Carbohydrate Research, 232 (1992) 125–130
Elsevier Science Publishers B.V., Amsterdam

The reaction of some dicarbonyl sugars with aminoguanidine *

Jan Hirsch [a,1], Eva Petrakova [b,1] and Milton S. Feather [a]

[a] *Department of Biochemistry, University of Missouri–Columbia, Columbia, Missouri 65211 (USA)*
[b] *NIDDK, National Institutes of Health, Bethesda, Maryland 20892 (USA)*

(Received December 13th, 1991; accepted February 18th, 1992)

ABSTRACT

The reactions of aminoguanidine (guanylhydrazine) with 3-deoxy-D-*erythro*-hexos-2-ulose (**1a**), 3-deoxy-D-*glycero*-pentose-2-ulose (**1b**), D-*erythro*-hexos-2-ulose (**1c**), and D-*glycero*-pentose-2-ulose (**1d**) were examined at 37° at a solution pH of 7.0 (phosphate buffer). For **1a** and **1b**, two major products were observed and shown respectively to be the 5- and 6-substituted 3-amino-1,2,4-triazine derivatives. The ratios of the products were independent of the amount of aminoguanidine present or the order of mixing the reagents prior to the experiments. For **1c** and **1d**, only the 5-substituted triazine derivatives were formed. No evidence for hydrazone or bishydrazone formation was observed.

INTRODUCTION

During the Maillard reaction, reducing sugars interact with amino groups to produce 1-amino-1-deoxy-2-ketose derivatives (Amadori compounds), which then undergo further degradation to give highly reactive dicarbonyl sugar derivatives as reaction intermediates. For a Maillard reaction involving D-glucose [1], the major dicarbonyl intermediate is 3-deoxy-D-*erythro*-hexos-2-ulose (**1a**) *. The extent to which the Maillard reaction proceeds in living systems via reducing sugars reacting with protein is not fully understood, but the initial stages of the reaction, the formation of Amadori compounds (protein glycation) have clearly been docu-

Correspondence to: Professor M.S. Feather, Department of Biochemistry, University of Missouri–Columbia, Columbia, MO 65211, USA.

* Journal paper No. 11570 of the Missouri Agricultural Experiment Station.

[1] On leave from the Institute of Chemistry, Slovak Academy of Sciences, 842 38 Bratislava, Czechoslovakia.

* Aldoketoses have also been named as "osones", a practice which is not recommended. Thus, **1a** = "3-deoxy-D-glucosone", **1b** = "3-deoxy-D-xylosone", **1c** = "D-glucosone", and **1d** = "D-xylosone".

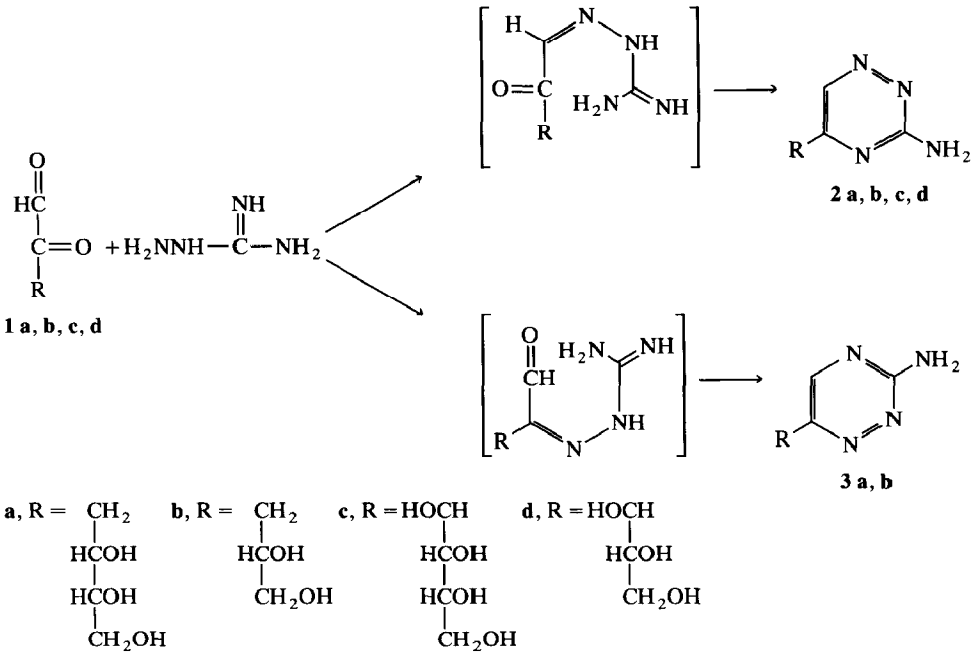
mented [2,3], and evidence for the presence of **1a** in human serum and urine has been reported [4]. Also associated with Maillard reactions in vivo are protein cross-linking [5] and the production of fluorescent materials [6,7]. Recently, a number of reports have appeared which show that aminoguanidine (guanylhydrazine) is capable of inhibiting Maillard reactions [8] in vitro in the sense that no protein cross-linking and other secondary degradation reactions are observed. This study reports an examination of the reactions of some relevant dicarbonyl sugar derivatives with aminoguanidine under physiological conditions. The dicarbonyl compounds used in the study are those that are produced from ascorbic acid [9] during its degradation. These include 3-deoxy-D-*glycero*-pentose-2-ulose (**1b**) and D-*glycero*-pentose-2-ulose (**1d**), as well as 3-deoxy-D-*erythro*-hexos-2-ulose (**1a**), which is produced from a D-glucose-derived Amadori compound [1] as well as from D-*erythro*-hexose-2-ulose (**1c**) itself.

