Reinvestigation of the Reaction between 2-Furancarboxaldehyde and 4-Hydroxy-5-methyl-3(2*H*)-furanone

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The reaction between 2-furancarboxaldehyde and 4-hydroxy-5-methyl-3(2*H*)-furanone was reinvestigated as a part of a systematic study on low molecular weight colored compounds from the Maillard reaction. In acetic acid/piperidine, besides 2-(2-furanylmethylene)-4-hydroxy-5-methyl-3(2*H*)-furanone (**1**) and 5-[2-(2-furanyl)ethenyl]-2-(2-furanylmethylene)-4-hydroxy-5-methyl-3(2*H*)-furanone (**2**), four novel compounds, **15a**, **15b**, **16a**, and **16b**, were isolated and characterized. These compounds are produced from two molecules of furanone **1** and one molecule of 2-furancarboxaldehyde, and a mechanism is proposed for their formation. Compounds **1**, **15a**, **15b**, **16a**, and **16b** are formed also by reacting 2-furancarboxaldehyde and 4-hydroxy-5-methyl-3(2*H*)-furanone in water at pH 3 and 2, whereas **2** was never detected. The formation of these compounds was studied also in xylose/lysine and xylose/glycine model systems.

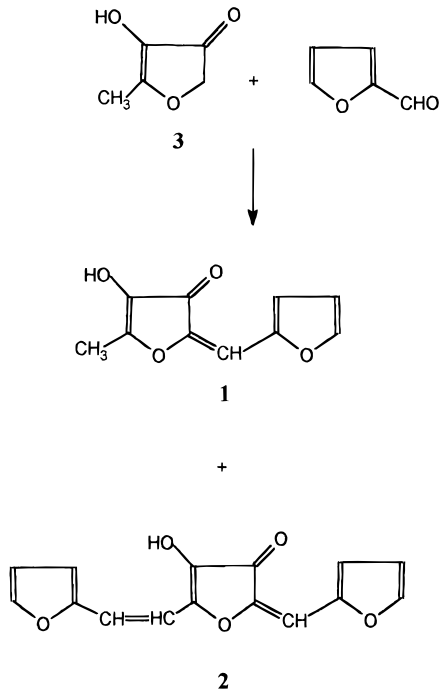
Keywords: *Maillard reaction; nonenzymic browning; colored compounds; heteronuclear two-dimensional NMR; 2-(2-furanylmethylene)-4-hydroxy-5-methyl-3(2H)-furanone; 4-hydroxy-5-methyl-3(2H)-furanone*

INTRODUCTION

The two most important sensory effects of the Maillard reaction in foods are the development of color and flavor. The formation of volatile compounds has been studied both in model systems and in foods by many authors, and several hundreds of papers have been published on this topic. In contrast to that, the data on the formation of colored compounds are amazingly scarce, mostly because the major colored compounds are melanoidins, polymers that appear to be particularly difficult to separate and purify. Until now the only colored compounds which have been fully characterized are some low molecular weight ones (Ledl and Severin, 1983; Nursten and O'Reilly, 1986; Banks et al., 1988; Ames and Nursten, 1989; Arnoldi et al., 1997; Wondrack, 1997; Hofmann, 1997, 1998a,b). Color dilution analysis (Hofmann, 1998b) permits assessment of their contribution to the overall color of reaction mixtures.

2-(2-Furanylmethylene)-4-hydroxy-5-methyl-3(2*H*)-furanone (**1**), isolated for the first time by Severin and Krönig (1972) from the reaction between xylose or arabinose and isopropylammonium acetate, belongs to this class. Ledl and Severin (1978) proposed that this compound derives from the condensation of 2-furancarboxaldehyde and 4-hydroxy-5-methyl-3(2*H*)-furanone (**3**) (Scheme 1), already isolated by them in pentose/amino acid reaction mixtures (Severin and Seilmeier, 1967), and synthesized it from the same precursors. In the meanwhile they demonstrated that 2-furancarboxaldehyde can condense further on the methyl group of compound **1** to give 5-[2-(2-furanyl)ethenyl]-2-(2-furanylmethylene)-4-hydroxy-5-methyl-3(2*H*)-furanone (**2**) (Scheme 1). Some years later, Nursten and O'Reilly (1983) isolated compound **1** from a xylose/glycine model system. Nevertheless, they could not detect compound **2** in their model systems at pH 8.2 or 6.0 and without pH control, but postulated that it could be formed at lower pH values. Ames et al. (1993) observed the formation of compound **1** in a xylose/lysine model system without pH control and, in lower amount, when the pH was kept constant at 5 by adding NaOH.

Scheme 1



As a part of a systematic study on low molecular weight colored compounds from the Maillard reaction, we decided to reinvestigate the reaction between 2-furancarboxaldehyde and 4-hydroxy-5-methyl-3(2*H*)-furanone

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3; we present here the structures and the physicochemical data of some novel compounds that were isolated from the reaction mixtures.

