Identification of New Heterocyclic Nitrogen Compounds from Glucose-Lysine and Xylose-Lysine Maillard Model Systems

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Aqueous sugar (glucose or xylose)-lysine model systems were heated at 80 °C for 6 h with the pH maintained at a predetermined value (3, 4, or 5). Selected compounds were isolated by combinations of solvent extraction and semipreparative HPLC, prior to identification by NMR and mass spectrometry. Two compounds were identified from the pH 5 glucose system and were identified as ϵ-[2-formyl-5-(hydroxymethyl)pyrrole-1-yl]-L-norleucine (pyrraline) and the new compound, 1-(5-carboxy-5-aminopentyl)-2-formyl-3-(1,2,3-trihydroxypropyl)pyrrole. A third compound was partially characterized. 2-Acetyl-5-hydroxymethyl-5,6-dihydro-4*H*-pyridinone was identified in the pH 3 xylose system, and the new compound, 8-furan-2-yl-methyl-5-hydroxymethyl-5,6-dihydro-indolizine-1,7-dione, was identified in the pH 4 xylose system. 2-Furfurylidene-4-hydroxy-5-methyl-3(2*H*)-furanone was identified in both xylose systems. Mechanisms of formation are proposed for the novel compounds.

Keywords: *Maillard reaction; colored compounds; pyrroles; indolizines; pyridinones; furanones*

INTRODUCTION

The Maillard reaction, the reaction between reducing sugars and compounds possessing a free amino group is important for food processing, as it leads to the development of substances that affect color and flavor. The colored products of the Maillard reaction fall into two classes, i.e., the low molecular mass (LMM) compounds, with masses below ∼1000 daltons, and the macromolecules known as melanoidins (Rizzi, 1997).

Following the isolation of the first LMM colored compound, 2-furfurylidene-4-hydroxy-5-methyl-3(2*H*)-furanone, from a xylose-glycine model system (Severin and Krönig, 1972), the structures of further colored Maillard products were elucidated throughout the 1980s and early 1990s (Ledl and Schleicher, 1990; Ames, 1992; Rizzi, 1997). More recently, substantial progress has been made in the elucidation of further structures of LMM colored Maillard reaction products (Arnoldi et al., 1997; Hofmann, 1997; 1998a; 1998b; 1998c; 1998d; 1998e; 1998f; 1998g; 1998h; Hofmann et al., 1999; Hofmann and Heuberger, 1999; Ames et al., 1999).

We recently reported the identification of 2-acetyl-6-(hydroxymethyl)-5,6-dihydro-4*H*-pyridinone from an aqueous xylose-glycine model system heated under reflux at pH 5 as well as the *cis*/*trans* ring isomers of 2-acetyl-6-hydroxy-7-(hydroxymethyl)-1,5,6,7-tetrahydro-4*H*-azepinone from the analogous system based on glucose (Ames et al., 1999). These compounds were all pale yellow. The aim of the work presented in this paper was to isolate and identify LMM compounds from glucose (or xylose)-lysine model systems heated at 80 °C.

MATERIALS AND METHODS

Materials. D-(+)-Xylose and lysine monohydrochloride were obtained as 99+% reagents, while D-(+)-glucose was an ACS grade chemical (Aldrich Chemical Co., Gillingham, U.K.). Glucose was used without further purification. Lysine monohydrochloride and xylose were recrystallized, respectively, from ethanol and water (10% ethanol, v/v), and from methanol and water (94% methanol, v/v), before use. Methanol for HPLC was obtained from Rathburn Chemicals Ltd. (Walkerburn, U.K.), and water for HPLC was prepared in the laboratory using a Purite Labwater RO50 unit (Purite Ltd., High Wycombe, U.K.). Ethyl acetate was HPLC grade from Rathburn (Walkerburn, U.K.).

HPLC Analyses. Analytical HPLC with diode array detection (DAD) was carried out using a reverse-phase ODS2 column (Hichrom Ltd, Reading, U.K.) and a water−methanol gradient, and DAD spectra were collected online (Bailey et al., 1996). Semipreparative HPLC was performed using an ODS2 column (Hichrom Ltd) and a water−methanol gradient, as described by Ames et al. (1999). The fractions from each day's collections were stored at −18 °C for up to 4 days prior to rotary evaporation to dryness at 40 °C. The solid residues were stored in a desiccator, over silica gel, at −18 °C.

Mass Spectrometry (MS). Chemical ionization (CI) mass spectra were obtained using ammonia as the reagent gas and a Micromass (Manchester, U.K.) Autospec double focusing magnetic sector mass spectrometer. Electrospray MS (ES-MS) was performed using loop-injections on a Perkin-Elmer LC series 200 coupled with an API-100 single quadrupole mass spectrometer (Sciex Instruments, Canada). A mixture of methanol and water, to which formic acid (2 mM) had been added, was the solvent. A probe voltage of 4700 V and a declustering potential of 50 V were used. The instrument was calibrated with the ammonium adduct of polypropylene glycol. Full-scan spectra were acquired from 100 to 600 amu using a step size of 0.5 amu and a dwell time of 4.2 ms.

