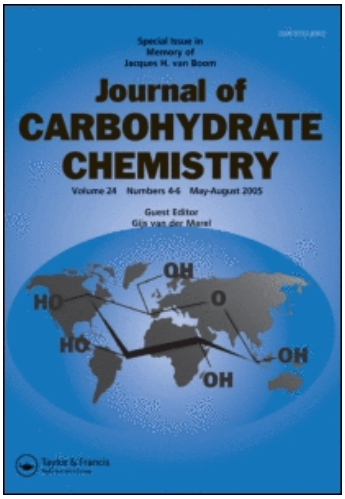

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Reaction of Some 1-Deoxy-2,3-Dicarbonyl Hexose Derivatives with Aminoguanidine (Guanylhydrazine)[1]

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REACTION OF SOME 1-DEOXY-2,3-DICARBONYL HEXOSE DERIVATIVES WITH AMINOGUANIDINE (GUANYLHYDRAZINE)[1]

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ABSTRACT

The reaction of 4-*O*-acetyl-1-deoxy-5,6-*O*-isopropylidene-2,3-D-*erythro* and (D-*threo*)-hexodiulose with aminoguanidine (guanylhydrazine) was investigated at pH 7.0 and 37 °C. The two dicarbonyl compounds reacted rapidly to give 6-methyl-5-substituted triazine derivatives, which were fully characterized. The compounds were deblocked in a stepwise manner to give, first the de-*O*-acetylated compounds and then (by removal of the isopropylidene groups) the free triazine derivatives which were fully characterized (GLC/MS, NMR and elemental analyses).

INTRODUCTION

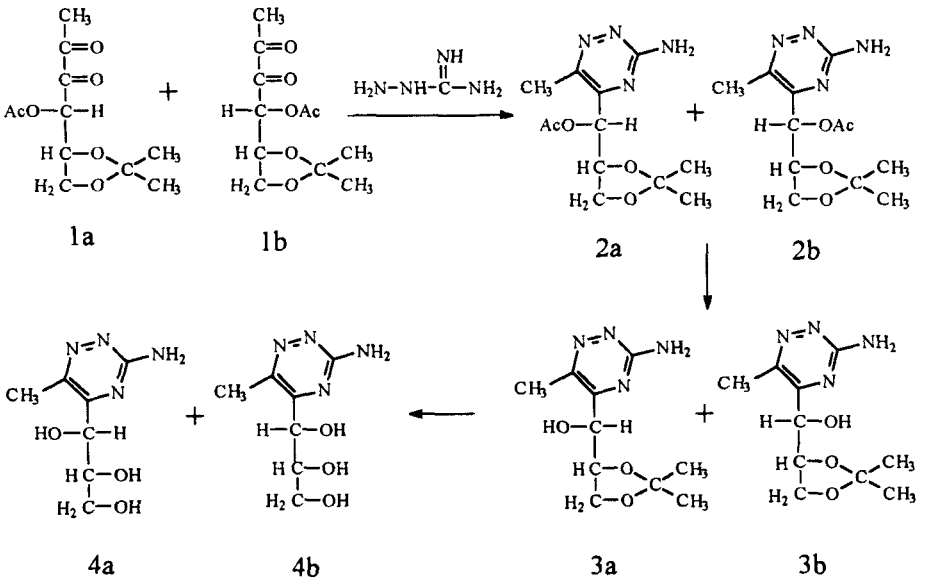
The Maillard reaction involves the reaction of aldoses with amino compounds to give 1-amino-1-deoxy-ketose derivatives (Amadori compounds), which are themselves unstable and undergo degradation to give highly reactive deoxy-dicarbonyl saccharide intermediates.[3] Because of the instability of these intermediates, a clear understanding of their reactivity and concentration is needed. It has now been established that for a reaction involving aldohexoses, 3-deoxy- and 1-deoxy-D-*erythro*-hexos-2-ulose are produced,[4] as well as 1,4-dideoxy-2,3-dicarbonyl intermediates that remain attached to the amino group.[5] Ledl's group

has published unequivocal data on the chemistry of both of the last intermediates,[4,5] and a synthesis of some derivatives of the 1-deoxy compound has also been published.[6] The latter synthesis resulted in the preparation of 5,6-*O*-isopropylidene derivatives of this intermediate.

