Analysis of Furanone, Pyranone, and New Heterocyclic Colored Compounds from Sugar−Glycine Model Maillard Systems

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Aqueous sugar (xylose or glucose)−glycine model systems were refluxed for 2 h with the pH maintained at 5. Reverse-phase HPLC of the total reaction products gave two resolved peaks (one of which was colored) for the xylose system and five resolved peaks (two of which were colored) for the glucose system. The components responsible for these peaks were isolated from the ethyl acetate extracts by semipreparative HPLC. Using mainly NMR, the colored compound from the xylose system was identified as the new 2-acetyl-6-(hydroxymethyl)-5,6-dihydro-4*H*-pyridinone. The colored compounds from the glucose system were most likely to be two novel *cis*/*trans* ring isomers of the related new compound 2-acetyl-6-hydroxy-7-(hydroxymethyl)-1,5,6,7-tetrahydro-4*H*-azepinone. These compounds are the first one-ring structures isolated from sugar−amino acid model systems that are reported to be colored. Two of the colorless components of the glucose system were identified, mainly by NMR experiments, as the related compounds 4-hydroxy-2-(hydroxymethyl)-5-methyl-3(2*H*)-furanone and 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyranone. The remaining compound from the glucose system and the colorless compound from the xylose system were identified as 5-(hydroxymethyl)furfural and 4-hydroxy-5-methyl-3(2*H*)-furanone, respectively.

Keywords: *Maillard reaction; colored compounds; 2-acetyl-6-(hydroxymethyl)-5,6-dihydro-4H-pyridinone; 2-acetyl-6-hydroxy-7-(hydroxymethyl)-1,5,6,7-tetrahydro-4H-azepinone; 4-hydroxy-2-(hydroxymethyl)-5-methyl-3(2H)-furanone; 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyranone; NMR*

INTRODUCTION

The Maillard reaction leads to the development of color when most foods are heated. The colored products of the reaction may be divided into the melanoidins, which are macromolecular, and the lower molecular weight colored reaction products (Rizzi, 1997). The first low molecular weight colored Maillard reaction product reported from a sugar−amino acid system, 2-furfurylidene-4-hydroxy-5-methyl-3(2*H*)-furanone, was isolated from xylose heated with either glycine or lysine (Severin and Krönig, 1972). Further colored sugar−amino acid reaction products have been characterized subsequently (Ledl and Severin, 1978; Nursten and O'Reilly, 1983, 1986; 'Banks et al., 1988; Ames et al., 1993; Arnoldi et al., 1997). All possess at least two rings. In addition, colored compounds in model systems based on sugar degradation products and amines simpler than amino acids have been described, in an attempt to better understand the role of colorless reaction intermediates (Hofmann, 1997; Rizzi, 1997).

In this paper we report the structures of the reaction products (colorless and colored) of aqueous xylose−glycine and glucose−glycine model systems heated under reflux with the pH maintained at 5, which were resolved from the total reaction mixture on an ODS2 high-performance liquid chromatography (HPLC) column using a water/methanol gradient.

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MATERIALS AND METHODS

Materials. The D-(+)-sugars and glycine were obtained from Aldrich Chemical Co. (Gillingham, U.K.). Xylose and glycine were 99+% grade and glucose was ACS grade. Glucose and glycine were used without further purification, whereas xylose was recrystallized 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.

HPLC Analyses. Analytical HPLC with diode array detection (DAD) was carried out using a reverse-phase ODS column and a water/methanol gradient as described by Bailey et al. (1996). DAD spectra were collected on-line during HPLC runs. Semipreparative HPLC was performed using a 25 × 0.775 cm, 5 μm particle size, Spherisorb ODS2 column (Hichrom Ltd., Theale, Reading, U.K.), with a methanol/water gradient from 5 to 25% methanol over 15 min, a flow rate of 2.5 mL/min, and an injection volume of 200 μL. The isolated compounds were obtained as solids by freeze-drying the HPLC eluates.