Nuclear Magnetic Resonance Spectroscopy (NMR). ^{1}H and ^{13}C NMR spectra were recorded on Bruker (Karlsruhe,

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10.1021/jf000722+ CCC: $19.00
Published on Web 10/21/2000

Germany) Avance DPX 250 and AMX 400 spectrometers at 250 and 62.8 or 400 and 100.6 MHz, respectively. DMSO-d_6, H_2O-d_2, and $CHCl_3$-d were used as solvents, and tetramethylsilane was used as the internal standard. Chemical shifts were expressed in parts per million (δ). Decoupling, distortionless enhancement through polarization transfer (DEPT) and ^{1}H-^{1}H and ^{1}H-^{13}C correlation spectroscopy (COSY) experiments were also performed. NMR calculations were carried out using the computer program NMR Simulator (Virginia Tech., VA).

Preparation of the Model Systems. Aqueous solutions (one molal with respect to xylose or glucose and lysine) were heated at 80 °C for 6 h. The pH was monitored using an autoclavable pH electrode and controlled at pH at 3, 4, or 5 by the automatic addition of sodium hydroxide solution (3M) or hydrochloric acid (25% v/v) according to Bailey et al., (1996). The cooled glucose system was extracted with ethyl acetate (3 × 170 mL) prior to semipreparative HPLC of the aqueous phase. The xylose systems were cooled and extracted with ethyl acetate (3 × 170 mL). The extracts were combined, washed with water (1 × 2 mL), dried with sodium sulfate, and evaporated to dryness on a rotary evaporator with the water bath at 40 °C. The residues were dissolved in water (ca. 2.0 mL), and the solution was used for semipreparative HPLC.

RESULTS AND DISCUSSION

The presence of large amounts of macromolecular material in the systems heated under reflux for 2 h (Bailey et al., 1996) made purification of the LMM compounds difficult. Heating at a lower temperature (80 °C) for a longer time (6 h) greatly facilitated purification of the target compounds because smaller amounts of macromolecular material were formed. Six components were isolated from three Maillard model systems: compounds **1**−**3** from the glucose system at pH 5, compounds **4** and **5** from the xylose system at pH 3 and 4, respectively, and compound **6** from the xylose system at both pH 3 and 4.

Compound 1. This compound was isolated from the pH 5 glucose system as a white solid. The 280 nm analytical HPLC chromatogram of the purified compound showed one peak with a retention time of 15 min, and it accounted for ∼18% of the total peak area before solvent extraction. About 10 mg was isolated. The DAD spectrum showed a maximum at 297 nm and was typical of a compound possessing a pyrrole-like structure (Bailey et al., 1996).

The ^{1}H NMR data and the suggested assignments are as follows (400 MHz, H_2O-d_2): δ 1.3−2.4 (m, 6, CH_2), 3.76 (t, 1, CH), 4.26 (t, 2, CH_2N), 4.66 (s, 2, CH_2OH), 6.32 (d, 1, CH), 7.04 (d, 1, CH), 9.20 (s, 1, CHO). The ^{13}C NMR spectrum revealed the presence of 12 carbons. The DEPT spectrum indicated five methylene carbons (at 22.7 ppm, C_{10}, 31.1 ppm, C_9 or C_{11}, 31.3 ppm, C_{11} or C_9, 45.6 ppm, C_8, and 55.8 ppm, C_7), three methine carbons (at 55.8 ppm, C_{12}, 111.7 ppm, C_4, and 127.1 ppm, C_3), and four quaternary carbons (at 132.3 ppm, C_2, 144.3 ppm, C_5, 175.5 ppm, C_{13}, and 181.6 ppm, C_6). ES-MS data were as follows: 255 (M + H^+), 277 (M + Na^+), 292 (M + K^+), 509 (2M + H^+), 531 (2M + Na^+), 547 (2M + K^+).

The UV, ^{1}H and ^{13}C NMR data for compound **1** compared well with the published data for ϵ-[2-formyl-5-(hydroxymethyl)pyrrole-1-yl]-L-norleucine or ϵ-pyrrolelysine (pyrraline) (Figure 1) first isolated by Nakayama et al. (1980), and the ES-MS data confirmed the molecular ion as 254 amu. Pyrraline is a well-known product of the Maillard reaction, formed by the condensation of 3-deoxyglucosone with lysine.