Aminoguanidine, a known inhibitor of the Maillard reaction,[7,8] has been shown to react with a number of 1,2-dicarbonyl sugar derivatives to give 3-aminotriazine derivatives,[9-12] suggesting that its role as an inhibitor may be to "trap" carbohydrate-derived dicarbonyl intermediates, by rapidly converting them into stable and relatively unreactive triazines, thus preventing them from participating further in subsequent degradation reactions. Because the triazine derivatives can be quantitatively measured by GLC and identified by GLC/MS, the possibility that aminoguanidine could be used as a reagent for trapping dicarbonyl intermediates in Maillard reactions as well as for following the progress of their formation is a possibility, if appropriate standards are made available. In this paper, we report the preparation and properties of the triazine derivatives obtained by reacting of aminoguanidine with 4-*O*-acetyl-1-deoxy-5,6-*O*-isopropylidene-2,3-D-*threo*-hexodiulose and 4-*O*-acetyl-1-deoxy-5,6-*O*-isopropylidene-2,3-D-*erythro*-hexodiulose.

RESULTS AND DISCUSSION

The recent verification that the predicted 1-deoxy-2,3-dicarbonyl intermediate is produced during a Maillard reaction[5] prompted the present investigation, since our studies have shown that the use of aminoguanidine may be useful in trapping such intermediates in these reactions. The chemistry of the reaction of other dicarbonyl compounds with aminoguanidine is reasonably well understood. In prior reports[10-12] we described the reactions of aminoguanidine with several 1,2-dicarbonyl sugar derivatives, some of which are known to be produced during the Maillard reaction. It is interesting to note that the 1,2-dicarbonyl derivatives having a deoxy substituent adjacent to the carbonyl group, e.g., 3-deoxy-1,2-dicarbonyl derivatives give two different triazine isomers, namely the 5- and the 6-substituted products, while dicarbonyl derivatives having an oxygenated substituent at C-3 gave only one isomer (the 5-substituted triazine). The starting material for the present study consisted of two C-4 epimers, 4-*O*-acetyl-1-deoxy-5,6-*O*-isopropylidene-2,3-D-*threo*-hexodiulose and 4-*O*-acetyl-1-deoxy-5,6-*O*-isopropylidene-2,3-D-*erythro*-hexodiulose (**1a** and **1b**, Scheme 1). Reaction with aminoguanidine gave (by TLC) 3-amino-5-[(D-*threo*)-1-*O*-acetyl-2,3-*O*-



Scheme 1

isopropylidene-1,2,3-trihydroxypropyl]-6-methyl-1,2,4-triazine and 3-amino-5-[D-*erythro*)-1-*O*-acetyl-2,3-*O*-isopropylidene-1,2,3-trihydroxypropyl]-6-methyl-1,2,4-triazine (**2a** and **2b**, Scheme 1), along with some spontaneously de-*O*-acetylated material, consisting of 3-amino-5-[(D-*threo*)-2,3-*O*-isopropylidene-1,2,3-trihydroxypropyl]-6-methyl-1,2,4-triazine and 3-amino-5-[(D-*erythro*)-2,3-*O*-isopropylidene-1,2,3-trihydroxypropyl]-6-methyl-1,2,4-triazine (**3a** and **3b**, Scheme 1). Compounds **3a** and **3b** were obtained as a mixture of crystals. Structures were confirmed by NMR spectra of the products and elemental analysis.

Finally, removal of the isopropylidene group gave 3-amino-5-[(D-*threo*)-1,2,3-trihydroxypropyl]-6-methyl-1,2,4-triazine and 3-amino-5-[(D-*erythro*)-1,2,3-trihydroxypropyl]-6-methyl-1,2,4-triazine (**4a** and **4b**, Scheme 1). It is also noteworthy that the TMS derivatives of **4a** and **4b** were separable by GLC, thus allowing a confirmation of their structures (by GLC/MS). This provides a method for the detection and analysis of **4a** and **4b** during the degradation of Amadori compounds in solution, since these dicarbonyl compounds react quickly and spontaneously with aminoguanidine to give the stable triazine derivatives which can then be quantitated. All of the triazine derivatives are UV absorbers and can be easily detected by UV light in conjunction with TLC on a florescent absorber.