Mass Spectrometry (MS). Low- and high-resolution electron impact (EI) analyses and low-resolution chemical ionization (CI) analyses were performed using a Finnigan MAT95 instrument (Thermoquest, Hemel Hempstead, U.K.). Significant operating conditions were as follows: electron energy, 70 eV; accelerating voltage, 5 kV; source temperature, 180 °C for CI, 200 °C for EI; probe temperature range, 20−1600 °C over 1.1 min; resolution, 1000 for low resolution, 7000 for high resolution.

Nuclear Magnetic Resonance Spectroscopy (NMR). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker (Karlsruhe, Germany) Avance DPX 250 spectrometer at 250 and 62.8 MHz, respectively. CH_3OH-d_4, DMSO-d_6, and $CHCl_3$-d were used as solvents, and tetramethylsilane was used as the internal standard. Chemical shifts were expressed in parts per million

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(δ). Decoupling, distortionless enhancement through polarization transfer (DEPT), and $^1H-^1H$ correlation spectroscopy (COSY) experiments were also performed.

Preparation and Extraction of the Model System. Aqueous solutions (1 molal with respect to xylose or glucose and glycine) were refluxed for 120 min with the pH controlled at 5 by the automatic addition of 3 M sodium hydroxide and continuous monitoring of the pH using an autoclavable pH electrode. Further details are given in Bailey et al. (1996). The cooled total reaction products were extracted with ethyl acetate (3×170 mL). The combined solvent extracts were washed with water (10 mL) and dried over anhydrous sodium sulfate (~5 g). After filtering, the solvent was removed on a rotary evaporator with the water bath at 40 °C. The residue was dissolved in water (1 mL) and stored at −18 °C, prior to separation by semipreparative HPLC.

RESULTS

After heating, the model systems were both dark brown. Analytical HPLC of the total reaction products revealed that most of the color was due to components that were unretained by the HPLC column (Bailey et al., 1996). Previous work involving separation of the total reaction products by ultrafiltration using a membrane with a nominal molecular mass cutoff of 3000 Da has shown that the material concerned is of high molecular weight and may be considered to be melanoidins (Royle et al., 1998). Although the unretained material dominated the HPLC chromatograms of both systems (Bailey et al., 1996), two components (one of which was colored) were resolved from the xylose system (compounds **1** and **5**) and five components (two of which were colored) were resolved from the system based on glucose (compounds **2**−**4**, **6**, and **7**).

Compound 1. This compound was isolated from the xylose system as a white solid. It had a retention time of 7.53 min on the 280 nm chromatogram obtained using the analytical HPLC conditions. Its purity was ~98% at 280 nm and ~6 mg was isolated. The electronic absorption spectrum had a maximum at 287 nm and was typical of a compound possessing a furanone-like structure (Bailey et al., 1996).

The low-resolution EI MS data were as follows: 115 (49, M + 1), 114 (74, M^+), 95 (3, M − [H + H_2O]), 81 (3, M − [CH_3 + H_2O]), 73 (8), 58 (8, $COCH_2O$), 55 (17, CH_3-CCO), 43 (100, CH_3CO).

The ^1H NMR data and the suggested assignments are as follows (250 MHz $CHCl_3$-*d*): δ 2.3 (s, 3, C*H*$_3$), 4.5 (s, 2, C*H*$_2$), 6.78 (s, 1, O*H*). The observed signals from the ^{13}C NMR spectrum of compound **1** are as follows: 13.55, due to a methyl carbon; 73.02, due to a methylene carbon; 135.25, due to a methyl-substituted double-bonded carbon; 175.02, due to a hydroxyl-substituted double-bonded carbon; 196.11, due to a carbonyl carbon.

The data obtained support the identification of this compound as 4-hydroxy-5-methyl-3(2*H*)-furanone. The data agree with those given in the literature (Peer et al., 1968; Tonsbeek et al., 1968); furthermore, the mass spectrum, the HPLC retention time, and the diode array spectrum were identical to those obtained for an authentic sample. The presence of this compound was expected because it is known to be formed in substantial amounts via 2,3-enolization of the Amadori rearrangement product (ARP) when the sugar is a pentose (Ledl and Schleicher, 1990).