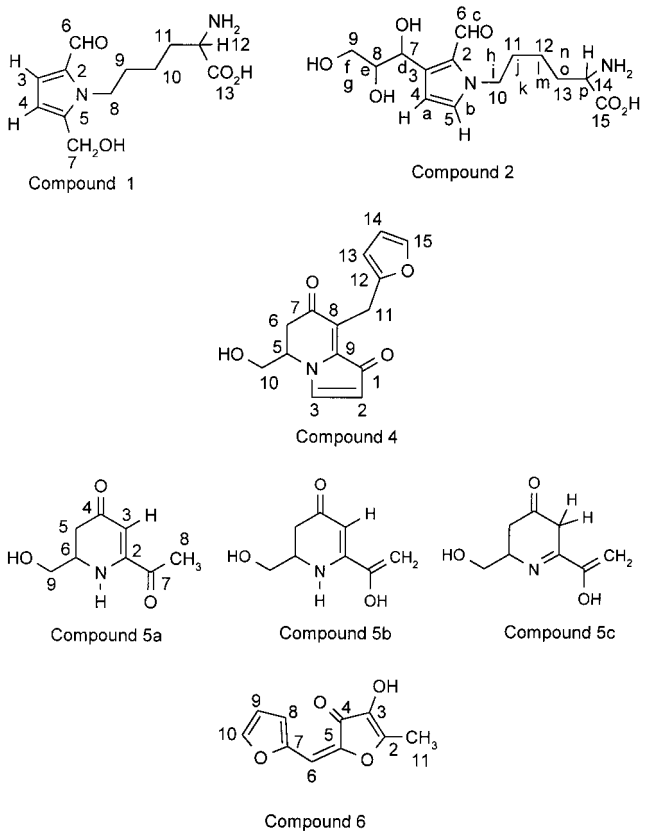


Figure 1. Structures of compounds **1**, **2**, and **4**−**6**.

Compound 2. The compound was isolated from the pH 5 glucose system as a white solid. It had a retention time of 5 min on the 280 nm chromatogram obtained using the analytical HPLC conditions and accounted for ∼27% of the total peak area before solvent extraction. Its purity was ∼100% at 280 nm and ∼10 mg was isolated. The electronic absorption spectrum had a maximum at 297 nm and was typical of a compound possessing a pyrrole-like spectrum (Bailey et al., 1996).

The ^{1}H NMR data and suggested assignments are as follows: (400 MHz DMSO-d_6): δ 1.30 (2H, m, 2 CH_2, 1.63 (m, 4, CH_2), 3.20 (t, 1, CH), 3.35 (dd, 1, H of CH_2-OH, $J_{f,g}$ =11.05 Hz, $J_{e,g}$ = 6.43 Hz), 3.44 (dd, 1, H of CH_2OH, $J_{e,f}$ = 4.13 Hz), 3.52 (m, 1, CHOH, $J_{d,e}$ = 6.27 Hz), 4.22 (m, 2, NCH_2), 4.78 (d, 1, CHOH), 6.17 (d, 1, CH, $J_{a,b}$ = 2.48 Hz), 7.13 (d, 1, CH, $J_{a,b}$ = 2.48 Hz), 9.69 (s, 1, CHO).

The ^{13}C NMR spectrum (100.6 MHz, DMSO-d_6) revealed 14 carbon atoms and the DEPT spectrum indicated five methylene carbons (at 21.1 ppm, C_{12}, 29.5 ppm, C_{11} or C_{13}, 30.0 ppm, C_{13} or C_{11}, 47.4 ppm, C_{10} and 62.1 ppm, C_9), five methine carbons (at 53.0 ppm, C_{14}, 66.4 ppm, C_7, 74.0 ppm, C_8, 107.7 ppm, C_4 and 129.7 ppm, C_5), and four quaternary carbons (at 126.2 ppm, C_3, 139.3 ppm, C_2, 169.5 ppm, C_{15} and 179.0 ppm, C_6). Data from the ^{1}H-^{13}C COSY spectrum and the carbon and proton assignments are tabulated in Table 1.

The NMR data indicated the presence of a disubstituted, five-membered heterocyclic ring and a hydrocarbon chain. A comparison of the NMR data with those in the literature (Olsson et al., 1977; Nakayama et al., 1980) suggested that compound **2** was a derivative of pyrraline. After accounting for the signals due to the pyrraline-like component of the structure, one methylene and two methine carbons remain, suggesting a three

