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Maillard Reaction of D-Glucose: Identification of a Colored Product with Conjugated Pyrrole and Furanone Rings

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Formation of colored compounds during the Maillard reaction of D-glucose with butylammonium acetate in aqueous solution has been investigated. Butylamine was used as a model compound analogous to the lysine side chains of proteins. The previously unknown, yellow product, 4-hydroxy-5-methyl-2-(*N*-butyl-3-hydroxy-5-(2-hydroxyethyl)pyrrolyl-2-methylidene)-2*H*-furan-3-one (**1a**), was isolated from the reaction mixtures and identified by spectroscopic data.

KEYWORDS: Maillard reaction; color formation; D-glucose

INTRODUCTION

When D-glucose or other reducing sugars are heated with amino acids, proteins, or simple primary amines in water or systems of low water activity, a yellow-orange color develops within a few minutes, which turns dark brown during prolonged heating. This process is of great importance in food chemistry because it proceeds during the preparation of several foods. Examples are the browning of bread crust and malt production. This well-known Maillard reaction has been investigated during the past decades by several groups. Many low molecular compounds have been isolated and characterized, but the structures of most of the products absorbing in the visible wavelength range are still unknown. Here, we report on the isolation and structural identification of a novel yellow-orange D-glucose/butylamine reaction product. Butylamine is a model compound representing the lysine side chain of proteins.

MATERIALS AND METHODS

Apparatus. ^{1}H nuclear magnetic resonance (NMR) (500 MHz), ^{13}C NMR (125 MHz), H,H-COSY (correlated spectroscopy), HMQC (heteronuclear multiquantum coherence), and HMBC (heteronuclear multibond correlation) spectra were recorded with a JEOL Eclipse+500 spectrometer. Chemical shifts are reported in parts per million relative to $(CH_3)_4Si$ as internal standard. Mass spectrometric analyses were obtained with an HP 5989A MS Engine, high-resolution fast atom bombardment mass spectra (HR-FAB-MS) were obtained with a JEOL MS station JMS 700 data, and HR-MS were obtained with a Finnigan MAT 95Q spectrometer. Thin-layer chromatography was performed using 20 cm × 20 cm glass plates coated with a 0.5 mm thickness of silica gel (Merck, Darmstadt, Germany). Column chromatography was performed on silica gel 230−400 mesh, 60 A (Merck), and applying slight pressure.

Isolation of 4-Hydroxy-5-methyl-2-(*N*-butyl-3-hydroxy-5-(2-hydroxyethyl)pyrrolyl-2-methylidene)-2*H*-furan-3-one (1a). A mixture of D-glucose (36 g, 0.2 mol), *n*-butylamine (30 mL, 0.3 mol), and acetic acid (21 mL, 0.35 mol) in 150 mL of water was heated for 30 min under reflux. After it was cooled, the solution was extracted with 250 mL of ethyl acetate. The organic layer was washed with 150 mL of water and concentrated under reduced pressure. The mixture was separated by column chromatography on silica gel (20 cm × 5.5 cm i.d, eluent ethyl acetate/methanol, 100:3). A dark brown fraction (Fr A, 30 mL) was followed by a light yellow fraction (Fr B, 20 mL) and an intense yellow-orange fraction (Fr C, 50 mL) in front of a light yellow fraction and a broad brown zone. Fr C was further separated by thin-layer chromatography, using acetone/trichloromethane (5:4) as eluent. An intense yellow zone of R_f 0.65 was extracted with acetonitrile/methanol (6:1) and concentrated under reduced pressure affording **1a** as a yellow-orange oil (approximately 0.1% yield). HR-FAB-MS: calcd for $C_{16}H_{22}NO_5$, 308.1498; found, 308.1520. ^{1}H NMR ($CDCl_3$; the arbitrary numbering of the carbon atoms refers to **Figure 1**): δ 0.94 (t, 3H, $^3J_{10,11}$ = 7.3 Hz, H-11); 1.39 (dt, 2H, $^3J_{9,10,11}$ = 7.3 Hz, H-10); 1.64 (dt, 2H,$^3J_{8,9,10}$ = 7.3 Hz, H-9); 2.34 (s, 3H, H-16); 2.87(t, 2H, $^3J_{6,7}$ = 6.4 Hz, H-6); 3.85 (m, 2H, H-8); 3.94 (t, $^3J_{6.7}$ = 6.4 Hz, H-7); 5.79 (s, 1H, H-4); 6.81 (s, 1H, H-1); 8,26 (s, 1H, HO-14); 12.23 (s, 1H, HO-3). ^{13}C, DEPT135, HMQC, HMBC: 12.25 (C-16); 13.76 (C-11); 20.13 (C-10); 30.86 (C-6); 32.97 (C-9); 43.46 (C-8); 60.91 (C-7); 101.23 (C-4); 112.01 (C-1); 117.51 (C-2);136.65 (C-12); 137.12 (C-14); 147.63 (C-5); 160.43 (C-15); 162.12 (C-3); 169.12 (C-13). UV−vis (CH_3OH): λ_{max} = 453 nm.