Compound 2. This compound was a white solid and was isolated from the glucose system. Its purity was 95% at 280 nm and ~5 mg was isolated. On the basis of the HPLC peak area data, the yield of compound **2** was ~60% of that of compound **3**. The DAD spectrum was typical of a furanone-like compound (Bailey et al., 1996), and it possessed a λ_{max} value at 287 nm. Its retention time was 6.04 min, using the analytical HPLC conditions of analysis.

The low-resolution EI MS data were as follows: 145 (2, M + 1^+), 144 (72, M^+), 126 (16), 114 (10), 113 (10), 101 (23), 84 (10), 55 (46), 43 (100), 31 (22). CI-MS gave a base peak at 145 *m*/*z*, confirming that the molecular ion was 144 amu.

The ^1H NMR data and the suggested assignments are as follows (250 MHz $CHCl_3$-*d*): δ 2.18 (s, 1, O*H*), 2.30 (s, 3, C*H*$_3$), 3.98 (m, 2, C*H*$_2$), 4.50 (m, 1, C*H*), 5.18 (s, 1, O*H*). However, the spectrum was non-first-order, making it difficult to analyze in detail, particularly the multiplets at δ 3.98 and 4.50. The chemical shifts and the coupling constants were altered by the addition of ~6% methanol-d_4 to the CH_3Cl-*d*, providing a spectrum that was more amenable to analysis. This unusual, and useful, sensitivity of the chemical shifts and coupling constants to small changes in the composition of the solvent has not been reported previously. Thus, the data are as follows (250 MHz $CHCl_3$-*d*/CH_3OH-d_4, 94:6): δ 2.25 (s, 3, C*H*$_3$), 3.88 (m, 1, C*H*$_2$ for proton A, of an ABX pattern, J_{AB} = 12.31 Hz), 3.97 (m, 1, C*H*$_2$ for proton B, of an ABX pattern, J_{AB} = 12.31 Hz), 4.48 (m, 1, C*H* for proton X of an ABX pattern, J_{AX} = 5.68 Hz, J_{BX} = 4.18 Hz). It was possible to analyze the ABX system, once the long-range coupling had been removed by decoupling and the AB coupling was estimated to be 12.31 Hz. The ABX system and coupling of 12.31 Hz suggested nonequivalent methylene protons in a six-membered ring. The ^1H NMR spectrum of compound **2** in DMSO-d_6 showed five one-proton signals, as required for the pyranone structure.

The ^{13}C spectrum of compound **2** showed signals for six carbon atoms, one of which was a carbonyl carbon. The observed signals and their assignments from the DEPT spectrum are as follows (62.8 MHz DMSO-d_6): 12.73 (methyl carbon), 60.20 (methylene carbon), 83.74 (methine carbon), 134.10 (quaternary carbon), 172.94 (quaternary carbon), 194.63 (carbonyl carbon). The combined spectral evidence suggested the pyranone structure 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one.

The ^1H NMR data obtained in $CHCl_3$-*d*/CH_3OH-d_4, 94:6, agree reasonably well with those published in the literature for this compound run in $CHCl_3$-*d* (Mills et al., 1970; Kim and Baltes, 1996). However, literature reports quote the data for the protons of the ABX system as three one-proton quartets. Computer simulation of the ABX system, based on the experimental data, gave calculated results that were very similar to the experimental values (δ 3.97, 3.88, and 4.48 ppm for protons A, B, and X, respectively; J_{AB}, J_{AX}, and J_{AB} = 12.31, 5.68, and 4.18 Hz, respectively). The MS data also showed some discrepancies with the literature. In particular, the intensity of the molecular ion was 43% of the base peak for compound **2**, but its relative abundance is only 20% for 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one, according to Kim and Baltes (1996). This difference could be due to differences in the ion source pressure between different instruments. When the ion source pressure is relatively high, the tendency is for protonation of the molecule by ion−molecule reaction.

Compound 3. This compound was isolated from the glucose system as a white solid. Using the analytical HPLC conditions, it had a retention time of 8.44 min on the 280 nm chromatogram. About 4 mg was isolated. The diode array spectrum showed a maximum at 297 nm and was typical of a compound possessing a furanone-like structure (Bailey et al., 1996). Its purity was 95% at 280 nm.

