-hydroxy-4a,6-dimethyl-2*H*,3*H*,4a*H*-furo[2,3-*b*]thiazine (**4**) in the reaction of acetylformoin (**3**) and L-cysteine.

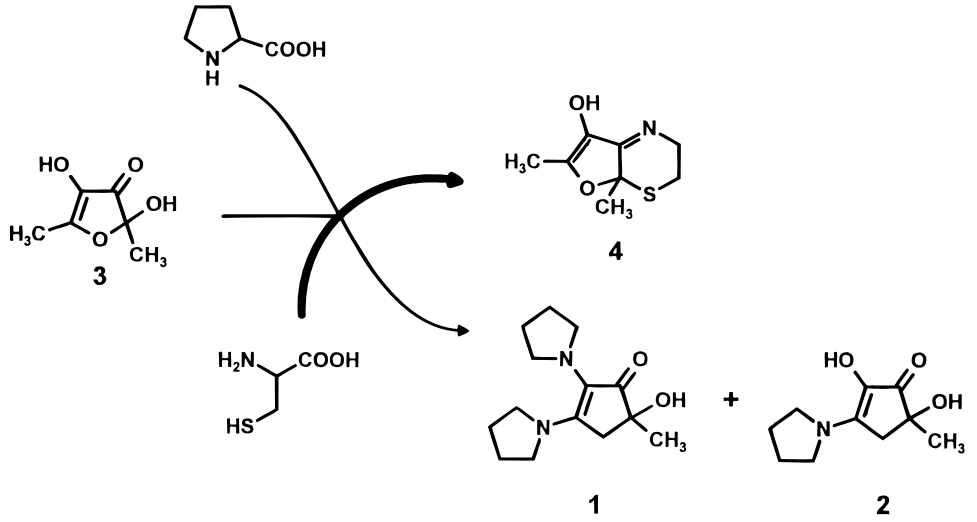


Figure 6. Blocking of formation of bitter-tasting aminohexose reductones **1** and **2** by formation of 7-hydroxy-4a,6-dimethyl-2*H*,3*H*,4a*H*-furo[2,3-*b*]thiazine (**4**) upon reaction of acetylformoin (**3**) with L-cysteine.

thereby confirming the assumed cysteamine moiety incorporated in a fixed ring structure. The NMR data of the thiazine ring system are in contrast to those found for the intense popcorn-smelling odorant 5-acetyl-2,3-dihydro-1,4-thiazine, which due to its dynamic change in conformation did not show two sets of diastereotopic protons but showed only two multiplets integrating for two protons each (Hofmann et al., 1995). The diastereotopic splitting of the methylene protons in structure **4** is, however, well in line with the neighbored chiral center at C(4a) and the fixed thiazine ring system.

Comparison of the ^{13}C data measured by ^{1}H broad band decoupled ^{13}C NMR spectroscopy with those obtained by distortionless enhancement of polarization transfer (DEPT) spectroscopy and, in addition, the $^{1}J_{C,H}$ correlations observed by means of a heteronuclear multiquantum correlation (HMQC) experiment confirmed the proposed methyl groups and both methylene groups of the cysteamine moiety and identified the ^{13}C resonance signals at 75.00, 133.96, 156.70, and 196.16 ppm as quarternary carbon atoms. Unequivocal assignment of these carbon atoms was performed by means of a heteronuclear multiple-bond correlation (HMBC) experiment enabling the observation of $^{2}J_{C,H}$, $^{3}J_{C,H}$, and $^{4}J_{C,H}$ correlations; for example, the ^{13}C signal at 156.70 ppm showed heteronuclear correlation with the diastereotopic protons of the nitrogen-bearing methylene group C(2) as well as with the protons of the methyl group C(9). In addition, the methylene protons H_a−C(2) and H_b−C(2) showed coupling with the carbon atom of the sulfur-bearing methylene group C(3) as well as the quaternary carbon C(7a). The proton H_b−C(2) showed additional correlation with the quaternary carbon resonating at 75.00 ppm, which, on the basis of its chemical shift, was assigned as the cyclic thiohemi ketal C(4a).

Taking all of these spectroscopical data into consideration, the structure of the novel Maillard reation product could be unequivocally identified as 7-hydroxy-4a,6-dimethyl-2*H*,3*H*,4a*H*-furo[2,3-*b*]thiazine (**4**). To further ascertain our proposal, compound **4** was synthesized by condensation and cyclization of acetylformoin and cysteamine in dimethylformamide and characterized by several 1D and 2D NMR experiments. The synthetic product obtained showed the identical spectroscopic and chromatographic data as compound **4** identified in the Maillard mixtures.

On the basis of these data a mechanism leading to the formation of **4** via the hexose intermediate acetylformoin (**3**) is proposed in Figure 5. Reaction of cysteine with the oxo function of acetylformoin (**3**), followed by decarboxylation and dehydratization, reveals a furanoic intermediate, which then subsequently undergoes ring closure to form the bicyclic furo[2,3-*b*]thiazine (**4**). In an alternative reaction route decarboxylation of cysteine in the course of a Strecker reaction may occur prior to its reaction with acetylformoin.

To ascertain that compound **4** is not able to generate bitter-tasting aminohexose reductones in the reaction with L-proline, a binary mixture of 7-hydroxy-4a,6-dimethyl-2*H*,3*H*,4a*H*-furo[2,3-*b*]thiazine (**4**) and L-proline was dry-heated. HPLC analysis of the reaction mixture revealed that the aminohexose reductones **1** and **2** were not formed, thereby indicating that the bitter-taste progenitor acetylformoin is indeed blocked by the reaction with cysteine as well as cysteamine.

CONCLUSIONS

The data presented indicate that in the presence of L-proline and L-cysteine, the hexose-derived Maillard intermediate acetylformoin (**3**) predominantly reacts with the sulfur-containing primary amino acid, leading to 7-hydroxy-4a,6-dimethyl-2*H*,3*H*,4a*H*-furo[2,3-*b*]thiazine (**4**), thereby blocking the L-proline-induced formation of bitter-tasting bispyrrolidinohexose reductones (**1**) and pyrrolidinohexose reductones (**2**) as outlined in Figure 6. The results of the present investigation provide useful information to extend the current knowledge on how to prevent the generation of bitter-tasting compounds by nonenzymatic reactions occurring during food processing.

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

Chen, W.-C. Ph.D. thesis, Technical University of Munich, 1979.

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

Hofmann, T. Acetylformoin—a chemical switch in the formation of colored Maillard reaction products from hexoses and primary and secondary amino acids. *J. Agric. Food Chem.* **1998**, *46*, 3918−3928.

Hofmann, T.; Hässner, R.; Schieberle, P. Determination of the chemical structure of the intense roasty, popcorn-like odorant 5-acetyl-2,3-dihydro-1,4-thiazine. *J. Agric. Food Chem.* **1995**, *43*, 2195−2198.

Papst, H. M. E.; Ledl, F., Belitz, H.-D. Bitter tasting compounds in heated proline/sucrose mixtures. *Z. Lebensm. Unters. Forsch.* **1984**, *178*, 356−360 (in German).

Papst, H. M. E.; Ledl, F., Belitz, H.-D. Bitter tasting compounds in heated mixtures of sucrose, maltose and L-proline. *Z. Lebensm. Unters. Forsch.* **1985**, *181*, 386−390 (in German).

Received for review March 10, 1999. Revised manuscript received August 12, 1999. Accepted August 31, 1999.

JF9903152