Maillard Reaction of Maltose—Isolation of 4-(Glucopyranosyloxy)-5-(hydroxymethyl)-2-methyl-3(2*H*)-furanone

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Heating of a neutral aqueous solution of maltose and a primary amine leads to the formation of 4-(glucopyranosyloxy)-5-(hydroxymethyl)-2-methyl-3(2*H*)-furanone (**6**). When a maltose/amine reaction mixture is separated by HPLC, compound **6** appears as a main product along with the previously described compounds **1**–**5**.

INTRODUCTION

Maillard reactions generally lead to the formation of numerous compounds with varied structures. Among these some sugar degradation products showing reducing properties are of special interest. Reductones and aminoreductones are obtained by interaction of carbohydrates with amino acids, proteins, or simple amines. Like ascorbic acid or other antioxidants, products of the Maillard reaction can prevent oxidative degradation and they act as stabilizing agents in several foods.

It is still unknown whether sugar-derived reducing compounds are resorbed by the human body or not, and it is speculative to assume that these reductones might have an antimutagenic effect, for instance, by reactions with radical oxygen. During the past two decades mutagenic as well as antimutagenic compounds have been isolated from Maillard reaction mixtures (Ledl and Schleicher, 1990). In this paper we describe isolation and identification of a cyclic reductone ether obtained by degradation of maltose in the presence of amine.

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 and semipreparative HPLC were carried out with a Merck-Hitachi Lichrograph 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 analysis was obtained with a Varian MAT CH7 (CI, NH_3) and +FAB spectrum with a Kratos MS 80 (Xe, 7 kV, 10 W). For UV spectra a Hitachi U 1100 spectrometer was used. CHN analysis was obtained with a Heraeus CHN Rapid instrument.

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.

High-Performance Liquid Chromatography (HPLC). For analytical chromatography about 1 mL of the sample was diluted with 4 mL of water and injected into the HPLC. Separation was performed on a column (250 mm × 4.6 mm i.d., 5-μm particle size), protected with a guard cartridge (25 mm × 4.6 mm i.d.), both packed with Nucleosil 5C-18. The eluents used were water (A) and acetonitrile (B) with a gradient 0–5 min, 100% A; 5–20 min, 100–50% A; 20–30 min, 0% A; and a flow rate of 0.8 mL/min. The substances were detected by a diode array detector from 210 to 360 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 RP 18 (Merck; 40–63-μm particle size) with an eluent of water–methanol (9:1) and a flow rate of 1.5 mL/min. Semipreparative HPLC was carried out with a column (250 mm × 10 mm i.d.) that was packed with Nucleosil RP 18 (Bischoff, Germany), an eluent of water–methanol (9:1), and a flow rate of 2.4 mL/min. At semipreparative and preparative HPLC the substances were detected with UV light at 280 nm.

Preparation of the Reference Compounds. 4-(α-D-Glucopyranosyloxy)-2-hydroxy-2-methyl-2*H*-pyran-3(6*H*)-one (**1**) was obtained by a method of Kramhöller et al. (1992). 4,5-Dihydroxy-2-(α-D-glucopyranosyloxy)-5-methyl-2-cyclopenten-1-one (**2**) was synthesized according to a method reported by Estendorfer et al. (1990). 1-[3-(α-D-Glucopyranosyloxy)-2-furanyl]-1-ethanone (**3**) was prepared according to a method reported by Goodwin (1983). 4-(α-D-Glucopyranosyloxy)-2-methyl-1-propylpyridinium-3-olate (**4**) was synthesized as described by Ledl et al. (1989). 5-(Hydroxymethyl)-1-propylpyrrol-2-aldehyde (**5**) was obtained by a method of Klein et al. (1992).

