Tetrahedron Letters, Vol. 34, No. 46, pp. 7389-7390, 1993
Printed in Great Britain

0040-4039/93 $6.00 + .00
Pergamon Press Ltd

Reaction of Glucose with Guanosine

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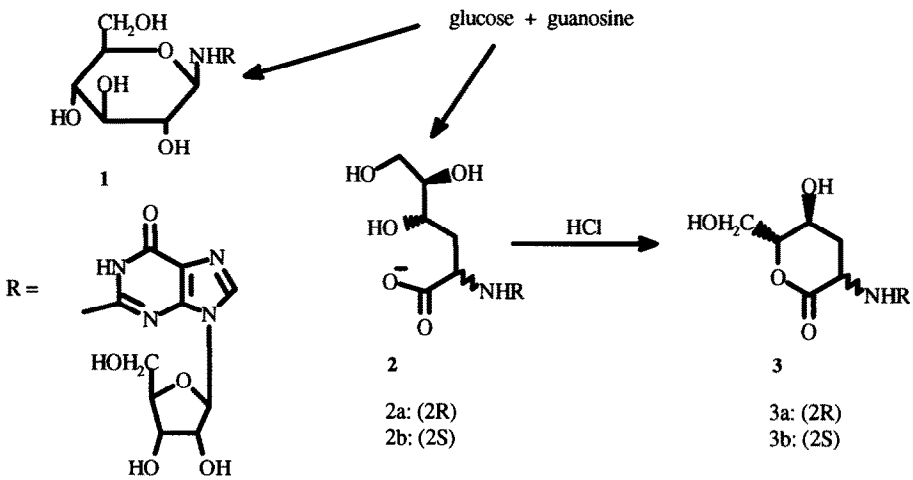
Abstract: Reaction of glucose with guanosine under physiological conditions leads to (2R,4S,5R)-2-(N^2-guanosyl)-4,5,6-trihydroxyhexanoic acid **2a** and (2S,4S,5R)-2-(N^2-guanosyl)-4,5,6-trihydroxyhexanoic acid **2b**.

In a series of investigations Cerami and coworkers have demonstrated that DNA can react with glucose, glucose-6-phosphate (G-6-P) or even more readily with G-6-P in the presence of lysine. Obviously a reactive intermediate formed by degradation of glucose or G-6-P adds to the amino groups of DNA [1]. On the basis of the results obtained it has been hypothesized that accumulation of sugar modified DNA may contribute to the biological aging process [1]. Recently Miksik et al. developped a HPLC-method for the separation of nucleotide glycation products [2]. But as far as we know the structures of compounds arising by glucose-DNA interaction remained unknown.



Previously we were able to isolate N^2-glycosylated guanosine **1** from a glucose-guanosine reaction mixture. These reactions were carried out at 100 °C [3]. In contrast it is possible to obtain two other products in high yields, when glucose, guanosine and propylamine are incubated at 37 °C for several days in concentrated phosphate buffered neutral aqueous solution. These products can be isolated as pure compounds by application of HPLC technique (reversed phase material, ammonium formate buffer as eluent). The structural assignment for **2a** and **2b**, which are diastereomers, is based on spectroscopic data:

For **2a** positive FAB-MS data confirm the molecular weight. The IR spectrum shows a broadened peak at 1668 cm $^{-1}$, the common signal of the two C=O double bonds. The UV-maximum (λ = 256 nm) is slightly shifted towards longer wavelenghts compared to guanosine. This shift is analogous to that observed for **1**. NMR spectra display the presence of the intact guanosine moiety. The ^{13}C chemical shift of the C^1 (179.8 ppm) is typical for carboxylic acids. C^2 and C^3 received a highfield shift compared to glucose. The ^{13}C signal for C^2 (55.6 ppm) is characteristic for amino acids (e.g. histidine: 55.1 ppm). ^1H-signals of **2a** and **2b** differ only for H-2 and H-3, which leads to the assumption that the two compounds are diastereomers. The H-3 signals in **2a** are neatly separated and are classified as a double double doublet (J = 2.5/5.5/12.5 Hz) and a double triplet (J = 8.8 and 13 Hz), whereas in **2b** the chemical shifts for these methylene protons are equal and a multiplet is observed [4].

