Tetrahedron Vol. 47, No. 45, pp. 9351-9356, 1991
Printed in Great Britain

0040-4020/91 $3.00+.00
Pergamon Press plc

5,6-DIHYDRO-3,5-DIHYDROXY-4H-PYRIDONES - NEW MAILLARD PRODUCTS FROM 6-O-SUBSTITUTED HEXOSES AND PRIMARY AMINES

Berthold Kettner, Franz Ledl*[a)], Holger Lerche, Theodor Severin*

Institut für Pharmazie und Lebensmittelchemie der Universität, Sophienstr. 10, 8000 München 2
Institut für Lebensmittelchemie und Analytische Chemie der Universität,[a)] Pfaffenwaldring 55, 7000 Stuttgart 80

(*Received in Germany* 28 *June* 1991)

Abstract - From reaction mixtures of n-propylamine and 6-O-substituted hexoses **1** (glucose-6-phosphate, isomaltose, 6-O-benzylgalactose) a previously unknown Maillard product, the 5,6-dihydro-3,5-dihydroxy-1-n-propyl-4H-pyridone (**7a**) was isolated in yields up to 20%.

INTRODUCTION

Reactions of reducing sugars with amino acids or proteins (Maillard-reaction) are of great interest in food chemistry and also in biochemistry and medical chemistry according to recent investigations.[1] Generally a vast variety of compounds is obtained when sugars degrade in the presence of primary or secondary amines. Among the products isolated so far there are heterocycles like furans, pyrroles, pyridines, pyrazines, pyrones or pyridones.

It is remarkable that different compounds are obtained from mono- and disaccharides. Maltol and galactosylisomaltol formed by thermal degradation of maltose and/or lactose are known for a long time.[2] More recently further compounds have been isolated and it is possible to describe the reaction pathways leading from disaccharides to several products.[3]

In the last years a new type of disaccharide has appeared in foods. To reduce calories and to avoid caries traditional sugars such as glucose or saccharose are partly replaced by 6-O-substituted hexoses **1**.[4]
Another very important compound of type **1** is glucose-6-phosphate which is

formed in cells immediately after the intake of glucose from the blood.

