On the Influence of the Carbohydrate Moiety on Chromophore Formation during Food-Related *Maillard* Reactions of Pentoses, Hexoses, and Disaccharides

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The influence of the carbohydrate moiety on the formation of 2-[4-oxo-3-(pyrrolidin-1-yl)cyclopent-2-en-1-ylidene]furan-3(2*H*)-one chromophores during food-related *Maillard* reactions from pentoses, hexoses, and disaccharides is reported. The orange compounds **1a**,**b** and **2a**,**b**, detected in a roasted xylose/L-proline mixture, were identified as (2*E*)/(2*Z*)-4-hydroxy-5-methyl-2-[4-oxo-3-(pyrrolidin-1-yl)cyclopent-2-ene-1-ylidene]furan-3(2*H*)-one and (2*E*)/(2*Z*)-5-methyl-2-[4-oxo-3-(pyrrolidin-1-yl)cyclopent-2-en-1-ylidene]-4-(pyrrolidin-1-yl)-furan-3(2*H*)-one, respectively, by 1D- and 2D-NMR, LC/mass, and UV/VIS spectroscopy, as well as by synthetic experiments. Studies on their formation revealed that **1a**, **b** and **2a**, **b** are formed upon condensation of pentose-derived 4-hydroxy-5-methyl- (**3**) and 5-methyl-4-(pyrrolidin-1-yl)furan-3(2*H*)-one (**4**), respectively, with 2-hydroxycyclopenta-2,4-dien-1-one (**5**) and L-proline (*Scheme 1*). Further condensation reaction of **1a**, **b** with furan-2-carbaldehyde yielded the red (2*Z*)-2-{(5*Z*)-5-[(2-furyl)methylidene]-4-oxo-3-(pyrrolidin-1-yl)cyclopent-2-en-1-ylidene}-4-hydroxy-5-methylfuran-3(2*H*)-one (**6**) as an additional novel *Maillard* chromophore. Replacement of the pentose by glucose in the mixture with L-proline led, after dry-heating, to the identification of the structurally related colored (2*Z*)/(2*E*)-2-[5-hydroxy-5-methyl-4-oxo-3-(pyrrolidin-1-yl)cyclopent-2-en-1-ylidene]-4-hydroxy-5-methylfuran-3(2*H*)-one (**7a**/**7b**) and to the characterization of 2,4,5-trihydroxy-5-methylcyclopent-2-en-1-one (**10**) and 5-hydroxy-5-methylcyclopent-3-ene-1,2-dione (**11**) as key intermediates in chromophore formation from hexoses. Comparative studies on disaccharides revealed that not **7a**/**7b**, but the colorless 4-(α-D-glucopyranosyloxy)-2-hydroxy-2-methyl-6*H*-pyran-3(2*H*)-one (**8**) and 2-(α-D-glucopyranosyloxy)-4,5-dihydroxy-5-methylcyclopent-2-en-1-one (**9**) were formed amongst the major degradation products of maltose (*Scheme 4*). The aglycons of **8** and **9** could not be liberated under food-related heating conditions, thus, inhibiting the formation of the color precursors **10** and **11** and, in consequence, of **7a**/**7b** (*Scheme 6*). These data strongly suggest that the 1,4-glycosidic linkage of disaccharides is responsible for their lower efficiency in browning development compared to pentoses or hexoses.

1. Introduction. – Besides the unique aroma, the typical brown color developing during thermal food processing mainly originates from interactions between reducing carbohydrates and amino compounds, known as the *Maillard* reaction. For instance, in coffee, marple syrup, bread crust, roasted meat, or kiln-dried malt, this browning is highly desirable and is intimately associated in consumers minds with a delicious, high-grade product. To further improve the quality of processed foods, *e.g.*, by controlling the non-enzymatic browning reaction more efficiently, a better understanding of the structures and the formation of chromophores from carbohydrates and amino acids is required.

Extensive model studies have been, therefore, performed to provide more detailed informations on the chemical nature of the chromophores formed, indicating condensation reactions between methylene-activated intermediates and carbonyl compounds as an important reaction type in nonenzymatic browning [1–4].

To gain more insight into the role of the amino-acid moiety in browning formation, one of us recently investigated amino-acid-specific, chromogenic degradation pathways of carbohydrates [5]. Several carbohydrate-derived intermediates could be successfully identified as chemical switches channeling the chromophore formation depending from the amino acid present. In the presence of primary amino acids, *e.g.*, furan-2-carboxaldehyde and acetylformoin (= 3,4-dihydroxyhex-2-ene-2,5-dione) were found as precursors of 3-oxo-1*H*-pyrrol-1-yl [6] and 2,4-dihydroxy-3-oxo-2*H*-pyrrol-1-yl chromophores [5], respectively, whereas the reaction with the secondary amino acid L-proline resulted in the formation of the yellow (2*E*,4*E*)-*N*,5-bis[(*S*)-2-carboxypyrrolidin-1-yl]-2-hydroxy-2,4-pentadien-1-iminepyrrolidin-1-yl)imine [6] and orange 4-[(alkylidene)methyl]-3-hydroxycyclopent-3-ene-1,2-diones [5].

It is long-known in the literature that, besides the amino acids, also the carbohydrates present in the food strongly influences the browning development. Recently [7], browning measurements on thermally treated solutions of various carbohydrates revealed pentoses as the most effective color precursors, closely followed by hexoses, whereas reducing disaccharides such as, *e.g.*, maltose or lactose produced browning products in comparatively low yields. The mechanisms determining the browning activities of carbohydrates are, however, as yet not entirely understood and require more detailed investigations at a molecular level.

The objectives of the present investigation were, therefore, to characterize chromophores formed from xylose, glucose, and maltose when heated in the presence of L-proline, and to study possible mechanisms determining the different efficiencies of mono- and disaccharides in chromophore formation.

2. Results and Discussion. – 2.1. *Pentoses.* Thermal treatment of xylose or ribose in the presence of L-proline under roasting conditions led rapidly to an intense browning of the reaction mixture. In a solvent extract prepared from each of these mixtures, four yellow-orange colorants could be detected by HPLC with either a diode-array detector (DAD) or an LC/MS. Two of these colored products exhibited an absorption maximum at 466 nm and showed a $[M+1]^+$ ion at *m/z* 262 (100%), whereas the other two chromophores showed an absorption maximum at 467 nm and a molecular ion at *m/z* 315 (100%). Because these compounds could be isolated from the carbohydrate/amino acid mixture only in low yields, an unequivocal assignment of the NMR data could not successfully be achieved. Based on the observation that alcoholic conditions might stabilize certain chromophores in *Maillard* reactions [8], and that L-proline is easily degraded to pyrrolidine at elevated temperatures [9][10], we studied whether the chromophores under investigation are formed in higher yields when xylose was reacted with pyrrolidine in MeOH solution. Monitoring the solvent-extractable reaction products by HPLC/DAD revealed that all four compounds were produced in significantly higher yields from xylose/pyrrolidine than from xylose/L-proline. These chromophores were, therefore, isolated from the xylose/pyrrolidine mixture and purified by chromatographic fractionation. Their structures were then determined by 1D- and 2D-NMR (*Tables 1* and *2*), LC/mass, and UV/VIS spectroscopy. The spectroscopic data of these compounds were consistent with the proposed structures for the (*E*)/(*Z*)-isomers **1a** and **1b** as well as for the (*E*)/(*Z*)-isomers **2a** and **2b**.

The isomers **1a** and **1b**, present in a ratio of 3 : 1, showed similar ^{1}H- and ^{13}C-NMR signal sets; in the following, only the structure determination of the predominant

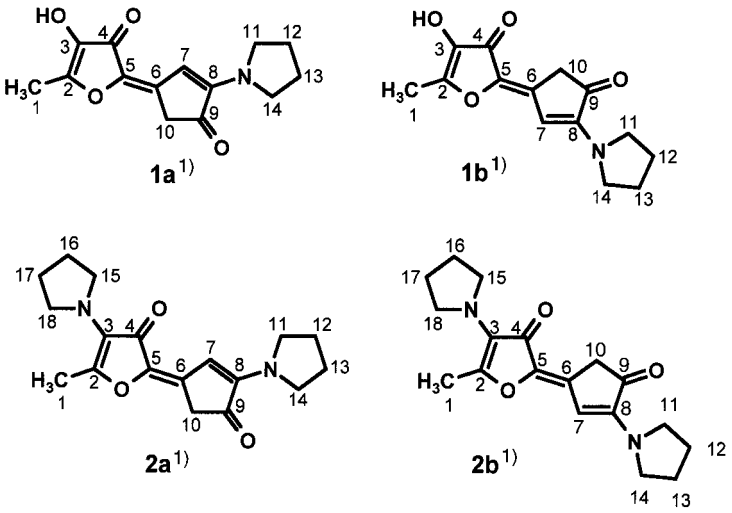


Table 1. *Assignment of ^{1}H-NMR Signals* (360 MHz, (D_6)DMSO) *of* **1a** *and* **1b**[a])

