5-Hydroxy-2-methyl-4-(alkylamino)-2*H*-pyran-3(6*H*)-one: A New Sugar-Derived Aminoreductone

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2,3-Dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one (**3**) is one of the main products obtained from glucose under the conditions of the Maillard reaction. Heating of **3** in the presence of primary amines leads to the formation of 5-hydroxy-2-methyl-4-(alkylamino)-2*H*-pyran-3(6*H*)-one (**11**). These new aminoreductones can also be isolated from glucose/amine reaction mixtures.

Keywords: *Maillard reaction; glucose; aminoreductone; HPLC*

INTRODUCTION

In spite of numerous investigations, the great complexity of Maillard reaction products is still a challenge for food chemists working in this field. It is well established that in addition to several other compounds reductones and aminoreductones are formed, but only a few substances of this type have been isolated and identified so far. Carbohydrate degradation products with reducing properties can stabilize foods, and they may prevent oxidative deterioration. Our knowledge about the physiological activity of Maillard reaction products is still very limited but under intense investigation. For instance, it is unknown whether sugar-derived reductones or aminoreductones are resorbed or connected with any effects in the human body.

Some cyclic reductone ethers have been isolated in pure form from Maillard reaction mixtures of several carbohydrates under appropriate conditions. 4-Hydroxy-5-methyl-3(2*H*)-furanone (**1**) has been obtained in high yield from D-ribose or D-xylose (Severin and Seilmeier, 1967). When glucose is heated with a primary amine in neutral aqueous solution, the furanone **2** and the dihydropyranone **3** are formed as main products (Knerr and Severin, 1993). Recently we were able to isolate the cyclic reductone **4** from a maltose/amine reaction mixture (Figure 1; Pischetsrieder and Severin, 1994).

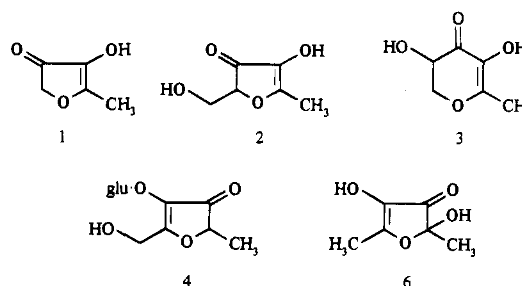

Figure 1. Reductones arising from pentoses, hexoses, and disaccharides.

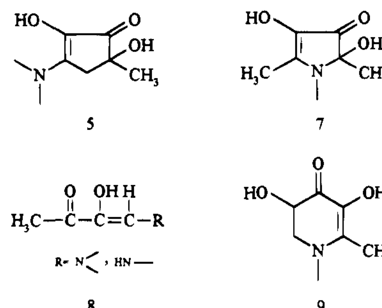

Figure 2. Aminoreductones.

Only a few sugar-derived aminoreductones have been described so far. When D-glucose is heated with piperidine, the cyclic reductone **5** is formed in considerable quantity (Weygand et al., 1959; Pabst et al., 1985). In contrast to expectation, this type of aminoreductone is not obtained when D-glucose reacts with a primary amine, and consequently compounds with the general structure **5** are only of limited interest in food chemistry (Figure 2) (Ledl et al., 1982). Acetylformoin **6**, a well-known sugar degradation product, is recognized as an intermediate which reacts with a secondary amine to give **5** (Weygand et al., 1959). On the other hand, condensation of **6** with primary amines leads to cyclic aminoreductones of general structure **7** (Ledl and Fritsch, 1984; Ledl et al., 1983). C_4-aminoreductones **8** have been detected at low levels after thermal degradation of Amadori compounds (Mills at al., 1970; Ledl and Severin, 1979). When hexoses with a 1,6 glycosidic link such as isomaltose, palatinose, or glucose 6-phosphate are heated with a primary amine, 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyridones **9** are formed in considerable quantity (Figure 2; Kettner et al., 1991) [formation of other aminoreductones see also: Tressl et al. (1993), Kramhöller et al. (1993), Estendorfer et al. (1990)]. In this paper we report on the isolation of a new aminoreductone from a D-glucose/amine reaction mixture.