Isolation of 4-(Glucopyranosyloxy)-5-(hydroxymethyl)-2-methyl-3(2*H*)-furanone (6). 1-Deoxypropylaminomaltulose (2.7 g, 5.8 mmol), which was synthesized according to a method of Hodge and Nelson (1961), was heated in 20 mL of dry *N*,*N*-dimethylformamide at 100 °C for 90 min. The solvent was removed under reduced pressure (40 °C) and the residue dissolved in 15 mL of ethyl acetate–methanol (2:1) and filtered. The solution was separated by column chromatography on silica gel (5.5 × 16 cm): fraction 1, 1 column volume; fraction 2, 20 mL; fraction 3, 300 mL. From fraction 3 the solvent was removed under reduced pressure (40 °C) and for separation by preparative HPLC the residue was diluted in 1 mL of eluent and injected into the system. The fraction between 87 and 114 min was collected, and the solvent was removed under reduced pressure (50 °C). Last purification was obtained by semipreparative HPLC: The residue was dissolved in 600 μL of eluent, and each time 150 μL of the sample was injected into the HPLC. The fractions between 4.5 and 9.2 min were collected, and the solvent was removed under reduced pressure (40 °C). Compound **6** was obtained as a colorless solid, a mixture of the diastereomeric compounds **A** and **B** (1:1): ^{1}H NMR (CD_3OD) δ 1.44 and 1.43 (2d, 3H, *J* = 7.34 Hz, C*H*$_3$CH A and B), 3.33–3.91 (m, 7H, C*H*OH$_{glu}$), 4.53 and 4.54 (2s, 2H, HOC*H*$_2$C=C, A and B), 4.60 (q, 1H, *J* = 7.34 Hz, C*H*CH$_3$), 5.42 and 5.50 (2d, 1H, *J* = 3.67 Hz, OC*H*O$_{glu}$ A and B); ^{13}C NMR (CD_3OD) δ 16.4 and 16.7 (*C*H$_3$CH A and B), 56.6 (*C*H$_2$OH), 62.2 (*C*H$_2$OH$_{glu}$), 71.2, 73.1, 74.4, and 74.7 (*C*HOH$_{glu}$), 82.2 (*C*HCH$_3$), 101.2 and 101.6 (*C*HO$_{glu}$ A and B), 134.2 and 134.4 (C=*C*O—glu A and B), 180.7 and 181.0 (C=*C*OCH), 201.0 (C=O); IR 3380.2, 2932.3, 1698.0, 1614.0, 1080.4, 1023.5 cm^{-1}; UV (CH_3OH) λ_{max} 281.1 nm (log ε = 3.6); CI-MS *m*/*z* 145 (M + 1 − $C_6H_{10}O_5$), 127 (M + 1 − $C_6H_{10}O_5$ − H_2O); FAB *m*/*z* (relative intensity) 307 (M + 1, 100), 289 (M + 1 − H_2O, 60). Anal. Calcd for $C_{12}H_{18}O_9$: C, 47.06; H, 5.92. Found: C, 46.92; H, 6.06. The p*K* value was determined by the method of

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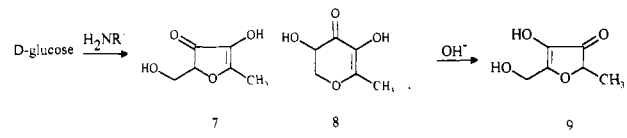

Figure 1. Maillard reaction of D-glucose with propylamine.

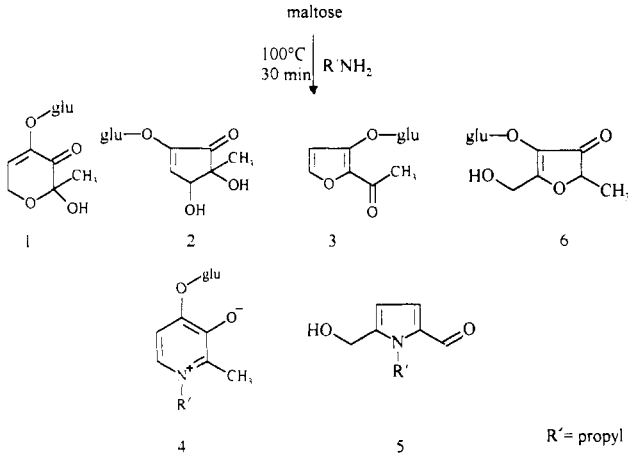

Figure 2. Products that arise from the reaction of maltose and propylamine.