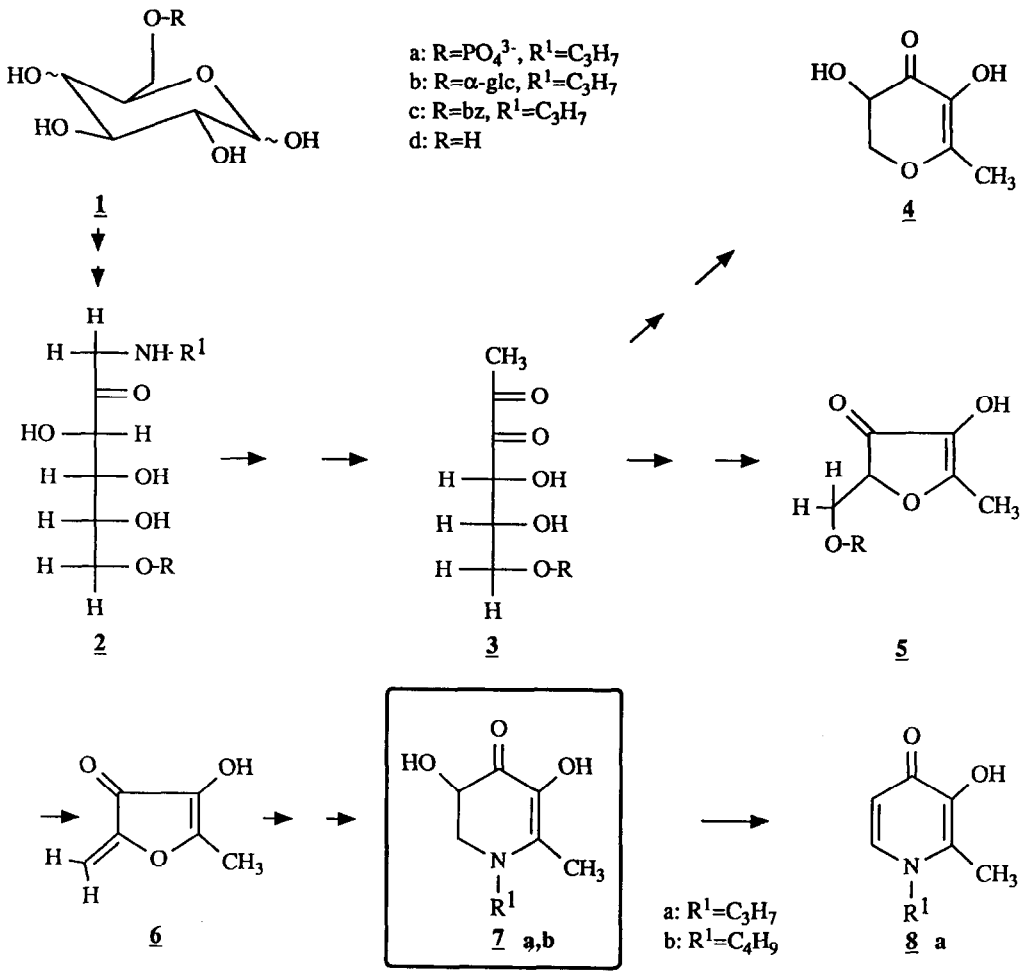
RESULTS AND DISCUSSION

Aqueous solutions of n-propylamine and glucose-6-phosphate, isomaltose or 6-O-benzylgalactose were heated at pH 7 for 30 minutes under reflux. From all reaction mixtures we obtained the dihydropyridone **7a** as a main product in yields from 5-20%. Under physiological conditions (pH 7.4, 37°C, 2 days) comparable amounts of the dihydropyridone can be isolated. The purification of **7a** was achieved by extraction, distillation and separation on reversed-phase-material. In comparable glucose reaction mixtures we did not detect compound **7a**; from fructose it was formed in small amounts (0.1%). Therefore the dihydropyridone **7** is a characteristic Maillard product of hexoses with the general structure **1**.

The structure of the previously unknown pyridone **7a** was derived from the spectral data. The maximum at 361 nm in the UV spectrum and the signal at 1613 cm^{-1} in the IR spectrum agree with the corresponding values of other enaminoketones. In the ^{1}H-NMR spectrum the coupling constant of 14 Hz is typical for antiperi-planar vicinal protons in 6-membered compounds. Substance **4**, a well-known hexose degradation product[5] shows the same pattern.

7a is transformed when heated in solution and this indicates that the amount of **7a** initially formed from **1** is higher than that determined in the reaction mixtures. Heating **7a** in an aqueous solution (buffered to pH 7) leads to pyridone **8a** as the main product. The compound **8a** has been isolated earlier from reaction mixtures of lactose or maltose with n-propylamine.[7] Like **4**, the dihydropyridone **7** is susceptible to oxidation reactions. With iron-III-salts blue complexes are formed but the solution quickly becomes colourless.

In the scheme a reaction pathway is proposed to explain the transformation of **1** into the dihydropyridone **7**. The intermediate furanone **5c** could be isolated. Results of model reactions support this pathway:



As expected we obtained 7a when 5c was heated with n-propylamine in neutral aqueous solution. We can exclude a pathway leading directly from the amino-ketose 2 to the dihydropyridone 7 without splitting off the amine residue. In reaction mixtures of 2c and n-butylamine (molar ratios a: 70:1, b: 1:1, c: 1:5) we found the pyridones 7a and 7b in nearly the same ratio. This is plausible only when the n-propylamine is split off during the reaction sequence.

At present we can not predict whether the formation of the dihydropyridone 7 is of importance as a reaction taking place in human cells with glucose-6-phosphate and proteins.