The low-resolution EI MS were as follows: 145 (3, M + 1^+), 144 (44, M^+), 118 (6), 103 (12), 101 (30), 76 (7), 73 (20), 72 (15), 61 (8), 58 (35), 55 (14), 44 (48), 43 (100), 28 (13). CI-MS gave a base peak at *m*/*z* 145, confirming that the molecular ion was 144 amu.

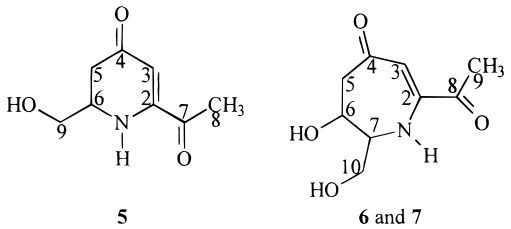
The ^{1}H NMR data and the suggested assignments are as follows (250 MHz $CHCl_3$-*d*): δ 2.10 (s, 3, C*H*$_3$), 3.30 (s, 1, O*H*), 4.02 (m, 1, C*H*), 4.45 (m, 2, C*H*$_2$), 4.92 (s, 1, O*H*). However, the spectrum was complex and non-first-order, making it difficult to analyze in detail, particularly the multiplets at δ 4.02 and 4.45. The chemical shifts and the coupling constants showed a previously unreported sensitivity to changes in solvent composition and were altered by the addition of ~10% methanol-d_4 to the CH_3Cl-*d*, providing a spectrum that was easier to interpret. Thus, the data are as follows (250 MHz $CHCl_3$-*d*/CH_3OH-d_4, 90:10): δ 2.70 (s, 3, C*H*$_3$), 4.07 (dd, 1, C*H* for proton A of an AMX system, J_{AM} = 10.515 Hz), 4.31 (dd, 1, C*H*$_2$ for proton M of an AMX system, J_{MX} = 5.2 Hz,), 4.40 (dd, 1, C*H*$_2$, for proton X of an AMX system, J_{AX} = 10.515 Hz). The methylene and methine protons appeared as an AMX system with second-order distortions. This splitting pattern could be accounted for by the coupling of the methylene protons of a hydroxymethyl group attached at C_5 of a furan ring with the methine proton attached to C_5.

The ^{13}C spectrum indicated six carbon atoms. The observed signals and their assignments from the DEPT spectrum of compound **3** are as follows (62.8 MHz DMSO-d_6): 15.31 (methyl carbon), 67.39 (methine carbon), 71.32, (methylene carbon), 131.31 (quaternary carbon), 157.73 (quaternary carbon), and 187.36 (carbonyl carbon).

The data obtained fit for 4-hydroxy-2-(hydroxymethyl)-5-methyl-3(2*H*)-furanone. They also agree reasonably well with the data reported for this compound (Ledl, 1979; Ames et al., 1997). In addition, an authentic sample of this compound gave an HPLC retention time and diode array spectrum that agreed exactly with the data for the isolated compound. Ledl (1979) reported this compound for the first time, and Hiebl et al. (1987), using MS and NMR, identified it among the reaction products of a glucose−glycine system. On the basis of the HPLC peak area data, the amount of compound **3** formed in the glucose−glycine system was ~12% of the corresponding furanone (compound **1**) formed from xylose−glycine.

Compound 4. This compound was isolated as a white solid from the glucose system. It possessed a retention time of 10.71 min using the analytical HPLC conditions and gave a diode array spectrum with maxima at 283 and 229 nm. It was identified as 5-(hydroxymethyl)-furfural (HMF) because its retention time and diode array spectrum matched exactly with those obtained for the standard compound. On the basis of the HPLC peak area data, the yield of compound **4** was ~30% of that of compound **3**.

Compound 5. Compound **5** was isolated as a yellow solid from the xylose system. It possessed a retention time of 10.45 min on the 360 nm chromatogram obtained using the analytical HPLC column, and its diode array spectrum showed a maximum at 371 nm. About 7 mg was isolated with a purity of ~95%. On the basis of the HPLC peak area data, the yield of compound **5** was ~3% of that of compound **1**.



