Formation of glucosyl-deoxyosones from Amadori compounds of maltose

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Bildung von Glucosyl-Desoxyosonen aus Amadoriverbindungen der Maltose

Zusammenfassung. Amadori-Verbindungen aus Maltose wurden mit *o*-Phenylendiamin umgesetzt. Als wesentliche Reaktionsprodukte konnten die Chinoxaline 5b, 6b und 7a isoliert und identifiziert werden. Die Versuche zeigten, daß bei der Maillard-Reaktion aus Maltose die Substanzen 2b, 3b und 4a als Zwischenstufen gebildet werden. Durch die Bestimmung von Folgeprodukten ließ sich zeigen, daß der Abbau von Glucose in Gegenwart von Propylamin nicht ausschließlich über das Amadoriprodukt 1a erfolgt. Erhitzen der Aminoketose 1d führte u. a. zur Bildung des Furanderivates 11a, was die Bildung von 4a bestätigt.

Summary. Amadori compounds obtained from maltose were heated with *o*-phenylenediamine. The main reaction products were found to be the quinoxalines 5b, 6b, and 7a. From the structures of the quinoxalines, it can be deduced that the deoxyosones 2b, 3b, and 4a are formed from maltose. From the determination of some transformation products, it was found that the degradation of glucose in the presence of propylamine does not proceed exclusively with Amadori compound 1a as an intermediate. On heating Amadori compound 1d, the furane derivative 11a was detected, confirming the formation of 4a.

Introduction

When reducing sugars and amines are heated in an aqueous solution (Maillard reaction), a multitude of compounds are formed. Many of these substances have also been detected in heated and stored foods. With the introduction of gas chromatography-mass spectrometry (GC-MS) the separation and identification of volatile low-molecular-weight products have been decisively improved. In contrast the isolation of non-volatile, polar sugar degradation products is more difficult. To a certain extent, it is possible to detect polar substances after derivatization using the combination GC-MS [M. Sengl, J. Beck, F. Ledl, and T. Severin, in preparation]. Not all of the products obtained by the usual derivatization methods (i.e., acylation, silylation or alkylation) are stable. Degradation and decomposition during separation with GC and HPLC systems can be observed.

Isolation and characterization of the unstable intermediates of the Maillard reaction would provide a better understanding of the reaction pathways. In order to obtain stable derivatives of these compounds, the reaction with *o*-phenylenediamine is suitable especially when α-dicarbonyl compounds are to be trapped. The degradation of sugars in alkaline and acidic media in the presence of *o*-phenylendiamine has already been studied [1]. The results cannot generally be explained by the Maillard reaction because there are indications that the sugar degradation under the influence of primary aliphatic amines, amino acids or proteins is quite different.

We recently reported on the degradation of Amadori compounds 1a and 1b, obtained from glucose and primary or secondary amines. With *o*-phenylenediamine as the trapping reagent the quinoxaline 5a could be isolated in a high yield, whereas only a relative small quantity of the expected quinoxaline 6a was obtained. From these results, it can be concluded that the degradation of the Amadori compounds leads to the formation of the α-dicarbonyl components 2a and 3a [2] (Scheme 1). Additional investigations with Amadori compounds of disaccharides will be presented in this paper. Furthermore, an attempt was made to find out the extent to which Amadori compounds are involved in sugar degradation.

Experimental section

General methods

Melting points (mp, uncorrected) were determined with a Büchi 510 apparatus. The IR spectra of the samples were recorded in KBr discs