EXPERIMENTAL SECTION

General

Melting points were determined on a Büchi 510 apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra (internal tetramethylsilane) were recorded with a Jeol 400 GSX spectrometer. IR spectra were measured with a Perkin Elmer 197 spectrometer. Thin-layer chromatography (TLC) was performed using aluminium sheets coated with 0.2 mm thickness of Merck silica gel 60 F_{254} (Merck, Art. 5554). Mass spectral analysis were obtained with a Varian MAT CH7 (EI: 70eV). GC/MS analysis were measured with a Hewlett Packard 5890 Series II combined with a MSD 5971A (GC: Column: Permabond OV-1701-DF-0.25, 25 m/0.25 mm ID, Machery-Nagel, Art. Nr. 723058; Method: Iso Time 1: 2.0 min, Temp. 1: 100°C, Temp. 2: 260°C, Rate: 6°C/min, Iso Time 2: 15 min). HPLC separations were performed with a Merck/Hitachi L-6000 pump, a L-4000 UV-detector and a D-2000 chromato-integrator on a Nucleosil C18 reversed-phase column (250 mm/10 mm, 10 µm packing, Fa. Bischoff, Part No.: 25801830; method a: flow rate 2.6 mL/min, detection at 360 nm, elution with water/methanol 75:25 and method b: flow rate 2.6 mL/min, detection at 290 nm, elution with water/methanol 50:50).

Isolation of 5,6-Dihydro-3,5-dihydroxy-1-n-propyl-4H-pyridone (7a)

A mixture of 6-O-benzylgalactose[6] (3.6875 g, 12.5 mmol), n-propylamine (neutralized with phosphoric acid; 5.1 ml, 62.5 mmol) and phosphate buffer pH 7.0 (4 ml) was refluxed for 30 min. Sand (50 g) was added and the suspension was dried under reduced pressure at 70°C. The resulting powder was extracted with ethyl acetate for 3 h. The solvent was removed and the remaining deep-brown syrup was distilled. The fraction boiling at 80-120°C and 0.1 Torr was separated on HPLC (HPLC method a). Fractions with RT 6.2 were collected and after removal of the elution solvents compound 7a was obtained as crystals (m.p. 100°C; yield 160 mg, 7%; RT 19.7 and silylated 21.75 in the GC/MS system).
In the same manner glucose-6-phosphate sodium salt (0.50 g, 1.7 mmol) and isomaltose (0.10 g, 0.29 mmol) were heated with n-propylamine. Yields were measured by quantitative HPLC analysis (method a, internal standard: p-nitroaniline) and were 20% for glucose-6-phosphate sodium salt and 5% for the isomaltose. Compound 7a was separated in the same way and identified after silylation by GC/MS analysis.

^{1}H NMR ($CDCl_3$): δ = 0.95 (t, 3H; $C\underline{H}_3$-CH_2-CH_2), 1.53-1.69 (m, 2H; CH_3-$C\underline{H}_2$-CH_2), 2.14 (s, 3H, $C\underline{H}_3$-C), 3.16-3.23 (m, 1H; CH_3-CH_2-$C\underline{H}_2$-N), 3.25-3.32 (q, $J_{vic,AX}$ = 14.1 Hz, $J_{gem,AB}$ = 12.4 Hz, 1H; N-$C\underline{H}_A H_B$-CH_XOH) 3.27-3.33 (m,1H; CH_3-CH_2-$C\underline{H}_2$-N), 3.45-3.49 (q, $J_{gem,AB}$ = 12.4 Hz, $J_{vic,BX}$ = 6 Hz, 1H; N-$CH_A\underline{H}_B$-CH_XOH), 4.17-4.22 (q, $J_{vic,AX}$ = 14.1 Hz, $J_{vic,BX}$ = 6 Hz, 1H; $CH_A H_B$-$C\underline{H}_X$OH-CO). - ^{13}C NMR (BB and DEPT; $CDCl_3$): δ = 11.06 ($\underline{C}H_3$-CH_2-CH_2), 13.33 ($\underline{C}H_3$-C), 21.85 (CH_3-$\underline{C}H_2$-CH_2), 53.16 (CH_3-CH_2-$\underline{C}H_2$-N), 54.11 (N-$\underline{C}H_2$-CHOH), 67.19 (CH_2-$\underline{C}$HOH-CO), 127.26 (CH_3-$\underline{C}$=C), 152.39 (-C=$\underline{C}$-CO), 180.97 (=C-$\underline{C}$O-CHOH). - MS (GC/MS) [m/z; relative %]: 185(M^+,79), 168(11), 156(100), 149(19), 140(14), 128(14), 114(6), 98(6), 84(7), 70(8), 55(11), 42(32). - MS (GC/MS; silylated): 329(M^+,16), 314,(100), 300(3), 270(5), 224(14), 212(6), 197(14), 182(15), 147(5(, 133(4), 73(54), 42(14). - IR (KBr) [cm^{-1}]: 3321, 2964, 1613, 1529, 1457, 1404, 1373, 1323, 1262, 1237, 1072, 856, 802, 688. - UV (MeOH): λ_{max} [nm] 361, (ϵ) (8500).