5 **6** and **7**

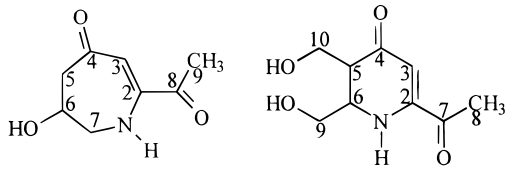
Figure 1. Structures of compounds **5**−**7**.

The low-resolution EI-MS data were as follows: 169 (18, M^+), 138 (100, M − CH_2OH), 110 (4, M − $HOCH_2$-CHNH), 96 (15, $CH_3COCCHCO$), 70 (2, CH_3COCNH), 68 (16, CH_3COCCH), 54 (16, CH_2COC), 43 (25, CH_3CO), 28 (5, CO). CI-MS, using either ammonia or methane as the reagent gas, gave spectra with the base peak at *m*/*z* 170, confirming *m*/*z* 169 as the molecular ion. High-resolution EI-MS of the molecular ion gave an accurate mass of 169.0738 amu and a best fit formula of C_8H_{11}-NO_3, with an error of 0.3 ppm.

The ^{1}H NMR data and suggested assignments are as follows (250 MHz $CHCl_3$-*d*/CH_3OH-*d*4, 9:1): δ 2.40 (m, 2, C*H*$_2$), 2.42 (s, 3, C*H*$_3$), 3.62 (m, 1, C*H*), 3.78 (m, 2, C*H*$_2$), 5.60 (s, 1, C*H*). ^{13}C NMR (62.8 MHz DMSO-d_6) revealed eight carbons, and the DEPT spectrum indicated one methyl carbon (at 25.48 ppm, C_8), two methylene carbons (at 37.83 ppm, C_5, and 63.22 ppm, C_9), two methine carbons (at 53.95 ppm, C_6, and 100.58 ppm, C_3), and three quaternary carbons (at 152.73 ppm, C_2, 195.70 ppm, C_4 or C_7, and 196.84 ppm, C_7 or C_4) (see Figure 1).

By considering all of the data, the most probable structure is that of 2-acetyl-6-(hydroxymethyl)-5,6-dihydropyridin-4*H*-one (see Figure 1). Assignment of the signal at δ 2.40 to the ring CH_2 group is in line with literature data for related compounds. For example, a signal at δ 2.45 was reported for the CH_2 group at C_5 for 2,3-dihydro-4(1*H*)-pyridinone in $CHCl_3$-*d* by Haider et al. (1975), and signals at δ 2.4 and 2.7 were assigned to the protons of the CH_2 group of *N*-propyl(or butyl)-3-hydroxy-6-(hydroxymethyl)-5,6-dihydro-4*H*-pyridinone in CH_3OH-d_4 by Schoetter et al. (1994). The carbons of the CH_2 at C_5 in the same compounds gave signals at 35.8 ppm (Haider et al., 1975) and at 36.8 ppm (Schoetter et al., 1994), respectively, that is, very close to the signal at 37.83 ppm observed for the analogous CH_2 group of compound **5**. Schoetter et al. (1994) state that a λ_{max} of 360 nm is characteristic of 3-hydroxy-5,6-dihydropyridinones. Therefore, a DAD λ_{max} of 371 nm is not unreasonable for a 5,6-dihydropyridinone with one double bond and two carbonyl groups in conjugation.

An alternative seven-membered ring structure for compound **5** (Figure 2 structure **A**) fits the empirical formula derived from the mass spectral data. However, the ^{13}C NMR data published by Goti et al. (1986), who synthesized 2-methyl-1,5,6,7-tetrahydro-4*H*-azepin-4-one, make structure **A** less probable. The ^{13}C NMR shifts of the two methylene carbons of compound **5**, at 37.8 ppm (C_5) and at 63.8 ppm (C_9), cannot be correlated with either of the methylene carbons of 2-methyl-1,5,6,7-tetrahydro(4*H*)azepin-4-one, which has shifts at 42.48



A **B**

Figure 2. Considered alternative structures for compounds **5**−**7**.

ppm (C_5) and at 46.54 ppm (C_7). Alternatively, if the hydroxyl group, placed at C_6 in structure **A**, is placed at C_7, a methylene group at C_6 is β to the carbonyl situated at C_4. The signal for C_6 should then be similar to that reported for C_4 of azepin-2-one, that is, at 23.2 ppm (Smalley, 1984), which is a long way from the signal at 63.2 ppm observed for compound **5**.

