Structure Determination of a Novel 3(6*H*)-Pyranone Chromophore and Clarification of Its Formation from Carbohydrates and Primary Amino Acids

O. Frank, S. Heuberger, and T. Hofmann*

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

An intensely orange compound, which has recently been evaluated as one of the main colored compounds formed in Maillard reactions of hexoses, could be unequivocally identified as (*Z*)-2-[(2-furyl)methylidene]-5,6-di(2-furyl)-6*H*-pyran-3-one (**1**) by application of several NMR and LC-MS experiments. To clarify its formation, the effectiveness of certain carbohydrate degradation products as precursors of **1** was studied in a quantitative experiment demonstrating hydroxy-2-propanone, furan-2-aldehyde, and 3-deoxy-2-hexosulose as precursors of the colorant. Site-specific labeling experiments with D-1-[^{13}C]glucose and D-6-[^{13}C]glucose, respectively, were performed to elucidate the formation pathway of **1** involving a cleavage of the hexose skeleton between carbon atoms C(5) and C(6). In addition, pentoses could be shown to generate **1** via a similar formation pathway involving the 3-deoxy-2-pentosulose.

Keywords: *Nonenzymatic browning; Maillard reaction; stable isotope labeling; color precursor; 3-deoxyosone*

INTRODUCTION

To further improve the quality of processed foods, for example, by controlling the nonenzymatic browning reaction (Maillard reaction) more efficiently, a better understanding of chromophore formation from carbohydrates is required. However, due to the complexity and multiplicity of the nonvolatile Maillard reaction products formed, surprisingly few studies have been aimed at identifying the structures of the compounds responsible for the typical brown color.

Very recently the so-called color activity concept was developed to evaluate the most intensely colored compounds in Maillard reaction mixtures by ranking them in their color contribution based on a dose/activity relationship (*1*). The striking advantage of this technique is that the key chromophores can be localized *without* knowledge of their chemical structures. This makes it possible to focus the challenging identification experiments on only these colorants, which have been found to contribute mainly to the overall color of the heated carbohydrate/amino acid mixtures (*2*).

By application of this color activity concept, an intensely orange compound was very recently evaluated as one of the key chromophores formed in an aqueous solution of L-alanine and hexoses, which was thermally treated in the presence of the carbohydrate degradation product furan-2-aldehyde (*3*). The structure of this colorant and its precursors as well as the formation route leading to this type of chromophore are, however, as yet not known.

In recent investigations, modern one- (1D) and two-dimensional (2D) NMR experiments as well as LC-MS spectrometry have been proven to be powerful tools to unequivocally determine the chemical structures of Maillard-generated colorants (*4*−*6*). Quantitative precursor studies and carefully planned labeling experiments with ^{13}C-labeled precursors were shown to enable the clarification of the reaction mechanisms converting the carbohydrate into the colored Maillard reaction products (*7*, *8*).

The objectives of the present investigation were, therefore, (i) to determine the chemical structure of this key chromophore, (ii) to identify its precursors by quantitative studies on certain Maillard reaction intermediates, and (iii) to unravel the reaction pathways governing the formation of the colorant from carbohydrates by application of ^{13}C-labeling experiments.

EXPERIMENTAL PROCEDURES

Chemicals. The following compounds were obtained commercially: D-glucose, D-1-[^{13}C]glucose, D-6-[^{13}C]glucose, L-alanine, furan-2-aldehyde, 1,3-dihydroxy-2-propanone, 2-oxopropanal (40% methylgloxal in water), glyceraldehyde, 2-propanone (Aldrich, Steinheim, Germany). Hydroxy-2-propanone (hydroxyacetone) was obtained from Fluka (Deisenhofen, Germany). Furan-2-aldehyde was distilled at 30 °C in high vacuum prior to use. Solvents were of HPLC grade (Aldrich). $CDCl_3$ was obtained from Isocom (Landshut, Germany).

Isolation of (*Z*)-2-[(2-Furyl)methylidene]-5,6-di(2-furyl)-6*H*-pyran-3-one (1) from Carbohydrate/L-Alanine Solutions Heated in the Presence of Furan-2-aldehyde. A mixture of glucose (1 mol) or xylose (1 mol), respectively, and L-alanine (0.2 mol) dissolved in phosphate buffer (1400 mL; 0.5 mol/L, pH 7.0) was refluxed for 1 h; then, furan-2-aldehyde (2 mol) was added, and heating was continued for another 3 h. After the mixture had cooled to room temperature, the pH was adjusted with aqueous hydrochloric acid (1 mol/L) to 5.0, and the solution was then extracted with ethyl acetate (10 × 100 mL). The combined organic layer was dried over Na_2SO_4 and concentrated to ∼200 mL at 25 °C in vacuo (100 mbar). To remove the volatiles, the extract was distilled in high vacuum (0.04 mbar) at 35 °C. The intensely colored

*Author to whom correspondence should be addressed (telephone +49-89-289 14170; fax +49-89-289 14183; e-mail Thomas.hofmann@lrz.tu-muenchen.de).