EXPERIMENTAL

General methods.—Melting points were determined with a Thomas–Hoover Unimelt apparatus in open capillary tubes and are uncorrected. Optical rotations were measured at 24° with a Perkin–Elmer automatic polarimeter Model 241 MC. TLC was performed on silica gel plates (Whatman K5F) using 7:3:0.3 (v/v) $CHCl_3$–MeOH–H_2O as irrigant. Detection was effected with UV light and by spraying with 5% H_2SO_4 in EtOH, followed by charring at 120°. Preparative column chromatography was performed using 200–400 mesh (ASTM) silica gel (Aldrich). ^{1}H- (500 MHz, H_2O, internal references) and ^{13}C-NMR (125 MHz, dioxane, external reference) spectra, and COSY and HETCOR experiments were obtained on a Bruker AMX 500 instrument. Nonequivalent geminal protons are denoted H_a when resonating at lower field and H_b when resonating at higher field. Mass spectra were obtained using a Kratos MS-25 spectrometer interfaced with a DS-55 data system by direct probe insertion at 70 eV and an ion source temperature of 200°. UV spectra were recorded with a Gilford spectrophotometer using aqueous solutions.

Preparation of dicarbonyl derivatives.—Compounds **1a** [10], **1b** [9], **1c** [11], and **1d** [12] were prepared as described in previous reports. All were obtained as syrups that were chromatographically homogeneous as evidenced by TLC.

General reaction conditions.—The experimental protocol was the same for all reactions. In a typical experiment, **1a–d** (500 mg) were dissolved in 25 mL of 0.2 M phosphate buffer at pH 7.0, a 1.2 molar equivalent of aminoguanidine (bicarbonate salt) was added to the solution, and the mixture was incubated at 37° in a water bath. Aliquots were removed at intervals and applied to TLC plates in order to examine the progress of the reaction. For **1a** and **1b**, the reaction was complete within 5 min with no trace of the starting compounds present (R_F 0.6 and 0.75, respectively), and two new spots having R_F 0.5, 0.45 and, 0.65, 0.6, respectively) were detectable as reaction products. For the cases of **1c** and **1d** (R_F 0.25 and 0.35,



Scheme 1.

respectively), the reactions proceeded more slowly but were complete within 8 h, with one major reaction product being produced (R_F 0.3 and 0.45, respectively). Other degradation products were also produced (R_F 0.0–0.1), but were not further examined. The reaction products were separated on 4 × 25 cm silica gel columns using the same irrigant as was used for TLC, and all were obtained as crystalline materials. The structures were assigned from the following data, where unprimed numbers refer to the triazine ring atoms, and primed numbers refer to the sugar-derived side chain atoms (R group, see Scheme 1).

