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Note

# Formation of amino acids by reaction of glucose and xylose with primary amines

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Reactions of reducing sugars with amino acids and proteins (Maillard reaction) are of great importance in food chemistry [1]. During past decades glucose–protein interactions have received significant attention due to their medical implications. For instance, Maillard reaction products are obviously involved in long term complications associated with diabetics [2]. In the first step, glycosylation of the lysine side chain of proteins or other primary amines leads to the formation of a glycosylamine which easily rearranges to give an Amadori compound. Degradation of the latter affords a great variety of compounds.

Little is known about the formation of amino acids through reactions of sugars with primary or secondary aliphatic amines. Previous work has shown that the interaction of glucose with lysine side chains of proteins leads to the ω-carboxymethyl derivative of the lysine residue [3]. More recently an ω-lactyl derivative of lysine has been detected [4]. In this communication we show that glucose reacts with primary aliphatic amines to give N-substituted alanine derivatives (**1–4**) and, in lower amounts, N-substituted 4,5,6-trihydroxy-α-aminohexanoic acid (**5**) (Scheme 1).

## 1. Results and discussion

When L-xylose or D-ribose are heated with propylamine in a phosphate buffered neutral or slightly alkaline aqueous solution at about 70 °C, *N*-propylalanine (**1**) is

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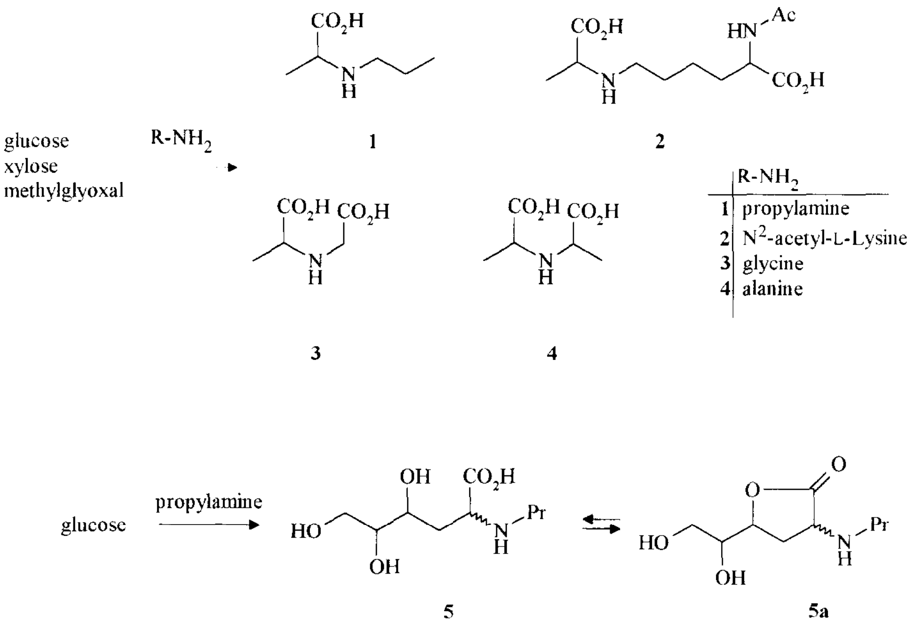


| | R-NH$_2$ |
|---|---|
| 1 | propylamine |
| 2 | N$^2$-acetyl-L-Lysine |
| 3 | glycine |
| 4 | alanine |

Scheme 1.

formed. The amino acid can be detected by GC–MS after esterification and acetylation. A reference substance is provided by reaction of pyruvic acid with propylamine and cyanoborohydride. Fig. 1 shows the GC-chromatogram of a xylose–propylamine reaction mixture after separation over a cation exchange resin and derivatization as described