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Table 1. Assignment of ^{1}H NMR Signals (360 MHz, $CDCl_3$) of (*Z*)-2-[(2-Furyl)methylidene]-5,6-di(2-furyl)-6*H*-pyran-3-one (1)

H at relevant C atom[a]	δ[b]	I[c]	M[c]	J[c] (Hz)	DQF-COSY[d]
H−C(8)	6.25	1	m		H−C(7), H−C(9)
H−C(7)	6.26	1	m		H−C(8), H−C(9)
H−C(17)	6.44	1	m		H−C(16), H−C(18)
H−C(12)	6.45	1	m		H−C(11), H−C(13)
H−C(14)	6.50	1	s		
H−C(5)	6.59	1	d	2.4	H−C(3)
H−C(11)	6.70	1	d	3.4	H−C(12), H−C(13)
H−C(16)	6.85	1	d	3.4	H−C(17), H−C(18)
H−C(9)	7.36	1	d	1.7	H−C(8)
H−C(18)	7.43	1	d	1.8	H−C(17)
H−C(3)	7.45	1	d	2.4	H−C(5)
H−C(13)	7.47	1	d	1.7	H−C(12)

[a] Numbering of carbon atoms refers to structure **1** in Figure 1. [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.

residue was dissolved in ethyl acetate (15 mL), and aliquots were then fractionated by column chromatography (30 × 500 mm) on silica gel (200 g, silica gel 60, Merck, Darmstadt, Germany). After application of an aliquot of the crude material (5 mL) onto the column conditioned with *n*-pentane, chromatography was performed using *n*-pentane (400 mL; fraction A), *n*-pentane/diethyl ether (9:1, v/v; 400 mL; fraction B), *n*-pentane/diethyl ether (8:2, v/v; 400 mL; fraction C), *n*-pentane/diethyl ether (7:3, v/v; 400 mL; fraction D), and *n*-pentane/diethyl ether (6:4, v/v; 400 mL; fraction E). Fractions C−E containing an orange colorant were combined and rechromatographed on silica gel using the same conditions described above. Elution with *n*-pentane/diethyl ether (8:2, v/v; 400 mL) affords a fraction containing a deep orange compound, which was freed from solvent in vacuo and dissolved in methanol/water (8:2, v/v; 2 mL). The colored compound was further purified by flash chromatography using an RP-18 stationary phase (15.0 g; Lichroprep 25−40 μm, Merck). The solution was placed onto the column (20 × 1.6 cm), which was conditioned with methanol/water (8:2, v/v). Flushing with the same solvent mixture (70 mL) afforded a fraction containing the orange colorant. After removal of the solvent, the aqueous phase was extracted with ethyl acetate (5 × 10 mL) and, after drying over Na_2SO_4, the orange colorant was isolated in 98% purity by preparative thin-layer chromatography on silica gel (20 × 20 cm; 0.5 mm; Merck) using *n*-pentane/diethyl ether (60:40, v/v) as the eluent. An orange band at $R_f = 0.63$ was scraped off and dissolved in ethyl acetate (20 mL). After filtration, the solvent was evaporated to dryness, affording **1** as an intensely orange oil (0.7 mmol; ~0.02% in yield): LC-MS(APCI$^+$), *m/z* 309 (100, $[M + 1]^+$); UV (in MeOH) $\lambda_{max1} =$ 430 nm ($\epsilon = 0.8 \times 10^4$ L mol^{-1} cm^{-1}), $\lambda_{max2} = 379$ nm; ^{1}H and ^{13}C NMR data are listed in Tables 1 and 2.