The structures of all compounds were confirmed by NMR spectroscopy. Proton signal assignments were done by COSY, and carbon signal assignments were based on heteronuclear shift-correlation 2D experiments. Compounds **3a** and **3b** have different $J_{1,2}$ coupling constants (4.4 and 7.6 Hz, respectively). These values are in good agreement with assignments in the literature for analogous structures, for example, a *threo* and *erythro* series of 1,2,3 triazoles,[13,14] the *ribo* series of hydroxyaminohydrazine thiosemicarbazides,[15] 2-(D-*arabino*-tetrahydroxybutyl) quinoxaline,[16] hydrazone derivatives of D-arabinose,[17] 2-(D-*arabino*-tetraacetoxybutyl) quinoxaline (and the deblocked derivative),[18] 4-(D-*arabino*-tetrahydroxybutyl)-2-phenyl-1,2,3-osotriazole (and the corresponding acetate).[19] Even though the NMR measurements were made in different solvents and the structures of **3a** and **3b** are more constrained, because of the isopropylidene group, the $J_{1,2}$ values are almost the same. Compounds **4a** and **4b**, which occurred in a ratio of 1:0.85, showed similar patterns for $J_{1,2}$. Compound **4a**, the major component, represents that *threo* form with $J_{1,2}$ = 3.3 Hz, while the minor component (**4b**) has an *erythro* configuration ($J_{1,2}$ = 7.8 Hz). Compounds **2a** and **2b** have nearly the same coupling constant ($J_{1,2}$ = 7.0 and 7.7 Hz), probably because of interactions between the nitrogen of the triazine ring and the blocked sugar chain on the molecule. Based on prior published NMR studies of these types of compounds, it seems clear that **2a**, **2b**, **3a**, **3b**, **4a** and **4b** all have a zig-zag, planar structure. The carbon chemical shifts of the 1,2,4-triazine ring are in good agreement with those reported for 1,2,4-triazine derivatives by Lancelot and co-workers.[20]

EXPERIMENTAL

General Procedures. Melting points were determined with a Thomas-Hoover Unimelt apparatus in open capillary tubes and are uncorrected. TLC was performed on silica gel (Whatman K5F) plates using the following irrigants: 5:1 (v/v) hexane-ethyl acetate (irrigant A); 3:1 (v/v) chloroform-acetone (irrigant B) or 10:1 (v/v) chloroform-methanol (irrigant C). Detection was accomplished either with UV light (for UV-absorbing compounds) or by spraying with 5% sulfuric acid in ethanol, followed by charring at 120 °C for 15 min. Preparative column chromatography was performed using 200 - 400 mesh silica gel (Aldrich). ^1H (500 MHz, D_2O, internal reference) and ^{13}C NMR (125 MHz, dioxane as external reference) spectra, and COSY and HETCOR experiments were obtained using a

Bruker AMX-500 spectrometer. Mass spectra were collected using a Kratos MS-25 spectrometer, interfaced with a DS-55 data handling system. GLC was performed using a Varian 3400 instrument in the split mode. GLC parameters: initial temperature 120 °C, followed by a 2 minute hold and then a ramp of 8 °C per min to a final temperature of 250 °C. The starting material **1**, a mixture of two isomers, 4-*O*-acetyl-1-deoxy-5,6-*O*-isopropylidene-D-*arabino* (**a**) and D-*ribo*-2,3-diulose (**b**) was synthesized as described by Feather and Eitelman.[6]