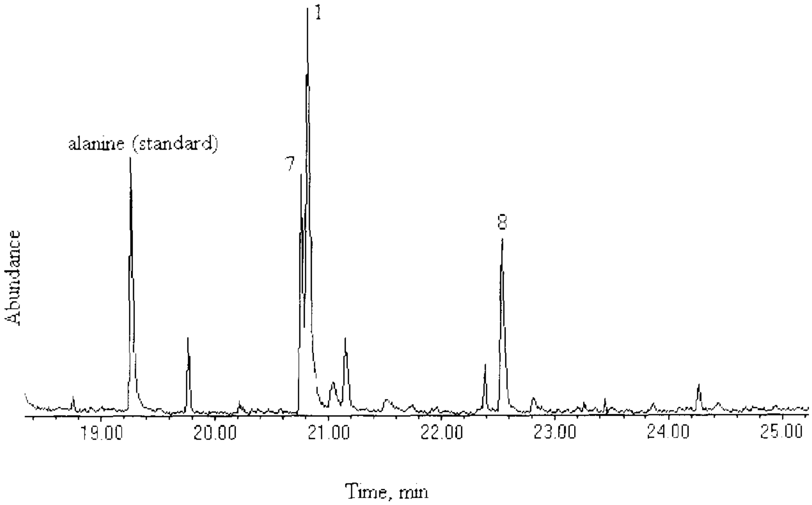


Fig. 1. Gas chromatogram of a Maillard reaction after extraction, ion exchange, esterification and acetylation.

below. Besides *N*-propylalanine (**1**), *N*-propylglycine (**7**) and ω-propylamino lactic acid (**8**) are produced as major products. It is well known that pentoses are more reactive than hexoses. Comparable yields of **1** are obtained from glucose only after prolonged heating. In analogous reactions the alanine derivatives **2** and **3** are formed when aldopentoses are heated with $N^2$-acetyl-L-lysine or glycine, respectively. The products were identified by GC–MS (see Experimental). When L-alanine reacts with xylose, the amino dicarboxylic acid **4** is obtained as a mixture of diastereoisomeres. Furthermore, *N*-carboxymethylalanine (**3**) was detected.

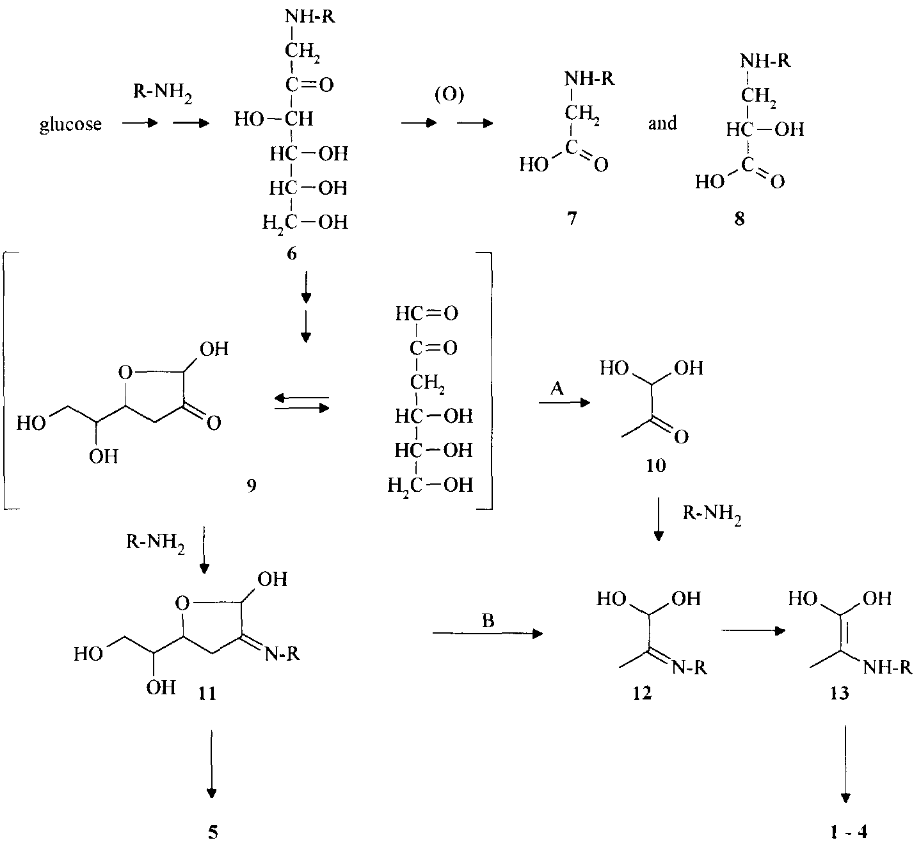
The yields of the alanine derivatives depend highly on the reaction conditions. In a typical experiment, an aqueous solution of equimolar amounts of xylose and propylamine and two molar equivalents of phosphate is adjusted to pH 7.5 and heated for two hours at 70 °C. During the reaction the solution turns deep brown and becomes slightly acidic (pH ~ 6.8). The yield of **1** after separation over a cation exchange resin and derivatization is approximately 0.7% and increases to 1% after several days. Prolonged heating at pH 8 leads to yields up to 2%. Heating for several days at 40 °C gave the same results.