	δ [ppm][b])		Multiplicity[c])	Connectivity[d]) with
	1a	**1b**		
H–C(12)	1.86–1.88	1.87–1.89	*m*	H–C(11), H–C(13)
H–C(13)	1.86–1.88	1.87–1.89	*m*	H–C(10), H–C(12)
Me(1)	2.17	2.17	*s*	
H–C(10)	3.28	3.29	*s*	
H–C(11)	3.34-3.37	3.51-3.56	*m*	H–C(12)
H–C(14)	3.34-3.37	3.51-3.56	*m*	H–C(13)
H–C(7)	7.12	6.40	*s*	

[a]) Arbitrary numbering according to *Formulae* **1a** and **1b**; for systematic names, see *Exper. Part.* [b]) δ(H) in relation to (D_6)DMSO. [c]) Determined from the 1D spectrum. [d]) Observed homonuclear ^{1}H,^{1}H connectivities by DQF-COSY.

isomer **1a** is described in detail. The spectroscopic data allowed to assign to **1a** and **1b** the structures of (*E*)- and (*Z*)-4-hydroxy-5-methyl-2-[4-oxo-3-(pyrrolidin-1-yl)cyclopent-2-ene-1-ylidene]furan-3(2*H*)-one, respectively. To the best of our knowledge, these orange chromophores, showing an extinction coefficient of 0.65×10^4 l mol^{-1} cm^{-1} (in H_2O, pH 7.0) have previously not been described in the literature. Although a structurally related colorant synthesized from diethylamine was reported earlier [12], we could show for the first time that this type of chromophore is formed from pentoses and L-proline under food-related roasting conditions.

The ^{1}H-NMR spectrum ((D_6)DMSO) of **1a** showed 7 resonance signals, (*Table 1*). The *m* at 1.86–1.88 (4 H) and 3.34–3.37 ppm (4 H) were in the range expected for the chemical shifts of the CH_2 protons in a pyrrolidine ring. Double-quantum-filtered homonuclear δ,δ-correlation spectroscopy (DQF-COSY) revealed these 8 protons to take part in a strongly coupled ^{1}H spin system, thus confirming the pyrrolidine moiety in structure **1a**. In addition, a *s* resonating at 2.17 ppm (3 H) was assigned to Me(1)[1]), fitting well the chemical shift

[1]) Arbitrary numbering, as depicted in the *Formulae*; for systematic names, see *Exper. Part.*

Table 2. *Assignment of ^{13}C-NMR Signals* (360 MHz, (D_6)DMSO) *of* **1a** *and* **1b** [a])

	δ [ppm][b])		DEPT[c])	Heteronuclear ^{1}H,^{13}C multiple-quantum coherence[d])	
	1a	**1b**		*via* ^1J(C,H)	*via* $^{2,3}J$(C,H)
C(1)	12.2	12.3	Me	Me(1)	
C(12)	25.1	25.2	CH_2	H–C(12)	H–C(11), H–C(13)
C(13)	25.1	25.2	CH_2	H–C(13)	H–C(12), H–C(14)
C(10)	37.4	37.4	CH_2	H–C(10)	H–C(7)
C(11)	48.2	48.3	CH_2	H–C(11)	H–C(12), H–C(13)
C(14)	48.2	48.3	CH_2	H–C(14)	H–C(12), H–C(13)
C(7)	115.4	115.4	CH	H–C(7)	H–C(10)
C(5)	125.2	125.0	C		H–C(7)
C(6)	129.0	128.2	C		H–C(7), H–C(10)
C(2)	137.5	136.6	C		Me(1)
C(8)	151.3	150.9	C		H–C(7), H–C(10)
C(3)	153.0	153.2	C		Me(1)
C(4)	181.2	181.0	C		
C(9)	197.0	197.8	C		H–C(7), H–C(10)

[a]) Arbitrary numbering according to *Formulae* **1a** and **1b**; for systematic names, see *Exper. Part.* [b]) δ(C) in relation to (D_6)DMSO. [c]) DEPT-135 spectroscopy. [d]) Assignments based on HMQC (^1J) and HMBC ($^{2,3}J$) experiments.

of the Me group of colored 2-alkylidene-4-hydroxy-5-methylfuran-3(2*H*)-ones [11]. A comparison of the ^{13}C-NMR spectrum of **1a**, in which 14 signals appeared, with the results of the DEPT-135 experiment revealed 7 signals corresponding to quarternary C-atoms (*Table 2*). Unequivocal assignment of the latter could be successfully achieved by means of heteronuclear multiple-bond coherence experiments (HMBC spectroscopy) optimized for ^2J(C,H) and ^3J(C,H) coupling constants (*Table 2*). Heteronuclear correlations between the quarternary C-atom resonating at 197.0 ppm with the olefinic proton H–C(7) and the methylene protons CH_2(10) led to its unequivocal assignment as the carbonyl group C(9)[1]). In addition, heteronuclear correlations were observed between the protons Me(1) and the quarternary C(2) and C(3), thus confirming the proposed furan-3(2*H*)-one partial structure of **1a**. Considering all these spectroscopic informations, the colorants **1a** and **1b** were proposed to be (*E*)/(*Z*)-isomers of 4-hydroxy-5-methyl-2-[4-oxo-3-(pyrrolidin-1-yl)cyclopent-2-en-1-ylidene]furan-3(2*H*)-one. The configuration of the C(5)=C(6) bond in **1a** and **1b** was unequivocally deduced from the most significant-chemical shift differences of the methine proton H–C(7), which was high-field shifted by 0.72 ppm in isomer **1b**. Because the (*E*)-configuration of the C(5)=C(6) bond brings H–C(7) in planarity with C(4)=O, this H-atom was strongly deshielded in **1a**. The fact that the magnetic anisotropy of the C=O function leads to abnormally strong deshielding of a proximate H-atom was recently reported also for pyrrol-3(2*H*)-ones [2] as well as for β-pyranones [7][8].

In comparison to **1a**,**b**, the chromophores **2a**,**b** showed nearly identical UV/VIS data, but a difference in molecular mass of 53 was calculated. This might be explained by the replacement of the OH group of **1a**,**b** by an additional pyrrolidine ring. This could be unequivocally confirmed by 1D- and 2D-NMR experiments (*Tables 3* and *4*) and established the structures of **2a** and **2b** to be (*E*)- and (*Z*)-5-methyl-2-[4-oxo-3-(pyrrolidin-1-yl)cyclopent-2-en-1-ylidene)-4-(pyrrolidin-1-yl)furan-3(2*H*)-one, respectively, which, to our knowledge, were as yet not reported in the literature.

Due to their structural similarity, the chromophores **1a**,**b** and **2a**,**b** were assumed to be formed *via* similar pentose-derived precursors. Since the structure of 4-hydroxy-5-methylfuran-3(2*H*)-one (**3**), a well-known dehydration product formed from pentoses [13][14], fits well with the furan-3(2*H*)-one motive of chromophores **1a**,**b**, this

Table 3. *Assignment of ^{1}H-NMR Signals* (360 MHz, (D_6)DMSO) *of* **2a** *and* **2b**[a])

	δ [ppm][b])		Multiplicity[c])	Connectivity[d]) with
	2a	**2b**		
H–C(12)	1.75–1.86	1.86–1.89	*m*	H–C(11), H–C(13)
H–C(13)	1.75–1.86	1.86–1.89	*m*	H–C(12), H–C(14)
H–C(16)	1.75–1.88	1.86–1.89	*m*	H–C(15), H–C(17)
H–C(17)	1.75–1.88	1.86–1.89	*m*	H–C(16), H–C(18)
Me(1)	2.27	2.29	*s*	
H–C(10)	3.26	3.26	*s*	
H–C(11)	3.13–3.16	3.13–3.16	*m*	H–C(12)
H–C(14)	3.13–3.16	3.13–3.16	*m*	H–C(13)
H–C(15)	3.13–3.16	3.13–3.16	*m*	H–C(16)
H–C(18)	3.13–3.16	3.13–3.16	*m*	H–C(17)
H–C(7)	7.12	6.65	*s*	

[a]) Arbitrary numbering according to *Formulae* **2a** and **2b**; for systematic names, see *Exper. Part.* [b]) δ(H) in relation to (D_6)DMSO. [c]) Determined from 1D spectrum. [d]) Observed homonuclear ^{1}H,^{1}H connectivities by DQF-COSY.