Isolation of 5,6-Dihydro-3,5-dihydroxy-1-n-butyl-4H-pyridone (7b)

In the same manner as 7a dihydropyridone 7b was prepared from 6-O-benzyl-galactose[6] (3.69 g, 12.5 mmol) and n-butylamine (6.1 ml, 62.5 mmol). After HPLC separation (method a) fractions with RT 9.2 were collected and

after removal of the elution solvents **7b** was obtained as a solid (m.p. 120°C, yield 150 mg, 6%; RT 20.4 and silylated 22.4 in the GC/MS system).

^{1}H NMR ($CDCl_3$): δ = 0.86 (t, 3H; $C\underline{H}_3$-CH_2-), 1.25-1.33 (m, 2H; CH_3-$C\underline{H}_2$-CH_2), 1.36-1.45 (m, 2H; CH_2-$C\underline{H}_2$-CH_2), 2.08 (s, 3H; $C\underline{H}_3$-C), 3.10-3.31 (m, 1H, $J_{vic,AX}$ = 14 Hz, $J_{gem,AB}$ = 12.5 Hz, 1H; N-$C\underline{H}_A\underline{H}_B$-$CH_X$OH and m, 2H; CH_2-$C\underline{H}_2$-N), 3.38-3.43 (q, $J_{gem,AB}$ = 12.5 Hz, $J_{vic,BX}$ = 6 Hz, 1H; N-$CH_A\underline{H}_B$-CH_XOH), 4.11-4.15 (q, $J_{vic,AX}$ = 14 Hz, $J_{vic,BX}$ = 6 Hz, 1H; $CH_A\underline{H}_B$-$C\underline{H}_X$OH-CO). - MS (Varian MAT CH7) [m/z; relative %]: 199 (M^+,47), 181(18), 170(41), 156(71), 150(29), 135(53), 128(47), 115(77), 100(82), 86 (83), 84(29), 73(88), 72(93), 60(88), 43(100). - MS (GC/MS; silylated): 343(M^+,26), 328(100), 300(6), 270(7), 238(20), 210(12), 182(20), 166(2), 147(45), 117(2), 100(17), 73(45), 45(9).

Isolation of 4-Hydroxy-2-(benzyloxymethyl)-5-methyl-3(2H)-furanone (5c)

A mixture of 6-O-benzylgalactose[6] (10 g, 37 mmol) and diethylamine (4 ml, 37 mmol) in 62 ml methanol was heated for 3 h under reflux. After dropwise adding of acetic acid the mixture was heated for a further 2.5 h. Methanol was removed, water was added to the residue and buffered to pH 5-6. The aqueous layer was extracted with diethyl ether and the residue of the organic layer was separated with HPLC (method b). The fractions with RT 7.27 were collected and after removal of the elution solvent compound **5c** was obtained as an oil (yield 170 mg, 2%).