Products **2a–d** *and* **3a** *and* **3b**.—3-Amino-5-[(2*S*,3*S*)-2,3,4-trihydroxybutyl]-1,2,4-triazine (**2a**); Yield 70 mg (11.3%); mp 146–147° (MeOH); $[\alpha]_D$ −66.4° (*c* 0.83, H_2O); R_F 0.5; λ_{max} 317.5 nm (ϵ 3360 M^{-1} cm^{-1}); ^{1}H-NMR data (D_2O): δ 8.34 (s, 1 H, H-6), 3.87 (ddd, 1 H, $J_{3',4'a}$ 9.6 Hz, $J_{3',4'b}$ 3.2 Hz, $J_{2',3'}$ 6.7 Hz, H-3′), 3.60 (dd, 1 H, $J_{1'b,2'}$ 3.0 Hz, $J_{1'a,1'b}$ 11.4 Hz, H-1′$_b$), 3.48 (m, 1 H, H-2′), 3.44 (dd, 1 H, $J_{1'a,2'}$ 6.8 Hz, H-1′$_a$), 2.85 (dd, 1 H, $J_{4'a,4'b}$ 14.4 Hz, H-4′$_b$), 2.61 (dd, 1 H, H-4′$_a$); ^{13}C-NMR data (^{1}H decoupled, confirmed by DEPT, D_2O): δ 38.1 (C-1′), 62.2 (C-4′), 70.1 (C-3′), 74.2 (C-2′), 141.4 (C-6), 162.1 (C-5) and 163.8 (C-3); MS data: *m/z* 182 (M − H_2O); 169 (M − CH_2OH), 123 (M − CHOH − CH_2OH − NH_2), 110 (M − CHOH − CHOH − CH_2OH + H); HRMS data: calcd for $C_7H_{12}N_4O_3$ − H_2O (M − H_2O), *m/z* 182.0813; found: *m/z* 182.0804.

3-Amino-6-[(2*S*,3*S*)-2,3,4-trihydroxybutyl]-1,2,4-triazine (**3a**): Yield 55 mg (8.9%); mp 141–142° (EtOH); $[\alpha]_D$ −61.8° (*c* 1, H_2O); R_F 0.45; λ_{max} 326 (ϵ 1891

M^{-1} cm^{-1}). ^{1}H-NMR data (D_2O): δ 8.13 (s, 1 H, H-5), 3.73 (ddd, 1 H, $J_{2',3'}$ 6.2 Hz, $J_{3',4'a}$ 9.6 Hz, $J_{3',4'b}$ 3.3 Hz, H-3'), 3.61 (dd, 1 H, $J_{1'b,2'}$ 2.3 Hz, $J_{1'a,1'b}$ 10.8 Hz, H-1'$_b$), 3.49 (m, 1 H, H-2'), 3.45 (m, 1 H, H-1'$_a$), 2.96 (dd, 1 H, $J_{3',4'b}$ 3.2 Hz, $J_{4'a,4'b}$ 14.6 Hz, H-4'$_b$), 2.69 (dd, 1 H, H-4'$_a$); ^{13}C-NMR data (^{1}H decoupled, confirmed by DEPT, D_2O): δ 35.1 (C-1'), 62.3 (C-4'), 70.8 (C-3'), 74.1 (C-2'), 149.9 (C-6), 153.2 (C-5), 161.4 (C-4); MS data: *m/z* 201 (M + H), 182 (M – H_2O), 169 (M – CH_2OH), 139 (M – CHOH – CHOH – CH_2OH), 123 (M – CHOH – CH_2OH – NH_2), 110 (M – CHOH – CHOH – CH_2OH + H); HRMS data: calcd for $C_7H_{12}N_4O_3$ (M^+), *m/z* 201.0980; found: *m/z* 201.0987.

3-Amino-5-[(2*S*)-2,3-dihydroxypropyl]-1,2,4-triazine (**2b**). Yield 40 mg (6.2%); λ_{max} 317 nm (ϵ 3130 M^{-1} cm^{-1}). The product was identical by ^{1}H- and ^{13}C-NMR spectroscopy, MS, and $[\alpha]_D$ with the compound described in a previous communication [13].

3-Amino-6-[(2*S*)-2,3-dihydroxypropyl]-1,2,4-triazine (**3b**). Yield 75 mg (11.6%); λ_{max} 326.5 nm (ϵ 2026 M^{-1} cm^{-1}). The product was identical by ^{1}H- and ^{13}C-NMR spectroscopy, MS, and $[\alpha]_D$ with the compound described in a previous communication [13].