Table 1. ^{1}H-^{13}C COSY Spectral Data and Assignments for Compound 2

carbon signal (ppm)	proton signal (ppm)	carbon assignment	proton assignment
21.1	1.30	C_{12}	l,m
29.5	1.63	C_{11} or C_{13}	j,k or n,o
30.0	1.63	C_{13} or C_{11}	n,o or j,k
47.4	4.22	C_{10}	h,i
53.0	3.20	C_{14}	p
62.1	3.44, 3.49	C_9	f,g
66.4	4.78	C_7	d
74.0	3.52	C_8	e
107.7	6.17	C_4	a
126.2		C_3	
129.7	7.13	C_5	b
139.3		C_2	
169.5		C_{15}	
179.0	9.69	C_6	c

carbon substituent on the pyrrole ring. Ledl and Schleicher (1990) have given examples of pyrrole derivatives containing the substituent $-CH(OH)CH(OH)CH_2OH$. Calculation of the ^{1}H NMR spectrum of this side chain, using the chemical shifts and coupling constants given above, gave the same splitting pattern as that observed in the experimental spectrum. However, the coupling constant of the heterocyclic protons was 2.48 Hz, a value typical of $J_{4,5}$ (or $J_{2,3}$) coupling and 2,3 (or 4,5)-disubstitution, rather than $J_{3,4}$ coupling (typically 4.0 Hz) and 2,5-disubstitution, as in pyrraline (Stierle and Faulkner, 1980). The ES-MS data were 315 ($M + H^+$), 267 ($M + H - H_2O - CHOH$) and 223 ($M + H - H_2O - CHOH - CO_2$). Ions corresponding to the sodium and potassium adducts of the monomer and dimer were also observed, confirming the molecular ion as 314 amu. Thus, compound **2** appears to be 1-(5-carboxy-5-aminopentyl)-2-formyl-3-(1,2,3-trihydroxypropyl)pyrrole (Figure 1). A Chemical Abstracts literature search established that compound **2** has not been reported previously in the literature.

Compound **2** may form by the reaction of the 3-deoxyosone with the Amadori product, to give a trisubstituted pyrrole (Farmer et al., 1988), followed by the loss of the substituent at position 2 of the pyrrole ring to form a disubstituted pyrrole (Ledl and Schleicher, 1990). A plausible mechanism is given in Scheme 1.

Compound 3. This compound was isolated as a white solid from the glucose system at pH 5. It accounted for ~15% of the total peak area (at 280 nm) before solvent extraction. The purified compound (~10 mg) gave one peak on the analytical HPLC chromatogram, with a retention time of 7 min. The DAD spectrum was typical of a pyrrole-like compound (Bailey et al., 1996), possessing a λ_{max} at 299 nm.

The ^{1}H NMR data were as follows (400 MHz, H_2O-d_2): δ 0.96 (m, 1, CH_2), 1.36 (m, 1, CH_2), 1.57 (m, 1, CH_2), 1.69 (m, 1, CH_2), 2.10 (m, 1, CH_2), 2.35 (m, 1, CH_2), 2.92 (t, 2, NCH_2, J = 7.63 Hz), 4.65 (d, 1, CH_2OH), pyrrole substituent, J = 14.10 Hz), 4.70 (d, 1, CH_2OH, J = 14.10 Hz), 6.45 (d, 1, CH, $J_{3,4}$ = 4.11 Hz), 7.22 (d, 1, CH, $J_{3,4}$ = 4.11 Hz), 9.35 (s, 1, CHO).

The ^{13}C NMR spectrum showed 11 carbon signals and the DEPT spectrum indicated five methylene carbons (at 23.4 ppm, C_{10}, 26.8 ppm, C_9, 31.9 ppm, C_{11}, 39.6 ppm, C_8, 55.8, C_7), two methine carbons (at 111.4 ppm, C_4, 127.5 ppm, C_3), and four quaternary carbons (at 132.3 ppm, C_2, 144.7 ppm, C_5, 177.5 ppm, C_{13}, 181.3 ppm, C_6).

The ^{1}H-^{1}H COSY spectrum showed five pairs of mutually coupled protons from the methylene groups, i.e., a set of proton pairs. Members of each pair were coupled to each other and also to other protons in the set, indicating a hydrocarbon chain.

ES-MS showed ions at m/z 237 ($M + H - H_2O$) and 219 ($M + H - H_2O - H_2O$). In addition, an ion series, with masses at m/z 277, 531, and 785 and the sodium adducts of the monomer, dimer, and trimer of pyrraline were observed.

The NMR data were very similar to those for compounds **1** and **2**, suggesting a pyrraline-like structure. The coupling constant of the heterocyclic protons was 4.11 Hz, a value typical of $J_{3,4}$ coupling and 2,5-disubstitution, as in pyrraline. The ES-MS data showed ions related to pyrraline and its sodium and potassium adducts. The sodium adducts of the monomers and dimers of compounds **1** and **2** were observed by ES-MS, but compound **3** was unique in exhibiting the sodium adduct of the trimer. The ions at m/z 277, 531, and 785 suggested a metal complex with up to three molecules of pyrraline and one sodium ion for this partially characterized reaction product.

Compound 4. Compound **4** was pale yellow in color and was isolated from the pH 3 xylose system. It had a retention time of 24.0 min on the analytical HPLC column, and the 280 nm chromatogram indicated a single peak. The DAD spectrum showed a single asymmetrical absorption band with a maximum at 342 nm, and the compound accounted for ~5% of the total peak area on the 360 nm chromatogram, before solvent extraction. Ammonia CI-MS gave a pseudomolecular ion at m/z 260.0926, fitting $C_{14}H_{14}O_4N$, with an error of 1.1534 ppm.