Acidification of the lyophilisized reaction mixture with methanolic HCl leads to lactonisation giving **3a** and **3b**. ^1H NMR spectra of these substances show a downfield shift for all ring protons of the glucose derived residue (0.3 to 0.7 ppm compared to **2**). H-3a in **3a** is diagnostic for the six-membered ring, because we observe a quartet with J = 11.5 ppm. Such a value is characteristic for diaxial protons in six-rings. In fact the geminal coupling constant is not larger than the vicinal in this product! IR spectrum of **3a** shows a signal at 1770 cm^{-1}, a rather high value for six-ring lactones, which can be explained by the electronegative α-substituent.

Considering the formation of **2a** and **2b** we assume that guanosine reacts with 3-deoxy-D-*erythro*-hexos-2-ulose, a well known glucose degradation product. The mechanism might be similar to that of the formation of meta saccharinic acid, which can be obtained from glucose under alkaline conditions [5].

References and Notes:

1. A. T. Lee, A. Cerami in P.A. Finot et al. (editors): *The Maillard reaction in Food Processing, Human Nutrition and Physiology*, 1990, Birkhäuser Verlag Basel, pp. 415-423.
2. I. Miksik, Z. Hodny, Z. Deyl, *J. Chromatogr.*, 1993, *612*, 57-61.
3. T. Knerr, S. Ochs, T. Severin, *Carbohydr. Res.*, 1993, submitted for publication.
4. *Ammonium (2R,4S,5R)-2-(N²-guanosyl)-4,5,6-trihydroxyhexanoate*: 83 mg (24%), m.p. 148°C (decomp.), ^1H-NMR (400 MHz, D_2O): δ = 7.94 (s, 1H, gua), 5.91 (d, J = 4.4, 1H, 1-H rib), 5.01 (t, J = 5.1, 1H, 2-H rib), 4.47 (t, J = 5.1, 1H, 3-H rib), 4.37 (t, J = 6.6, 1H, 2-H), 4.20 (m, J = 5.0, 1H, 4-H rib), 3.95 (dd, J = 3 and 12, 1H, 5a-H rib), 3.80-3.90 (m, 3H, 4-H and 6a-H, 5b-H rib), 3.70 (m, 2H, 5-H and 6b-H), 2.27 (ddd, J = 2.5, 5.5 and 12.5, 1H, 3a-H) and 1.94 (dt, J = 8.8 and 13, 1H, 3b-H).-^{13}C-NMR (400 MHZ, D_2O, DEPT): δ = 179.8 (s, C-1), 159.0 (s, C-6 gua), 152.0 (s, C-2 gua), 151.4 (s, C-4 gua), 139.1 (d,C-8 gua), 116.8 (s, C-5 gua), 88.9 (d, C-1 rib), 84.7 (d, C-4 rib), 74.9 (d, C-5), 72.6 (d, C-2 rib), 70.3 (2 C, d, C-3 rib and C-4), 62.7 (t, C-6), 61.7 (t, C-5 rib), 55.6 (d, C-2), 34.9 (t, C-3).- IR: ν = 3358 (br), 1668, 1605 .- UV (H_2O, pH 7): λ_{max} <nm> = 255.6, 278.5 (sh).- FAB-MS (Xe, [7 kV, 10 W], glycerol): 446 ($M + H^+$) for the free acid.- $C_{16}H_{26}N_6O_{10}$: calc. C 41.56%, H 5.67%, N 18.17%, found C 41.57%, H 6.12%, N 17.71%.
5. Rodd's Chemistry of Carbon Compounds, Vol. I, Part F, 2nd ed., 1967, Elsevier Publishing Comp, Amsterdam, 251-260.

(Received in Germany 4 August 1993; accepted 15 September 1993)