Isolation of 4-Acetoxy-5-methyl-2-(*N*-butyl-3-hydroxy-5-(2-hydroxyethyl)pyrrolyl-2-methylidene)-2*H*-furan-3-one (1b). The yellow product in Fr C was acetylated with an excess of acetyl imidazole in methylenechloride for 1 h at room temperature. After concentration of the solvent under reduced pressure, ethyl acetate was added and the organic solution was washed three times with water. The product was purified by thin-layer chromatography on silica gel plates using ethyl acetate as eluent. An intense yellow zone (R_f 0.40) was eluted with acetonitril/methanol (6:1). After it was evaporated, **1b** was obtained as a yellow oil. HR-MS: calcd for $C_{18}H_{23}NO_6$, 349.1577; found, 349.1551. ^{1}H NMR ($CDCl_3$; the arbitrary numbering of the carbon atoms refers to **Figure 1**): δ 0.96 (t, 3H, $^3J_{10,11}$ = 7.5 Hz, H-11); 1.30 (s, HO-7); 1.35 (dt, 2H, $^3J_{9,10,11}$ = 7.5 Hz, H-10); 1.62 (dt, 2H, $^3J_{8,9,10}$

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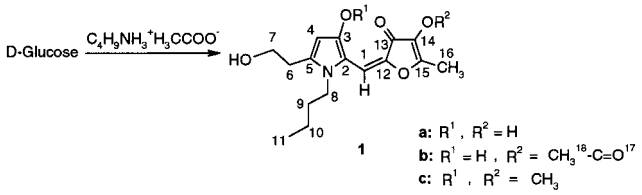

Figure 1. Formation of the colored compound **1a** from D-glucose and butylammonium acetate in aqueous solution with arbitrary numbering of the carbon atoms.

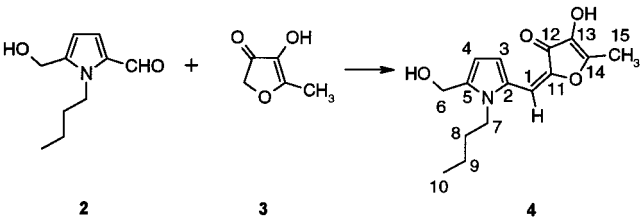

Figure 2. Formation of the colored compound **4** from pyrrole aldehyde **2** and furanone **3**.

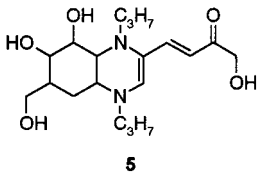

Figure 3. Structure of the colored compound **5** from D-glucose and propylamine in alcoholic solution.

= 7.5 Hz, H-9); 2.27 (s, 3H, H-16); 2.33 (s, 3H, H-18);2.86 (t, 2H, $^3J_{6,7}$ = 6.5 Hz, H-6); 3.85 (m, 2H, H-8); 3.93 (t, 2H, $^3J_{6,7}$ = 6.5 Hz, H-7); 5.75 (s, 1H, H-4); 6.82 (s, 1H, H-1); 12.37 (s, 1H, HO-3). ^{13}C, DEPT135, HMQC, HMBC: 12.89 (C-16); 13.75 (C-11); 20.14 (C-10); 20.37 (C-18); 30.63 (C-6); 32.87 (C-9); 43.40 (C-8); 60.86 (C-7); 101.19 (C-4); 112.45 (C-1); 118.04 (C-2); 131.55 (C-14); 136.92 (C-12); 148.45 (C-5); 160.51 (C-15); 164.15 (C-3); 167.80 (C-17); 168.93 (13). UV−vis (CH_3OH): λ_{max} (log ϵ) 457 nm (4.06).