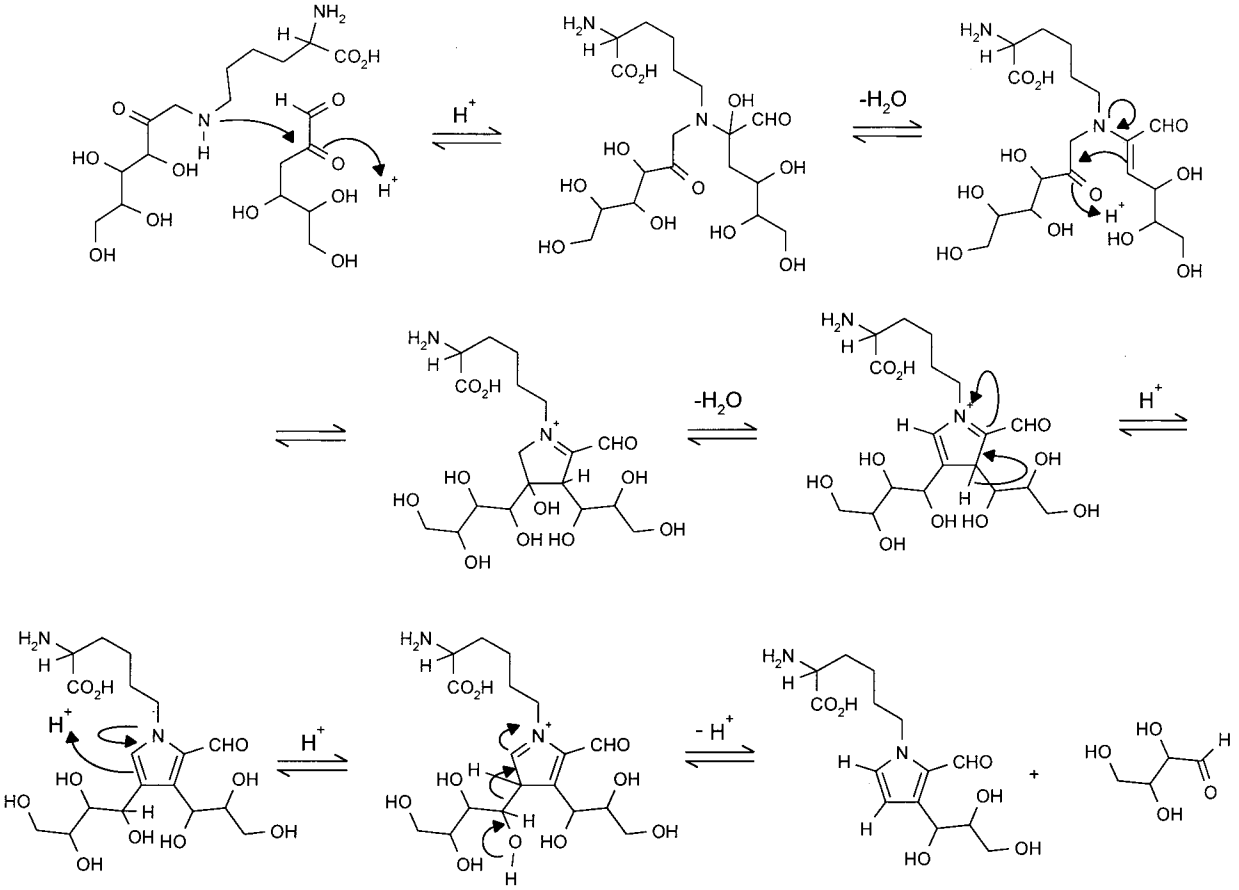
The ^{1}H NMR data and suggested assignments are as follows: (250 MHz, DMSO-d_6): δ 2.66 (dd, 1, CH_2CO), 3.00 (dd, 1, CH_2CO), 3.74 (d, 1, CH_2OH), 3.82 (s, 2, CH_2), 4.02 (d, 1, CH_2OH), 4.95 (m, 1, CH), 5.25 (t, 1, CH$_2$OH), 6.20 (dd, 1, CH, J_{MX} = 3.16, J_{AX} = 0.87 Hz), 6.40 (dd, 1, CH, J_{MX} = 3.16, J_{AM} = 1.88), 7.31 (s, 1, CH), 7.56 (dd, 1, CH, J_{AM} = 1.88, J_{AX} = 0.87 Hz), 7.87 (s, 1, CH).