Quantification of (*Z*)-2-[(2-Furyl)methylidene]-5,6-di-(2-furyl)-6*H*-pyran-3-one (1) in Maillard Reaction Mixtures. After cooling of the reaction mixtures, detailed in Table 3, the aqueous solution was extracted with ethyl acetate (5 × 10 mL), and the combined organic layers were dried over Na_2SO_4 and then distilled in high vacuum (0.04 mbar) at 35 °C. The residue was dissolved in ethyl acetate (5 mL) and then applied onto the top of a glass column (500 × 30 mm) filled with silica gel (200 g, silica gel 60, Merck), which was conditioned with toluene. Chromatography was performed using toluene (800 mL), followed by toluene/ethyl acetate (80:20, v/v; 400 mL), affording an intensely orange fraction, which was freed from solvent under vacuo at 25 °C and then taken up in methanol (20 mL). After membrane filtration, the fraction was analyzed by RP-HPLC. Identification of colorant **1** was performed by comparison of the LC-MS and UV−vis spectra as well as the retention time ($t_R = 44.4$ min) with those obtained for the reference compound. Quantification of **1** was performed by comparing the peak area obtained at 430 nm with that of a defined standard solution of the reference compound in methanol. The results given in Table 3 are the mean of duplicates.

Table 2. Assignment of ^{13}C NMR Signals (360 MHz, $CDCl_3$) of (*Z*)-2-[(2-Furyl)methylidene]-5,6-di(2-furyl)-6*H*-pyran-3-one (1)

H at relevant C atom[a]	δ[b]	DEPT[c]	heteronuclear ^{1}H,^{13}C multiple-quantum coherence[d] via ^1J(C,H)	via $^{2,3}J$(C,H)
C(5)	75.8	CH	H−C(5)	H−C(3), H−C(7)
C(14)	95.8	CH	H−C(14)	H−C(16)
C(7)	108.9	CH	H−C(7)	H−C(5), H−C(8), H−C(9)
C(8)	110.5	CH	H−C(8)	H−C(7), H−C(9)
C(17)	112.6	CH	H−C(17)	H−C(16), H−C(18)
C(12)	113.2	CH	H−C(12)	H−C(11), H−C(13)
C(16)	113.8	CH	H−C(16)	H−C(14), H−C(17), H−C(18)
C(11)	119.8	CH	H−C(11)	H−C(12), H−C(13)
C(3)	121.2	CH	H−C(3)	H−C(5)
C(4)	128.0	C		H−C(3), H−C(5), H−C(11)
C(9)	143.1	CH	H−C(9)	H−C(7), H−C(8)
C(18)	143.3	CH	H−C(18)	H−C(16), H−C(17)
C(13)	146.8	CH	H−C(13)	H−C(11)
C(1)	147.2	C		H−C(14)
C(15)	150.2	C		H−C(14), H−C(16), H−C(17)
C(10)	150.2	C		H−C(11), H−C(12)
C(6)	151.2	C		H−C(5), H−C(7), H−C(9)
C(2)	186.0	C		H−C(3), H−C(5), H−C(14)

[a] Numbering of carbon atoms refers to structure **1** in Figure 1. [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 ($^{2,3}J$) experiments.

Table 3. Influence of C_3 Carbohydrate Degradation Products on the Amounts of 1 Generated from Glucose/Alanine in the Presence of Furan-2-aldehyde[a]

C_3 compound	amount of **1** mg	%
(no additive)	10.5	0.05
2-propanone	10.1	0.05
2-oxopropanal	10.6	0.05
glyceraldehyde	12.0	0.06
1,3-dihydroxy-2-propanone	16.0	0.08
hydroxy-2-propanone	19.7	0.10

[a] A mixture of glucose (66.0 mmol) and L-alanine (13.0 mmol) was heated in phosphate buffer (90 mL; 0.5 mol/L, pH 7.0) under reflux for 1 h; then, the C-3 compound (5 mmol) and furan-2-aldehyde (132 mmol) were added, and heating was continued for another 2 h.

Stable Isotope Labeling Experiments. A solution of D-1-[^{13}C]glucose (6.6 mmol) or d-6-[^{13}C]glucose (2.0 mmol), respectively, and L-alanine (1.3 or 0.4 mmol) in phosphate buffer (9 or 3 mL; 0.5 mol/L, pH 7.0) was refluxed for 1 h in a closed vial. A mixture of hydroxy-2-propanone (0.5 or 0.16 mmol) and furan-2-aldehyde (13.2 or 4.0 mmol) was added, and heating was continued for another 3 h. The reacted mixture was cooled to room temperature, the pH was adjusted to 5.0, and the aqueous solution was extracted with diethyl ether (5 × 5 mL). The organic layer was dried over Na_2SO_4, the volatiles were removed in high vacuum, and the orange colorant was isolated by chromatography using silica gel and RP-18 material as described above for the nonlabeled colorant **1**.