3-Amino-5-[(D-*threo*) and (D-*erythro*)-1-*O*-acetyl-2,3-*O*-isopropylidene-trihydroxypropyl]-6-methyl-1,2,4-triazine (2a and 2b). A mixture of **1a** and **1b** (1.6 g, 6.55 mmol), which was present in a ratio of approximately 1:1 (R_f = 0.5 and 0.45, irrigant A) was reacted with aminoguanidine bicarbonate salt (1.34 g, 9.8 mmol) in 70 mL of methanol-water (2:1, v/v) at 37 °C. TLC (irrigant B) showed that the reaction was complete within 12 hours as evidenced by the appearance of two new compounds in approximately equal ratios and having R_f = 0.45 and 0.2 (irrigant B) and the complete disappearance of the starting compound. After filtration and solvent evaporation, the reaction products were separated on a 2.6 by 26 cm silica gel column using irrigant B as the eluent. The faster moving component (0.72 g, 38.9%) was obtained as a colorless syrup, and was characterized by NMR spectroscopy as a mixture of **2a** and **2b.** For NMR assignments, primed numbers refer to atoms that make up the triazine ring, while unprimed numbers to atoms of the carbohydrate-derived side chain. ^{1}H NMR ($CDCl_3$),- **2a**, δ 5.65 (d, 1 H, $J_{1,2}$ = 7.0 Hz, H-1), 5.54 (bs, 2 H, NH_2), 4.56 (ddd, 1 H, $J_{2,3b}$ = 5.2 Hz, $J_{2,3a}$ = 6.8 Hz, H-2), 3.92 (dd, 1 H, $J_{3a,3b}$ = 9.0 Hz, H-3_a), 3.79 (dd, 1 H, H-3_b), 2.59 (s, 3 H, CH_3 - triazine methyl), 2.08 (s, 3 H, $COCH_3$), 1.27 (s, 3 H, CH_3 - isopropyl), 1.25 (s, 3 H, CH_3 - isopropyl); ^{13}C NMR ($CDCl_3$), **2a**, δ 169.88 ($COCH_3$), 161.46 (C-3′), 156.17 (C-5′), 148.43 (C-6′), 110.35 (C - isopropyl), 75.72 (C-2), 72.07 (C-1), 65.00 (C-3), 26.20 (CH_3 - isopropyl), 25.00 (CH_3 - isopropyl), 20.63 ($COCH_3$), 18.30 (CH_3 - triazine methyl); ^{1}H NMR ($CDCl_3$), **2b**, δ 5.54 (s, 2 H, NH_2), 5.45 (d, 1 H, $J_{1,2}$ = 7.7 Hz, H-1), 4.43 (ddd, 1 H, H-2), 4.13 (dd, 1 H, $J_{2,3a}$ = 6.4 Hz, $J_{3a,3b}$ = 9.0 Hz, H-3_a), 3.99 (dd, 1 H, $J_{2,3b}$ = 5.4 Hz, H-3_b), 2.60 (s, 3 H, CH_3 -triazine methyl), 2.04 (s, 3 H, $COCH_3$), 1.36 (s, 3 H, CH_3 - isopropyl), 1.24 (s, 3 H, CH_3 - isopropyl); ^{13}C NMR ($CDCl_3$), **2b**, δ 169.60 ($COCH_3$), 161.82 (C-3′), 157.32 (C-5′), 148.54 (C-6′), 110.14 (C - isopropyl), 76.00 (C-2), 71.90 (C-1), 66.69 (C-3), 26.34 (CH_3 - isopropyl), 24.76 (CH_3 - isopropyl), 20.51 ($COCH_3$), 18.07 (CH_3 - triazine methyl).

The slower moving component (0.65 g, 41.3%) was found to be the deacetylated form of **2a** and **2b** (see below).

3-Amino-5-[(D-*threo*) and (D-*erythro*)-2,3-*O*-isopropylidene-1,2,3 trihydroxypropyl]-6-methyl-1,2,4-triazine (3a and 3b). A mixture of **2a** and **2b** (0.5 g, 1.77 mmol) was de-*O*-acetylated by treatment with 10 mL of anhydrous methanol containing a trace of sodium methoxide. After 30 min at room temperature, the solution was neutralized with Dowex 50 (hydrogen form) ion exchange resin, filtered and concentrated to give **3a** and **3b** (0.4 g, 94%, $R_f = 0.2$, irrigant B) as crystalline material, mp, 148 - 150 °C(ethanol); ^{1}H NMR ($CDCl_3$), **3a**, δ 5.51 (s, 2 H, N*H*$_2$), 4.68 (d, 1 H, $J_{1,2} = 4.4$ Hz, H-1), 4.45 (m, 1 H, H-2), 4.02 (m, overlapped, 1 H, H-3$_a$), 3.94 (dd, 1 H, $J_{2,3a} = 6.0$ Hz, $J_{3a,3b} = 8.7$ Hz, H-3$_b$), 2.55 (s, 3 H, C*H*$_3$ - triazine methyl), 1.29 (s, 3 H, C*H*$_3$ - isopropyl), 1.26 (s, 3 H, C*H*$_3$ - isopropyl); ^{13}C NMR ($CDCl_3$), **3a**, δ 160.91 (C-3′), 158.75 (C-5′), 147.74 (C-6′), 110.06 (C - isopropyl), 76.55 (C-2), 69.41 (C-1), 65.39 (C-3), 26.07 (*C*H$_3$ - isopropyl), 25.01 (*C*H$_3$ - isopropyl), 18.17 (*C*H$_3$ - triazine methyl); ^{1}H NMR ($CDCl_3$), **3b**, δ 5.51 (s, 2 H, N*H*$_2$), 4.60 (d, 1 H, $J_{1,2} = 7.6$ Hz, H-1), 4.12 (m, 2 H, H-2, H-3$_a$), 4.02 (dd, 1 H, $J_{2,3b} = 7.0$ Hz, $J_{3a,3b} = 13.3$ Hz, H-3$_b$), 2.56 (s, 3 H, C*H*$_3$ - triazine methyl), 1.41 (s, 3 H, C*H*$_3$ - isopropyl), 1.24 (s, 3 H, C*H*$_3$ - isopropyl); ^{13}C NMR ($CDCl_3$) - compound **3b**, δ 161.22 (C-3′), 160.06 (C-5′), 148.25 (C-6′), 110.09 (C - isopropyl), 78.99 (C-2), 70.41 (C-1), 67.30 (C-3), 26.25 (*C*H$_3$ - isopropyl), 24.78 (*C*H$_3$ - isopropyl), 18.17 (*C*H$_3$ - triazine methyl).