Table 4. *Assignment of ^{13}C-NMR signals* (360 MHz, (D_6)DMSO) *of* **2a** *and* **2b**[a])

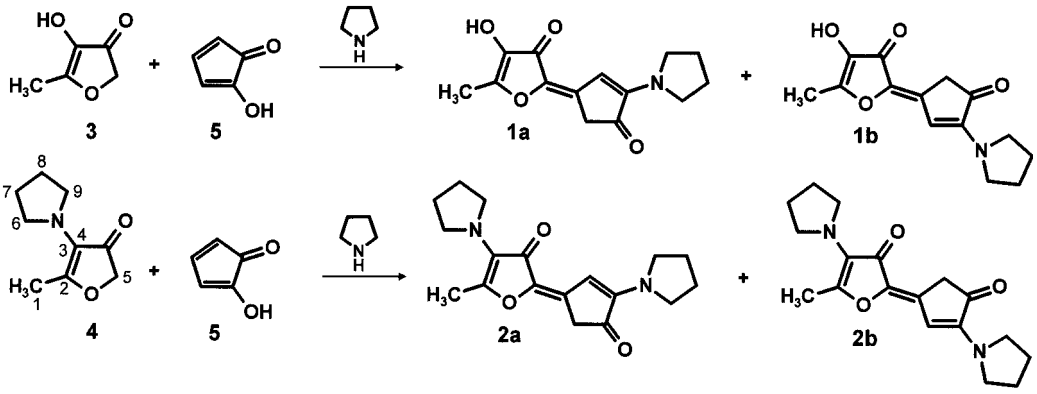
	δ [ppm][b])		DEPT[c])	Heteronuclear ^{1}H,^{13}C multiple-quantum coherence[d])	
	2a	**2b**		*via* ^1J(C,H)	*via* $^{2,3}J$(C,H)
C(1)	13.8	13.9	Me	Me(1)	
C(12)	24.4	24.5	CH_2	H–C(12)	H–C(11), H–C(13)
C(13)	24.4	24.5	CH_2	H–C(13)	H–C(12), H–C(14)
C(16)	24.4	24.5	CH_2	H–C(16)	H–C(15), H–C(17)
C(17)	24.4	24.5	CH_2	H–C(17)	H–C(16), H–C(18)
C(10)	37.3	37.3	CH_2	H–C(10)	H–C(7)
C(11)	50.1	50.2	CH_2	H–C(11)	H–C(12), H–C(13)
C(14)	50.1	50.2	CH_2	H–C(14)	H–C(12), H–C(13)
C(15)	50.1	50.2	CH_2	H–C(15)	H–C(16), H–C(17)
C(18)	50.1	50.2	CH_2	H–C(18)	H–C(16), H–C(17)
C(7)	112.9	112.9	CH	H–C(7)	H–C(10)
C(5)	128.4	128.3	C		H–C(7)
C(6)	128.9	128.0	C		H–C(7), H–C(10)
C(2)	134.3	133.3	C		Me(1)
C(8)	150.7	150.2	C		H–C(7), H–C(10)
C(3)	163.0	163.4	C		Me(1)
C(4)	181.4	181.2	C		
C(9)	196.7	198.6	C		H–C(7), H–C(10)

[a]) Arbitrary numbering according to *Formulae* **2a** and **2b**; for systematic names, see *Exper. Part.* [b]) δ(C) in relation to (D_6)DMSO. [c]) DEPT-135 spectroscopy. [d]) Assignments based on HMQC (^1J) and HMBC ($^{2,3}J$) experiments.

carbohydrate intermediate was suggested as one of the precursors. The pyrrolidine-substituted furan-3(2*H*)-one system in chromophore **2a**,**b** was assumed to be formed *via* the corresponding 5-methyl-4-(pyrrolidin-1-yl)furan-3(2*H*)-one (**4**). This hypothesis could be further corroborated by the successful isolation of **4** from the *Maillard* mixture of xylose and pyrrolidine, and its identification by ^{1}H- and ^{13}C-NMR and mass spectrometry. In addition to the furan-3(2*H*)-ones **3** and **4**, the 4-oxo-3-(pyrrolidin-1-

yl)cyclopent-2-en-1-ylidene moiety of the chromophores was assumed to be formed from 2-hydroxycyclopenta-2,4-dien-1-one (**5**) and proline-derived pyrrolidine. To confirm these intermediates as effective precursors of **1a**,**b** and **2a**,**b**, **5** was synthesized by oxidation of cyclopent-2-en-1-one with selenium(IV) oxide, and then treated with **3** or **4** in the presence of pyrrolidine (*Scheme 1*). Monitoring the reaction products by HPLC/DAD revealed that the chromophores **1a**,**b** and **2a**,**b** were formed in high yields, thereby confirming pyrrolidine and compounds **3**–**5** as key intermediates in the chromophore formation from pentoses.

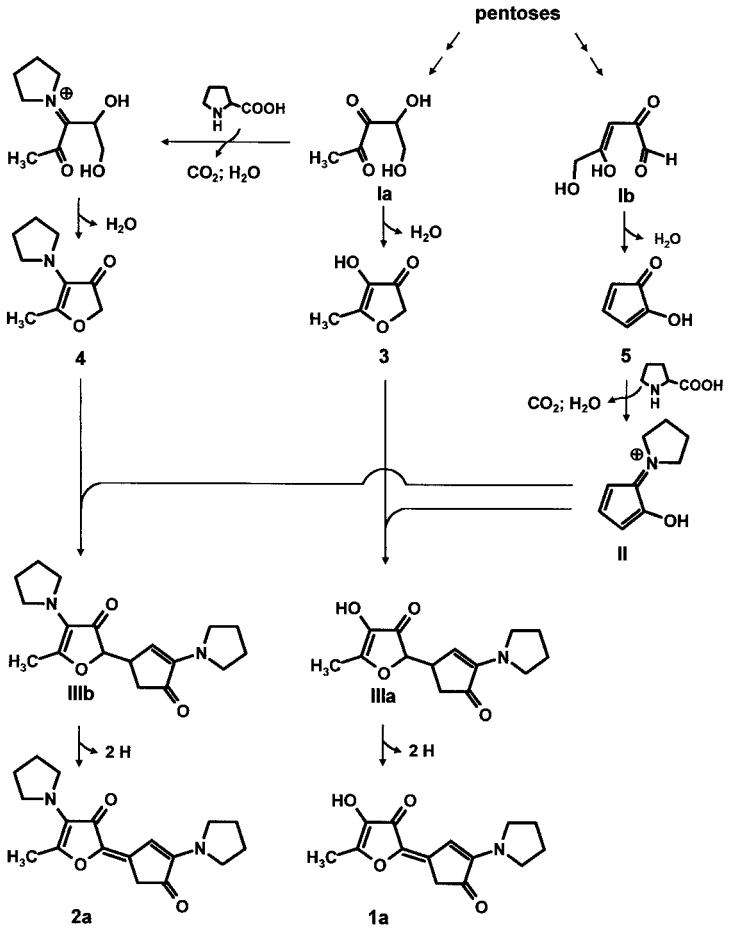
Scheme 1. *Preparation of Chromophores* **1a**,**b** *and* **2a**,**b**



On the basis of these results, a reaction pathway leading to the formation of the chromophores **1a**,**b** and **2a**,**b** from pentoses and L-proline is proposed in *Scheme 2*. Cyclization and H_2O elimination of the 1-deoxypentodiulose **Ia**, formed by an amino-acid-assisted degradation of pentoses [13], affords the furan-3(2*H*)-one **3** amongst the major reaction products [13][14]. In the presence of L-proline, *Strecker*-type reactions involving the 1-deoxypentenodiulose **Ia** lead to the formation of the corresponding furan-(2*H*)-one **4**, which could be successfully isolated from the *Maillard* reaction mixture. In competition to the formation of **Ia**, 3-deoxypentenosulose **Ib** is liberated from reducing carbohydrates [13][15]. Upon enolization, cyclization, and dehydratization, this intermediate gives rise to cyclopentadienone **5**, which, upon *Strecker*-type reaction with L-proline, is converted into the electrophilic iminium ion **II**. Reaction of the acidic methylene group of the furan-3(2*H*)-ones **3** and **4** with **II** leads to the colorless leuco bases, *i.e.*, to 4-hydroxy- and 4-(pyrrolidin-1-yl)-substituted furan-3(2*H*)-ones **IIIa** and **IIIb**, respectively, which, upon oxidation, give rise to the chromophores **1a** and **2a**, respectively, as well as to the minor isomers **1b** and **2b**. Quantitative studies performed in the absence or presence of air O_2 and/or copper ions clearly pointed out that O_2 and/or transition-metal ions are not involved in this oxidation step (data not shown). Therefore, reactive *Maillard* reaction intermediates such as, *e.g.*, di- and trioxo compounds acting as redox partners [13], have to be taken into consideration for this oxidation step, and the mechanism of such oxidations has to be elucidated by future investigations.