Experiment with D-1-[^{13}C]Glucose. The purified ^{13}C-enriched 2-[(2-furyl)methylidene]-5,6-di(2-furyl)-6*H*-pyran-3-one (**e-1**) was dissolved in $CDCl_3$ and then analyzed by ^{1}H broad band decoupled ^{13}C NMR spectroscopy as well as LC-MS. Comparison of the LC-MS data with those of natural ^{13}C abundant **1** demonstrates the incorporation of one ^{13}C atom in **e-1**. LC-MS (APCI$^+$): 310 (100, $[M + 1]^+$).

Experiment with D-6-[^{13}C]Glucose. The purified target compound was analyzed by HPLC-MS showing MS data identical with those obtained for the colorant **1** isolated from a mixture of natural ^{13}C abundant glucose. LC-MS (APCI$^+$): 309 (100, $[M + 1]^+$).

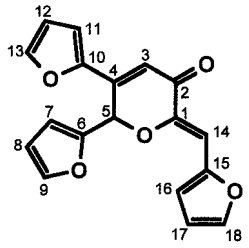

Figure 1. Chemical structure of the orange (*Z*)-2-[(2-furyl)-methylidene]-5,6-di(2-furyl)-6*H*-pyran-3-one (**1**).

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 $\times$ 250 mm, flow rate = 0.8 mL/min).

Liquid Chromatography—Mass Spectrometry (LC-MS). An analytical HPLC column (Nucleosil 100-5C18, Macherey and Nagel, Dürren, Germany) was coupled to an LCQ-MS (Finnigan MAT GmbH, Bremen, Germany) using electrospray ionization (APCI). After injection of the sample (5.0 μL), analysis was performed using a gradient starting with a 10:90, v/v, mixture of methanol and water and increasing the methanol content to 100% within 55 min.

UV—Vis Spectrocopy. UV−vis spectra were obtained using a U-2000 spectrometer (Colora Messtechnik GmbH, Lorch, Germany).

Nuclear Magnetic Resonance Spectroscopy (NMR). ^{1}H, ^{13}C, DEPT-135, DQF-COSY, TOCSY, HMQC, and HMBC experiments were performed on Bruker-AC-200 and Bruker-AM-360 spectrometers (Bruker, Rheinstetten, Germany) using the acquisition parameters described recently (*4*). ^{1}H broad band decoupled ^{13}C NMR was performed on a Bruker-AM-360 spectrometer using the following acquisition parameters: transmitter frequency, 90.56 MHz; spectral width, 25000 Hz; pulse length, 10.4 μs; recorded with 64K data points; repetition time, 2.5 s; 1 Hz line broadening; 12500 scans; processing was done by multiplication with a Lorentz−Gaussian function prior to transformation. The sample was dissolved in $CDCl_3$, chemical shifts expressed in parts per million (δ) were measured from residual $CDCl_3$ (77.0 ppm).

RESULTS AND DISCUSSION

By application of the color activity concept on an aqueous solution of glucose thermally treated in the presence of L-alanine and furan-2-aldehyde, an orange compound was found to contribute significantely to the total color of the Maillard mixture (*3*). This intensely colored reaction product exhibiting absorption maxima at 379 and 430 nm was, therefore, isolated by using several chromatographic separation techniques, and its chemical structure was then unequivocally identified by using several 1D and 2D NMR techniques, and, in addition, by LC-MS, and UV−vis spectroscopy. The spectroscopic data obtained were consistent with structure **1** outlined in Figure 1.