Anal. Calcd for $C_{10}H_{16}O_3N_4$ (240.26): C, 49.49; H, 6.71; N, 23.32. Found: C, 49.64; H, 6.66; N, 23.12.

3-Amino-5-[(D-*threo*) and (D-*erythro*)-1,2,3-trihydroxypropyl]-6-methyl-1,2,4-triazine (4a and 4b). A mixture of **3a** and **3b** (0.3 g, 1.23 mmol) was stirred in 5% hydrochloric acid in methanol for 20 min at room temperature, when TLC (irrigant C) showed the absence of starting material ($R_f = 0.65$) and the appearance of the deblocked products (**4a** and **4b**, $R_f = 0.1$). After neutralization with anion exchange resin (hydroxide form), filtration and solvent evaporation, a mixture of **4a** and **4b** was obtained as a yellow syrup (0.24 g, 96%), which was further purified by silica gel column chromatography (irrigant C) and obtained as a colorless foam. ^{1}H NMR (D_2O), **4a**, δ 4.79 (s, 1 H, $J_{1,2} = 3.3$ Hz, H-1), 3.89 (m, 1 H, H-2), 3.63 (m, 1 H, H-3$_a$), 3.52 (dd, 1 H, $J_{2,3b} = 6.8$ Hz, $J_{3a,3b} = 11.5$ Hz, H-3$_b$), 2.33 (s, 3 H, C*H*$_3$ - triazine methyl); ^{13}C NMR (D_2O), **4a**, δ 164.00 (C-3′), 163.16 (C-5′), 147.83

(C-6′), 72.93 (C-2), 70.08 (C-1), 63.04 (C-3), 17.72 (*C*H_3 - triazine methyl); ^{1}H NMR (D_2O), **4b**, δ 4.71 (d, 1 H, $J_{1,2}$ = 7.8 Hz, H-1), 3.84 (m, 1 H, H-2), 3.72 (dd, 1 H, $J_{2,3a}$ = 2.9 Hz, $J_{3a,3b}$ = 12.0 Hz, H-3_a), 3.63 (m, 1 H, H-3_b), 2.37 (s, 3 H, C*H*$_3$ - triazine methyl); ^{13}C NMR (D_2O), **4b**, δ 162.56 (C-3′), 162.11 (C-5′), 149.00 (C-6′), 74.34 (C-2), 70.19 (C-1), 63.02 (C-3), 17.87 (*C*H_3 - triazine methyl).

Anal. Calcd for $C_7H_{12}O_3N_4$ (200.20): C, 41.99; H, 6.04; N, 27.99. Found: C, 41.87; H, 6.06; N, 27.45.

Trimethylsilyl ether derivatives of 4a and 4b. A mixture of **4a** and **4b** (1 mg) was converted to the trimethylsilyl derivative using pyridine (0.1 mL) and *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA, Pierce Chemical, Rockford, IL) (0.1 mL) at 70 °C for 30 min in a 3 mL reactivial. A 1 μL aliquot was injected on a 0.25 mm by 25 m Quadrex OV-17 GLC column. The GLC retention times for these compounds were 15.4 and 15.55 min and, for both compounds, the MS showed peaks at *m/z* 488 (M) and at 473 (M - CH_3).

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