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Note

Reaction of guanosine, 2′-deoxyguanosine and guanosine-5′-monophosphate with glucose

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Reactions of reducing sugars with amino acids, proteins or simple amines have been thoroughly investigated. This area of research is of great interest not only in food chemistry but also in medical biochemistry. Evidence has been accumulated that nonenzymatic glycosylation is of relevance in diabetic complications and probably in age related changes[1,2].

In contrast, little is known concerning the interaction of reducing sugars with nucleotides[3]. Glucose and glucose-6-phosphate were shown to react with nucleotides or DNA under physiological conditions, giving rise to spectral changes similar to those observed for nonenzymatic glycosylation products of proteins[4,5]. The hypothesis was established that amino groups on DNA bases could also participate in the reaction with reducing sugars, thereby resulting in DNA modification and subsequent DNA damage. It was argued that glycosylation of DNA could play a role in DNA strand breaking and may contribute to some age related changes[4]. To the best of our knowledge products with a definite structure have not been isolated so far[5]. We have investigated the glucosylation of guanosine, 2′-deoxyguanosine and some simple amino pyrimidines as model compounds.

When glucose and guanosine are heated in a phosphate buffered (pH 7) aqueous solution for some hours at 100°C, the glucosyl derivative **1** is formed as the main product (Fig. 1). The new compound was assigned the structure **1** on the basis of spectroscopic data. The ^{13}C NMR signal associated with C-1 of the glucosyl residue at 81.4 ppm is consistant with an N,O-acetal structure[6]. The evidence for a β-glycosidic bond comes from ^{1}H NMR which shows a coupling constant of 8.8 Hz for H-1. Likewise the "model compounds", 2-amino-4,6-dimethyl-pyrimidine (**2**) and 2-amino-6-methyl-3*H*-pyrimidin-4-one (**3**), react with

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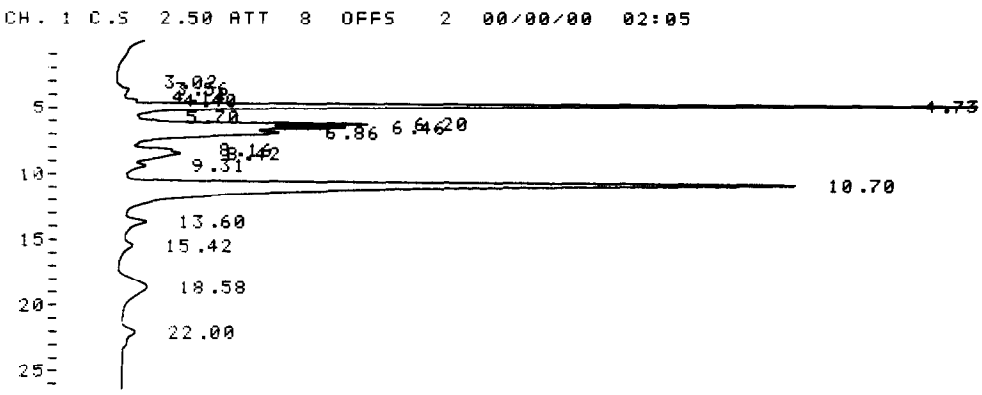


Fig. 1. HPL-chromatogram of the mixture of glucose and guanosine (4 h, 100°C, pH 7.0, system 1a; λ, 260 nm) after filtration: t_R 4.73 min, **1**; t_R 10.70 min, guanosine.