Isolation of 4-Methoxy-5-methyl-2-(*N*-butyl-3-methoxy-5-(2-hydroxyethyl)pyrrolyl-2-methylidene)-2*H*-furan-3-one (1c). The yellow product in Fr C was methylated with an excess of potassium carbonate and methyl iodide in acetone by heating under reflux for 1 h. Isolation and purification were achieved as described for the acetyl derivative **1b**. The dimethyl derivative of **1a** was obtained as a yellow oil. HR-MS: calcd for $C_{18}H_{25}NO_5$, 335.1732; found, 335.1777. ^{1}H NMR ($CDCl_3$; the arbitrary numbering of the carbon atoms refers to **Figure 4**): δ 0.92 (t, 3H, $^3J_{10,11}$ = 7.5 Hz, H-11); 1.25 (s, 1H, HO-7); 1.32 (dt, 2H, $^3J_{9,10,11}$ = 7.5 Hz, H-10); 1.61 (dt, 2H, $^3J_{8,9,10}$ = 7.5 Hz, H−9); 2.27 (s, 3H, H-16); 2.87 (t, 2H, $^3J_{6,7}$ = 6.5 Hz, H-6); 3.82 (s, 3H, CH_3O-3); 3.90 (s, 3H, CH_3O-14); 3.91 (t, 2H, $^3J_{6,7}$ = 6.5 Hz, H-7); 4.04 (m, 2H, H-8); 5.78 (s, 1H, H-4); 6.79 (s, 1H, H-1). ^{13}C, DEPT135, HMQC, HMBC: 12.42 (C-16); 13.82 (C-11); 19.98 (C-10); 30.47 (C-6); 33.45 (C-9); 44.42 (C-8); 57.91 (CH_3O-3); 59.95 (CH_3O-14); 61.27 (C-7); 94.85 (C-4); 101.82 (C-1); 111.81 (C-2); 136.70 (C-5); 138.28 (C-12); 139.08 (C-14); 154.46 (C-3); 163.15 (C-15); 180.10 (C-13). UV−vis (CH_3OH): λ_{max} (log ϵ) 444 nm (4.12).

Synthesis of *N*-Butyl-2-formyl-5-hydroxymethyl-pyrrole (2). A mixture of reactants as described for the preparation of **1a** in 150 mL of methanol was heated for 30 min under reflux. After it was cooled, the solution was concentrated under reduced pressure, diluted with water, and extracted with ethyl acetate. The products in the organic layer were separated by column chromatography on silica gel (20 cm × 2.5 cm i.d.; eluent ethyl acetate). A nearly colorless zone behind the front of the solvent afforded the pyrrole aldehyde **(2)** in a purity of more than 90%. ^{1}H NMR ($CDCl_3$): δ 0.93 (t, 3H, ^3J = 7.5 Hz, CH_3); 1.37 (dt, 2H, ^3J = 7.5 Hz, CH_2); 1.70(dt, 2H, ^3J = 7.5 Hz); 4.34 (m, 2H, N−CH_2); 4.67 (s, 2H, O−CH_2); 6.21 (d,1H, ^3J = 4.0 Hz, CH); 6.86 (d, ^3J = 4.0 Hz, CH); 9.49 (s,1H, CHO). This raw product was reacted with 4-hydroxy-5-methyl-2*H*-furan-3-one **(3)**.

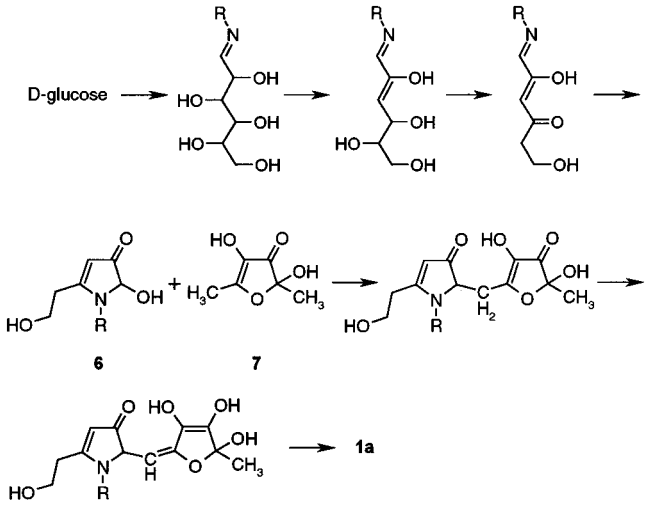

Figure 4. Proposed reaction mechanism leading from D-glucose to **1a**.