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using a Perkin-Elmer 197 spectrometer. NMR spectra (internal standard: tetramethylsilane) were recorded with a Varian A-60 spectrometer at 60 MHz. Mass spectra were produced with a Varian MAT CH7 spectrometer equipped with a probe inlet at 70 eV. Silica gel 60 F_{254} (2 mm, Merck, 5717; 0.5 mm, Merck, 5744) was used for thin-layer chromatography (TLC) and silica gel 60 (Merck, 9385) for column chromatography. Low pressure chromatography was achieved with a reversed phase (RP)-column (Merck, 10625, 40–60 µm, type B). High-performance liquid chromatography (HPLC) was performed on a Merck-Hitachi system (pump L-6000, UV detector L-4000 and a chromato-integrator D-2000) with RP-columns (Merck, 50994, 7 µm, 250 × 10 mm i.d.; Bischoff, 25461855, 5 µm, 250 × 4.6 mm i.d.). Gas chromatography (GC) was performed with a Perkin-Elmer 8320 instrument equipped with a flame-ionization detector and a quartz capillary column (25 m × 0.25 mm i.d.; permaphase, Perkin-Elmer 698346) coated with dimethylsilicone; injection and detection ports at 260 °C; temperature program 100 °–200 °C at 6 °C/min. Iso time 1/0 min; iso time 2/15 min. The mass spectra of the volatile compounds were obtained from a GC-MS system: column: permaphase DMS (25 m × 0,32 mm i.d., Perkin-Elmer 698349). MS: Finnigan MAT 8230, 70 eV. GC: Carlo Erba 5160.

Isolation and identification of quinoxalines 5b and 7a

A 4.09 g sample (0.01 *mol*) of 1-deoxy-1-piperidinomaltulose (1d) [3] and a 1.08 g (0.01 *mol*) sample of *o*-phenylenediamine were heated for 10 h in water (50 ml) at a pH range of 5.5–8 under reflux. The cooled solution was neutralized and extracted with methylene chloride (3 × 30 ml). The residue of the organic layer was fractionated by TLC (2 mm) with acetonitrile/water (7 + 1, v/v). From a band with an R_f value between 0.2 and 0.3, quinoxaline 7a was eluted with methanol. Further purification was performed on a low-pressure RP-column with water/methanol (1 + 1, v/v) adjusted to pH 3.8 with hydrogen chloride, using a flow rate of 4 ml/min. Fractions of 20 ml were collected. After 17 min, quinoxaline 7a was visible (TLC control, acetonitrile/water/NH_3; 7 + 1 + 1 v/v/v, R_f 0.7). Fractions containing 7a were neutralized, concentrated and extracted with methylene chloride. The organic solvent was dried with anhydrous sodium sulphate, filtered and methylene chloride extensively removed under reduced pressure. Quinoxaline 7a remained as a syrupy oil (200 mg, 5% relative to 1d). The MS data obtained were as follow: m/z 301 (5%, M^+), 270 (10), 218 (85), 184 (10), 169 (15), 159 (100), 158 (98), 98 (95), 84 (99), 55 (60).

In order to obtain ^{1}H-NMR spectral data with greater validity, 50 mg of 7a was acetylated with pyridine (1 ml) and acetic anhydride (1 ml) overnight at room temperature. Purification was performed by vacuum destillation.

7a (acetylated): bp 150°–160 °C at 0.1 Torr; ^{1}H-NMR ($CDCl_3$): δ 1.53 (m, 6 H), 2.03 (s, 3 H), 2.13 (s, 3 H), 2.53 (m, 4 H), 3.60 (d, 2 H, J 6 Hz), 3.90 (dd, 2 H, J 4 Hz and J 1 Hz), 4.46 (m, 2 H), 6.00 (m, 1 H), 8.00 (m, 4 H). – IR: 3050, 2920, 1730, 1355, 1220, 1030, 750 cm^{-1}. – MS: m/z 385 (1%, M^+), 301 (50), 243 (7), 199 (10), 183 (70), 169 (65), 158 (50), 98 (50), 84 (95), 77 (10), 55 (20), 43 (100).

From the aqueous layer of the reaction mixture, the water was removed under diminished pressure and the residue was dissolved in hot methanol. The solution was filtered and concentrated. After cooling overnight in the refrigerator, quinoxaline 5b was obtained as a crystalline substance (2.45 g, 60% relative to 1d). 5b mp 197 °C (recrystallized from methanol). Elemental analysis calculated for C, H, N and O was as follows: C, 54.54%; H, 6.11%; N, 7.06%. The percentages actually found were: C, 54.31%; H, 6.32%; N, 6.94%. MS: m/z: 396 (1%, M^+), 336 (2), 318 (5), 287 (2), 218 (10), 203 (25), 187 (50), 174 (60), 159 (60), 158 (100), 157 (75), 143 (40), 130 (15), 117 (50), 102 (30), 89 (50), 76 (45), 43 (60). – ^{1}H-NMR (DMSO): δ 2.93 (s, 3 H), 3.2–5.3 (m, 17 H), 8.1 (m, 4 H). In order to obtain ^{1}H-NMR spectral data with greater validity, 50 mg of 5b was acetylated as described above for 7a.