MATERIALS AND METHODS

Materials. Solvents and reagents were used without any purification. Anhydrous solvents were prepared with usual procedures. Methanol for HPLC was purchased from Baker, and water for HPLC was produced with a Milli-Q Water purification System (Millipore). Samples for HPLC were filtered through disposable nylon 66 filters (0.45 μm, Alltech).

HPLC. Analyses were conducted on an HP-1050 quaternary pump fitted with a Rheodyne injector (20 μL loop) and equipped with an HP-1050 diode array detector (HPLC-DAD). The system was controlled by an HP ChemStation (DOS series, Hewlett-Packard). Spectral data were recorded from 220 to 550 nm for peaks of interest (peak width = 0.05 min, threshold = 1.00 mAU).

The column was a Lichrosorb RP-18 (5 μm, 250 × 4 mm, Merck, Darmstadt, Germany), the flow rate 1 mL/min, and the gradient from 5:95 methanol/water to 100:0 methanol/water over 30 min and then 5 min isocratic.

Preparation of a UV−Visible Spectral Library. A spectral library was prepared with the purpose to obtain a fast suggestion on the possible structures of the peaks detected in HPLC. It was prepared by injecting in the HPLC-DAD methanol solutions of 40 standards, selected because they contain the most important chromophores of the Maillard reaction products. The analytical conditions were the same to be used for the analysis of the model systems. The acquired UV−visible spectra were properly selected and stored in a user spectral library.

Mass Spectrometry (MS). Direct introductions were performed on a Finnigan-MAT TSQ70 with an ICIS data system. The spectra were obtained by electron impact (EI).

LC/MS was performed on a Perkin-Elmer LC series 200 connected with a 785A UV−vis detector and coupled with an API-100 single-quadrupole mass spectrometer (Perkin-Elmer Sciex Instruments, Canada). A flow of 40 μL/min was used from the LC eluent into the spray ion source. A probe voltage of 4700 V and a clustering potential of 50 V were applied. The instrument mass-to-charge ratio (*m*/*z*) was calibrated with ions of the ammonium adduct of propylene glycol. Full-scan spectra were acquired from 100 to 700 *m*/*z* using a step size of 0.5 *m*/*z* and a dwell time of 4.201 ms. The samples were added with trifluoroacetic acid to favor ionization.

Nuclear Magnetic Resonance Spectroscopy (NMR). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-600 spectrometer at 600.1 and 150.9 MHz, respectively. $CDCl_3$ and DMSO-d_6 were used as solvents, and tetramethylsilane was used as internal standard. Chemical shifts were expressed in parts per million (δ). Heteronuclear two-dimensional ^{1}H−^{13}C correlations one-bond, HMQC (Bax and Morris, 1981), and multiple-bond, HMBC (Bax and Summers, 1986), were carried out in the ^{1}H-detected mode with broad-band decoupling in the ^{13}C domain.

Reaction of Furanone 3 and 2-Furancarboxaldehyde in Anhydrous Conditions. Compound **3** (0.52 g, 4.56 mmol) was dissolved in ethanol (4.6 mL) and warmed at 50 °C. In sequence 2-furancarboxaldehyde (0.38 mL, 4.56 mmol), piperidine (95 μL), and acetic acid (95 μL) were added, and the mixture was reacted at 50 °C for 2 h. Purification by flash chromatography on silica gel 60, using hexane/ethyl acetate 6:4, gave compound **1** (60 mg, 6.8% yield) and compound **2** (160 mg, 13% yield).

In another experiment, compound **3** (0.1 g, 0.88 mmol), 2-furancarboxaldehyde (58 μL, 0.70 mmol), piperidine (18 μL), and acetic acid (18 μL) were reacted as above following the formation of compounds **1** and **2** by HPLC to maximize compound **1**. After evaporation of the solvents, the residue was purified by flash chromatography using hexane/ethyl acetate from 6:4 to 2:8. In this way it was possible to obtain compound **1** (42.5 mg, 31.6% yield) and compound **2** (only trace). Besides these expected products, it was possible to isolate some novel compounds: **15a,b** (8 mg, 4% yield), a double peak with t_R = 15.21 and 15.40 min, and **16a,b** (10 mg, 5% yield), a double peak with t_R = 16.71 and 16.90 min.

Compound **1** has R_f 0.26 in hexane/ethyl acetate 6:4; λ_{max} 354 nm (methanol), 364 nm (water); IR 3400, 1635 cm^{-1}; EI-MS, *m*/*z* (%) 192 (94), 177 (5), 125 (5), 123 (4), 121 (100), 97 (16), 95 (11), 85 (11), 83 (12), 81 (13).

Compound **2** has R_f 0.19 in hexane/ethyl acetate 6:4; λ_{max} 312 and 407 nm (methanol); IR 3350, 1635 cm^{-1}; EI-MS, *m*/*z* (%) 270 (44), 213 (4), 185 (5), 157 (5), 129 (4), 121 (100), 105 (6), 93 (8), 78 (10).

Compound **15** has R_f 0.11 in hexane/ethyl acetate 6:4; λ_{max} 215 and 275 nm (methanol); EI-MS, *m*/*z* (%) 306 (trace), 261 (8), 233 (4), 192 (100), 163 (7), 135 (10), 106 (7); LC/MS, *m*/*z* 307 (M + 1) accompanied by an intense M + Na adduct at 329 *m*/*z* and very scarce fragments at 217 and 193 *m*/*z*.