Isolation of 4-Hydroxy-5-methyl-2-(*N*-butyl-5-hydroxymethyl)-pyrrolyl-2-methylidene)-2*H*-furan-3-one (4). The pyrrole aldehyde (**2**) was condensed with **3** (*1*) following a procedure described in the literature (*2*). Compound **4** was obtained in 35% yield as a yellow solid. MS: (EI, 277, M^+). ^{1}H NMR ($CDCl_3$; the arbitrary numbering of the carbon atoms refers to **Figure 2**): δ 0.94 (t, 3H, $^3J_{9,10}$ = 7.5 Hz, H-10); 1.26 (s, 1H, HO-6); 1.36 (dt, 2H, $^3J_{8,9,10}$ = 7.5 Hz, H-9); 1.69 (dt, 2H, $^3J_{7,8,9}$ = 7.5 Hz, H-8); 2.35 (s, 3H, H-15); 4.08 (m, 2H, H-7); 4.65 (s, 2H, H-6), 6.27 (d, 1H, $^3J_{3,4}$ = 4.0 Hz, H-4); 6.72 (s, 1H, H-1); 7.03 (d, 1H, $^3J_{3,4}$ = 4.0 Hz, H-3); 7.2−7.6 (s,1H, HO-13). ^{13}C, DEPT135, HMQC, HMBC: 12.45 (C-15); 13.67 (C-10); 20.16 (C-9); 34.19 (C-8); 43.94 (C-7); 56.93 (6); 103.30 (C-1); 111.87 (C-4); 118.15 (C-3); 126.71 (C-2); 136.43 (C-13); 137.40 (5); 142.34 (C-11); 160.73 (C-14); 180.48 (C-12). UV−vis (CH_3OH): λ_{max} (log ϵ) 414 nm (4.22).

RESULTS AND DISCUSSION

When D-glucose and butylammonium acetate were heated in a nearly neutral aqueous solution, a dark brown mixture of products was obtained. Several colored compounds could be extracted with ethyl acetate. By chromatography on a silica gel column followed by a separation on silica gel plates, a deep yellow fraction containing compound **1a** was obtained. Further purification of the main colored product **1a** was not successful, because the substance was unstable and slowly degraded under ambient conditions. In contrast, a solution in ethyl acetate could be stored for a few days at 0 °C or below. The limited stability of compound **1a** is probably due to the hydroxy group at the pyrrole nucleus. Stable derivatives of the yellow compound were obtained by acylation or methylation. Acetylation with acetyl imidazole at room temperature led to a monoacetyl derivative, which could be purified on silica gel thin-layer plates. Reaction with methyl iodide and potassium carbonate afforded a dimethyl derivative. The structures of the colored compound **1a**, the acetyl derivative **1b**, and the methylation product **1c** were derived from the MS spectra and the NMR spectroscopic data. The structure of **1b** was identified as follows: the two saturated side chains of the pyrrole ring were recognized by the chemical shift and coupling patterns of the signals in the ^{1}H NMR data, which were confirmed by COSY. In DMSO-d_6, the HOC-7 showed a triplet at 4.8, which coupled with the quartet at 3.67, representing the adjacent methylene group. The structure and substitution pattern of the pyrrole ring could be estabished by the HMQC and HMBC data. Couplings via two and three bonds in HMBC of C-5 with the protons H-4, H-6, and H-8 and couplings of

C-2 with the protons H-4, H-8, and H-1 established the pyrrole ring with an adjacent hydroxyethyl and methine group in the α and α′ positions. H-1 was connected with the carbon atoms C-2 and C-3 of the pyrrole ring and the carbon atoms C-12 and C-13 of the furanone part.

Evidence for the hydroxypyrrole ring system was obtained by HMBC connection in DMSO-d_6 of HOC-3 with C-3 and C-4. The C-16 methyl group was connected with C-15 and C-14 of the furanone system. Further verification of the conjugated pyrrole furanone structure was obtained by comparison of the spectral data with those of a synthetic product (**4**).