Methylglyoxal can be considered an intermediate during the formation of the alanine derivatives by the reaction of pentoses or hexoses with amines. When methylglyoxal and propylamine react in a buffered solution at 70 °C for about 1 h, *N*-propylalanine is generated as one of the major products (yield ~ 3%).

Under the conditions applied so far, *N*-substituted 4,5,6-trihydroxy-*α*-aminohexanoic acids could be detected in low amounts. A reference compound (**5a**) was synthesized starting with glucometasaccharinic acid lactone by triflation and propylamine exchange. Acidic hydrolysis of the ketal group and alkali-induced ring opening provided the *N*-propyl-4,5,6-trihydroxy-α-aminohexanoic acid **5**. Ring opening is reversed by warming **5** with acid and **5a** can be detected by GC–MS after acetylation. When 3-deoxyhexosulose, obtained by degradation of glucose, is heated with propylamine in neutral solution, the aminolactone **5a** can be detected in the reaction mixture but only in very low yield.

Much effort has been devoted to isolate comparatively stable 'advanced glycosylation end products' (AGEs). One of the few hitherto known compounds which meets these criteria is carboxymethyllysine (CML) and methods have been developed to detect CML in reaction mixtures [5]. CML has already been shown to be a valuable indicator of advanced glycosylation [6]. As can be derived from Fig. 1, the alanine derivatives are of equal importance.

The amino acids identified as sugar degradation products are formed by different reaction routes. Oxidative degradation of the corresponding Amadori compound probably affords carboxymethyl substituted amines **7** [7] (Scheme 2). On the other hand the formation of the alanine derivatives can be explained without an oxidation step as shown in Scheme 2. Degradation of the Amadori compound **6** generates two main products, namely 1-deoxyhexosulose and 3-deoxyhexosulose (**9**) [1]. An essential step during the formation of *N*-propylalanine is a retroaldol reaction of the dicarbonyl compound **9**. This CC-cleavage may proceed either before (A) or after (B) generation of a Schiff base. Tautomerisation of the glyoxal ketimine **12** affords the amino acids **1**–**4**. On the other hand an intramolecular redox reaction of the Schiff base **11** would lead directly to the



Scheme 2.

trihydroxy-α-aminohexanoic acid **5**. An analogous reaction scheme does explain the degradation of xylose.

The formation of *N*-carboxymethylalanine from a pentose and glycine deserves special comment. The Strecker degradation of α-amino acids induced by reducing sugars is one of the best documented reactions in Maillard systems leading to an oxidative decarboxylation of the amino acid, ultimately resulting in the formation of aldehydes. This process is explained mechanistically by assuming a sugar-derived α-dicarbonyl compound as an intermediate. The results discussed above show that the intermediate **9** may rearrange in an alternative process to give the amino dicarboxylic acids **3**. The previously reported formation of carboxymethylalanine from glyoxal and alanine [8] follows an analogous reaction route.

$N^6$-(1-Carboxyethyl)lysine and the amino dicarboxylic acids **3** and **4** have been isolated previously as naturally occurring amino acids [9]. The corresponding amino acid and pyruvic acid should be viewed as biosynthetic precursors, whereas the formation of alanine derivatives from amino acids and a reducing sugar represents a new type of reaction.

## 2. Experimental

*Apparatus.*—$^1$H and $^{13}$C NMR spectra (internal standards; tetramethylsilane and [$d_6$]-acetone, respectively) were recorded with a JEOL 400 GSX spectrometer. Chemical shifts are reported in ppm. Mass spectral analyses were obtained with a Varian MAT CH7 (CI with $CH_4$, 70 eV).