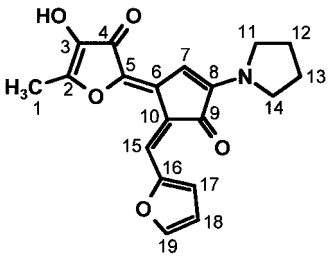
Because it is commonly believed that high-molecular melanoidins are formed by multiple condensation reactions of low-molecular-weight carbohydrate degradation

Scheme 2. *Formation Pathway Leading to Chromophores* **1a**,**b** *and* **2a**,**b** *from Pentoses and* L-*Proline*



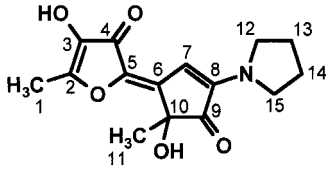
products, we examined, in addition, whether the chromophoric system of **1a**,**b** might be extended by further reactions with additional *Maillard*-reaction intermediates. The furan-3(2*H*)-ones **1a**,**b** bear an activated CH_2 group, which might act as a bridge extending the chromophoric system upon condensation with *Maillard*-generated oxo compounds. To study this in more detail, an aqueous solution of **1a**,**b** was heated in the presence of furan-2-carbaldehyde, a well-known major pentose dehydration product [13][14], whilst the color of the reaction mixture turned rapidly into deep red. An intensely colored product **6** exhibiting absorption maxima at 471 and 361 nm was isolated, purified by TLC, and analyzed by ^{1}H-NMR and LC/mass spectroscopy. The data of **6** (^{1}H-NMR: *s* at 2.24 ppm, no signal for $CH_2(10)$; abnormally strong deshielding of H–C(7) and H–C(17), *cf.* **1a**) clearly indicated that a condensation reaction had occurred between the aldehyde function of furan-2-carbaldehyde and the activated $CH_2(10)$ of **1a**,**b**, and established its structure to be the previously unknown (2*Z*)-2-

{(5*Z*)-5-[(2-furyl)methylidene]-4-oxo-3-(pyrrolidin-1-yl)cyclopent-2-en-1-ylidene}-4-hydroxy-5-methylfuran-3(2*H*)-one.

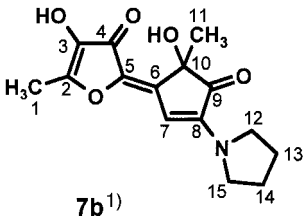


6 [1)]

2.2. *Hexoses.* To gain insight into chromogenic reaction pathways of hexoses, the xylose in the *Maillard* mixture was replaced by glucose or fructose, respectively, and the reaction products formed were monitored for colored compounds by means of HPLC (diode-array detection). An orange compound **7**, exhibiting an absorption maximum at 466 nm, was detected and analyzed by LC/MS revealing an $[M+1]^+$ ion at *m/z* 292 (100%). Due to the identical UV/VIS data as observed for **1a,b** and a difference in molecular mass of 30, we assumed a similar structure of the chromophore with an additional OH function as well as a Me group. Comparison of the ^{1}H-NMR data of chromophore **7** (*Table 5*) with the data of **1a,b**: ^{1}H-NMR (**7**): no CH_2 at 3.28/3.29 ppm (*cf. Table 1*)); additional Me signal at 2.50/2.51 ppm) indicated the presence of 5:1 mixture of (2*Z*)- and (2*E*)-4-hydroxy-2-[5-hydroxy-5-methyl-4-oxo-3-(pyrrolidin-1-yl)cyclopent-2-en-1-ylidene]-5-methylfuran-3(2*H*)-one (**7a** and **7b**, resp.). Although this type of chromophore is structurally related to a known synthetic piperidine derivative [12], its formation from hexoses and amino acids under food-related conditions has as yet not been demonstrated.



7a [1)]



7b [1)]

Assuming a similar formation as that of **1a**,**b**, a reaction pathway leading to chromophore **7a**,**b** from hexoses and L-proline is outlined in *Scheme 3*. Recent studies demonstrated that the furan-3(2*H*)-one **3** is formed from hexoses *via* 4-hydroxy-2-(hydroxymethyl)-5-methylfuran-3(2*H*)-one (**V**), a furanoid cyclization product of 1-deoxyhexenulose (**IV**), upon *retro-Michael* cleavage of formaldehyde [16]. Formation of the 2,4,5-trihydroxy-5-methylcyclopent-2-en-1-one (**VI**) *via* cyclization of the 1,5-dideoxyhexenodiulose and H_2O elimination from the vinylogous hydrate gives rise to 5-hydroxy-5-methylcyclopent-3-ene-1,2-dione (**VII**). Reaction with pyrrolidine, liberated by *Strecker*-type reactions of L-proline, yields the iminium ion **VIII**, thereby

Table 5. *Assignment of ^{1}H-NMR Signals* (360 MHz, (D_6)DMSO) *of* **7a** *and* **7b**[a])

	δ [ppm][b])		Multiplicity[c])	Connectivity[d]) with
	7a	**7b**		
H–C(13)	1.80–1.84	1.87–1.91	*m*	H–C(12), H–C(14)
H–C(14)	1.80–1.84	1.87–1.91	*m*	H–C(13), H–C(15)
Me(1)	2.26	2.25	*s*	
H–C(11)	2.50	2.51	*s*	
H–C(12)	3.17–3.20	3.17–3.20	*m*	H–C(13)
H–C(15)	3.17–3.20	3.18–3.20	*m*	H–C(14)
H–C(7)	7.05	6.59	*s*	

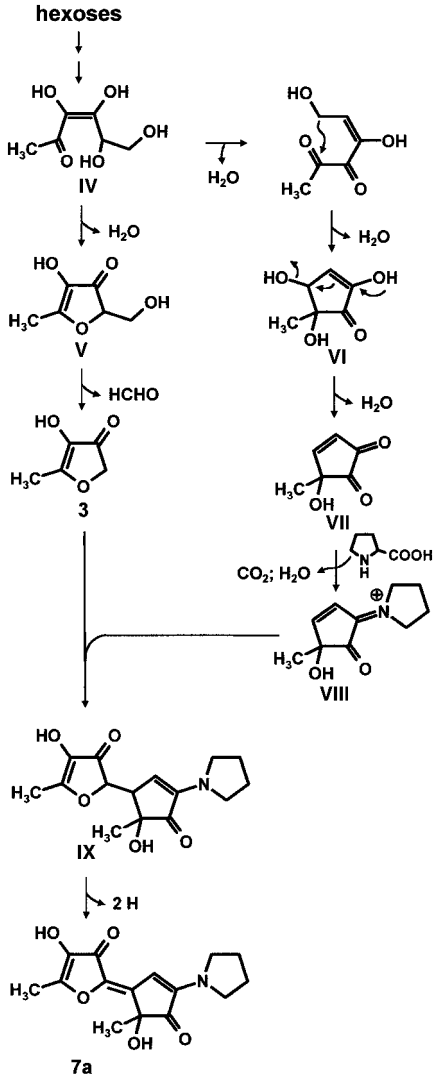
[a]) Arbitrary numbering according to *Formulae* **7a** and **7b**; for systematic names, see *Exper. Part.* [b]) δ(H) in relation to (D_6)-DMSO. [c]) Determined from 1D spectrum. [d]) Observed homonuclear ^{1}H,^{1}H connectivities by DQF-COSY.

activating the unsaturated, cyclic dione for nucleophilic addition reactions. Reaction with the methylene-activated furan-3(2*H*)-one **3** reveals the leuco base 4-hydroxy-2-[5-hydroxy-5-methyl-4-oxo-3-(pyrrolidin-1-yl)cyclopent-2-en-1-yl]-5-methylfuran-3(2*H*)-one (**IX**), which is finally oxidized to form the major isomer **7a**, besides the minor isomer **7b**.

2.3. *Disaccharides.* In comparison to reducing pentoses and hexoses, thermal treatment of reducing disaccharides such as, *e.g.*, maltose with amino acids led only to a relatively weak browning of the reaction mixture [7]. This is well in line with our finding that 2-[4-oxo-3-(pyrrolidin-1-yl)cyclopent-2-en-1-ylidene]furan-3(2*H*)-one-type chromophores, such as, *e.g.*, **1a**,**b** or **7a**,**b** formed by *Maillard* reactions from pentoses and hexoses, were not detectable by HPLC/MS and HPLC/DAD analysis of a maltose/proline reaction mixture. Instead of such chromophores, two colorless products, both showing an $[M+1]^+$ ion at *m/z* 307, were detected. To examine whether the formation of these colorless compounds might be somehow related to the lack of chromophore formation, both products were isolated from a heated solution of the synthetic *Amadori* product *N*-(1-deoxy-D-maltulos-1-yl)-L-proline (**X**; in *Scheme 4*), the primary reaction product formed from maltose and L-proline. By comparison of the LC/MS and the ^{1}H-NMR data, as well as the retention times (reversed-phase HPLC (*RP-18*)) with those obtained for the synthetic reference compounds, the two colorless products were identified as 4-(α-D-glucopyranosyloxy)-2-hydroxy-2-methyl-2*H*-pyran-3(6*H*)-one (**8**) and 2-(α-D-glucopyranosyloxy)-4,5-dihydroxy-5-methylcyclopent-2-en-1-one (**9**; see *Scheme 4*). HPLC Analysis of reacted solutions of the *Amadori* product **X**, varying in the heating time, revealed an equilibrium between the two glucosides **8** and **9**, with the pyranone **8** predominating at the beginning of the thermal treatment, whereas **8** was preferentially produced after longer heating periods, thereby confirming the observations reported in [17].