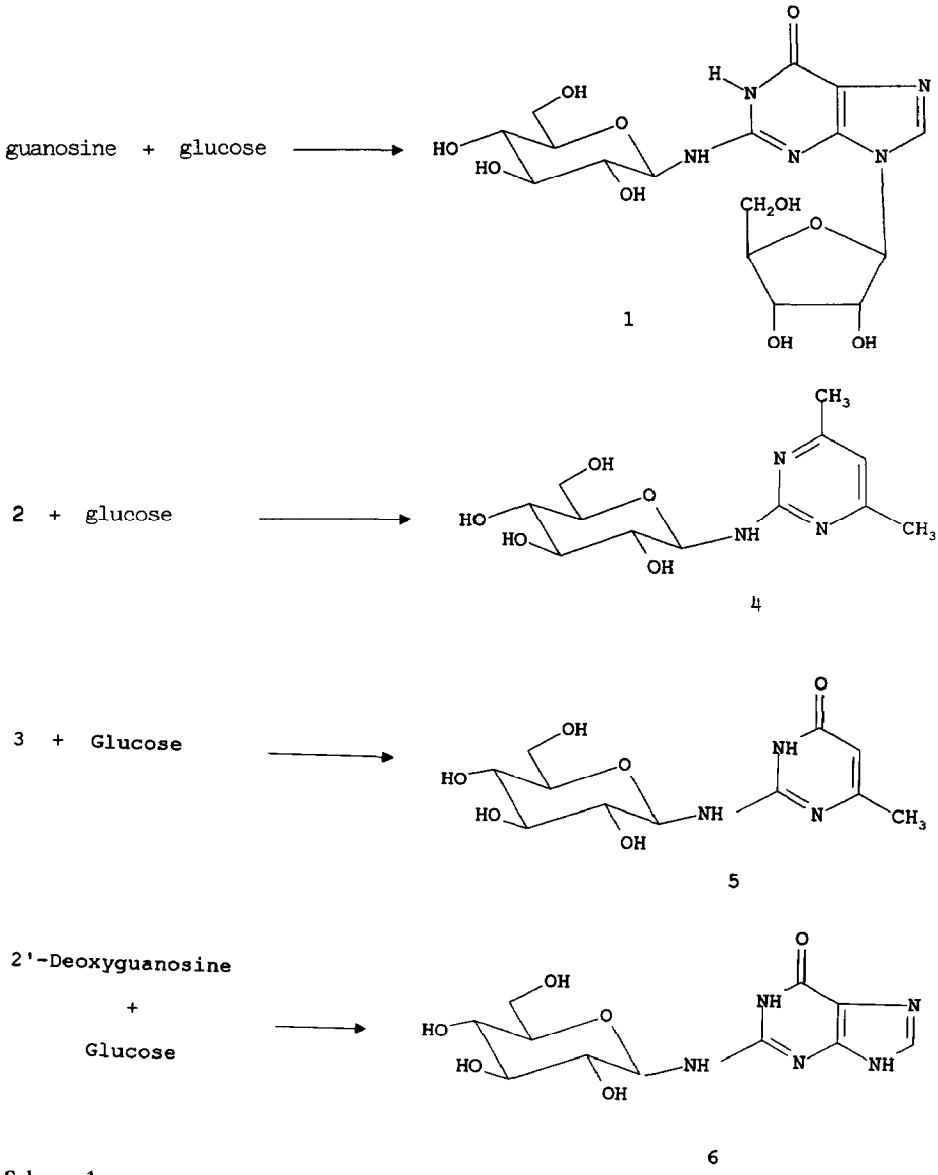
glucose under similar conditions (pH 7, 100°C) to give the glucosyl derivatives **4** and **5**, respectively. As expected the spectroscopic data of compounds **1**, **4**, and **5** show striking similarities. It must be recognized that compounds of type **1** do not readily undergo Amadori-rearrangement, but the same is true for the pentose residue in nucleosides.

When 2′-deoxyguanosine and glucose are heated in unbuffered aqueous solution for several hours the solution turns slightly acidic and the glycosyl derivative **6** is formed as the main product. The structure of **6** can be derived from ^{1}H NMR spectral data. It is well established that hydrolytic cleavage of 2′-deoxyguanosine occurs more readily than solvolytic degradation of guanosine[7]. When 2′-deoxyguanosine and glucose are heated in phosphate buffer a more complex reaction mixture is obtained which is still under investigation.

The glycosylation reactions described so far were conducted at 100°C in water or in *N*,*N*-dimethylformamide (DMF). At ca. 70°C the same results are obtained, but longer reaction times are required. At this temperature, **1** is the first detectable product. After 9 days guanosine had nearly completely degraded to give a great variety of products among which **1** can be detected by HPLC (Fig. 2). Compound **1** seems to be less stable than compounds X and Y which accumulate during the reaction, the structures of which are still unknown. Lowering the temperature is limited by the low solubility of guanosine. On the other hand, guanosine-5′-phosphate (GMP) reacts with glucose even at 40°C. To elucidate the structure of the main product the yield was improved by heating GMP and glucose in a buffered solution at 100°C. After enzymatic hydrolysis by application of alkaline phosphatase, one product is obtained which is, according to chromatographic behaviour and UV spectral data, identical with the glucosylated guanosine **1**. The results show that glycosylation of nucleotides may be of importance under physiological conditions.