Compound **16** has R_f 0.07 in hexane/ethyl acetate 6:4; λ_{max} 270 and 360 nm (methanol); EI-MS, *m*/*z* (%) 304 (14), 275 (7), 261 (100), 247 (13), 233 (43), 192 (65), 177 (22), 162 (32), 135 (33), 114 (26), 106 (56); LC/MS, *m*/*z* 305 (M + 1) accompanied by an intense M + Na adduct at 327 *m*/*z* and an M + K adduct at *m*/*z* 343.

Reaction of Furanone 3 and 2-Furancarboxaldehyde in Water. Compound **3** (50 mg, 0.438 mmol) was dissolved in distilled water (22 mL), 2-furancarboxaldehyde (36.5 μL, 0.44 mmol, 1:1 ratio, or 146 μL, 1.75 mmol, 4:1 ratio) was added, and the mixture was heated at 100 °C under reflux for 2 or 4 h. The reaction mixtures were cooled and analyzed in HPLC, without any treatment.

Maillard Model Systems with pH Control. The amino acid (0.1 mol) and xylose (0.1 mol) were dissolved in distilled water (100 mL) in a flask equipped with a pH electrode to monitor the pH during the heating time. The pH was adjusted to the desired value by addition of HCl or NaOH. The solution was heated for 2 h at 100 °C under reflux. The resulting brown mixture was extracted with ethyl acetate (3 × 50 mL). The combined extracts were dried with sodium sulfate, and the solvent was evaporated under vacuum at 40 °C. The residue was dissolved in methanol and analyzed by HPLC-DAD.

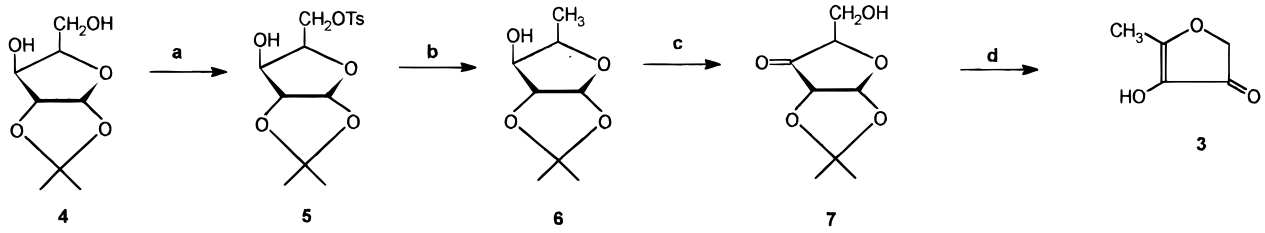
Maillard Model Systems without pH Control. They were prepared in the same way without addition of a base to keep the pH constant. During the heating time, the pH value dropped from 5.18 to 1.87 in the case of lysine and from 5.23 to 3.62 in the case of glycine.

RESULTS AND DISCUSSION

Synthesis of 4-Hydroxy-5-methyl-3(2*H*)-furanone (3). Compound **3** is the key intermediate for the synthesis of **1** and **2**. Two reaction sequences have been proposed in the literature for its synthesis. Following procedure A (Scheme 2) 1,2-*O*-isopropiliden-D-xylofuranose (**4**) is transformed in the tosyl derivative **5** by treatment with *p*-toluenesulfonyl chloride and pyridine in chloroform (Snyder and Serianni, 1987). This compound is then reduced with lithium aluminum hydride in tetrahydrofuran to the 5-deoxy derivative **6** (Snyder and Serianni, 1987), the secondary alcoholic group of which is oxidized to a keto group (compound **7**) with pyridinium chlorochromate (PCC) (Shono et al., 1983; Hollenberg et al., 1978). Deprotection of the isopropylidene group with aqueous acetic acid produces directly compound **3** (Shono et al., 1983).