3-Amino-5-[(1*R*,2*S*,3*S*)-1,2,3,4-tetrahydroxybutyl]-1,2,4-triazine (**2c**): Yield 80 mg (13.2%); mp 141–142° (MeOH), $[\alpha]_D$ −81.1° (*c* 0.85, H_2O); R_F 0.3; λ_{max} 319 (ϵ 2968 M^{-1} cm^{-1}); ^{1}H-NMR data (confirmed by COSY, D_2O): δ 8.62 (s, 1 H, H-6), 4.74 (d, 1 H, H-1'), 3.78 (dd, 1 H, H-2'), 3.73 (m, 2 H, H-3',4'$_b$), 3.55 (dd, 1 H, $J_{3',4'a}$ 5.7, $J_{4'a,4'b}$ 11.7 Hz, H-4'$_a$); ^{13}C-NMR data (^{1}H decoupled, confirmed by HETCOR, D_2O): δ 62.85 (C-4'), 70.6 (C-3'), 70.9 (C-1'), 72.9 (C-2'), 138.9 (C-6), 162.0 (C-5), 165.8 (C-3); MS data: *m/z* 217 (M + H), 199 (M – H_2O + H), 187 (M – CHOH + H), 126 (M – CHOH – CHOH – CH_2OH + H).

Anal. Calcd for $C_7H_{12}N_4O_4$: C, 38.89; H, 5.59; N, 25.91. Found: C, 38.99; H, 5.85; N, 25.36.

3-Amino-5-[(1*R*,2*S*)-1,2,3-trihydroxypropyl]-1,2,4-triazine (**2d**): Yield 95 mg (15.1%); mp 140–141° (MeOH); $[\alpha]_D$ −88.6° (*c* 1.0, H_2O), R_F 0.45; λ_{max} 319.5 (ϵ 3350 M^{-1} cm^{-1}); ^{1}H-NMR data (confirmed by COSY, D_2O): δ 8.61 (s, 1 H, H-6), 4.63 (d, 1 H, $J_{1',2'}$ 3.3 Hz, H-1'), 3.93 (m, 1 H, H-2'), 3.63 (dd, 1 H, $J_{2',3'b}$ 5.3, $J_{3'a,3'b}$ 11.6 Hz, H-3'$_b$), 3.54 (dd, 1 H, $J_{2',3'a}$ 7.0 Hz, H-3'$_a$); ^{13}C-NMR data (^{1}H decoupled, confirmed by DEPT and HETCOR, D_2O): δ 62.0 (C-3'), 71.4 (C-1'), 73.3 (C-2'), 138.7 (C-6), 161.9 (C-5), 164.8 (C-3); MS data: *m/z* 186 (M), 155 (M – CH_2OH), 126 (M – CHOH – CH_2OH + H), 95 (M – CHOH – CHOH – CH_2OH).

Anal. Calcd for $C_6H_{10}N_4O_3$: C, 38.71; H, 5.41; N, 30.09. Found: C, 38.19; H, 5.46; N, 30.05.

RESULTS AND DISCUSSION

The recent detection of 3-deoxy-D-*erythro*-hexos-2-ulose (**1a**) in urine and serum of both normal and diabetic human subjects suggests that it is produced in

significant quantities as a result of Maillard reactions [4] in vivo. In addition, Kato's group have reported that **1a** causes in vitro protein cross-linking at rates much faster than those observed for D-glucose [14,15]. Thus, **1a** appears to be a valid candidate for a source of protein cross-linking in vivo. A number of reports are now in print that show that aminoguanidine inhibits the Maillard reaction, based on experiments in vitro. Other workers (via experiments in vitro) have shown that a Maillard reaction involving albumin and **1a** is strongly inhibited by aminoguanidine [16,17]. Other reports suggest that the primary source for the inhibition is the initially formed Amadori compound, which could also react further with aminoguanidine [18].

Dicarbonyl sugar derivatives other than **1a** may also be of significance in such reactions. Maillard reactions involving L-ascorbic acid have been observed for eye lens preparations, and it has been suggested that these may contribute to cataract formation (high levels of L-ascorbic acid are present in the lens) [19]. We have recently shown that both **1b** and **1d** are produced as intermediates during the degradation of L-ascorbic acid in solution and in the presence of oxygen [9]. Thus, it is possible that these compounds cause the cross-linking of lens proteins during such reactions.