LC-MS with positive atmospheric pressure chemical ionization ($APCI^+$) showed an intense $[M + 1]^+$ ion at *m*/*z* 309 (100%), fitting well with the structure of **1**. The ^{1}H NMR spectrum measured in $CDCl_3$ showed 12 resonance signals corroborating the presence of 12 hydrogen atoms in structure **1**. Further NMR data, fitting well with the structure **1**, are given in Table 1. A total of three furan rings, each substituted at the 2-position, was deduced from the characteristic coupling pattern of the hydrogens H−C(7)/H−C(8)/H−C(9), H−C(11)/H−C(12)/H−C(13), and H−C(16)/H−C(17)/H−C(18). This was further confirmed by double quantum filtered homonuclear δ,δ-correlation experiments (DQF-COSY) indicating the expected strongly coupled ^{1}H spin system in the furan rings. In addition, the COSY technique revealed a connectivity between the doublets resonating at 6.59 and 7.45 ppm and exhibiting allylic coupling of 2.4 Hz. These signals were assigned as the hydrogen atoms H−C(5) and H−C(3). The chemical shift of the signal at 6.50 ppm is in the range expected for olefinic hydrogen atoms, but no coupling with other hydrogens could be observed. Heteronuclear multiple-bond coherence experiments (HMBC) optimized for $^2J_{C,H}$ and $^3J_{C,H}$ coupling constants (Table 2), however, revealed a correlation between this hydrogen assigned as H−C(14) with the furan carbon atoms C(15) and C(16) as well as with the quaternary carbon atoms C(1) and C(2) resonating at 147.2 and 186.0 ppm. These data demonstrate that the furan ring C(15−18) is directly linked at the 2-position via a methylidene-group to C(1) as proposed for structure **1**. Due to the $^3J_{C,H}$ coupling between H−C(14) and C(2), the carbonyl group must be linked to the C(1). The ^{1}H and ^{13}C chemical shifts of the structure fragment C(2)−C(1)=C(14−18) are well in line with those found for (2-furyl)methylidene groups connected to 2*H*-furan-3-ones (*9*, *10*), thus confirming the proposed partial structure of **1**. If the double bond C(1)=C(14) would be *E*-configured, then the furan hydrogen H−C(16) should be strongly deshielded due to the magnetic anisotropy of the carbonyl function. Such "proximity" interactions with the carbonyl function, having generally very pronounced effects on the chemical shifts of *peri* protons (*4*), could, however, not be observed in the NMR spectra, giving evidence that the (2-furyl)methylidene group is linked to the 6*H*-pyran-3-one ring in the *Z*-configuration.

A comparison of the ^{13}C NMR spectrum of **1**, in which 18 signals appeared, with the results of the DEPT-135 experiment revealed 12 signals corresponding to ternary and 6 signals corresponding to quaternary carbon atoms (Table 2). Unequivocal assignment of these quaternary carbon atoms could be successfully achieved by means of HMBC spectroscopy optimized for $^2J_{C,H}$ and $^3J_{C,H}$ coupling constants (Table 2). Heteronuclear correlations between the quaternary carbon atom resonating at 186.0 ppm and the methylidene proton H−C(14) and the hydrogens H−C(3) and H−(5) as well as the C,H-coupling between the latter protons and C(4) led to the unequivocal assignment of a 3(6*H*)-pyranone ring system. Further correlations between H−C(11) and C(4) as well as between H−C(7) and C(5) enable a more detailed insight into the structure of **1**, demonstrating that the two 2-furyl moieties C(10−13) and C(6−9) are directly connected with the heterocyclic core at the positions C(4) and C(5).

In summary, the obtained spectroscopical data are consistent with the proposed structure of **1** as (*Z*)-2-[(2-furyl)methylidene]-5,6-di(2-furyl)-6*H*-pyran-3-one (Figure 1). To our knowledge, the intensely orange compound **1**, showing an extinction coefficient of 0.8×10^4 L mol^{-1} cm^{-1} at 430 nm, has previously not been described in the literature.

Formation of Colorant I from Hexoses. It is obvious from the structure of **1** (Figure 1) that either three molecules of furan-2-aldehyde or other C_5 reaction intermediates are involved in the formation of (*Z*)-2-[(2-

furyl)methylidene]-5,6-di(2-furyl)-6*H*-pyran-3-one and that the remaining three carbon atoms should derive from a C_3 carbohydrate degradation product. To elucidate the origin of these three carbon atoms, in a first set of experiments, the influence of several C_3 compounds, which are well-known to be formed from carbohydrates, on the yields of **1** has been studied. To achieve this, the Maillard mixture was thermally treated in the presence of either 2-propanone, hydroxy-2-propanone, 1,3-dihydroxy-2-propanone, 2-oxopropanal, or glyceraldehyde, and the amounts of **1** formed were quantified and compared with those generated in a control experiment, in which the mixture was heated without addition of any C_3 compound. The results, given in Table 3, demonstrated that, in comparison to the control experiment, a 2-fold higher amount of **1** was produced in the Maillard mixtures containing hydroxy-2-propanone. The favored production of **1** in the presence of hydroxy-2-propanone, which was recently identified as one of the major degradation products of hexoses (*11*), documented this C_3 compound as a penultimate precursor in the formation of the chromophore.