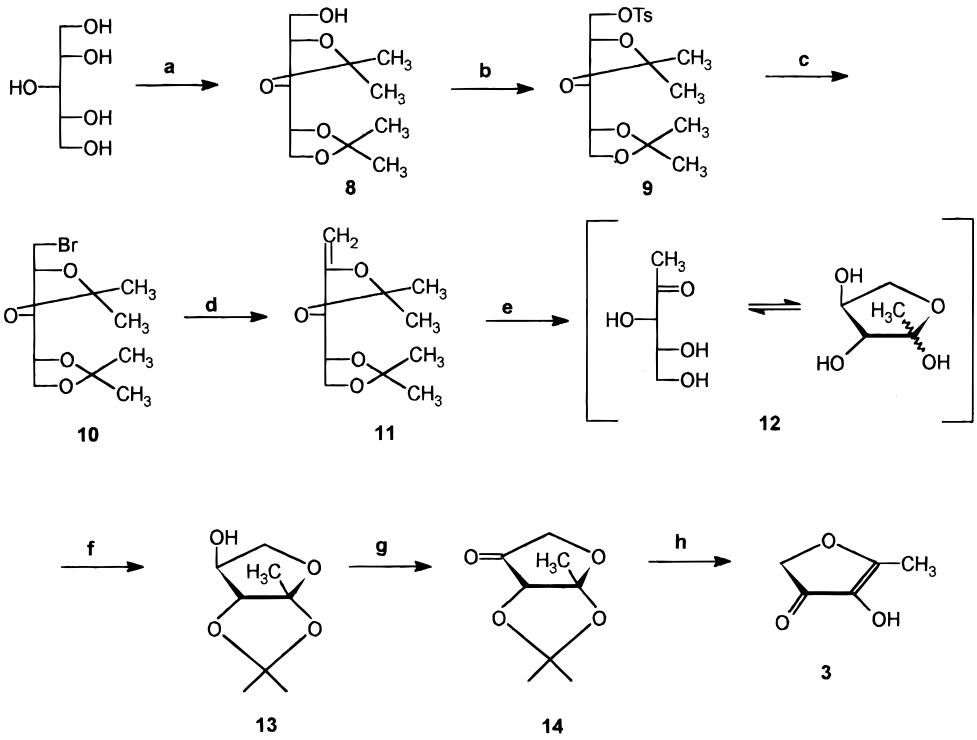
Shono et al. (1983) have proposed an alternative route starting from the inexpensive xylitol (Scheme 3), which is protected as bis(acetonide) (Hann et al., 1944) by treatment with acetone, anhydrous $CuSO_4$, and H_2SO_4. Theoretically, two isomers can be formed, but only that with a free 5-OH is obtained. This alcoholic group is tosylated (Hann et al., 1944) and then transformed in bromide **10** (Shono et al., 1983) by treatment with LiBr

Scheme 2[a]



[a] a, TsCl, pyridine (90% yield); b, $LiAlH_4$, dry THF (84% yield); c, PCC, dry CH_2Cl_2 (67% yield); d, 80% CH_3COOH (90% yield).

Scheme 3[a]



[a] a, anhydrous $CuSO_4$, acetone (96% yield); b, TsCl, pyridine (70% yield); c, LiBr, DMF (83% yield); d, KOH; e, 80% CH_3COOH; f, anhydrous $CuSO_4$, H_2SO_4, acetone (27% yield); g, PCC, dry CH_2Cl_2 (57% yield); h, 80% CH_3COOH.

in dimethylformamide (DMF). Dehydrohalogenation by distillation on pulverized KOH produces the unsaturated compound **11**. Deprotection with acetic acid produces a 2-keto derivative **12** in equilibrium with the cyclic form, which is protected as acetonide **13**. Oxidation of the secondary OH to ketone with PCC and deprotection give the expected compound **3** (Shono et al., 1983).

In our hands both routes allowed us to obtain compound **3**; whereas the former requires only four steps with good yields, the latter requires seven steps and sometimes the yields are not satisfactory. In particular, the dehydrohalogenation of compound **10** to **11** proceeds only in part, and the separation of the two compounds is rather difficult, by either distillation or column chromatography. Therefore, the sequence depicted in Scheme 2 is certainly more favorable.

Reaction of Furanone 3 with 2-Furancarboxaldehyde in Anhydrous Conditions. To obtain samples of the colored compounds **1** and **2** to submit to NMR analysis and to use as standards for quantification in model systems, the condensation of furanone **3** with 2-furancarboxaldehyde was conducted in the presence of piperidine and acetic acid in ethanol (Ledl and Severin, 1978). In a first experiment, with equimolar

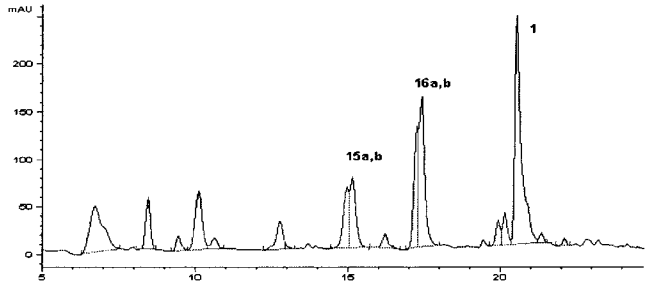


Figure 1. Chromatogram at 280 nm of the reaction between 2-furancarboxaldehyde and furanone **3** in acetic acid/piperidine.

reagents and after 2 h of heating, **2** was formed in a 2:1 ratio with respect to compound **1**. The separation of the two compounds was easily accomplished by flash chromatography. Their NMR data fit very well with those reported in the literature (Hofmann, 1998b).