Compounds 6 and 7. These two compounds were isolated as yellow solids from the glucose system. They possessed retention times of 9.54 and 10.71 min, respectively, using the analytical HPLC conditions. Their diode array spectra were very similar, showing λ_{max} values at 373 and 371 nm, respectively. The diode array spectra of these two compounds and compound **5** were virtually superimposable. Compounds **6** and **7** were each isolated with a purity of ∼95%, and ∼5 mg of compound **6** and ∼8 mg of compound **7** were available for analysis. On the basis of the HPLC peak area data, the yields of compounds **6** and **7** were ∼6 and ∼10%, respectively, of that of compound **3**.

Low-resolution EI-MS gave the following data for compound **6**: 199 (10, M^+), 168 (5, M − CH_2OH), 138 (100, M − CH_2OH and CHOH), 128 (15, M − CH_3CO and CO), 111 (8, M − $HOCH_2CHCHCH_2OH$), 109 (8), 97 (25, $CH_3COCH{=}CCH_2OH$), 69 (5), 68 (5, CH_3COCN), 54 (16, CH_2COC), 43 (25, CH_3CO), 28 (5, CO). Similar data were collected for compound **7**: 199 (10, M^+), 168 (5), 138 (78), 128 (63), 111 (16), 109 (18), 97 (100), 84 (10), 69 (18), 55 (10), 43 (25), 41 (21), 28 (20). The major fragments were of the same mass but of different intensities for the two compounds. The molecular ion was confirmed as *m*/*z* 199 for both compounds by CI-MS, using ammonia as the reagent gas, which gave a prominent ion at *m*/*z* 200. High-resolution EI-MS of the molecular ion gave the same result for both samples. An accurate mass of 199.0842 amu was observed for a best fit formula of $C_9H_{13}NO_4$ and an error of 1.1 ppm. Therefore, compounds **6** and **7** are isomers and differ from compound **5** by CH_2O.

The ^{1}H NMR data (250 MHz $CHCl_3$-*d*/CH_3OH-d_4; 80:20) for compound **6** are as follows: δ 2.46 (s, 3, C*H*$_3$), 2.52 (m, 2, C*H*$_2$), 3.65 (m, 2, C*H*), 3.80 (m, 2, C*H*$_2$), 5.60 (s, 1, C*H*). Those for compound **7** are as follows: δ 2.48 (s, 3, C*H*$_3$), 2.50 (m, 2, C*H*$_2$), 3.70 (m, probably 4, two C*H*$_2$ and two C*H*), 5.60 (s, 1, C*H*). The data for both compounds are very similar, showing many similarities with those for compound **5**, and indicate closely related structures. The multiplet at δ 3.70 for compound **7** was less clear than the two multiplets at δ 3.65 and 3.80 for compound **6.** However, this could be due to methine and methylene protons from macromolecular material, which was more difficult to remove from compound **7** than from compound **6**. A ^{1}H−^{1}H COSY experiment on compound **7** confirmed that the methylene protons at δ 2.50 were coupled to the large multiplet at δ 3.70. No other coupling involving the methylene protons at δ 2.50 was apparent, suggesting that the signal for the methine proton on C_4 did occur in this region.

^{13}C NMR data (in $CHCl_3$-*d*/CH_3OH-d_4; 80:20) were obtained for compound **6** and **7**. For compound **6**, nine signals were observed of varying intensity. The DEPT spectrum showed the presence of one methyl carbon (at 25.48 ppm, C_9), two methylene carbons (at 36.94 ppm, C_5, and 63.59 ppm, C_{10}), three methine carbons (at 54.81 ppm, C_7, 70.91 ppm, C_6, and 100.32 ppm, C_3), and three quaternary carbons (at 153.02 ppm, C_2, 195.74 ppm, C_4 or C_8, and 197.41 ppm, C_8 or C_4), the last two being carbonyl carbons. A very similar DEPT spectrum was obtained for compound **7**, with signals at 24.70, 37.72, 54.08, 63.00, 71.93, 99.22, 152.72, 194.93, and 196.52 ppm.