The ^{13}C NMR spectrum revealed 14 carbons, and the DEPT spectrum showed three methylene carbons (at 27.1 ppm, C_{11}, 35.3 ppm, C_5 and 61.57 ppm, C_{10}), six methine carbons (at 63.13 ppm, C_6, 106.68 ppm, C_{13}, 110.03 ppm, C_{14}, 119.35 ppm, C_8, 129.67 ppm, C_9 and 141.50 ppm, C_{15}), and five quaternary carbons (at 123.03 ppm, C_3, 141.46 ppm, C_2, 151.46 ppm, C_{12}, 170.37 ppm, C_7, and 190.14 ppm, C_4).

By considering all of the data, the most likely structure is that for compound **4** in Figure 1. The quaternary carbons at 170.37 and 190.14 ppm were assigned to the two carbonyl groups. The ^{1}H NMR showed a broad one-proton triplet at δ 5.25 that was removed by H_2O-d_2, and this peak was assigned to the hydroxyl group of the hydroxymethyl substituent at C_{10}.

Comparing the chemical shifts of the carbon atoms at 106.68, C_{13}, 110.03, C_{14}, 141.50, C_{15}, and 151.46 ppm, C_{12}, with those for the carbon atoms of 2-methylfuran (Levy et al., 1980), allowed their assignment to a monosubstituted furan ring. The ^{13}C-^{1}H COSY data and proton−proton coupling constants showed the protons at δ 6.20, 6.40, and 7.56 to be the AMX system of a monosubstituted furan. The chemical shift of the methylene carbon at 27.10 ppm (C_{11}) correlated well with the methylene carbon atom of 2-furfurylidene-10-methyl-*trans*-1-decalone (Stoessl and Stothers, 1975) which was paired with the two-proton singlet at δ 3.82.

Scheme 1. Plausible Mechanism for the Formation of Compound 2



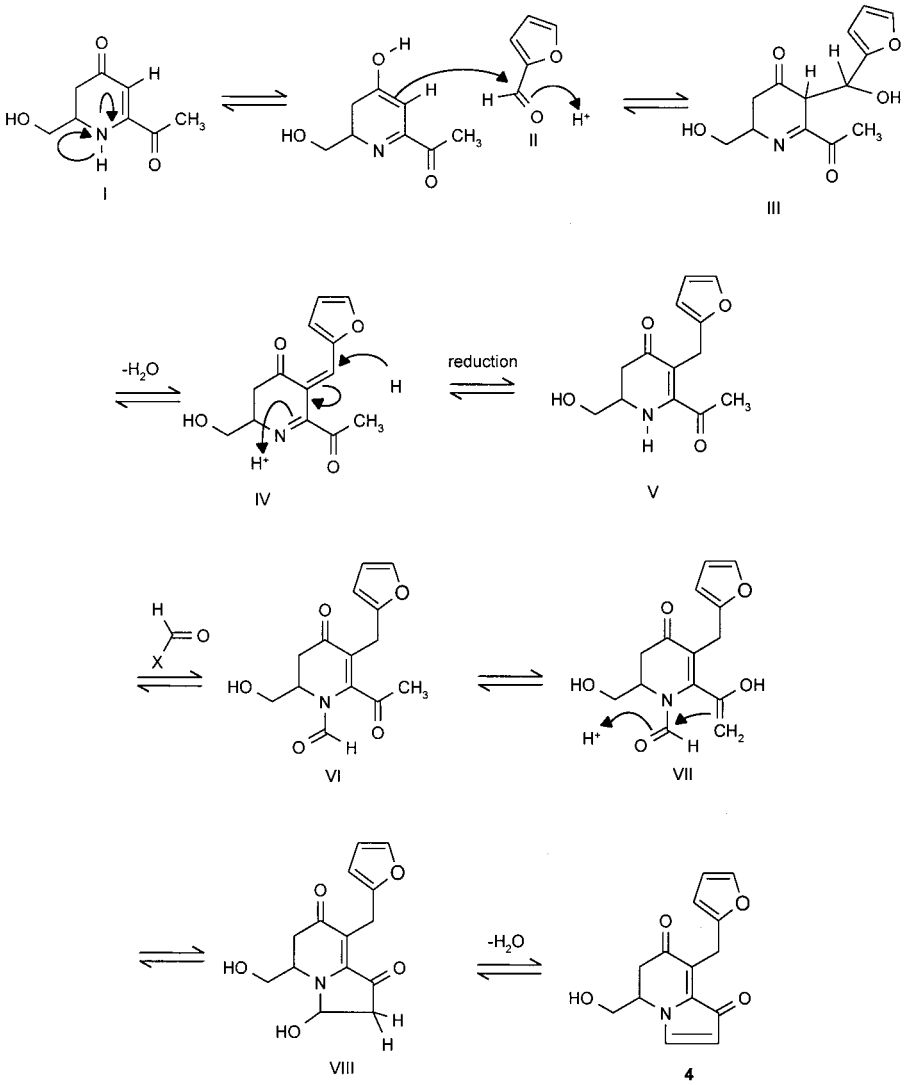
The chemical shifts of the protons at δ 2.66 and 3.00 and the carbon at 35.30 ppm (C_5) are close to the respective shifts of the ring methylene of 2-acetyl-5-hydroxymethyl-5,6-dihydro-4*H*-pyridinone (Ames et al., 1999), so these signals were assigned to the ring methylene group at C_5. The doublet of doublet splitting of each of these protons suggested an AMX system in a ring. Addition of H_2O-d_2 to the original DMSO-d_6 solution simplified the broadened "AB-like" patterns at δ 3.74 and 4.02 to two doublets of doublets, showing these protons to be part of a hydroxymethyl group. Decoupling the broad one-proton multiplet at δ 4.95 collapsed the doublet of doublets further to two doublets, suggesting hydroxymethyl substitution of a methine carbon. This decoupling also collapsed each of the protons on the C_5 doublet of doublets to doublets, showing that the proton on C_6 was coupled to the methylene groups on C_5 and C_{10}. This suggested the $-COCH_2CHCH_2OH$ substructure and a ring similar to that for 2-acetyl-5-hydroxymethyl-5,6-dihydro-4*H*-pyridinone. Four carbon atoms remained. The two quaternary carbon atoms, at 123.30 and 141.46 ppm, were assigned to the double bond in the dihydropyridinone ring. The ^{13}C-^{1}H COSY data showed the methine carbons at 119.35 (C_8) and 129.67 (C_9) ppm to be coupled to protons at δ 7.31 and 7.87, respectively. Although not in exactly similar environments, the chemical shifts of C_5 of 4-pyrrolin-2-one and C_3 of 3-pyrrolin-2-one, respectively, (Fronza and Mondelli, 1977) support the assignment of these carbon atoms to another heterocyclic ring, suggesting 8-furan-2-yl-methyl-5-hydroxymethyl-5,6-dihydroindolizine-1,7-dione for compound **4** (see Figure 1). A search of the literature confirmed this compound to be new.