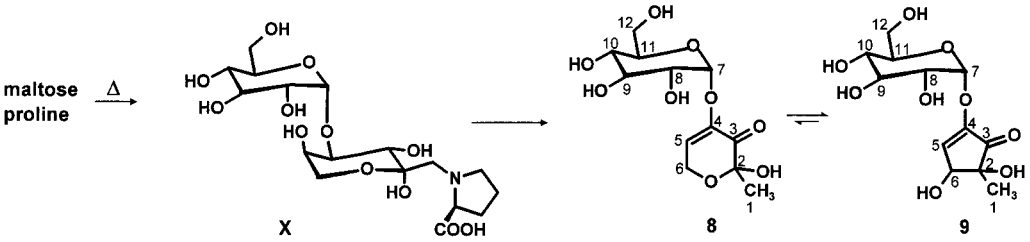
The elucidation of the structure of **9** clearly demonstrated the aglycon to be the 2,4,5-trihydroxy-5-methylcyclopent-2-en-1-one (**10**), which was proposed as a key intermediate in the formation of **7a**,**b** from hexoses (see **VI** in *Scheme 3*). Due to the 1,4-glycosidically linked glucose monomers in maltose, the position of the glycosidic bond in the aglycon is assumed to be C(4) of the reducing part of the maltose. To establish the aglycon **10** as a precursor of **7a**,**b**, and to examine whether the glucosidic

Scheme 3. *Reaction Pathways Leading to the Chromophores* **7a**,**b** *from Hexoses and* L-*Proline*



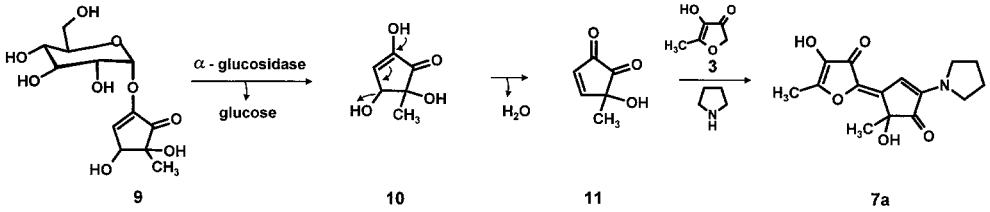
bond in **8** or **9** is responsible for the inhibition of the chromophore formation, an aqueous solution of glucoside **8** or **9** was incubated with α-glucosidase at 37° in a first experiment. A rapid release of the aglycone **10** (*Scheme 5*) from the glucosides could be monitored by GC/MS. Additional heat treatment of this enzymatically hydrolyzed solution led to a spontaneous dehydration, thus converting the primary aglycon **10** to 5-hydroxy-5-methylcyclopent-3-ene-1,2-dione (**11**), which was proposed as the penultimate precursor of chromophore **7a**,**b** (see **VII** in *Scheme 3*). Reaction of either **10** or **11** with 4-hydroxy-5-methylfuran-3(2*H*)-one (**3**) and pyrrolidine produced the chromophore **7a**,**b** in yields of *ca.* 15%, thereby confirming **10** and **11** as key precursors

Scheme 4. *4-(α-D-Glucopyranosyloxy)-2-hydroxy-2-methyl-2*H*-pyran-3(6*H*)-one* (**8**) *and 2-(α-D-Glucopyranosyloxy)-4,5-dihydroxy-5-methylcyclopent-2-en-1-one* (**9**) *Formed from the* Amadori *Product* **X** *of Maltose and* L-*Proline*



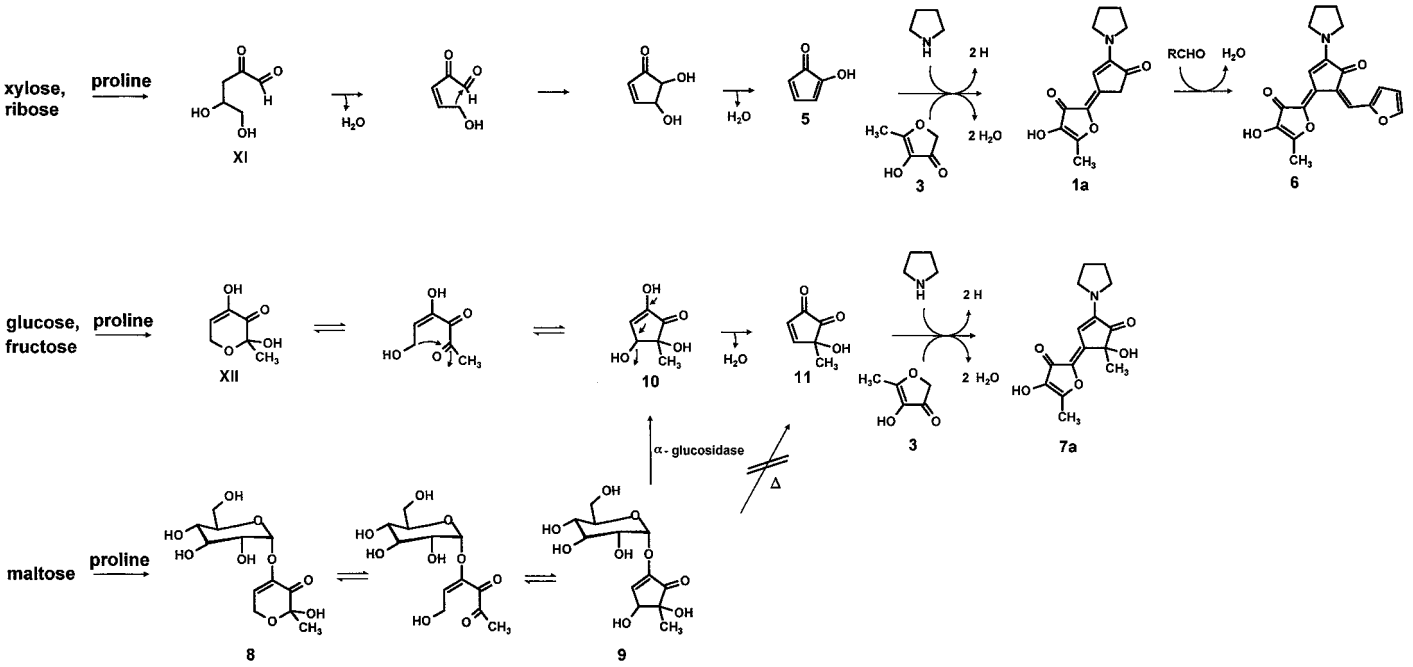
of the hexose-derived chromophore (*Scheme 5*; *cf.* **VI** and **VII** in *Scheme 3*). In a comparative experiment, **3** and pyrrolidine were treated with an aqueous solution of the glucoside **9** or **8**, which were not pre-exposed to α-glucosidase. In this case, HPLC analysis demonstrated that the colorant **7a**,**b** was not formed to any extent when the precursors **10** and **11** were not enzymatically generated from the glucosides, indicating that the non-hydrolyzable 1,4-glycosidic link in maltose prevents the formation of **7a**,**b**-type chromophores during thermal food processing.

Scheme 5. *Generation of 2,4,5-Trihydroxy-5-methylcyclopent-2-en-1-one* (**10**) *by α-Glucosidase Treatment of 2-(α-D-Glucopyranosyloxy)-4,5-dihydroxy-5-methylcyclopent-2-en-1-one* (**9**) *and Formation of Chromophore* **7a**,**b** via *5-Hydroxy-5-methylcyclopent-3-ene-1,2-dione* (**11**) *as a Key Intermediate*



3. Conclusion. – The data presented indicate that in the presence of L-proline, pentoses generate colorants of type **1a** (and **1b**) *via* the 3-deoxypentosulose(**XI**)-derived 2-hydroxycyclopenta-2,4-dien-1-one (**5**), 4-hydroxy-5-methylfuran-3(2*H*)-one (**3**), and proline-mediated pyrrolidine (*Scheme 6*). In the presence of 5-methyl-4-(pyrrolidin-1-yl)furan-3(2*H*)-one (**4**), chromophores of type **2a**,**b** are formed in a similar reaction pathway. However, these pentose-derived colorants are not stable end products of *Maillard* reactions and undergo further condensation reactions with oxo compounds to form chromophores of type **6**. Hexoses produce structurally related chromophores, with an additional Me and OH group, *via* the 1,5-dideoxyhexenodiulose(**XII**)-derived 2,4,5-trihydroxy-5-methylcyclopent-2-en-1-one (**10**), which, upon dehydration, is rapidly converted to the penultimate color precursor 5-hydroxy-5-methylcyclopent-3-ene-1,2-dione (**11**; *Scheme 6*). Condensation reaction of this unsaturated dione with **3** and pyrrolidine then leads to chromophores of type **7a**,**b**. Due to the lack of a methylene group, this colorant cannot undergo further condensation reactions to extend the chromophoric system, being well in line with

Scheme 6. *Influence of Carbohydrate Moiety on the Formation of 2-(4-Oxo-3-(pyrrolidin-1-yl)cyclopent-2-en-1-ylidene)furan-3(2*H*)-one Chromophores from Pentoses, Hexoses, and Disaccharides*



the higher browning activity found for pentoses. Also 1,4-glycosidically linked disaccharides such as, *e.g.*, maltose, are able to form the carbocyclic cyclopentenone **10**, but only glucosidically bound as 2-(α-D-glucopyranosyloxy)-4,5-dihydroxy-5-methylcyclopent-2-en-1-one (**9**), the latter being in an equilibrium with the 4-(α-D-glucopyranosyloxy)-2-hydroxy-2-methyl-2*H*-pyran-3(6*H*)-one (**8**), most likely *via* the open-chain form (*Scheme 6*). Because the glucose acts as a protecting group of the OH function at C(4) (open form), **9** is not able to liberate the color precursor **11** upon food-related thermal treatment, thus preventing the formation of chromophore **7a**,**b** from disaccharides. However, incubation with α-glucosidase prior to thermal treatment generates **10** and, upon dehydration, **11**, thereby inducing the hexose-specific reaction pathways to **7a**,**b**. These data strongly suggest that the 1,4-glycosidic linkage of disaccharides is responsible for their lower efficiency in browning development compared to pentoses or hexoses. Oligo- and polysaccharides that are connected by a 1,4-glycosidic link, such as maltodextrins or starch, can be expected to react in a similar way.