A comparison of the MS data for compounds **5**−**7** indicates that all three compounds are most likely structurally related. The high-resolution data indicate that compounds **6** and **7** differ from compound **5** by CH_2O. The low-resolution spectra reveal several fragment ions that are common to two or more of these compounds. In particular, *m*/*z* 138 is the base peak for compounds **5** and **6** and the second most prominent ion for compound **7**. A comparison of the ^{1}H and ^{13}C NMR data for compounds **5**−**7** supports the similarity in structure of the three compounds. Equivalent carbons have very similar chemical shifts in all three compounds. An additional signal, at 70.91 ppm in compound **6** and at 71.93 ppm in compound **7**, accounts for C_6. The proposed seven-membered ring structure is that of the new compound, 2-acetyl-6-hydroxy-7-(hydroxymethyl)-1,5,6,7-tetrahydro-4*H*-azepinone (see Figure 1). It is the only structure that fits the complete set of NMR data for compounds **6** and **7**. The ^{13}C NMR chemical shifts of carbons 2−5 of 2-methyl-1,5,6,7-tetrahydro-4*H*-azepin-4-one (Goti et al., 1986) and the corresponding carbon atoms of compounds **6** and **7** agree reasonably well. The ^{13}C chemical shifts of the remaining carbon atoms of **6** and **7** are within normal ranges. The ^{13}C NMR data for compounds **6** and **7** were so similar that the only possible explanation is that they are *cis*/*trans* ring isomers. 2-Acetyl-6-hydroxy-7-(hydroxymethyl)-1,5,6,7-tetrahydro-4*H*-azepinone is structurally very similar to 2-acetyl-6-(hydroxymethyl)-5,6-dihydro-4*H*-pyridinone, proposed for compound **5**, thus explaining the very consistent NMR and mass spectral data for all three compounds **5**−**7**. The only slight anomaly concerns the diode array data. The diode array spectra for compounds **5**−**7** were virtually superimposable, but this would not be expected because the seven-membered ring structures proposed for compounds **6** and **7** are nonplanar (Smalley, 1984), in contrast to the six-membered planar structure proposed for compound **5**. The close similarity of the DAD spectra for compounds **5**−**7** is presumably coincidental.

Alternative six-membered ring structures for compounds **6** and **7** (Figure 2, structure **B**) fit the empirical formula derived from the mass spectral data and preserve their close similarity to the structure proposed for compound **5**. However, the overall fit of the data is less good than that for the seven-membered ring structures proposed because the ^{13}C NMR chemical shift at 36.91 (or 37.72) ppm, assigned to the methylene carbon, C_{10}, in structure **B** is outside the normal range, 45−72 ppm, for the carbon atom of a hydroxymethyl group (Günther, 1980). DEPT spectra for the 3,5-, 3,6-, 5,5-, and 6,6-di(hydroxymethyl) isomers of structure **B** would

each have revealed three methylene carbons, one methine, and four quaternary carbons and can thus be ruled out.

From the NMR, MS, and diode array data obtained for all three compounds, it is certain that compounds **6** and **7** differ from compound **5** only by CH_2O and that the most likely structures are those given in Figure 1.

DISCUSSION

All of the furans and the pyranone identified are established as products of the Maillard reaction and all, apart from HMF, form via 2,3-enolization of the ARP (Ledl and Schleicher,1990).

In the past, there has been some debate in the literature concerning the correct identification of 4-hydroxy-2-(hydroxymethyl)-5-methyl-3(2*H*)-furanone (compound **3**) and its isomeric pyranone (compound **2**), but it has been clearly shown that both compounds can form in the same system (Hiebl et al., 1987). Identification of these compounds has been based on MS and ^{1}H NMR data. We have found it very difficult to discriminate between the two compounds without the aid of ^{13}C NMR (used here) or 2D NMR experiments (Ames et al., 1997), together with ^{1}H NMR in solvents of different composition. To our knowledge, this paper is the first reporting ^{13}C NMR data and the use of ^{1}H solvent shift data for these compounds.