Compound **4** could be formed from 2-acetyl-5-hydroxymethyl-5,6-dihydro-4*H*-pyridinone, as shown in Scheme 2. The furan substituent probably comes from furfural. Furfural is a prominent peak in the 280 nm chromatograms of xylose-lysine reacted for 6 h at 80 °C and pH 3. 2-Acetyl-5-hydroxymethyl-5,6-dihydro-4*H*-pyridinone, **I**, and furfural, **II**, may react to give the intermediate **III**, which dehydrates to **IV**. Transfer of a hydride ion to **IV** allows the positively charged nitrogen atom to regain neutrality, giving **V**. Intermediate **V** is attacked by a reagent capable of donating a one-carbon unit. One candidate for this reagent is formaldehyde which would yield the intermediate alcohol derivative and which in turn would oxidize to give the *N*-formyl derivative **VI**. Enolization of **VI** to **VII** and ring closure gives the heterocyclic ring **VIII**, which would dehydrate to compound **4**. Since electrophilic attack on indolizines is strongly directed toward position 3 of the ring, reaction of 2-acetyl-5-hydroxymethyl-5,6-dihydro-4*H*-pyridinone with formaldehyde, followed by attack of the resulting 5-hydroxymethyl-5,6-dihydroindolizine-1,7-dione by furfural, seems less likely.

Compound 5. Compound 5 was isolated from the pH 4 xylose system. Its retention time was 11.5 min on the analytical HPLC column. Its DAD spectrum revealed a maximum at $\lambda_{max} = 371$ nm, and the compound accounted for ~10% of the 360 nm chromatogram peak area, before solvent extraction. Ammonia CI-MS gave a pseudomolecular ion at *m/z* 170.0822, fitting $C_8H_{12}NO_3$ with an error of 2.9 ppm.

The ^{1}H (250 MHz, DMSO-d_6) and ^{13}C (62.8 MHz, DMSO-d_6) spectra showed all the signals obtained previously for 2-acetyl-5-hydroxymethyl-5,6-dihydro-4*H*-pyridinone (Figure 1, compound **5a**), previously identi-

Scheme 2. Plausible Mechanism for the Formation of Compound 4



fied from an aqueous xylose-glycine model system (Ames et al., 1999). However, the ^{13}C and ^{1}H NMR spectra showed signals which suggested that the substance isolated on this occasion was a mixture of tautomeric species (compounds **5a**−**c**). The ^{13}C NMR spectrum showed two additional methylene groups (at 23.74 ppm, C_3 and 30.75 ppm, C_5) and a new carbonyl group at 186.05 ppm. The ^{1}H NMR spectrum showed an additional doublet of AB quartets, centered at δ 4.44, which was collapsed to a single AB ($J_{AB} = 13.76$ Hz) by H_2O-d_2 and two singlets at δ 5.48 and δ 5.68, respectively, corresponding to protons attached to a double bond. Finally, addition of H_2O-d_2 showed three exchangeable protons, a result consistent with two hydroxyl and one imino group in the tautomeric mixture. The isolation of 2-acetyl-5-hydroxymethyl-5,6-dihydro-4*H*-pyridinone and its tautomers in the xylose-lysine system provides supporting evidence for the mechanism shown in Scheme 2, since the closure of the ring in **VI** depends on the tautomerization of the acetyl group as shown in **VII**.