Subsequently, with the aim to minimize the formation of **2**, it was decided to use different furanone/aldehyde ratios and to monitor the formation of **1** and **2** by HPLC-DAD. In particular, using an excess of furanone **3**, after 1 h only a very small amount of **2** was formed, but some novel compounds were detected (Figure 1). The first two, **15a,b**, appeared as a double peak with $t_R = 15.21$ and

Table 1. NMR Data of Compounds 16a and 16b in $CDCl_3$

^1H chemical shift[a] (ppm)	integral	multiplicity	hydrogen coupling constants *J* (Hz)	chemical shift of directly connected carbon	$^1H-^{13}C$ multiple-bond correlation
7.67	1	d	1.8	146.9	113.1−120.4−145.7
7.66	1	d	1.8	146.9	113.1−120.4−145.7
7.59	1	d	3.5	120.4	113.1−145.7
7.50	1	d	3.5	120.4	113.1−145.7
6.59	1	dd	1.8−3.5	113.1	145.7−146.9
6.57	1	dd	1.8−3.5	113.1	145.7−146.9
4.69	1	q	1	78.5	175.7−195.8
4.68	1	q	1	78.5	175.9−195.8
2.39	3	s		10.7	140.8−149.2−196.6
2.34	3	s		10.7	140.8−149.2−196.6
2.06	3	d	1	13.2	135.5−175.7
2.03	3	d	1	13.2	135.5−175.9

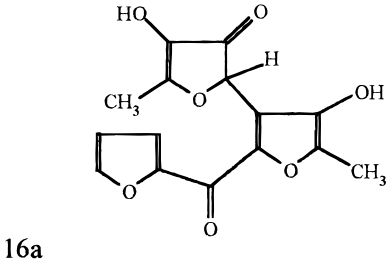
[a] Couples of peaks deriving from compounds **16a** or **16b**.

Table 2. NMR Data of Compounds 15a and 15b in $CDCl_3$

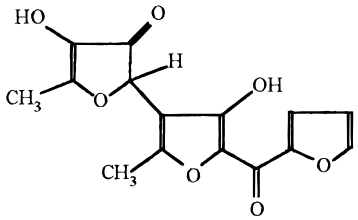
^1H chemical shift (ppm)	component[a]	integral	multiplicity	hydrogen coupling constants *J* (Hz)	chemical shift of directly connected carbon	$^1H-^{13}C$ multiple-bond correlation
7.36	I	1	d	1.7	143.0	110.1−146.8
7.35	II	1	d	1.7	143.4	110.1−146.8
6.34	I	1	d	3.5	110.6	143.0
6.31	II	1	d	3.5	110.6	143.4
6.24	I	1	dd	1.7−3.5	110.1	143.0
6.14	II	1	dd	1.7−3.5	110.1	143.4
4.75	II	1	s		81.2	195.4
4.55	I	1	s		82.4	194.8
4.32	I	1	s		46.8	142.7−149.3
3.85	II	1	s		45.5	143.5−148.6
2.26	II	3	s		13.4	135.8−176.1
2.23	I	3	s		13.4	135.8−174.3
1.92	I	3	s		13.0	46.8−143.7−148
1.90	II	3	s		13.0	45.5−143.7−148

[a] Peak 15 contains two compounds: **I** is the larger peak, **II** is the smaller.

Scheme 4

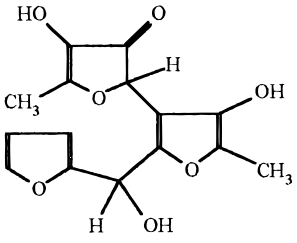


16a

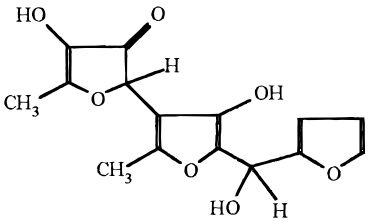


16b

Scheme 5



15a



15b

15.40 min, the other two, **16a,b**, as a double peak with $t_R = 16.71$ and 16.90 min. Compounds **15a,b** and **16a,b** were separated by flash chromatography on silica gel, and their structures were assigned by careful consideration of the NMR (Tables 1 and 2) and MS data.

The mixture **16a,b** was submitted to EI-MS and LC/MS. The EI spectrum shows the molecular peak at *m/z* 304 and the base peak at *m/z* 261, corresponding to a loss of 43. There is also an intense fragment at *m/z* 192 that corresponds to a monoadduct between 2-furancarboxaldehyde and furanone **3**. The molecular weight is formally 192 + 114 − 2 and indicates that one molecule of 2-furancarboxaldehyde and two of **3** have participated in its formation. The LC/MS confirmed the molecular weight, because the base peak is at *m/z* 305 (M + 1) and there are M + Na and M + K adducts at *m/z* 327 and 343, respectively.