*Gas chromatography (GC)–mass spectrometry (MS).*—Solutions were prepared according to the described procedures and analyzed by GC–MS using a 25 m × 0.25 μm i.d. fused silica capillary column coated with Permabond OV-1701-DF in a Hewlett Packard gas chromatograph, Model 5890/2, equipped with a Hewlett Packard MS computerized system, (Model 5971A). Temperature was programmed as follows: $\theta_1 = 40$ °C; Isotime 1 = 4 min; Ramp Rate 1 = 4 °C/min. $\theta_2 = 80$ °C; Isotime 2 = 0 min; Ramp Rate 2 = 20 °C/min. $\theta_3 = 260$ °C; Isotime 3 = 15 min. The mass spectrometer was operated in scan mode for compound identification, and mass spectra were recorded in the electron impact mode.

*Sample derivatization.*—(A) The alanine derivatives (**1–4**) (1 mg each) were esterified with 1 mL of an 1 M $SOCl_2$–EtOH solution for 1 h at 100 °C and filtered. After evaporation in vacuo the products were acetylated with 0.2 mL acetic anhydride in 0.4 mL anhydrous pyridine overnight and filtered. (B) (4*S*, 5*R*)-5,6-Dihydroxy-2-propylamino-γ-hexanolactone (**5a**) (1 mg) was acetylated as described above. (C) *N*-Propyl-alanine (**1**) and (4*S*, 5*R*)-5,6-dihydroxy-2-propylamino-γ-hexanolactone (**5**) (100 μg each) were silylated in 100 μL *N,O*-bis-(trimethylsilyl)-trifluoroacetamide (45 min, at 100 °C) and filtered.

*Sample preparation.*—Alanine derivatives: Sugar or methylglyoxal (0.1 mmol) were heated with amine (0.1 mmol) (for propylamine propylammonium acetate) in 1 mL of 0.2 M phosphate buffer (pH 7.5) for 3 h in a sealed tube. To a sample (0.2 mL) of this solution was added alanine (0.005 mmol) as internal standard. The resulting solution was extracted with ethyl acetate and acidified with concd HCl. It was added to a Dowex 50 ($H^+$-form) column and rinsed with distilled water until the washings were colorless. The cationic compounds were eluted with 1 N ammonium hydroxide. The lyophilizate was treated as described above and analyzed by GC–MS.

(4*S*, 5*R*)-5,6-Dihydroxy-2-propylamino-hexanolactone (**5a**): 3-Deoxy-2-glucosulose, obtained by the method of Madson and Feather [10] (80 mg, 0.5 mmol) and propylamine (60 mg, 1 mmol) were heated in 1 mL of a 1 M phosphate buffer (pH 6) for 4 h at 70 °C. An aliquot (0.2 mL) was dried under vacuum, suspended in 1 mL of MeOH–concd HCl (97:3) and allowed to stand for 1 h. After evaporation, the residue was treated as described above and analyzed by GC–MS.

*Preparation of reference compounds.*—Synthesis of *N*-propylalanine (**1**), $N^2$-acetyl-$N^6$-(1-carboxyethyl)-L-lysine (**2**), N-(carboxymethyl)-alanine (**3**) and N-(1-carboxyethyl)-alanine (**4**). These compounds were synthesized via reductive amination according to ref. [11] with minor changes. A solution of sodium pyruvate (330 mg, 3 mmol) and propylammonium chloride (315 mg, 3.3 mmol) or the corresponding amino acid (2 mmol) and $NaBH_3CN$ (160 mg, 2.5 mmol) in 30 mL of methanol were stirred for 24 h at room temperature. The solution was acidified with concd HCl and evaporated in vacuo. The residue was dissolved in 5 mL of $H_2O$, applied to a Dowex 50 ($H^+$-form) column (50–100 mesh) and the column was washed with amounts of 5 mL of 1 N ammonium hydroxide.