Compound 6. Compound 6 was isolated from the pH 3 and 4 xylose systems. It possessed a retention time of 39.0 min on the analytical HPLC column. It was bright orange in color and possessed a maximum at 360 nm, according to the DAD spectrum, and ~13 mg was isolated from the pH 3 system. Prior to solvent extraction, it accounted for <5% of the total peak area of the 360 nm chromatogram of the pH 3 system. Ammonia CI-MS gave a pseudomolecular ion at *m*/*z* 193 for the compound isolated from both systems, confirming the molecular mass as 192 amu.

NMR experiments were performed on the same sample isolated from the pH 3 system. The ^{1}H NMR data and the suggested assignments are as follows: (250 MHz, DMSO-d_6): δ 2.28 (s, 3, C*H*$_3$), 6.62 (1, s, C*H*), 6.69 (dd, 1, C*H*, J = 3.48, 1.79 Hz), 7.06 (d, 1, C*H*, J = 3.48 Hz), 7.87 (d, 1, C*H*, J = 1.59 Hz), 8.85 (s, 1, O*H*). The ^{13}C NMR spectrum indicated 10 carbon atoms, while the DEPT spectrum showed one methyl carbon (at 12.16 ppm, C_{11}), four methine carbons (at 99.41 ppm, C_6, 113.23 ppm, C_8, 117.65, C_9 and 146.46 ppm, C_{10}), and five quaternary carbons (at 135.55 ppm, C_2, 141.86 ppm, C_5, 147.86 ppm, C_7, 162.04 ppm, C_3, and 180.58 ppm, C_4). The ^{1}H NMR spectrum was close to the literature (Severin and Krönig, 1972; Nursten and O'Reilly, 1983) spectra for 4-hydroxy-5-methyl-2-furfurylidene-3(2*H*)-furanone, and compound **6** (Figure 1) was assigned to this structure.

Although the observed coupling pattern of the furan ring protons (d, dd, and d) agrees with the coupling patterns found in the literature for compound **6**, it is not the pattern expected for the AMX system of a monosubstituted furan ring (i.e., dd, dd, dd). This

problem was resolved by irradiating the broadened and unsymmetrical signal at δ 6.62, as this restored the AMX pattern, i.e., δ A = 7.87 (dd), M = δ 7.06 (dd), and X = δ 6.69 (dd), and J_{MX} = 3.36, J_{AX} = 1.67, J_{AM} = 0.67 Hz. Thus, the doublets at δ 6.69 and δ 7.87 had been broadened by unresolved long-range coupling to the proton at δ 6.62.

In summary, three colorless compounds have been isolated and characterized from the glucose system at pH 5. Compound **1** is pyrraline, an established product of the Maillard reaction. Compound **2** is the 3-(1,2,3-trihydroxypropyl) derivative of pyrraline and is reported here for the first time. A plausible mechanism of formation is proposed involving 3-deoxyglucosone and the Amadori product. Compound **3** also possesses a pyrraline-like structure but is not fully elucidated. All three compounds possess reactive groups that would allow their further involvement in the Maillard reaction, possibly including the formation of colored materials. Compounds **4**−**6** are colored and were isolated from the pH 3 and/or 4 xylose system. To the best of our knowledge, compound **4** has not been reported previously in the literature. It is a derivative of compound **5**, previously reported by us from xylose heated with glycine (Ames et al., 1999), and a mechanism of formation is proposed. The well-known colored Maillard product, 4-hydroxy-5-methyl-2-furfurylidene-3(2*H*)-furanone, is compound **6**. The reactive groups of compounds **4**−**6** also suggest their involvement in further steps of the Maillard reaction.

ABBREVIATIONS USED

DAD, diode array detector; MS, mass spectrometry; NMR, nuclear magnetic resonance; ES-MS, electrospray mass spectrometry; CI, chemical ionization; DEPT, distortionless enhancement through polarization transfer; COSY, correlation spectroscopy.

ACKNOWLEDGMENT

We acknowledge Mr. P. Heath (Department of Chemistry, The University of Reading) for running the NMR spectra, Mr. A. Dodson (School of Food Biosciences, The University of Reading) for help in isolating the compounds, Dr. V. Fogliano and Dr. S. Monti (Università di Napoli Federico II, Italy) for running ES-MS samples, and the European Union for funding the work. This paper is dedicated to the late Professor G. Randazzo, Università di Napoli Federico II, Italy.

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Received for review June 9, 2000. Revised manuscript received September 6, 2000. Accepted September 7, 2000.

JF000722+