The results of the present investigation provide useful informations extending the knowledge on chromogenic reaction pathways of pentoses, hexoses, and disaccharides on a molecular level and will help to construct a route map of reactions enabling a better understanding of browning development during food processing.

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Experimental Part

General. The following compounds were obtained commercially: xylose, ribose, glucose, fructose, maltose, L-proline, furan-2-carbaldehyde, selenium(IV) oxide, ethylenediaminetetraacetic acid (EDTA), piperidine, AcOH (*Aldrich*, Steinheim, Germany), α-glucosidase (*Sigma*, Steinheim, Germany). Furan-2-carbaldehyde was distilled at 30° under high vacuum prior to use. Solvents were HPLC-grade (*Aldrich*, Steinheim, Germany). (D_6)DMSO, $CDCl_3$, and CD_3OD were obtained from *Isocom* (Landshut, Germany). Column chromatography (CC): silica gel *60* (*Merck*, Darmstadt, Germany); H_2O-cooled glass column (40 × 2.5 cm). Thin-layer chromatography (TLC): silica gel × 20 cm, 0.5 mm, *Merck*, Darmstadt, Germany). Gas chromatography/mass spectroscopy (GC/MS): type-*5160* gas chromatograph (*Fisons Instruments*, Mainz, Germany), *SE-54* capillary (3 × 0.32 mm, 0.25 μm; *J&W Scientific*, *Fisons Instruments*, Mainz, Germany) coupled with an *MD-800* mass spectrometer (*Fisons Instruments*, Mainz, Germany); sample application (0.5 μl) by on-column injection at 35°. HPLC/Diode array detector (HPLC/DAD): HPLC system (*Rheodyne* injector 100-μl loop, gradient mixture *M800*, two pumps of type *422*) and DAD (type *540*) from *Kontron* (Eching, Germany); SiO_2 material (*Hypersil*, 5 μm, 10 nm, *Shandon*, Frankfurt, Germany) for anal. (250 × 4.6 mm, flow rate 0.8 ml/min), gradient toluene/AcOEt 70:30 → AcOEt within 40 min; *RP-18* material (*ODS-Hypersil*, 5 mm, 10 nm, *Shandon*, Frankfurt, Germany) for anal. (250 × 4.6 mm, flow rate 0.8 ml/min), isocratic elution with H_2O/MeOH 75:25; t_R in min. HPLC/MS: anal. HPLC coupled with an *LCQ-MS* (*Finnigan MAT GmbH*, Bremen, Germany), positive atmospheric pressure chemical ionization ($APCI^+$). UV/VIS: spectrometer *U-2000* (*Colora Messtechnik GmbH*, Lorch, Germany); λ_{max} in nm, ε in 1 mol^{-1} cm^{-1}. 1D- and 2D-NMR experiments: *Bruker-AC-200* spectrometer, at 297 K, for DQF-COSY, HMQC, and HMBC experiments; *Bruker-AM-360* spectrometer, at 297 K, for ^1H, ^{13}C, and DEPT-135 experiments; for HMQC and HMBC measurements, the protons connected to ^{12}C were suppressed by a 'BIRD' pulse according to *Bax* and *Summers* [18]; parameters used for data aquisition as reported recently [2]; evaluation of the experiments with 1D- and 2D-WIN-NMR software (*Bruker*); sample (*ca.* 5–10 mg) in (D_6)DMSO in a *Wilmad-535-PP* tube; chemical shifts in ppm measured from residual (D_5)DMSO (2.49 ppm) in the ^1H dimension and with the ^{13}C signal of (D_6)DMSO (39.5 ppm) in the ^{13}C dimension.

*Detection of (2*E*)- and (2*Z*)-4-Hydroxy-5-methyl-2-[4-oxo-3-(pyrrolidin-1-yl)cyclopent-2-en-1-ylidene]furan-3(2*H*)-one* (**1a** and **b**, resp.) *and (2*E*)- and (2*Z*)-5-Methyl-2-[4-oxo-3-(pyrrolidin-1-yl)cyclopent-2-en-1-ylidene]-4-(pyrrolidin-1-yl)furan-3(2*H*)-one* (**2a** and **b**, resp.) *in Roasted Mixtures of Hexoses and* L*-Proline.* A mixture of xylose (10 mmol) or ribose (10 mmol) and L-proline (10 mmol) was dry-heated for 10 min at 205°. After cooling to r.t., the mixture was taken up in H_2O (250 ml), extracted with AcOEt (50 ml, 5 times), the combined org. layer dried (Na_2SO_4), concentrated to *ca.* 2 ml *in vacuo*, and then separated by prep. TLC (toluene/AcOEt 1:2); 4 orange bands at R_f 0.40 (**1a**), 0.42 (**1b**), 0.68 (**2a**), and 0.70 (**2b**), which were scraped off and dissolved in MeOH. After filteration, the fractions were analyzed by HPLC/DAD.

1a,**b**: t_R 11.97 min. UV/VIS: 466.

2a,**b**: t_R 6.98 min UV/VIS: 467 nm.

*Furan-3(2*H*)-one* **1a**,**b** *and from Xylose and Pyrrolidine* **2a**,**b**. A soln. of xylose (0.2 mol) and pyrrolidine (0.2 mol) in MeOH (180 ml) was refluxed for 3 h. Then, AcOH (0.2 mol) was added, and heating was continued for additional 2.5 h. After cooling, the solvent was evaporated and the residue taken up in H_2O (250 ml) and extracted with AcOEt (100 ml, 5 ×). The combined org. layers were dried (Na_2SO_4), concentrated to 5 ml and then fractionated by CC (silica gel, conditioning with toluene/AcOEt 8:2, then toluene/AcOEt 8:2 (400 ml), 6:4 (400 ml), and 5:5 (800 ml)). The intensely colored fraction obtained with toluene/AcOEt 5:5 was further fractionated by a second CC (silica gel, conditioning with pentane/Et_2O 8:2, then pentane/Et_2O 8:2 (200 ml), 6:4 (200 ml), 4:6 (200 ml), 2:8 (800 ml), and Et_2O (400 ml)). The fractions obtained with pentane/Et_2O 2:8 and Et_2O were further fractionated by prep. TLC (toluene/AcOEt 1:2). Four orange colored bands at R_f 0.40 (**1a**), 0.42 (**1b**), 0.68 (**2a**), and 0.70 (**2b**) were extracted with AcOEt/MeOH 1:1 (20 ml) affording **1a** (6 mmol, 0.3%), **1b** (2 mmol, 0.1%), **2a** (6 mmol, 0.3%), and **2b** (2 mmol, 0.1%) with purities of *ca.* 96%. Deep red solids.

1a,**b**: UV/VIS (H_2O, pH 7): 466 ($0.65 \cdot 10^4$). ^{1}H-NMR (360 MHz, (D_6)DMSO): *Table 1*. ^{13}C-NMR (360 MHz, (D_6)DMSO): *Table 2*. LC/MS: 262 (100, $[M+1]^+$).

2a,**b**: UV/VIS (H_2O, pH 7): 467 ($0.69 \cdot 10^4$). ^{1}H-NMR (360 MHz, (D_6)DMSO): *Table 3*. ^{13}C-NMR (360 MHz, (D_6)DMSO): *Table 4*. LC/MS: 315 (100, $[M+1]^+$).