MATERIALS AND METHODS

Apparatus. For analytical liquid chromatography a Merck L-6200 gradient pump and a Merck programmable photodiode array detector, Model D-6500, with Merck DAD-Manager Software and a NEC pinwriter P60, were used. Preparative HPLC was carried out with a Merck-Hitachi Lichograph chromatograph Model L-6000, equipped with a Merck-Hitachi chromato-integrator Model D-2500, interfaced with an UV detector L-4000. IR spectra were recorded in a KBr disk with a Perkin-Elmer 197 spectrometer. NMR spectra (internal standard tetramethylsilane) were recorded with a JEOL 400 GSX spectrometer. Mass spectral analyses were obtained with a Varian MAT CH7 (EI).

Reagents. HPLC grade solvent (acetonitrile) was used without further purification. The water used for HPLC was distilled and filtered through a 0.45-μm nylon membrane. All solvents were degassed with helium. Thin-layer chromatography (TLC) was performed using 20 cm × 20 cm glass plates

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coated with a 0.5-mm thickness of silca gel 60 F_{254}. α-*N*-Acetyllysine was synthesized as described by Hardy et al. (1976).

High-Performance Liquid Chromatography (HPLC). The samples were diluted with methanol (1:10), filtered, and injected into the HPLC. Separation was performed on a Nucleosil 5 C_{18} column (250 mm × 4.6 mm i.d., 5-μm particle size), protected with Nucleosil 5 C_{18} guard cartridge (25 mm × 4.6 mm i.d.). The eluents used were water (A) and acetonitrile (B) with the following gradient: 0–20 min, 100–50 % A; 20.1–30 min, 0% A. The flow rate was 0.8 mL/min. The substances were detected by a diode array detector from 220 to 320 nm. Identification of the samples was achieved by comparison of the retention times and UV spectra with those of the synthesized reference compounds.

For preparative HPLC a lobar glass column was used (Merck; 310 mm × 25 mm i.d.) packed with LiChroprep RP18 (Merk; 40–63-μm particle size) with an eluent of acetonitrile–water (1:9) and a flow rate of 0.86 mL/min. The substance was detected with UV light at 280 nm.

Preparation of the Reference Compounds. 5-(Hydroxymethyl)-1-propylpyrrole-2-carbaldehyde **(10)** was obtained according to a method of Klein et al. (1992). 2,3-Dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one **(3)** was synthesized as described by Van den Ouweland and Peer (1970). 4-Hydroxy-2-(hydroxymethyl)-5-methyl-3(2*H*)-furanone **(2)** was prepared according to the procedure of Knerr and Severin (1992).

Isolation of 5-Hydroxy-2-methyl-4-(propylamino)-2*H*-pyran-3(6*H*)-one (11a) from a Reaction Mixture of Glucose and Propylammonium Acetate. Propylamine (3.3 g, 55.9 mmol) and acetic acid (3.3 g, 55 mmol) were added to a solution of glucose (1 g, 5.6 mmol) in 20 mL of dry *N,N*-dimethylformamide. After 1 h of heating at 100 °C, the solvent was removed under reduced pressure at 40 °C. The residue was fractionated by column chromatography on silica gel (3 × 8 cm): fraction 1, 250 mL of ethyl acetate; fraction 2, 50 mL of ethyl acetate–methanol (1:1); fraction 3, 100 mL of ethyl acetate–methanol (1:1). Fraction 3 was evaporated at 40 °C and purified by flash chromatography on RP-18 silica gel [Merk; LiChroprep; 40–63-μm particle size, 3 × 16 cm; eluent acetonitrile–water (1:9)]: fraction 1, 150 mL; fraction 2, 120 mL. Fraction 2 was evaporated and separated by preparative HPLC: The substance was diluted in 1 mL of eluent and injected into the system. The fraction between 217 and 246 min was collected and dried under reduced pressure (40 °C). **11a** was obtained as a slightly yellow solid, and spectra were identical with those of **11a** described below.