N-*Propylalanine* (**1**) *(177 mg, 45%)*.—$^1$H NMR ($CD_3OD$): δ 1.0 (t, 3 H, *J* 7.2 Hz, $CH_3$–$CH_2$), 1.48 (d, 3 H, *J* 7.0 Hz, H-3), 1.71 (s, 2 H, *J* 7.5 Hz, $CH_3$–$CH_2$), 2.9 (m, 2 H, $CH_2$–NH), 3.48 (q, 1 H, *J* 7.1 Hz, H-2); $^{13}$C ($D_2O$): δ 12.63 ($CH_3$–$CH_2$), 17.5 (C-3), 21.84 ($CH_3$–$CH_2$), 50.25 ($CH_2$–NH), 60.20 (C-2), 177.83 (C-1); GC–MS-data (after esterification and acetylation): $t_{ret}$ = 20.81 min; $m/z$ = 201 (1%, $M^+$), 158 (6), 128 (41), 86 (100), 56 (13), 43 (16); [after silylation (under the present conditions mainly monosilylation occurred)]: $t_{ret}$ = 16.7 min; $m/z$ 203 (0.2%, $M^+$), 188 (3), 103 (9), 86 (100), 75 (15), 73 (13), 56 (20), 44 (9).

$N^2$-*Acetyl*-$N^6$-*(1-carboxyethyl)*-L-*lysine* (**2**) *(39.6 mg, 9.1%)*.—$^1$H NMR ($D_2O$): δ 1.43 (m, 2 H, H-4), 1.46 (d, 3 H, *J* 7.1 Hz, CH–$CH_3$), 1.74 (m, 3 H, H-5 + H-$3_a$), 1.89 (dq, 1 H, *J* 6 Hz, H-$3_b$), 2.05 (s, 3 H, $CH_3$–CO), 3.03 (t, 2 H, *J* 7.3 Hz, H-6), 3.65 (q, 1 H, *J* 7.1 Hz, $CH$–$CH_3$), 4.40 (dd, 1 H, *J* 5 Hz, H-2); $^{13}$C ($D_2O$): 15.42 ($CH_3$–CH), 21.84 ($CH_3$–CO), 22.48 (C-4), 25.57 (C-3), 30.02 (C-5), 46.14 (C-6), 53.14 + 58.11 (C-2 + $CH_3$–$CH$), 173.68 + 174.45 + 174.70 (2 × $CO_2H$ + CONHR); GC–MS-data (after esterification and acetylation): $t_{ret}$ = 35.1 min: $m/z$ = 358 (2%, $M^+$), 315 (10), 285 (10), 243 (100), 225 (24), 184 (37), 156 (30), 130 (19), 110 (27), 84 (24), 56 (13), 43 (23).

N-*(Carboxymethyl)-alanine* (**3**) *(65 mg, 22%)*.—$^1$H NMR data corresponded to those in ref. [12]. $^1$H NMR ($D_2O$): δ 1.50 (d, 3 H, *J* 7 Hz, $CH_3$), 3.75 (s, 2 H, $CH_2$), 3.85 (q, 1 H, *J* 7 Hz, $CH$); $^{13}$C ($D_2O$): δ 15.06 ($CH_3$), 47.96 ($CH_2$), 57.86 (CH), 171.91 + 175.31 (2 × $CO_2H$); GC–MS-data (after esterification and acetylation): $t_{ret}$ = 21.9 min: $m/z$ = 245 (1%, $M^+$), 202 (5), 200 (4), 130 (100), 102 (5), 56 (25), 43 (15).

N-*(1-Carboxyethyl)-alanine* (**4**) *(77 mg, 24%)*.—$^1$H NMR data corresponded to those in ref. [12]. $^1$H NMR ($D_2O$): δ 1.45 (d, 6 H, *J* 7 Hz, 2 × $CH_3$), 3.51 (q, 1 H, *J* 7 Hz, CH), 3.6 (q, 1 H, *J* 7 Hz, CH); $^{13}$C ($D_2O$): δ 15.27 + 15.97 ($CH_3$), 56.78 + 57.47 (CH), 175.51 + 175.56 (2 × $CO_2H$); GC–MS-data (after esterification and acetylation): $t_{ret}$ = 22.08 min + 22.16 min: $m/z$ 259 (1%, $M^+$), 216 (7), 186 (10), 144 (100), 70 (20), 43 (11).