*5-Methyl-4-(pyrrolidin-1-yl)furan-3(2*H*)-one* (**4**) *from Xylose or 4-Hydroxy-5-methylfuran-3(2*H*)-one* (**3**) *and Pyrrolidine.* A soln. of xylose (0.1 mol) and pyrrolidine (0.1 mol) in MeOH (90 ml) was refluxed for 3 h. Then AcOH (0.1 mol) was added, and heating was continued for additional 2 h. Similarly, 4-hydroxy-5-methylfuran-3(2*H*)-one (**3**; 0.2 mmol) [14], pyrrolidine (0.2 mmol), and AcOH (0.2 mmol) were refluxed in MeOH (5 ml) for 2 h. After cooling, the solvent was evaporated, the residue taken up in H_2O (100 ml) and extracted with AcOEt (100 ml, 5 ×), the combined org. phase extracted with 0.1M aq. NaOH (3 × 50 ml), dried (Na_2SO_4), and evaporated, and the residue fractionated by CC (Al_2O_3 basic, activity III–IV (*Merck*, Darmstadt, Germany), conditioning in hexane, then hexane (200 ml), hexane/Et_2O 7:3 (400 ml), 3:7 (400 ml), and Et_2O (400 ml)). The fraction obtained with Et_2O yielded 0.9 mmol (0.9%) of **4** from xylose (only trace amount of **4** from **3**). Colorless oil (purity of 99%). ^{1}H-NMR (360 MHz, $CDCl_3$; arbitrary numbering, see *Scheme 1*): 1.82 (*m*, 2 H–C(7), 2 H–C(8)); 2.23 (*s*, Me(1)); 3.11 (*m*, 2 H–C(6), 2 H–C(9)); 4.38 (*s*, 2 H–C(5)). ^{13}C-NMR (360 MHz; $CDCl_3$; arbitrary numbering, see *Scheme 1*): 14.2 (C(1)); 24.7 (C(7), C(8)); 50.6 (C(6), C(9)); 72.9 (C(5)); 126.3 (C(2)); 183.0 (C(3)); 198.9 (C(4)). GC/EI-MS: 42 (100), 167 (95), 54 (93), 96 (76), 124 (74).

2-Hydroxycyclopenta-2,4-dien-1-one (**5**). A mixture of cyclopent-2-en-1-one (12 mmol) and selenium(IV) oxide (12 mmol) in dioxane/H_2O 8:2 (10 ml) was heated for 2 h at 90°. After cooling and evaporation, H_2O (50 ml) was added and the soln. extracted with AcOEt (4 × 50 ml). The combined org. phase was dried (Na_2SO_4), treated with EDTA (5 mmol), and then distilled *in vacuo:* 6 mmol (50%) of **5**. Pale-yellow oil. Data incomplete because **5** polymerized rapidly upon concentration. GC/EI-MS: 96 (49), 68 (100), 39 (63), 40 (39), 42 (25).

*Furan-3(2*H*)-one* **1a**, **b** *from* **3** *and* **5**, *and, Furan-3(2*H*)-one* **2a**,**b** from **4** and **5**. A soln. of **3** (1.0 mmol; for **1a**,**b**) or **4** (1.0 mmol; for **2a**,**b**) was reacted with **5** (1.0 mmol), pyrrolidine (1 mmol), and AcOH (1 mmol) in 0.5M phosphate buffer (pH 7.0; 10 ml) for 60 min at 80°. After cooling, the mixture was extracted with AcOEt (4 × 5 ml), the org. layer dried (Na_2SO_4) and evaporated, and the residue fractionated by prep. TLC (toluene/AcOEt 1:2). The bands at R_f 0.40 (**1a**), 0.42 (**1b**), 0.68 (**2a**), and 0.70 (**2b**) were extracted with AcOEt/MeOH 1:1, (20 ml) to yield 0.2 mmol (20%) of **1a**,**b**, and 0.2 mmol (20%) of **2a**,**b**. Red solids. LC/MS and NMR: identical with those obtained from **1a**,**b** (*Tables 1* and *2*) and **2a**,**b** (*Tables 3* and *4*) isolated from the *Maillard* reactions of xylose and L-proline or pyrrolidine.

*(2*Z*)-2-[(5*Z*)-5-[(2-Furyl)methylidene]-4-oxo-3-(pyrrolidin-1-yl)cyclopent-2-en-1-ylidene]-4-hydroxy-5-methylfuran-3(2*H*)-one* (**6**). A soln. of **1a**, **b** (0.2 mmol) and furan-2-carbaldehyde (0.5 mmol) in 0.5M phosphate buffer (pH 7.0; 5 ml) was heated for 1 h at 70°. After cooling, the dark red mixture was extracted with AcOEt

(3×5 ml), the org. layer dried (Na_2SO_4) and evaporated, and the colored product isolated by prep. TLC (toluene/AcOEt 3:6). The dark-red band at R_f 0.61 was extracted with MeOH (10 ml) to yield 0.11 mmol (57%) of **6**. Dark red solid. UV/VIS (H_2O, pH 7): 471, 361. ^{1}H-NMR (360 MHz, (D_6)DMSO; arbitrary numbering, see *Formula* **6**): 1.89–1.91 (*m*, 2 H–C(12)); 1.89–1.91 (*m*, 2 H–C(13)); 2.24 (*s*, Me(1)); 3.17–3.18 (*m*, 2 H–C(11)); 3.17–3.18 (*m*, 2 H–C(14)); 6.79 (*dd*, *J*(18,17) = 3.5, *J*(18,19) = 1.7, H–C(18)); 7.36 (*s*, H–C(7)); 7.84 (*s*, H–C(15)); 8.02 (*d*, *J*(19,18) = 1.7, H–C(19)); 8.18 (*d*, *J*(17,18) = 3.5, H–C(17)). LC/MS: 340 (100, $[M+1]^+$).

*Detection of (2Z)- and (2*E*)-4-Hydroxy-2-(2-Hydroxy-2-methyl-3-oxo-4-(pyrrolidin-1-yl)cyclopent-4-en-1-ylidene)-5-methylfuran-3(2*H*)-one (***7a** *and* **7b**, *resp.) in Roasted Mixtures of Hexoses and* L*-Proline.* A mixture of glucose (10 mmol) or fructose (10 mmol), and L-proline (10 mmol) was dry-heated for 15 min at 205°. After cooling to r.t., the mixture was taken up in H_2O (250 ml) and extracted with AcOEt (50 ml, 5 times), the combined org. phase dried (Na_2SO_4), concentrated to *ca.* 1 ml *in vacuo*, and then separated by prep. TLC (toluene/AcOEt 3:7). An orange band at R_f 0.40 was suspended in MeOH (3 ml), the mixture filtered, and the filtrate analyzed by HPLC/DAD and HPLC/MS: **7a**,**b** at λ 466 nm and at *m*/*z* 292 (100, $[M+1]^+$).

*Furan-3(2*H*)-ones* **7a**,**b** *from Glucose and Pyrrolidine.* A soln. of glucose (0.4 mol) and pyrrolidine (0.4 mol) in EtOH (300 ml) was refluxed for 1.5 h. Then AcOH (0.4 mol) was added and heating was continued for additional 20 h. After cooling and evaporation, the residue was taken up in H_2O (500 ml) and extracted with AcOEt (10×200 ml), the org. layer dried (Na_2SO_4) and evaporated, and the residue fractionated by CC (silica gel, conditioning with toluene/AcOEt 8:2, then toluene/AcOEt 8:2 (400 ml), 7:3 (400 ml), 5:5 (400 ml), 3:7 (400 ml), and AcOEt (400 ml)). The orange fraction obtained from toluene/AcOEt 3:7 was further fractionated by prep. TLC (toluene/AcOEt 3:7). An orange, broad band at R_f 0.40 was extracted with AcOEt/MeOH 2:1, (10 ml) to yield 2.4 mmol of **7a/7b**. Deep red solid. UV/VIS (H_2O, pH 7): 466 ($0.70 \cdot 10^4$); ^{1}H-NMR (360 MHz, (D_6)DMSO): *Table 5*. LC/MS: 292 (100, $[M+1]^+$).

N*-(1-Deoxy-*D*-maltulos-1-yl)-*L*-proline* (= N*-[1-Deoxy-4-*D*-(α-*D*-glucopyranosyl)-β-*D-arabino*-hex-2-ulopyranosyl]-*L*-proline*). A mixture of maltose (60 mmol) and L-proline (50 mmol) was refluxed for 120 min in anh. MeOH (500 ml). After cooling to −18°, the mixture was filtered, the filtrate evaporated, and the residue taken up in H_2O (20 ml). Aliquots (5 ml) of the crude material were then fractionated by anion-exchange CC (*Dowex* (*Serva*, Heidelberg, Germany), acetate form). After washing the column with deionized H_2O (1.2 l), the target compound was eluted with 2M aq. AcOH (1.0 l). The acidic fraction was extracted with CH_2Cl_2 (10×200 ml) and the aq. layer freeze-dried: 8.5 mmol (14.2%) of *N*-(1-deoxy-D-maltulos-1-yl)-L-proline. Pale amorphous powder, purity 95%. LC/MS: 440 (100, $[M+1]^+$), 422 (38, $[M+1-H_2O]^+$), 462 (9, $[M+Na]^+$).