If all three furan rings should originate from furan-2-aldehyde, then the reaction between hydroxy-2-propanone and furan-2-aldehyde should generate the colorant **1**. Independent from the reaction conditions, the target colorant was, however, not generated upon heating of aqueous solutions of these compounds (data not shown). These data indicate that besides furan-2-aldehyde and hydroxy-2-propanone, another C_5 intermediate derived from the carbon skeleton of the hexose is most likely involved in the formation of **1**. It is obvious that the cleavage of one carbon atom from the hexose skeleton is a necessary prerequisite to form the colorant **1** from glucose. Recent investigations (*7*) on chromophore formation from hexoses revealed 3-deoxy-2-hexosulose as an effective color precursor involving the liberation of a C_5 intermediate by cleavage of the C_6 skeleton between carbon atoms C(5) and C(6).

To study whether the C(1) or the C(6) is split off from the hexose skeleton and to gain insights into the pathway of how the carbon backbone is incorporated into **1**, D-1-[^{13}C]glucose and L-alanine were heated in the presence of hydroxy-2-propanone and furan-2-aldehyde, and the fate of the anomeric carbon atom of the hexose during its incorporation into the colorant was followed by means of ^{1}H broad band decoupled ^{13}C NMR spectroscopy (Figure 2). Comparing the ^{13}C NMR spectrum of the site-specific ^{13}C-enriched isotopomer of the colorant (**e-1**; A in Figure 2) with the spectrum of nonlabeled **1** (B in Figure 2) exhibits the signal at 128.0 ppm to be ^{13}C enriched. Heteronuclear correlation experiments (HMQC, HMBC) led to the assignment of the increased signal as C(4) in structure **1** (Figure 1). The position of the ^{13}C-enriched carbon atom at C(4) in **e-1** clearly demonstrates that the furan ring linked to carbon C(4) cannot originate from furan-2-aldehyde itself but from a C5 intermediate of the glucose degradation.

These data clearly showed that the hexose backbone is cleaved between C(5) and C(6). For further confirmation of this finding, D-6-[^{13}C]glucose and L-alanine were heated in the presence of the precursors hydroxy-2-propanone and furan-2-aldehyde, and the colorant formed was isolated and purified. LC-MS analysis of this colorant revealed identical MS data for the colorant produced from D-6-[^{13}C]glucose as for **1** formed from natural ^{13}C abundant glucose. These results clearly

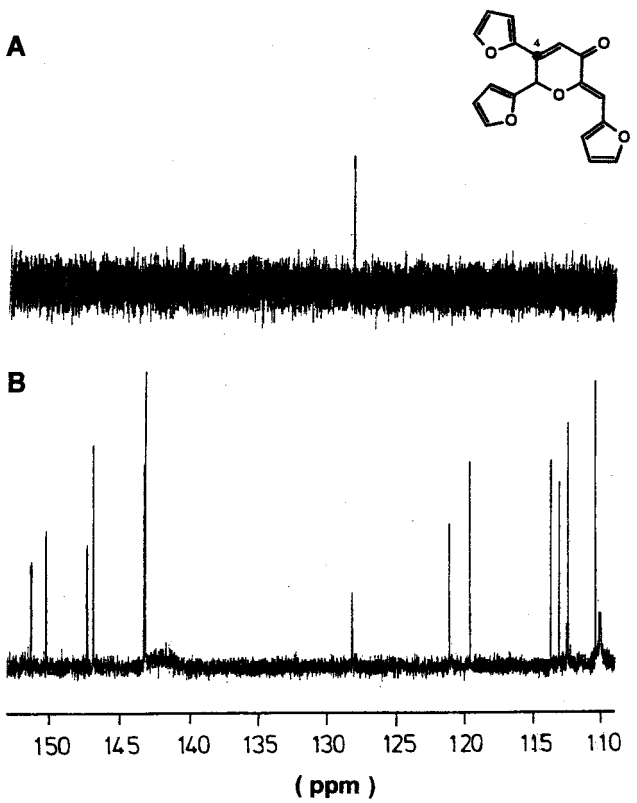

Figure 2. Excerpt of the ^{13}C NMR spectrum (360 MHz; $CDCl_3$) of **1** formed from (A) D-1-[^{13}C]glucose and (B) glucose with natural ^{13}C abundance, respectively.

demonstrated that in the formation of **1** from hexoses the carbon atom C(6) is cleaved from the carbohydrate skeleton.