Isolation of 11a from a Reaction Mixture of 3 with Propylammonium Acetate. One hundred eighty milligrams of the raw product of **3** (about 80%, 1 mmol), 60 mg of propylamine, and 60 mg of acetic acid (1 mmol each) were dissolved in 1 mL of THF and refluxed for 20 min. The dark brown solution was chromatographed on silica gel: column, 3 cm i.d. × 20 cm; eluent, methanol–ethyl acetate (28:72); fractions of 15 mL; fractions 10–14 contain **11a.** Further cleaning was achieved by TLC (same eluent, R_f = 0.45) and colorless crystals were obtained: 77 mg (42%), mp 172–174 °C (dec); ^{1}H NMR ($CDCl_3$, COSY) δ 0.93 (t, J = 7.3 Hz, 3H, CH_2CH_3), 1.34 (d, J = 6.6 Hz, 3H, $CHCH_3$), 1.67 (s, J = 7.3 Hz, 2 H, CH_2CH_2), 3.02 (t, J = 7.3 Hz, 2 H, NCH_2), 4.06 (d, J = 16.1 Hz, 1H, OCH_aH_b), 4.06 (q, J = 8 Hz, 1H, H-2), 4.11 (d, J = 16.1 Hz, 1H, OCH_aH_b); ^{13}C NMR (CD_3OD, COSY, DEPT) δ 9.1 (q, CH_2CH_3), 16.4 (q, $CHCH_3$), 17.9 (t, CH_2CH_2), 49.6 (t, NCH_2), 67.8 (t, C-6), 74.4 (d, C-2), 106.2 (s, C-4), 182.5 (s, C-5), 185.4 (s, C-3); MS, *m/z* 186 (75%, M + 1^+), 185 (80%, M^+), 170 (50%, M − methyl), 156 (90%, M − ethyl,), 114 (50%), 43 (100%); IR 2800–3000 (br, chelated OH), 1500–1600 (br, C=C), 1380, 1120 cm^{-1}; UV λ_{max} 272 (water pH 7.0, lg ε = 4.30), 270 (water pH 1.0, lg ε = 4.25), 312 nm (water, pH 13.0, lg ε = 4.18). Anal. Calcd for $C_9H_{15}NO_3$: C, 58.36; H, 8.16; N, 7.56. Found: C, 58.50; H, 7.92; N, 7.64.

Isolation of 2-(Acetylamino)-6-[5-hydroxy-2-methyl-3(6*H*)-oxo-(2*H*-pyran-4-ylamino)]hexanoic Acid (11b). 3 (100 mg, 0.7 mmol), α-*N*-acetyllysine (520 mg, 2.8 mmol), and acetic acid (160 mg, 2.7 mmol) were heated for 4 h at 100 °C. The mixture was suspended in 5 mL of methanol–ethyl acetate (3:2) and centrifuged. The solution was decanted and separated by flash chromatography on silica gel [2 × 16 cm, eluent methanol–ethyl acetate (3:2)]: fraction 1, 60 mL; fraction 2, 40 mL. Fraction 2 was evaporated at 40 °C and separated by TLC with methanol–ethyl acetate (3:2). From a band with a R_f value of 0.31–0.45 was eluted compound **11b** with methanol: ^{1}H NMR (D_2O) δ 1.35–1.42 (m, 5H, CH_3CH and CH_2-4-lys), 1.63–1.68 (m, 3H, CH_2-5-lys and CH_a-3-lys), 1.73–1.79 (m, 1H, CH_b-3-lys), 1.99 (s, CH_3-CO), 3.09–3.13 (t, 2H, CH_2-6-lys), 4.08–4.11 (dd, 1H, CH-2-lys), 4.23 (s, 2H, CH_2O), 4.27–4.33 (q, 1H, $CHCH_3$); ^{13}C NMR (D_2O) 15.6 (CH_3CH), 22.1 (CH_3CO), 22.3 (C-4-lys), 24.9 (C-5-lys), 31.3 (C-3-lys), 49.0 (C-6-lys), 55.1 (C-2-lys), 68.3 (CH_2O), 75.5 ($CHCH_3$), 108.0 (C=CN), 173.7 (CONH), 179 (COOH), 184.6 (O=CCH), 187.6 (=$COCH_2$) (the identification of the signals of the ^{1}H NMR and ^{13}C NMR was completed by H–H COSY, C–H COSY, and DEPT spectra); UV λ_{max} 269.5 (lg ε = 4.2); IR 3415 (br), 2926, 2854, 1545, 1408 cm^{-1}. Anal. Calcd for $C_{14}H_{22}N_2O_6$: C, 53.49; H, 7.05; N, 8.91. Found: C, 53.51; H, 7.04; N, 8.71.