^{1}H NMR ($CDCl_3$): δ = 2.20 (s, 3H; $C\underline{H}_3$-C), 3.58-3.62 (dd, $J_{gem,AB}$ = 11 Hz, $J_{vic,AX}$ = 7 Hz, 1H; O-$C\underline{H}_A\underline{H}_B$-$CH_X$), 3.82-3.85 (dd, $J_{gem,AB}$ = 11 Hz, $J_{vic,BX}$ = 2 Hz, 1H ; O-$CH_A\underline{H}_B$-CH_X), 4.47-4.56 (2d, J=12 Hz, 2H; $CH_A\underline{H}_B$-O-$C\underline{H}_2$-C_6H_5, 4.56-4.58 (q, $J_{vic,AX}$ = 7 Hz, $J_{vic,BX}$ = 2 Hz, 1H; $CH_A\underline{H}_B$-$C\underline{H}_X$-O), 7.18-7.28 (m, 5H; $C_6\underline{H}_5$-CH_2). - MS (Varian MAT CH7): 234(M^+,37), 216(17), 204(6), 191(4), 181(4), 175(9), 168(6), 161(4), 157(9), 145(10), 141(9), 128(46), 126(37), 107(43), 105(37), 91(100), 77(53), 65(49), 55(46), 43(63).

Formation of 7a from Furanone 5c and n-Propylamine

An aqueous solution (0.2 ml, buffered to pH 7) of furanone **5c** (0.138 g, 0.59 mmol) and n-propylamine (0.048 ml, 0.59 mmol) was kept at 100°C for 15 min. HPLC analysis (method a) and mass spectral data (GC/MS) indicated dihydropyridone **7a** as a main product.

Formation of 7a and 7b from Aminoketose 2c and n-Butylamine

A mixture of 1-n-propyl-1-desoxy-6-O-benzyl-fructose oxalic salt **2c** (4.45 g, 12.5 mmol), n-butylamine (neutralized with phosphoric acid; a: 0.018 ml, 0.18 mmol, molar ratio 70:1; b: 1.23 ml, 12.5 mmol, molar ratio 1:1; c: 6.1 ml, 62.5 mmol, molar ratio 1:5) and phosphate buffer pH 7.0 (4 ml) was refluxed for 10 min. HPLC analysis (method a) showed nearly the same peak area ratios for **7a** and **7b** in relationship to the molar ratios (a: 85:1; b: 27:24; c: 7:43).

Formation of Pyridone 8a from Dihydropyridone 7a

An aqueous solution (5 ml, buffered to pH 7) of **7a** (200 mg, 1.1 mmol) was stirred under reflux for 1 h. As main degradation product pyridone **8a** could be isolated. Spectral data were in agreement with those of the reference substance.[7]

REFERENCES

1. F.Ledl, E. Schleicher, *Angew. Chem., Int. Ed. Engl. 29(1990) 565.*
2.a. J.E. Hodge, *E.C. Nelson, Cereal. Chem. 38(1961) 207.*
   b. J. Brand, *Ber. Dtsch. Chem. Ges. 27(1894) 806.*
   c. S. Patton, *J. Biol. Chem. 184(1950) 131.*
3. F. Ledl, G. Ellrich, H. Klostermeyer, *Z. Lebensm. Unters. Forsch. 182(1986) 19.*
4. H. Schiwieck, *Lebensmittelchem. Gerichtl. Chem. 41(1987) 49.*
5.a. T. Severin, W. Seilmeier, *Z. Lebensm. Unters. Forsch. 137(1968) 4*
   b. F.D. Mills, D. Weisleder, *Tetrahedron Lett. 1970, 1243.*
6. E.F.L.J. Anet, *Carbohydr. Res., 7(1968) 84.*
7. F. Ledl, H. Osiander, O. Pachmayr, T. Severin, *Z. Lebensm. Unters Forsch. 188(1989) 207.*