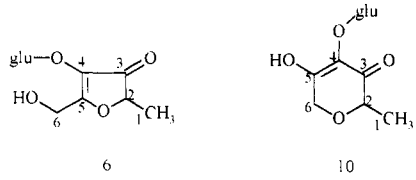

Figure 3. Theoretically possible structures for the new compound.

Flexer et al. (1935), measuring at a pH value of 7.85 and at λ = 305 or 280 nm, respectively.

Enzymatic Hydrolysis of 6. 6 (6.7 mg) was dissolved in 2.5 mL of citrate buffer (pH 6.6), and 6 μL of α-glucosidase (Boehringer, Germany; 50 units/mg) was added. The solution was allowed to stand for 1 day at room temperature and was then injected into the HPLC without dilution. Compound **9** was synthesized according to the method of Mills (1978).

Degradation of Maltose. Maltose monohydrate (100 mg, 0.28 mmol) and propylamine (16.5 mg, 0.28 mmol) were dissolved in 2 mL of phosphate buffer (1 M, pH 7.0), and the pH was adjusted to pH 7.0 with acetic acid. The solution was heated for 30 min under reflux.

RESULTS AND DISCUSSION

When D-glucose is heated with a primary amine in neutral aqueous solution, the furanone **7** and the dihydro-γ-pyrone **8** are formed as main products (Figure 1), which can be separated by HPLC (Knerr and Severin, 1993). Both compounds are cyclic reductone ethers, and they are readily oxidized, for instance, by a solution of iodine (Severin and Seilmeier, 1968; Mills et al., 1970; Hiebl et al., 1987). The D-glucose (and D-fructose) degradation products **7** and **8** have been detected in several foods, and they must be considered as typical products of Maillard-type reactions (Ledl et al., 1976). An isomeric substance, **9**, has been obtained by the action of alkali on the γ-pyrone **8**, conditions which are unrealistic for food chemistry (Mills, 1978).

Recent investigations have shown that monosaccharides and disaccharides with 1,4-glycosidic bonds give different products when heated with primary or secondary amines. From a maltose/propylamine reaction mixture previously the compounds **1–5** have been isolated and identified (Figure 2) (Kramhöller et al., 1993). After short reaction times, the cyclopentenone **2** and the β-pyranone **1** are predominant. After prolonged heating, **1** and **2** are transformed into the more stable D-glucosylisomaltol (**3**) (Kramhöller et al., 1992). More detailed investigations revealed that one important maltose degradation product has previously been overlooked. Under suitable HPLC conditions the β-pyranone **1** is separated from a compound to which structure **6** is assigned. The new compound is obtained as a main product when the propylamine-derived Amadori compound of maltose is heated in DMF.

The structure of **6** is derived from spectroscopic data, where the problem of deciding between the furanoid (**6**) and pyranoid (**10**) isomers arises (Figure 3). The ^{1}H NMR spectrum in CD_3OD shows the signals of both diastereomeric compounds **A** and **B** in a ratio of 1:1. Besides the signals that belong to the sugar residue, three signals occur in the spectrum. A pair of doublets at δ 1.44 (3H, J = 7.34 Hz) indicates the presence of the methyl groups of **A** and **B** at C-1, which couple with the proton of C-2. The methine group appears as a quartet at δ 4.6 (1H, J = 7.34 Hz). The methylene group at C-6 is represented by a pair of sharp singlets at 4.54 due to both diastereomeric forms. If these protons are part of the pyranoid structure, the protons should represent an A–B pattern with geminal coupling, and therefore they should appear as two well-separated doublets that couple with a large coupling constant of about 15 Hz (Mills et al., 1970; Hiromichi et al., 1984). In the ^{13}C NMR the signal for the carbonyl carbon appears at 201 ppm, which is in accordance with expectation for the carbonyl carbon of the furanone structure, whereas signals of unsaturated cyclohexanone derivatives are shifted to higher field (Kalinowski et al., 1984; Breitmaier and Voetler, 1987; Hiromichi et al., 1984). Except for the predominant band at 3380 cm^{-1} due to the hydroxy groups of the sugar residue, the IR spectrum shows two important bands at 1698 and 1614 cm^{-1}, which indicate the reductone structure. Whereas six-membered ring reductones, such as 2-alkoxy-3-hydroxy-2-cyclohexenones, show two characteristic absorptions at 1635 and 1590–1615 cm^{-1} and a predominant one at 1565 cm^{-1} (Adler et al., 1979), the