The ^1H NMR and ^{13}C NMR data of **16a,b** are given in Table 1. HPLC shows the presence of two products in a ratio 1:1 that have very similar signals, which suggests that they are isomers. Some substructures were easily assigned. The hydrogen signals at 7.67 (7.66), 7.59 (7.50), and 6.59 ppm (6.57) (values in parentheses are of the second component), the chemical shifts of the corresponding carbon atoms, and the coupling constants are typical of a furan ring. The hydrogen at 4.69 ppm (4.68) has a typical vinylic value and a long-range coupling with an allylic CH_3 at 2.06

Table 3. Reaction of 2-Furancarboxaldehyde and Furanone 3 in Water

				peak areas[b]					
				products				starting reagents	
entry	molar ratio[a]	pH	time (h)	**1**	**2**	**15a,b**	**16a,b**	**3**	2-furancarboxaldehyde
1	1:1	2	2	1313	nd	10506	2000	20906	42380
2	1:4	2	2	2455	nd	28358	2832	9394	102050
3	1:4	2	4	4851	nd	28867	9862	2782	82695
4	1:1	3	2	1706	nd	3000	1140	38169	55378
5	1:4	3	2	1491	nd	9728	1394	27849	32386
6	1:4	3	4	trace	nd	9188	9698	26289	7330

[a] Millimoles of furanone **3** per millimole of 2-furancarboxaldehyde. [b] Areas of the peaks in the HPLC chromatograms (mean value of two experiments), using 254 nm for compounds **3** and 2-furancarboxaldehyde and 360 nm for compounds **1**, **15a,b**, and **16a,b**.

Table 4. Analysis of the Compounds Formed in Xylose/Glycine and Xylose/Lysine Model Systems at Different pH Values (Amounts Expressed in Millimoles)

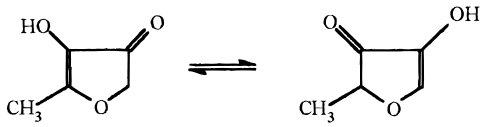
	pH						
compound	2	3	4	5	6	7	without control
			Xylose/Glycine Model Systems				
3		0.097	0.213	0.235	0.092	0.040	0.135
2-furancarboxaldehyde		1.175	trace	trace	nd	nd	0.311
1		0.195	0.047	nd	nd	nd	0.090
1 + **3**		0.292	0.260	0.235	0.092	0.040	0.225
			Xylose/Lysine Model Systems				
3	0.036	0.118	0.220	0.222	0.019		0.020
2-furancarboxaldehyde	18.056	0.603	trace	nd	nd		6.110
1	0.229	0.122	0.030	nd	nd		0.153
1 + **3**	0.265	0.240	0.250	0.222	0.019		0.173

ppm (2.03). Similar chemical shifts and coupling constant (1 Hz) were reported for compounds similar to furanone **3** [see, for example, Blank et al. (1997)]. There is also another singlet methyl group on a double bond at 2.34 ppm (2.39).

The ^{13}C NMR showed some quaternary carbons that could be assigned easily through $^{1}H-^{13}C$ heteronuclear correlations: two carbonyl groups at 195.8 and 196.6 ppm; the quaternary carbon of the furan ring (145.7 ppm); a quaternary carbon at 135.5 ppm bonded to CH_3 at 13.2 ppm; and a C═*C*H(OH)C═O at 175.7 ppm, suggesting the presence of a 2-substituted furanone for each product. Heteronuclear correlations were not able, however, to indicate how the fragments were connected because of the presence of four quaternary carbon atoms at 127.9, 140.8, 147.2, and 149.2 ppm that, giving no correlations, could not be assigned without any doubt. Nevertheless, taking into account the structure and reactivity of the starting materials, the two isomeric structures **16a** and **16b** (Scheme 4) seemed to be compatible with all spectroscopic data even if they must be considered as tentative. Unluckily, the NMR data are so similar that it was not possible to decide which signals belong to **16a** and which to **16b**. The presence of two furanone residues is explained by the excess of compound **3** that was used in these experiments.

The mixture **15a,b** was submitted to EI-MS and LC/MS analysis, too. The EI-MS spectrum showed the molecular ion at *m*/*z* 306. The molecular weight was confirmed in LC/MS by a clear M + 1 at *m*/*z* 307 and an intense M + Na adduct at *m*/*z* 329. The base peak in EI-MS is at *m*/*z* 192, and it could correspond, as described above, to a monoadduct between 2-furancarboxaldehyde and furanone **3**. The difference to 306 corresponds formally to the addition of another molecule of furanone. The ^{1}H NMR and ^{13}C NMR data (Table 2) confirmed the presence of two products formed in slightly different amounts. The presence of a furan ring having hydrogens at 7.36 (7.35), 6.34 (6.31), and 6.24 ppm (6.14) is evident (the values in parentheses are of the minor product); the ^{13}C chemical shifts and the values of the coupling constants are like those reported in the literature. There are, also, two methyl groups on double bonds and two aliphatic singlet hydrogens at 4.32 and 4.55 ppm. Comparing the NMR data of compounds **16a,b** and **15a,b**, it appears that they are very similar, but **15a,b** lack a carbonyl group, which is substituted by a singlet H at 4.32 ppm (3.85) [the carbonyl group is at 194.8 ppm (195.4)]. The hydrogen and the carbon in position 3 of the furan ring are at 6.34 ppm (6.31) and 110.6 ppm (110.6) in **15a,b** and at 7.59 ppm (7.50) and 120.3 ppm (120.3) in **16a,b**, respectively. At 146.8 ppm there is the quaternary carbon of the furan ring, at 174.3 ppm (176.1) the C═*C*H(OH)C═), and at 135.8 ppm the quaternary carbon bonded to CH_3 at 13.4 ppm. Also with compound **15a,b**, it was not possible to indicate without any doubt how the fragments are connected because of the presence of quaternary carbon atoms at 142.7 ppm (143.5), 143.7, 148, and 148.6 ppm (149.3), which could not be assigned without any doubt. Neverthless, considering all these data, it was possible to assign to **15a** and **15b** the tentative structures shown in Scheme 5. As in the case of **16a**,**b**, it was not possible to discriminate compound **15a** from **15b**.