The probable pathways for the formation of the triazine derivatives are shown in Scheme 1. The 3-deoxy derivatives (**1a** and **1b**) reacted very rapidly (in less than 5 min), while the 3-hydroxylated compounds (**1c** and **1d**) were slower in reacting (requiring up to 8 h for completion). In contrast (based on qualitative TLC), D-glucose is unreactive under these conditions and reaction times. Wolfrom's group reported the formation of the bishydrazone when **1c** is reacted with aminoguanidine [20], but the conditions used were much more drastic than those employed herein. During this study, we prepared the bishydrazone derivative of **1c** employing the conditions used in the earlier study [20] and examined our reaction mixtures for the presence of this compound using TLC. No evidence could be found for its presence under the conditions employed in this study.

The formation of the 6-substituted triazine derivatives (**3a** and **3b**) can be explained by the initial formation of a hydrazone at C-1 of the dicarbonyl derivative, followed by cyclization of the guanylhydrazone, and reaction of the imidamido nitrogen at C-2, followed by rearrangement to the triazine. Conversely, the 5-substituted triazines (**2a–d**) can be formed by initial hydrazone formation at C-2 of the sugar, followed by analogous cyclization. It is noteworthy that, while all four of the dicarbonyl sugars react to form the 5-substituted triazine, only **1a** and **1b** give the 6-substituted isomers **3a** and **3b**. This may be due to the C-3 hydroxyl groups of **1c** and **1d** preventing the approach of the aminoguanidine to the C-2 carbonyl group via steric hindrance. Also noteworthy is the fact that all of the triazines are strong UV absorbers, a fact that may be useful in the detection of these compounds in experiments in vivo.

ACKNOWLEDGMENT

This work was supported by grant No. 190821 from the Juvenile Diabetes Foundation International.

REFERENCES

1 E.F.L.J. Anet, *Adv. Carbohydr. Chem.*, 19 (1964) 181–218.
2 H.F. Bunn and P.J. Higgins, *Science*, 213 (1981) 222–224.
3 R. Sapiro, M.J. McManus, C. Zalut, and H.F. Bunn, *J. Biol. Chem.*, 255 (1980) 3120–3127.
4 K.J. Knecht, M.S. Feather, and J.W. Baynes, *Arch. Biochem. Biophys.*, in press.
5 A.S. Eble, S.R. Thorpe, and J.W. Baynes, *J. Biol. Chem.*, 258 (1983) 9406–9412.
6 F.G. Njoroge, A.A. Fernandes, and V.M. Monnier, *J. Biol. Chem.*, 263 (1988) 10646–10652.
7 S. Pongor, P.C. Ulrich, F.A. Bencsath, and A. Cerami, *Proc. Natl. Acad. Sci. U.S.A.*, 81 (1984) 2684–2688.
8 M. Brownlee, H. Vlassara, A. Kooney, P. Ulrich, and A. Cerami, *Science*, 232 (1986) 1629–1632.
9 D.B. Shin and M.S. Feather, *Carbohydr. Res.*, 208 (1990) 246–250.
10 M.A. Madson, and M.S. Feather, *Carbohydr. Res.*, 94 (1981) 183–191.
11 S. Bayne, *Methods Carbohydr. Chem.*, 2 (1963) 421–423.
12 L.L. Salomon, J.J. Burns, and O.G. King, *J. Am. Chem. Soc.*, 74 (1952) 5161–5162.
13 J. Hirsch, J.W. Baynes, J.A. Blackledge, and M.S. Feather, *Carbohydr. Res.*, 220 (1991) c5–c7.
14 H. Kato, R.K. Cho, A. Okitani, and F. Hayase, *Agric. Biol. Chem.*, 51 (1987) 683–689.
15 H. Kato, D.B. Shin, and F. Hayase, *Agric. Biol. Chem.*, 51 (1987) 2009–2011.
16 M. Oimomi, N. Igaki, M. Sakai, T. Ohara, S. Baba, and H. Kato, *Agric. Biol. Chem.*, 53 (1989) 1727–1728.
17 M. Oimomi and N. Igaki, *Diabetes Res. Clin. Pract.*, 6 (1989) 311–313.
18 B.S. Lewis and J.J. Harding, *Exp. Eye Res.*, 50 (1990) 463–467.
19 M. Prabhakaram and B.J. Ortwerth, *Exp. Eye Res.*, 53 (1991) 261–268.
20 M.L. Wolfrom, H.S. El Khadem, and H. Alfes, *J. Org. Chem.*, 29 (1964) 3074–3076.