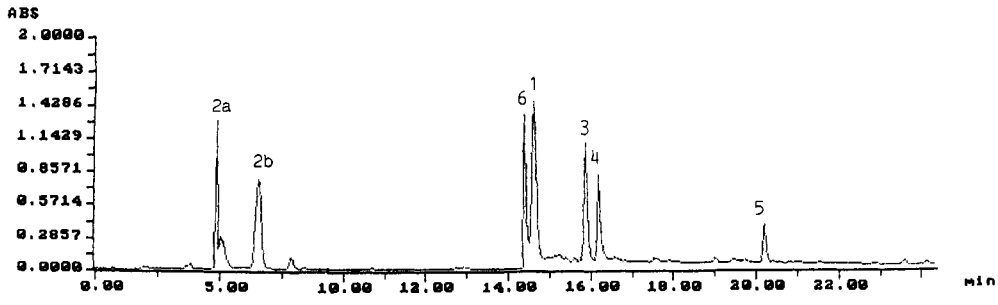

Figure 4. HPLC chromatogram for the degradation of maltose with propylamine: detection, 0–10 min, UV 242 nm, then 262 nm. Numbers on tops of peaks refer to structures in Figure 2.

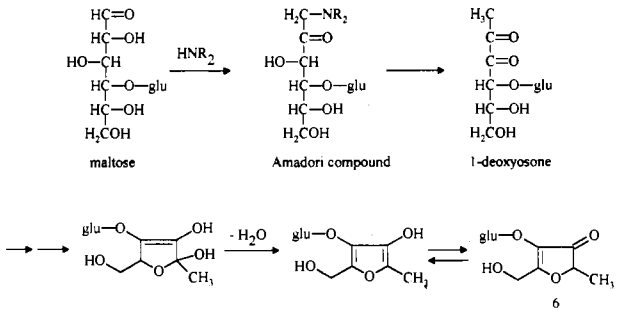

Figure 5. Proposed reaction mechanism of the formation of **6**.

C=O and C=C stretching bands are observed at about 1690–1700 and 1600–1610 cm^{-1} for 3-furanone derivatives (Ledl, 1979; Miyagi, 1964). For compound **6** a pK value of 9.0 was found, which is according to expectation for 3-furanone derivatives; a compound of structure **10** should react as an acid, and for similar molecules pK values between 3 and 4 are given in literature (Hesse, 1978). In addition to this result, the UV spectrum in neutral or weak alkaline (pH 9) solution was identical to the UV spectrum at acid pH. Acid reductones, for example, ascorbic acid, show under neutral conditions the UV spectrum of the monoanion form (Moss, 1971). Finally, the enzymatic hydrolysis of **6** released a compound which was identified by HPLC as **9**.

The formation of **6** is highly dependent on reaction conditions. The HPLC of a typical reaction mixture, obtained by heating of maltose and propylammonium acetate in aqueous solution at 100 °C is shown in Figure 4. Compounds **1–5** have been isolated previously, and **2a** and **2b** are probably two diasteriomeric compounds. At lower temperatures the relative amount of **1** is increasing. As a cyclic reductone ether, similar to the monosaccharide-derived compounds **7** and **8**, the furanone **6** is easily oxidized, for example, by iodine in aqueous solution. A reasonable reaction mechanism for the formation of **6** is shown in Figure 5. It should be assumed that lactose reacts under similar conditions to give a D-galactosyl derivative of 5-(hydroxymethyl)-2-methyl-3(2H)-furanone, and further investigations will be carried out in this direction.

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