Although colorless themselves, compounds **1**−**4** all possess reactive groups, and it is well-known that they participate in further steps of the Maillard reaction with the formation of a range of colored and colorless compounds, depending on the system (Ledl and Schleicher, 1990; Ames, 1992). For example, compound **3** was first isolated from a glucose−glycine system by Hiebl et al. (1987), who showed that both compounds **2** and **3** yield the same pyrrolinone on reaction with primary amine (glycine methyl ester) at pH 5.6. We were surprised to identify compounds **5** as a pyridinone and compounds **6** and **7** as azepinones and to find no evidence of pyrrolinones in this study. If pyrrolinones are formed, they are either removed from the system by further reaction or they are not resolved by the chosen HPLC conditions and are probably unretained by the stationary phase. Royle et al. (1998) analyzed the same xylose−glycine and glucose−glycine model systems by capillary electrophoresis, with detection at 200 nm. About 30 peaks superimposed on a broad band of high molecular weight material for the xylose−glycine system and the glucose−glycine system showed a similar degree of complexity. None of the compounds responsible for these peaks has been identified, but it is possible that some of them are pyrrolinones.

At pH 7, in phosphate buffer, compound **3** gives compound **1** in 10% yield by retro-aldol reaction (Hiebl et al., 1987). Compound **1** can react with furfural (not identified in the xylose−glycine system) to give 2-furfurylidene-4-hydroxy-5-methyl-3(2*H*)-furanone. Although this last compound was not detected using the HPLC running conditions chosen for this study, a small peak was detected during solvent re-equilibration, isolated, and analyzed by ^{1}H NMR (A. Ravegli, The University of Milan, personal communication, 1998). Its spectrum matched that of 2-furfurylidene-4-hydroxy-5-methyl-3(2*H*)-furanone, which was first reported from a xylose−glycine system by Severin and Krönig (1972), and its identification confirms the formation of furfural in our xylose−glycine system.

The pyridinone and azepinone derivatives identified in this study have not been reported previously in the literature. However, other pyridinones, such as 5,6-dihydro-3-hydroxypyridinone, have been isolated from disaccharide-containing model systems (Schoetter et al., 1994; Pischetsrieder and Severin, 1996). Also, methylpyridinols have been reported from a glucose−glycine system (Nyhammar et al., 1983) and glucose/fructose−isoleucine systems by gas chromatography−mass spectrometry of an ether extract (Tressl et al., 1995). Cyclopent[*b*]azepin-8(1*H*)-ones have been reported as proline-specific Maillard products (Tressl et al., 1985; Chen et al., 1997), whereas hexahydroimidazo[4,5-*b*]-azepinyl derivatives were identified when glucose was reacted with butylamine and guanidine derivatives (Lederer et al., 1998).

Because compounds **5**−**7** have more carbon atoms than the sugars from which they were formed, they must have been formed by the linkage of smaller units. Compound **5** might be formed by, for example, the reaction of a three-carbon unit with a five-carbon unit by carbonium ion addition, followed by hydride transfer, dehydrations, ring closure, and nucleophilic substitution by ammonia or an amine, to give the required heterocyclic ring. Compounds **6** and **7** could be formed by an analogous route from a three-carbon unit and a six-carbon unit, resulting in seven-membered ring structures. The detailed mechanisms of formation of compounds **5**−**7** require further investigation.

Like compounds **1**−**4**, the pyridinone (compound **5**) and the azepinones (compounds **6** and **7**) all possess several reactive groups and may be expected to participate in further reactions in Maillard systems, for example, attack at the methylene group by aldehydes to give further colored compounds, including, ultimately, melanoidins.

It is of interest that 2-pyridinones are being investigated as potential non-nucleoside reverse-transcriptase inhibitors for possible use as anti-HIV agents (Tucker et al., 1996). Also, the antimicrobial activities of 2- and 4-pyridinones are being studied (Eliopoulos et al., 1996; Erol and Yulug, 1994). The pharmacological properties of compounds **5**−**7** have not been investigated.

ABBREVIATIONS USED

DAD, diode array detector; MS, mass spectrometry; NMR, nuclear magnetic resonance spectroscopy; DEPT, distortionless enhancement through polarization transfer; COSY, correlation spectroscopy; HMF, 5-(hydroxymethyl)furfural.

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