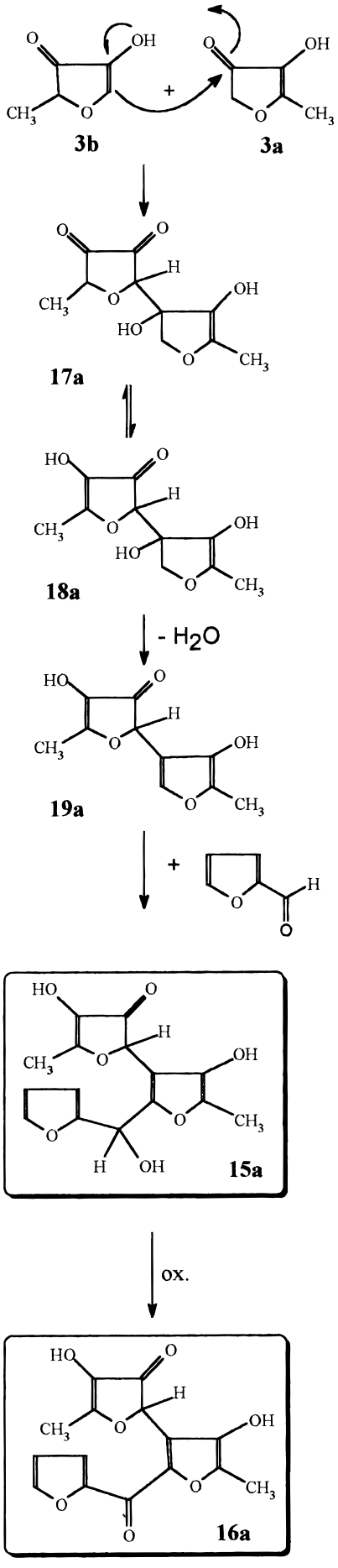
Scheme 6



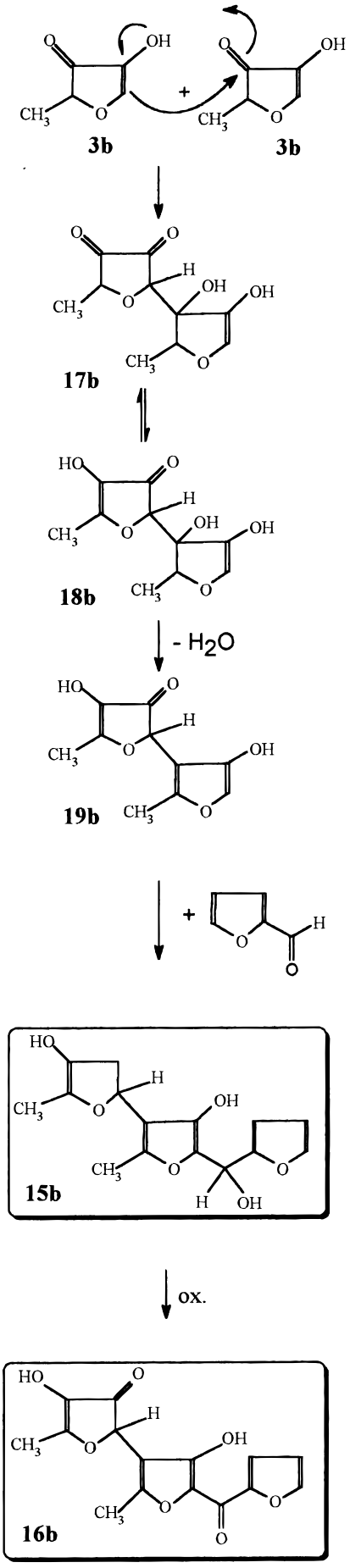
3a 3b

Reaction of 2-Furancarboxaldehyde and 4-Hydroxy-5-methyl-3(2*H*)-furanone 3 in Water. Compound **2** has never been observed in model systems, although Nursten and O'Reilly (1983) have suggested that its formation could be favored by low pH values. To find the best conditions for the formation of compounds **1** and **2** in water, solutions containing different molar ratios (1:1 and 4:1) of 2-furancarboxaldehyde and

Scheme 7



Scheme 8



4-hydroxy-5-methyl-3(2*H*)-furanone **3** were heated at 100 °C. The experiments were performed at pH 2 and 3, at which the formation of **2** should be favored, because 2-furancarboxaldehyde is the major Maillard reaction product at low pH. Most of the reaction mixtures were heated for 2 h, but in some cases the heating was continued for up to 4 h. HPLC analysis of the reaction mixtures without any purification or concentration permitted quantification of **1**, **2**, and the novel compounds **15a**, **15b**, **16a**, and **16b** (Table 3).