*Identification of 4-(α-*D*-Glucopyranosyloxy)-2-hydroxy-2-methyl-2*H*-pyran-3(6*H*)-one* (**8**) *and 2-(α-*D*-Glucopyranosyloxy)-4,5-dihydroxy-5-methylcyclopent-2-en-1-one* (**9**) *in a Heated Solution of Maltose and* L*-Proline or* N*-(1-Deoxy-*D*-maltulos-1-yl)-*L*-proline.* A soln. of maltose (8 mmol) and L-proline (8 mmol) or *N*-(1-deoxy-D-maltulos-1-yl)-L-proline (8 mmol) in 0.5M phosphate buffer (pH 7.0; 2 ml) was heated at 60° for 4.5 h. Reversed-phase HPLC/MS: t_R 4.2 and 3.5 for **8** and **9**, resp., identified by comparison (MS) with the synthetic reference compounds. LC/MS of **8**: 307 (100), 289 (45). LC/MS of **9**: 307 (100), 289 (39).

Preparation of **8** *and* **9**. Following a reported procedure [19], piperidine (0.25 mol) and AcOH (0.25 mol) were simultaneously added dropwise to a soln. of maltose (0.125 mol) and Et_3N (13 ml) in EtOH (37 ml), which was stirred at 75°. Heating was then continued for additional 2 h at 75°. After cooling to r.t., EtOH (100 ml) was added and the soln. stored at −18°: 25 mmol (20%) of piperidinomaltulose. Colorless crystals. LC/MS: 422 (100, $[M+1]^+$), 404 (32, $[M+1-H_2O]^+$), 444 (7, $[M+Na]^+$).

Following known procedures [17][20] with some modifications, piperidinomaltulose (10 mmol) in 0.5M phosphate buffer (pH 7.0; 20 ml) was heated at 60° for 4.5 h. The H_2O was evaporated at 40°, the residue taken up in MeOH (40 ml), the soln. filtered and evaporated, and the residue fractionated by CC (silica gel, conditioning with AcOEt, then AcOEt (500 ml), AcOEt/MeOH 9:1 (300 ml), 8:2 (300 ml), 6:4 (300 ml), 5:5 (300 ml), and 4:6 (300 ml)). The fractions obtained with AcOEt/MeOH 6:4 and 5:5 afforded a white powder, which was dissolved in AcOEt/MeOH 1:1 and further fractionated by prep. TLC (AcOEt/MeOH 1:1). Each plate was developed 3×, efficiently separating the material into two bands at R_f 0.59 (**8**) and 0.47 (**9**), which were then extracted with MeOH (20 ml) to yield 0.9 mmol (0.9%) of **9** and 0.4 mmol (0.4%) of **8**. White solids.

Data of **8**: ^{1}H-NMR (360 MHz, (D_6)DMSO; arbitrary numbering, see *Scheme 4*): *diasteroisomer A:* 1.10 (*s*, Me(1)); 3.15–3.54 (*m*, H–C(8), H–C(9), H–C(10), H–C(11), 2 H–C(12)); 4.39 (*m*, 2 H–C(6)); 5.32 (*d*, *J*(7,8) = 3.4, H–C(7)); 6.51 (*d*, *J*(5,6) = 3.0, H–C(5)); *diastereoisomer B:* 1.10 (*s*, Me(1)); 3.15–3.54 (*m*, H–C(8), H–C(9), H–C(10), H–C(11), H–C(12)); 4.39 (*m*, 2 H–C(6)); 5.37 (*d*, *J*(7,8) = 3.4, H–C(7)); 6.52 (*d*, *J*(5,6) = 3.0, H–C(5)). LC/MS: 307 (100, $[M+1]^+$), 289 (45, $[M+1-H_2O]^+$).

Data of **9**: ^{1}H-NMR (360 MHz; CD_3OD; arbitrary numbering, see *Scheme 4*): *diastereoisomer A:* 1.38 (*s*, Me(1)); 3.42–3.90 (*m*, H–C(8), H–C(9), H–C(10), H–C(11), H–C(12)); 4.67 (*m*, *J*(6,5) = 2.6, H–C(6)); 5.30 (*d*, *J*(7,8) = 3.4, H–C(7)); 6.67 (*d*, *J*(5,6) = 2.6, H–C(5)); *diastereoisomer B:* 1.38 (*s*, Me(1)); 3.42–3.90 (*m*, H–C(8), H–C(9), H–C(10), H–C(11), H–(12)); 4.77 (*d*, *J*(6,5) = 2.6, H–C(6)); 5.35 (*d*, *J*(7,8) = 3.4, H–C(7)); 6.68 (*d*, *J*(5,6) = 2.6, H–C(5)). LC/MS: 307 (100, $[M+1]^+$), 289 (39, $[M+1-H_2O]^+$).

Generation of 2,4,5-Trihydroxy-5-methylcyclopent-2-en-1-one (**10**) *and 5-Hydroxy-5-methylcyclopent-3-ene-1,2-dione* (**11**) *from Glucosides* **8** *and* **9**. A soln. of **9** (0.1 mmol) or **8** (0.1 mmol) in 0.1M phosphate buffer (pH 6.8; 1 ml) was incubated for 2 h at 37° in the presence of α-glucosidase (10 mg; 4.5 units/mg). The soln. was extracted with CH_2Cl_2 (4 × 1 ml), dried (Na_2SO_4), and analyzed by GC/MS. GC/EI-MS of **10**: 43 (100), 45 (52), 60 (32), 126 (25), 144 (6). Upon thermal treatment (5 min, 90°) in aq. soln., **10** was rapidly converted into **11**. GC/EI-MS of **11**: 43 (100), 45 (87), 60 (25), 126 (10). No NMR spectra of **10** and **11**, because they were rapidly degraded upon concentration.

Reaction of **8** *and* **9** *with* **3** *and Pyrrolidine before/after Incubation with* α*-Glucosidase.* A soln. of **9** (0.2 mmol) or **8** (0.2 mmol) in 0.1M phosphate buffer (pH 6.8; 2 ml) was incubated at 38° in the presence or absence of α-glucosidase (10 mg, 4.5 units/mg), respectively. After 2 h, **3** (0.2 mmol), pyrrolidine (0.2 mmol), and AcOH (0.2 mmol) dissolved in 0.5M phosphate buffer (pH 7.0; 2 ml) were added, and the mixture was heated for 30 min at 80°. After cooling, the mixture was extracted with AcOEt (4 × 3 ml), the org. layer dried (Na_2SO_4) and analyzed by HPLC. Exclusively in the mixture containing the α-glucosidase, an orange colorant (0.03 mmol, 15% yield) was detected, which was isolated by prep. TLC (toluene/AcOEt 1:2). The LC/MS and ^{1}H-NMR data were identical with those obtained for **7a**,**b** (*Table 5*) isolated from the *Maillard* mixture of glucose and pyrrolidine. In the model mixture, which was not enzymatically digested prior to the thermal treatment, colorants **7a**,**b** were not detectable.

REFERENCES

[1] A. Arnoldi, E. A. Corain, L. Scaglioni, J. M. Ames, *J. Agric. Food Chem.* **1997**, *45*, 650.
[2] T. Hofmann, *Helv. Chim. Acta* **1997**, *80*, 1843.
[3] T. Hofmann, *J. Agric. Food Chem.* **1998**, *46*, 3902.
[4] A. Ravagli, G. Boschin, L. Scaglioni, A. Arnoldi, *J. Agric. Food Chem.* **1999**, *47*, 4962.
[5] T. Hofmann, *J. Agric. Food Chem.* **1998**, *46*, 3918.
[6] T. Hofmann, *J. Agric. Food Chem.* **1998**, *46*, 932.
[7] S. Heuberger, Diploma thesis, Technical University of Munich, Germany, 1998.
[8] T. Hofmann, *J. Agric. Food Chem.* **1998**, *46*, 3912.
[9] H. M. E. Papst, F. Ledl, H.-D. Belitz, *Z. Lebensm.-Unters. Forsch.* **1984**, *178*, 356.
[10] H. M. E. Papst, F. Ledl, H.-D. Belitz, *Z. Lebensm.-Unters. Forsch.* **1985**, *181*, 386.
[11] T. Hofmann, *Carbohydr. Res.* **1998**, *313*, 203.
[12] F. Ledl, U. Krönig, T. Severin, H. Lotter, *Z. Lebensm.-Unters. Forsch.* **1983**, *177*, 267.
[13] F. Ledl, E. Schleicher, *Angew. Chem.* **1990**, *102*, 597; *Angew. Chem., Int. Ed.* **1990**, *29*, 565.
[14] T. Hofmann, P. Schieberle, *J. Agric. Food Chem.* **1998**, *46*, 235.
[15] E. F. L. J. Anet, *Aust. J. Chem.* **1965**, *18*, 240.
[16] J. Hiebl, F. Ledl, T. Severin, *J. Agric. Food Chem.* **1987**, *35*, 990.
[17] B. Kramhöller, F. Ledl, H. Lerche, T. Severin, *Z. Lebensm.-Unters. Forsch.* **1992**, *194*, 431.
[18] A. Bax, M. F. Summers, *J. Am. Chem. Soc.* **1986**, *108*, 2093.
[19] J. Hodge, E. Nelson, *Cereal Chem.* **1961**, *38*, 207.
[20] S. Estendorfer, F. Ledl, T. Severin, *Tetrahedron* **1990**, *46*, 5617.

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