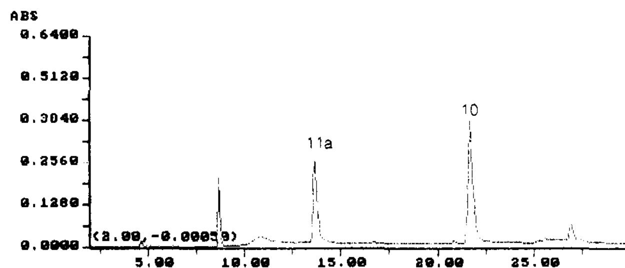

Figure 3. HPLC chromatogram of sample A, detection at UV 272 nm. Numbers at tops of peaks refer to structures in Figure 4.

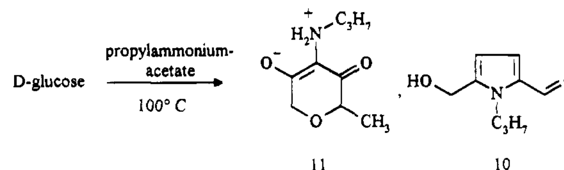

Figure 4. Degradation of D-glucose in a melt.

Preparation of the Samples. *A.* D-Glucose (50 mg, 0.28 mmol), 10 mg of Na_2HPO_4 × 2 H_2O (0.056 mmol), 320 mg of propylamine (5.4 mmol), and 525 mg of acetic acid (8.7 mmol) were heated for 1 h at 100 °C.

B. D-Glucose (50 mg, 0.28 mmol), 165 mg of propylamine (2.8 mmol), and 165 mg of acetic acid (2.75 mmol) were dissolved in 0.33 mL of water and heated for 1 h at 100 °C.

RESULTS AND DISCUSSION

When glucose is heated with propylammonium acetate in a melt, in DMF or in concentrated aqueous solution at about 100 °C, the mixture turns dark brown. Two main reaction products absorbing in the UV region can be detected by HPLC (Figure 3). One compound has been identified as the well-known 5-(hydroxymethyl)-1-propylpyrrole-2-carbaldehyde **(10)** (Jurch and Tatum, 1970). For the other main product the structure of the previously unknown aminoreductone **11** could be established (Figure 4).