The conversion of the reagents in these conditions is not very high, and traces of compound **2** were observed only after 4 h of heating. The consumption of the starting substrates is faster at pH 2 than at pH 3. At pH 2, compound **1** is formed more with a 4:1 than with a 1:1 reagent ratio and continues to accumulate during further heating; at pH 3, on the contrary, compound **1** is formed more when the reagent ratio is 1:1 and tends to decrease at longer times. Either **15a,b** or **16a,b** are favored by a lower pH. With respect to **15a** and **15b**, compounds **16a** and **16b** increase by prolonging the heating time: this suggests that **15a** and **15b** are spontaneously converted to **16a** and **16b** by air oxidation. This was confirmed by heating aqueous solutions of pure **15**.

Maillard Reaction Model Systems. The formation of these compounds was studied also in xylose/lysine and xylose/glycine model systems. The targets were fundamentally two: (a) to check whether compound **2** is formed or not at low pH and (b) to obtain evidence of the formation of the novel compounds **15a**, **15b**, **16a**, and **16b**.

As it is common knowledge that pH has a critical effect on the formation of Maillard reaction products, it was decided to investigate the pH range 2−6 and to keep its value constant during the heating by the addition of a base. It must be emphasized that the pH values indicate the actual value read on the pH-meter at 100 °C. The reaction mixtures were not analyzed directly, because the chromatograms showed only large amounts of unretained compounds, perhaps melanoidins (Bailey et al., 1996), but were extracted with ethyl acetate. A check of the extraction efficacy on a standard solution showed that this procedure produces a modification of the relative amounts of colored compounds, because **1**, **2**, **16a**, and **16b** are extracted efficiently, whereas **15a** and **15b** remain in part in water; however, it was the only procedure that permitted us to obtain useful chromatograms.

The compounds that could be detected in these conditions are 2-furancarboxaldehyde, furanone **3**, and the colored compound **1**. They were quantified by the external standard method; the data are collected in Table 4. Compounds **15a**, **15b**, **16a**, **16b**, and **2** were never observed, the last not even at the lowest pH, at which the aldehyde is very abundant.

A low pH value is very favorable for the formation of 2-furancarboxaldehyde. At the same pH, this compound was detected in higher amounts in glycine/xylose than in lysine/xylose. Also, furanone **3** is detected in the model system glycine/xylose, but it has a maximum between pH 4 and 5, whereas at low pH it decreases. Compound **1** is particularly dependent on the formation of the aldehyde; therefore, it is favored in acidic conditions. Table 4 contains also the sum of the millimoles of compounds **1** and **3**, which decreases slightly as the pH increases until pH 5 and then decreases rapidly.

CONCLUSIONS

This paper confirms that compound **2** is not formed in xylose/lysine and xylose/glycine model systems, not even in very acidic conditions, at which 2-furancarboxaldehyde is very abundant. Moreover, the reaction of furanone **3** with a large excess of the aldehyde in water does not produce detectable amounts of compound **2**. This means that the aldehyde concentration is not the limiting factor for its formation, but possibly the reaction medium. Actually, according to our experience, compound **2** was observed only by reacting the substrates in acetic acid/piperidine mixtures, that is, in anhydrous conditions. The condensation of compound **1** with a second molecule of aldehyde requires that the methyl group in position 5 of the furanone residue becomes in some way electrophilic. This is possible only with an enolization of the 3-keto group, which, apparently, is possible only in the presence of acetic acid/piperidine mixtures, but not in water.

The formation of compounds **15a**, **15b**, **16a**, and **16b**, on the contrary, takes place either in water or in anhydrous conditions, and the accumulation of **16a** and **16b** after prolonged heating suggests that they derive from **15a** and **15b** by air oxidation (see the data of Table 3).

Although the structure assignment to **15a**, **15b**, **16a**, and **16b** is only tentative, it is worthwhile to make some considerations on their formation. It seems unlikely that **15a**, **15b**, and **1** derive from a common intermediate, because the double bond of **1** indicates that the elimination of water from the intermediate alcohol deriving from the condensation of furanone **3** and 2-furancarboxaldehyde is very easy. Therefore, a mechanism involving the condensation of two furanone rings as primary interaction seems more likely in the case of **15a** and **15b**.

The ^{1}H NMR of pure furanone **3** indicates that the stable form in solution is **3a** (Scheme 6), but in principle it is possible that it isomerizes to the tautomer **3b**. Two molecules of furanone **3** (one as **3a** and the other as **3b**) can condense to give an intermediate **17a**, which enolizes to **18a** and eliminates one molecule of water to give **19a**, which reacts with the aldehyde to give **15a**. This compound can, in part, be oxidized to **16a** (Scheme 6). In the meanwhile, two molecules of **3b** can condense to give **17b** that, following a similar pathway, gives rise to the isomers **15b** and **16b** (Scheme 8).

It is not clear whether compounds **15** and **16** can be formed in Maillard model systems; even if we were not able to detect them in the ethyl acetate extracts of lysine/xylose and glycine/xylose model systems, their amounts could be very low or their peaks could be covered by other major compounds.

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