Condensation of the CH-acidic furanone **3** with the pyrrole aldehyde **2** by a procedure that has been described previously (*2*) led to the compound **4**, which possesses a similar conjugated pyrrole furanone structure. NMR spectroscopic data of both compounds agree in several relevant details, if the influence of substituents on the chemical shifts is taken into account.The furanone **3** is the predominant Maillard product of pentoses (*1*), whereas pyrrole aldehydes of type **2** are formed by reaction of D-glucose with primary amines (*3*, *4*).

Colored Maillard products with a structure similar to **4** have been isolated from pentose/amine or pentose/amino acid reaction mixtures (*2*, *5*). They are formed by condensation of the pentose-derived CH-acidic furanone **3** with *N*-alkyl-pyrrole aldehydes. So far, a similar reaction scheme could not be established for hexose browning reactions. To provide insight as to which Maillard products might be involved in color formation of hexoses, furfural and hydroxymethylfurfural have been added to Maillard reaction mixtures. Several colored products incorporating the furan nucleus have been identified (*5*−*7*). However, furfural and hydroxyfurfural are formed only in low concentrations during the Maillard rection so that the contribution of these condensation products to the browning still has to be established.

When D-glucose and butylammonium acetate are heated in methanol or in systems with low water activity, a yellow compound of structure **5** is formed (*8*). In aqueous solution, on the other hand, colored compounds of different structures are predominant. Among the products extractable with organic solvents, the new substance **1a** is one of the main products, but the absolute concentration is low. Several other compounds contribute to the color in the yellow to orange range, many of which are formed only in extremely low amounts. Two major products are at the present under investigation, but separation and purification are difficult. After extraction of the butylamine/D-glucose reaction mixture with organic solvents, dark brown products remain in the water layer. The structures of these compounds are so far unknown.

On the basis of the results obtained so far, a stringent reaction mechanism explaining the formation of compound **1a** cannot be described. At the first glance, one might assume that compound **1a** stems from two intact C_6 moieties. However, it is well-known that hexoses give several short chain degradation products under the conditions of the Maillard reaction. Thus, the formation of compound **1a** from scission products must be considered. A reaction mechanism as proposed in **Figure 4** is therefore speculative. The condensation of two C_6 moieties is in its essentials a type of Mannich reaction, where a CH-acidic methyl group of compound **7** condenses with an electrophilic amino alcohol **6**. Acetylformoin **7** is a well-known sugar degradation product.

The Maillard reaction of D-glucose leads to a variety of colored products. Structure **1a** was identified as a reaction product of D-glucose with butylamine with an intensive yellow color. Other products that are more hydrophilic are at present under investigation.

LITERATURE CITED

(1) Severin, T.; Seilmeier, W. Studien zur Maillard-Raktion. *Z. Lebensm. Unters. Forsch.* **1967**, *134*, 230.
(2) Ledl, F.; Severin, T. Browning rections of pentoses with amines. *Z. Lebensm. Unters. Forsch.* **1978**, *167*, 410−413.
(3) Sengl, M.; Ledl, F.; Severin, T. Reaktion von Rinderserumalbumin mit Glucose. *J. Chromatogr.* **1989**, *463*, 119.
(4) Henle, T.; Bachmann, A. Synthesis of pyrroline reference material. *Z. Lebensm. Unters. Forsch.* **1996**, *202*, 72−74.
(5) Hofmann, T. Identification of novel colored compounds containing pyrrole- and pyrrolinone- structures formed by Maillard reactions of pentoses and primary amino acids. *J. Agric. Food. Chem.* **1998**, *46*, 3902−3911.
(6) Ledl, F.; Hiebl, J.; Severin, T. The formation of colored *β*-pyranones from pentoses and hexoses. *Z. Lebensm. Unters. Forsch.* **1983**, *177*, 353−355.
(7) Frank, O.; Heuberger, S.; Hofmann, T. Structure determination of a novel 3(6*H*)-pyranone chromophore and clarification of its formation from carbohydrates and primary amino acids. *J. Agric. Food Chem.* **2001**, *49*, 1595−1600.
(8) Knerr, T.; Lerche, H.; Pischetsrieder, M.; Severin, T. Formation of a novel colored product during the Maillard reaction of D-glucose. *J. Agric. Food. Chem.* **2001**, *49*, 1966−1970.

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