On the basis of the quantitative studies and the results of the ^{13}C-labeling experiments, the reaction pathway, displayed in Figure 3, was proposed for the formation of **1**. To follow the fate of the ^{13}C label of the hexose throughout its incorporation into the colorant, the ^{13}C-enriched atoms are dotted in the structures outlined in Figure 3. Aldol-type reaction between 3-deoxy-2-hexosulose (**I**; Figure 3) and hydroxy-2-propanone forms the endiol ether **II**, which, upon condensation with one molecule of furan-2-aldehyde and water elimination, leads to the oxonium intermediate **III**. Well in line with the C(5)/C(6) sission of the hexose skeleton observed in the labeling experiment, the electron-withdrawing effect of the oxonium ion (**III**) subsequently induces retro-Aldol cleavage of formaldehyde, thereby giving rise to the furanoid compound **IV** (Figure 3). Elimination of water and enolization yield the methylene-active 5,6-di(2-furyl)-6*H*-pyran-3-one (**V**), giving rise to the colorant **1** upon condensation with one molecule of furan-2-aldehyde.

Formation of Colorant I from Pentoses. On the basis of these data, it was suggested that colorant **1** might be also formed directly via the 3-deoxy-2-osulose of pentoses. To check this assumption, an aqueous solution of xylose and L-alanine was heated in the presence of furan-2-aldehyde, and the reaction products were monitored by HPLC. After isolation and chromatographic purification, an orange colorant was isolated showing identical spectroscopic (NMR, LC-MS, UV−vis) as well as chromatographic data as found for the novel (*Z*)-2-[(2-furyl)methylidene]-5,6-di(2-furyl)-6*H*-pyran-3-one (**1**) isolated from the hexose system.