Separation and purification of **11a** were achieved by chromatographic procedures. The assignment of the structure of **11a** is based on spectroscopic data: CHN analysis and MS confirm that **11a** is a condensation product of propylamine and **3** by loss of one molecule of water. The ^{1}H-NMR spectrum shows the signals expected for the propyl substituent. For the glucose-derived residue it displays a multiplet at 4 ppm that can be read as two doublets with a high coupling

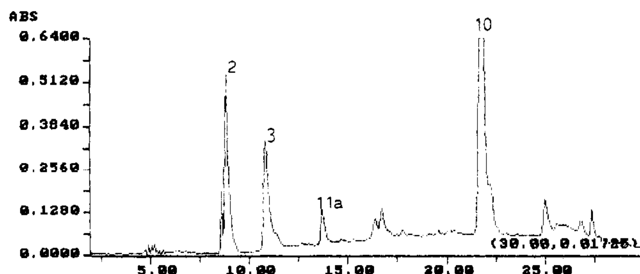

Figure 5. HPLC chromtogram of sample B, detection at UV 271 nm. Numbers at tops of peaks refer to structures in Figures 1 and 4.

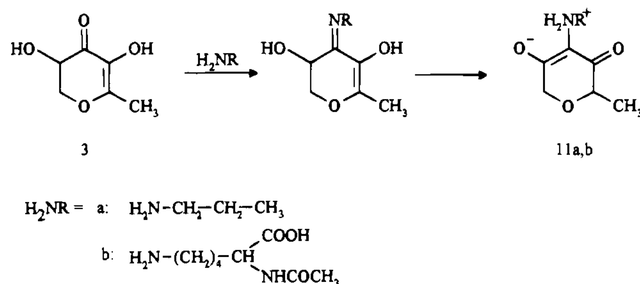

Figure 6. Proposed reaction mechanism of the formation of **11**.

constant ($J = 16$ Hz) and a quartet each integrating for one proton. At 1.4 ppm a diagnostic doublet (3H) is found. ^{13}C-NMR spectra (including ^{1}H$-^{13}$C COSY) affirm the presence of the methine, methylene, and methyl groups (74, 68, and 16 ppm plus signals for the propyl chain). Additionally, three singlets are observed. Two of them are located in the region of carbon esters and six-membered ring enones: 186 and 183 ppm. The third is shifted highfield to 108 ppm, a rather low value for N-substituted olefinic carbons. Considering that the compound is a betaine (Euler and Eistert, 1957; Eistert et al., 1968), this phenomenon can be explained: The carbon atoms C-3 and C-5 differ only with respect to the α-methyl group, corresponding to the difference in the chemical shift of 3 ppm. C-4 is rich in electrons due to the mesomeric effect of the enolate. Acylated α-aminoreductones are known to show similar behavior (Wilson et al., 1990). Additional confirmation is supplied by the UV absorption: The UV maximum ($\lambda = 272$ nm in neutral aqueous solution) is not greatly influenced by addition of acid ($\lambda_{max} = 270$ nm at pH 1) but did shift to longer wavelengths at higher pH values ($\lambda_{max} = 314$ nm at pH 13). This verifies that the amino group is protonated in neutral solution and that only under alkaline conditions is a proton abstracted by the solvent. The IR spectrum shows broadened signals at 2800–3000 and 1450–1600 cm^{-1} in accordance with the structure proposed (Eistert et al., 1968).

The amount of **11a** obtained from D-glucose is highly dependent on the reaction conditions: When glucose is heated with propylammonium acetate in DMF or in a melt at 100 °C, **11a** arises in addition to **10** as a UV-absorbing main product (Figure 3). In aqueous solution, however, its relative quantity diminishes and in addition **2** and **3** appear in the HPLC chromatogram (Figure 5).

Further investigations gave insight into the reaction mechanism leading to **11.** Dihydropyranone **3** is a product typical for the Maillard reaction of glucose. This substance has already been detected in several foods (Ledl et al., 1976). When **3** is heated with propylamine in THF, the aminoreductone **11a** is obtained as the main product in comparatively high yield.

On the basis of these results a reaction mechanism can be drawn as shown in Figure 6. The reactions of glucose described here have been performed with several amines, for example, propylamime and α-*N*-acetyllysine. In consequence, there is little doubt that other primary amines, for instance, lysine side chains of proteins, react in the same way.

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