5b (acetylated): mp 166 °C, ^{1}H-NMR ($CDCl_3$): δ 1.90 (s, 3 H), 2.00 (s, 3 H), 2.03 (s, 3 H), 2.06 (s, 3 H), 2.09 (s, 3 H), 2.12 (s, 3 H), 2.93 (s, 3 H), 4.20–5.70 (m, 11 H), 7.96 (m, 4 H). – IR: 3050, 2950, 1730, 1360, 1210, 1015, 750 cm^{-1}. – MS: m/z 648 (1%, M^+), 588 (3), 528 (1), 504 (4), 488 (2), 446 (2), 347 (4), 331 (20), 302 (70), 301 (70), 243 (65), 242 (70), 229 (40), 200 (70), 199 (65), 187 (60), 183 (65), 171 (55), 169 (60), 143 (45), 127 (40), 109 (70), 43 (100).

A 0.16 g sample of 5b (0.4 m*mol*) and 20 µl of α-glucosidase (Boehringer, 105414) were dissolved in water (50 ml, adjusted to pH 6.6 with sodium acetate and acetic acid). After incubation overnight at room temperature, the solution was extracted three times with n-butanol (5 ml). The organic solvent was dried with anhydrous sodium sulphate, centrifuged, filtered and concentrated. The residue was dissolved in hot methanol and after cooling, 5a was obtained as a crystalline substance. Melting point and spectral data were identical to those obtained from a substance isolated from a reaction mixture of 1b and *o*-phenylenediamine [3].

Isolation and identification of quinoxaline 6b

1-Deoxy-1-propylaminomaltulose (1c, oxalate, 2.37 g, 0.005 *mol*), prepared according to the synthesis of 1a [5], and *o*-phenylenediamine (0.54 g, 0.005 *mol*) were heated in water (50 ml, phosphate buffer, pH 7) for 10 h under reflux. The extraction of the reaction mixture was carried out as described for 1-deoxy-1-piperidinomaltulose (1d). The residue of the aqueous solution was fractionated on silica gel. A fraction including 5b and 6b was eluted with methanol. A further separation was performed by HPLC. The products, eluted with methanol/water (4 + 6, v/v, detection at 320 nm) were collected and reinjected for purification on the analytical column (elution and detection as above). With a flow rate of 1 ml/min, the R_t value of 5b was 3.88 and that of 6b was 4.67. The fraction eluted at R_t 4.67 was collected (repeated injections). From the ^{1}H-NMR spectral data, it could be seen that a mixture of 6b and the benzimidazole of maltonic acid was eluted. After incubation with α-glucosidase and acteylation as described for 5b, compound 6a (acetylated) was identified using GC-MS (the retention time and the mass spectrum were identical to those obtained from a substance isolated from a reaction mixture of 1a and *o*-phenylenediamine) [2].

Determination of 8, 9, and 10 formed from 1a

An aqueous solution (10 ml, phosphate buffer pH 7) of 1a [4] (oxalate, 1.35 g, 5 m*mol*) was heated for 3 h under reflux. The solution was extracted with ethyl acetate and the residue was silylated with 0.5 ml pyridine, 0.2 ml trimethylchlorosilane and 0.2 ml hexamethyldisilazane. The volatile products were separated by GC (retention time of 8 was 13.24 min and that of 9 was 11.56 min). The relative amounts of these compounds were determined by comparing the peak areas with that of added maltol. 10 was not detected.

Determination of 8, 9, and 10 formed in a glucose propylamine reaction mixture

An aqueous solution (50 ml, phosphate buffer pH 7) of glucose (9 g, 0.05 *mol*) and propylamine (2.85 g, 0.05 *mol*) was heated for 3 h under reflux. The reaction mixture was extracted with ethyl acetate and the residue of the organic layer treated as described above. The retention time of 10 was 12.93 min.