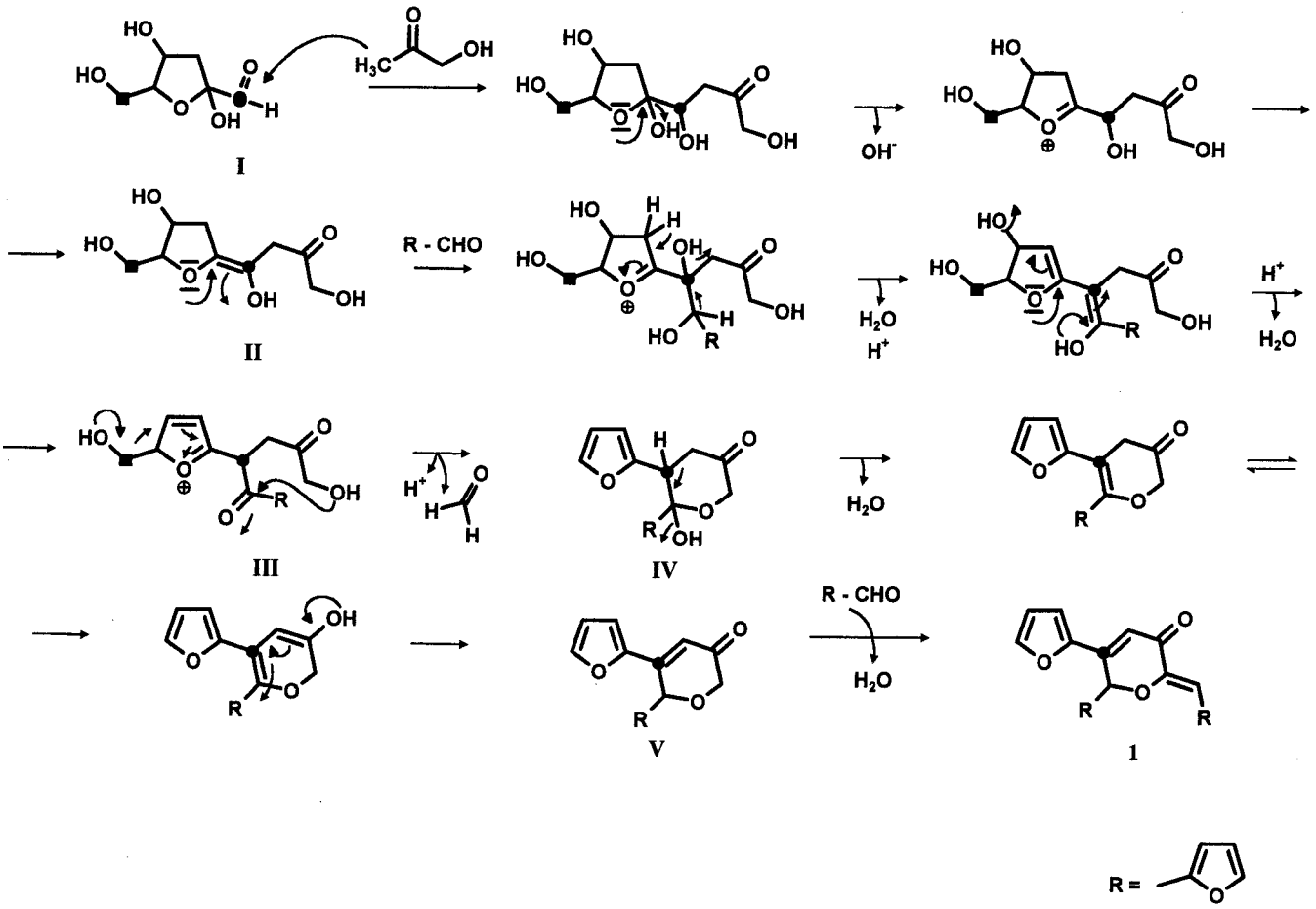

Figure 3. Formation pathway leading to chromophore **1** from hexoses (●, ^{13}C-labeled carbon atom from D-1-[^{13}C]glucose; ■, ^{13}C-labeled carbon atom from D-6-[^{13}C]glucose).

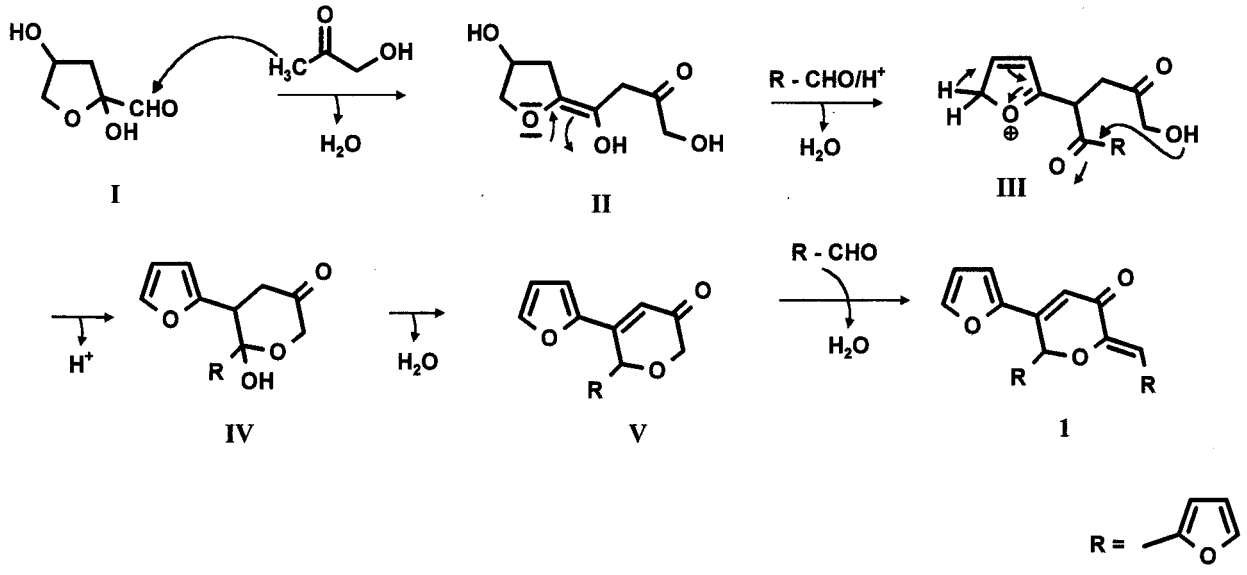

Figure 4. Formation pathway leading to chromophore **1** from pentoses.

These data clearly demonstrate that colorant **1** can also originate directly from the C_5 skeleton of a pentose. A brief reaction pathway leading to the formation of **1** from pentoses is displayed in Figure 4. In analogy to the colorant formation from hexoses, Aldol-type reaction between the 3-deoxy-2-osulose (**I**; Figure 4) and hydroxy-2-propanone leads to the enediol **II**, which forms the oxonium intermediate **III** upon condensation with furan-2-aldehyde. Aromatization by proton abstraction, water elimination, and enolization yield the methylene-active 5,6-di(2-furyl)-6*H*-pyran-3-one (**V**), giving rise to colorant **1** upon condensation with furan-2-aldehyde.

Conclusion. These data demonstrate that the identification of key chromophores by means of modern spectroscopical techniques and the clarification of precursors by quantitative studies, followed by the elucidation of reaction pathways using ^{13}C-labeling experiments, provide a suitable strategy to unravel the

puzzling network of nonenzymatic browning reactions. Details, obtained thereof, will help to construct a route map of chromogenic reactions providing a better understanding of thermally induced browning at a molecular level. On the basis of this information, the browning development during food processing might be controlled more efficiently.

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