*Synthesis of the diastereomeric mixture of (4*S*, 5*R*)-5,6-dihydroxy-2-propylamino-γ-hexanolactone* (**5a**).—(A) Synthesis of the diastereomeric mixture of (4*S*, 5*R*)-5,6-*O*-isopropylidene-2-hydroxy-γ-hexanolactone. A stirred suspension of glucometasaccharinic acid lactone, obtained by the method of Wood and Fletcher [13], in boiling dioxane (300 mL) and 2,2-dimethoxypropane (40 mL) was treated with anhydrous stannous chloride (100 mg) and the mixture was heated under reflux for 2 h. The cooled mixture was treated with pyridine (0.1 mL) and concentrated in vacuo. Chromatography of the residue on silica gel, eluting with 3:1 (v/v) EtOAc–hexane, gave 6.7 g (90%) of the acetal as an oil. $^{13}$C NMR ($CD_3OD$): δ 26.5 and 24.9 ($CH_3$), 33.3 (C-3), 66.3 (C-6), 67.9 (C-4), 76.5 (C-5), 76.6 (C-2), 110.3 [$(CH_3)_2C(O)_2$], 177.0 (C-1); MS-CI: $m/z$ = 203 (100%, M + $1^+$), 145 (11), 117 (8), 99 (19).

(B) Synthesis of the diastereomeric mixture of (4*S*, 5*R*)-5,6-*O*-isopropylidene-2-propylamino-γ-hexanolactone. A solution of trifluoromethanesulfonic acid anhydride (0.4 mL, 2.4 mmol) in 4 mL dichloromethane was added dropwise over 0.5 h to a cooled (−50 °C), stirred solution of the acetal described in the proceeding section (440 mg, 2 mmol) in pyridine (0.7 mL, 8 mmol) and dichloromethane (40 mL). The reaction was allowed to proceed at − 10 °C for 1 h, after which time the mixture was washed with 1 N HCl (10 mL). The layers were separated and the organic phase was dried over $Na_2SO_4$ and used without further purification. Pyridine was added (0.2 mL, 2.2 mmol) and propylamine (120 mg, 2 mmol) was added dropwise to the stirred, cooled (0 °C) solution of the triflate. After stirring for 2 h, the mixture was filtered and concentrated in vacuo. Chromatography of the residue on silica gel, eluting with 3:1 (v/v) EtOAc–hexane, gave 370 mg (76%) of the protected amino acid lactone as an oil. $^1H$ NMR ($CD_3OD$): δ 0.91 (t, 3 H, *J* 7.3 Hz, $CH_2$–$C\mathit{H}_3$), 1.28 and 1.21 [s, 3 H, $(C\mathit{H}_3)_2C(O)_2$], 1.63 (m, 2 H, $C\mathit{H}_2$–$CH_3$), 1.84 (m, 1 H, H-$3_b$), 2.11 (m, 1 H, H-$3_a$), 2.88 (t, *J* 7.3 Hz, 2 H, N–$CH_2$), 3.66 (m, 1 H, H-2), 3.95–3.77 (m, 3 H, H-5, H-$6_{a,b}$), 4.51 (m, 1 H, H-4); $^{13}C$ NMR ($CD_3OD$): 10.3 ($CH_2$–$\mathit{C}H_3$), 20.5 ($\mathit{C}H_2$–$CH_3$), 26.9 and 25.4 [$(\mathit{C}H_3)_2C$], 37.4 (C-3), 41.3 (N–$CH_2$), 60.5 (C-2), 62.7 (C-6), 74.4 (C-4), 77.4 (C-5), 109.5 [$(CH_3)_2\mathit{C}$], 180.2 (C-1). MS-CI: $m/z = 244$ (100%, M + $1^+$), 216 (28), 186 (11), 149 (19).

(C) Removing the protective group. The protected amino acid lactone was dissolved in 2 mL of THF 1 N HCl (1:1). After standing overnight at room temperature the solution was concentrated in vacuo. The resulting residue was used after acetylation or silylation for gas chromatographic analysis. GC–MS-Data for **5a**: $t_{ret}$ (acet.) = 32.66 min; $m/z = 392$ (10%, $M^+$), 287 (4), 258 (35), 216 (15), 198 (13), 184 (40), 154 (52), 142 (13), 124 (44), 114 (46), 43 (100); $t_{ret}$ (silyl.) = 23.42 and 23.46 min; $m/z = 304$ (12), 201 (15), 200 (15), 147 (21), 114 (31), 98 (100), 73 (81).

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