Isolation of 11a

A 20.45 g sample (0.05 mol) of 1b [5] was dissolved in 6*N*-hydrogen chloride (50 ml) and heated for 12 h under reflux. The pH of the solution was adjusted to pH 7 with sodium hydroxide and extracted with ethyl acetate. The residue of the organic layer was distilled at

120 °C at 0.1 Torr. The volatile products were separated on TLC with a mixture of ethyl acetate/methanol (8+2, v/v). From a band with an R_f value between 0.4 and 0.5, compound 11a was eluted with methanol. 11a: colourless oil with b.p. 110–120°C/0.1 Torr, ^{1}H-NMR ($CDCl_3$): δ 1.52 (m, 6 H), 2.54 (m, 4 H), 3.62 (s, 2 H), 6.53 (m, 1 H), 7.38 (d, 1 H, J 3.5 Hz) 7.72 (d, 1 H, 2 Hz). IR: 3400, 1700, 1595 cm^{-1}. MS: m/z 193 (12%, M^+), 112 (22), 99 (71), 98 (100), 95 (65), 84 (48), 70 (42), 69 (47).

Formation of 11a from 1d

A 4.09 g sample 0.01 *mol* of 1d was heated in water (100 ml, phosphate buffer pH 7) for 3 h under reflux. The solution was extracted with ethyl acetate and the residue of the organic fraction was distilled at 120 °C at 0.1 Torr. The volatile compounds were separated on a GC-MS system. The retention time (19.72 min) and mass spectral data of a substance were identical to those obtained from the isolated compound 11a.

Discussion

It can be assumed that Amadori compounds of type 1 are partly formed when glucose is degraded in the presence of amines. Aminoketoses (1) have been detected in dried and heated foods and also in foodstuffs stored over long periods [6].

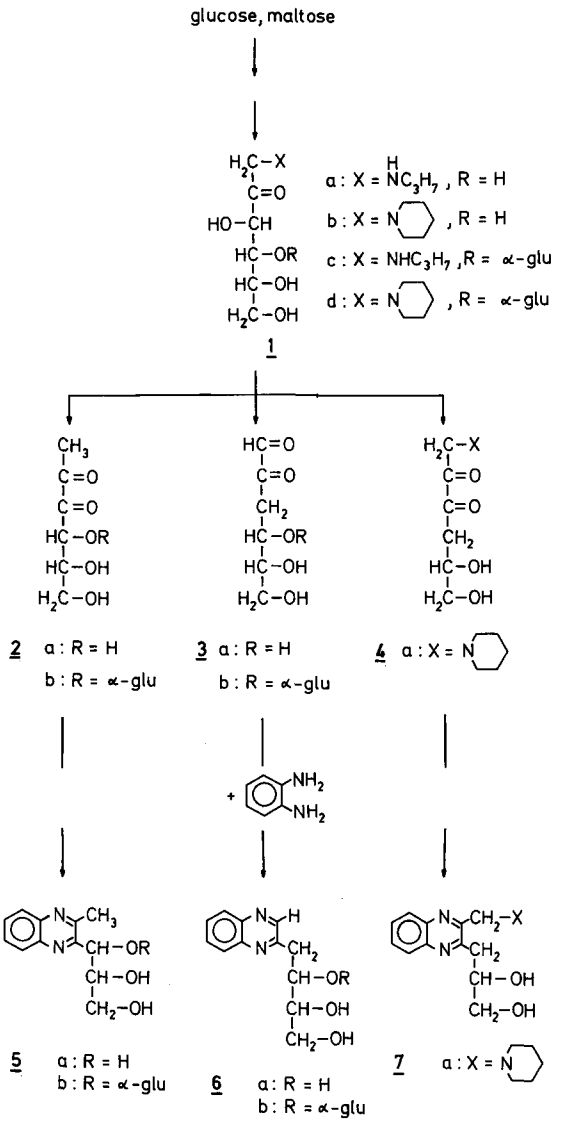
Compounds having the structure of 1 are formed in the human body by the reaction of glucose with the side-chain amino groups of lysine residues in proteins [7]. It has been suggested that the deoxyosones 2 and 3 are important degradation products of the aminoketoses.

Some years ago , Anet and Kato [8] succeeded in detecting 3a in the reaction mixtures of monosaccharides with amines; however, in spite of several attempts the isomeric dicarbonyl compound 2a could not be isolated from the appropriate sugar degradation products. 1-Deoxyosones are of special interest in the Maillard reaction. From the structures of characteristic products, it can be deduced that 1-deoxyosones must be intermediates in the sugar degradation process.

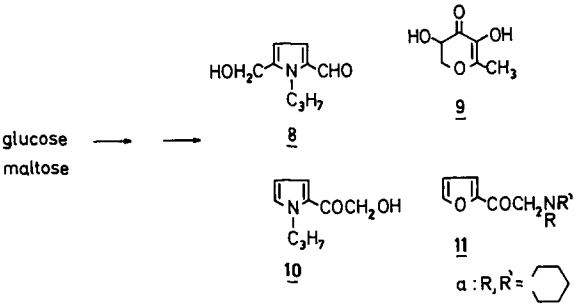
On heating an aqueous solution of 1-deoxy-1-piperidinofructose (1b) in the presence of *o*-phenylenediamine, a high yield of the quinoxaline 5a was obtained [2]. This result indicates that the degradation of the Amadori compound 1b leads to the production of 1-deoxyosone 2a as a main product.

Under similar conditions of reaction, 1-deoxy-1-propylaminofructose (1a) is transformed into the quinoxalines 5a and 6a with the latter as a minor component.

We also studied the degradation of the aminoketoses of disaccharides when heated in the pH range 4–7. In the presence of *o*-phenylenediamine we obtained from piperidinomaltulose 1d the quinoxalines 5b and 7a, and from propylaminomaltulose 1c the quinoxalines 5b and 6b. The formation of 5b and 6b during acid catalysed disaccharide degradation has been reported,



Scheme 1



Scheme 2

but the structures of 5d and 6d were only assigned tentatively, by interpretation of the mass spectral data of the silylated compounds [1c]. We obtained a high yield of 5b as a crystalline substance from the reaction mixture.

The results indicate that the Amadori compounds of glucose and maltose react in a similar way, giving the 1-deoxyosones 2a and 2b as the main reaction products and the 3-deoxyoson 3a and 3b as the minor products, respectively. Furthermore, the existence of the previously unknown 1-amino-1,4-dideoxy hexosulose 4a could be proven. Compared with acid catalysed sugar degradation, the deoxyosone 2b is obtained in essentially larger amounts from Amadori compounds 1c and 1d.

In further experiments, we compared the amounts of the Maillard products 8 and 9 formed in a glucose propylamine reaction mixture, with the concentration of these substances obtained from Amadori compound 1a. It is well known that 8 is a degradation product of the deoxyosone 3a [9] and the formation of 9 can be best explained from deoxyosone 2a. On heating the aminoketose 1a we found a ratio of 5:1 for compounds 9 and 8. In the glucose propylamine system, the ratio was 1:2. From these different ratios, it can be deduced that the sugar degradation in the presence of amines does not proceed exclusively through intermediate Amadori compounds. This result was confirmed by the separation of a considerable amount of pyrrole 10 in the glucose propylamine mixture. 10 was not detected in the heated solution of 1a.

It is well known that the treatment of Amadori compounds of type 1 with strong acids partly leads to the formation of furans with structure 11 [10]. Deoxyosones of general structure 4 are supposed to be intermediate substances in this acid catalysed degradation. However, it is unknown whether compounds of type 11 are formed in heated foods or more generally under the conditions of the Maillard reaction (pH range 5–7). We heated maltose and piperidine in an aqueous solution under nearly neutral conditions. Among the volatile products, 11a could be detected with the GC-MS system. Furane 11a was unstable in the pH range 5–7. The detection of 11a was only possible when the separation and the purification were started immediately after the reaction mixture was heated.

In future experiments, we would vice to investigate whether the formation of 4a and 11a is restricted to reaction systems of disaccharides and secondary amines or if the same products are formed from monosaccharides and primary amines.

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