EXPERIMENTAL

General methods.—^{1}H NMR and ^{13}C NMR spectra (external standard tetramethylsilane) were recorded with a Jeol 400 GSX spectrometer. Positive-ion FABMS were obtained with a Kratos MS 80 RFA spectrometer. pH-Measurement was with a WTW pH 253. For analytical chromatography a Merck L-6200 gradient



Scheme 1.

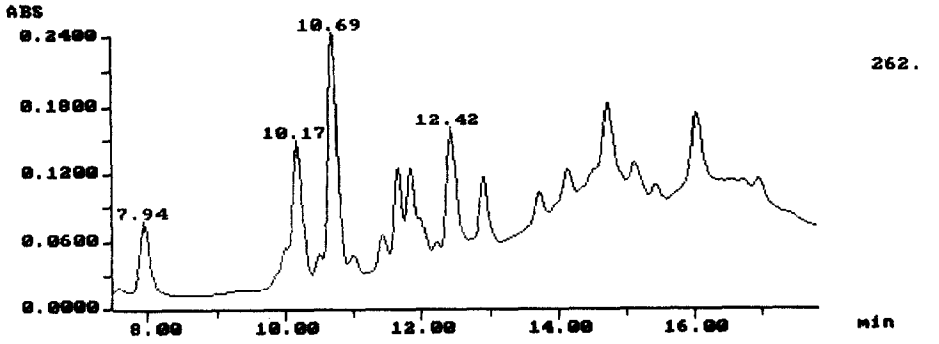


Fig. 2. HPL-chromatogram of the mixture of glucose and guanosine after 9 days incubation at 70°C (system 1b; λ, 262 nm): t_R 7.94 min, 1; t_R 10.17 and t_R 10.69 min comparatively stable products X and Y of unknown structure; t_R 12.42 min, guanosine.

pump, a Merck programmable photodiode array detector (DAD), Model D-6500 with Merck DAD-Manager Software, and a NEC pinwriter P60 were used. UV-spectra were taken directly from this system. Preparative HPLC was performed with a Merck L-6000 pump, a Merck L-4000 UV-detector, and a Merck D-2500 Chromato Integrator. HPLC grade solvent (MeOH) was used without further purification. The water used for HPLC was distilled and filtered through a nylon membrane of 0.45 μm. All solvents were degassed with He. System 1 (analytical): column: 250 × 4.6 mm i.d., RP 18 LiChrosorb, 5 μm with guard cartridge (25 × 4.6 mm i.d.); UV-detection between 220 and 300 nm (DAD); 1a eluent: 0.05 M triethylammonium acetate in 1:9 MeOH methanol–water, pH 7.0, at a flow rate of 1.0 mL/min; 1b: gradient elution starting with 0.05 M triethylammonium acetate buffer pH 7.0 ending with 1:4 buffer–MeOH. System 2 (preparative): column: Hibar LiChrosorb RP 18 (250 × 10 mm i.d., 7-μm particle size, Merck 50994); UV-detection at λ = 270 nm; 2a eluent: 5 mM ammonium formate (pH 7.0) at a flow rate of 3.5 mL/min; 2b eluent: 1:4 MeOH–water at a flow rate of 3.5 mL/min; 2c eluent: 1:19 MeOH–water at a flow rate of 3.5 mL/min. Thin layer chromatography (TLC) was performed using 20 × 20 cm glass plates coated with 2.0-mm thickness of Silica Gel 60 F-254 (Merck).

2-(β-D-Glucopyranosylamino)-1,9-dihydro-9-(β-D-ribofuranosyl)-6-purinone (**1**). —Guanosine (1.129, 4 mmol, Fluka 51050) and glucose (7.2 g, 40 mmol) in 5 mL of 2 M phosphate buffer (pH 7.0) were kept at 100°C for 4 h. The solution was diluted to 50 mL, put into an ultrasonic bath for 15 min, and filtered. The filtrate was concentrated nearly to dryness under reduced pressure. Portions of 1 mL each containing 500 mg of the mixture were chromatographed by TLC using 170:28:3 EtOH–water–acetic acid as eluting agent. The zone of R_f 0.15–0.25 (guanosine, R_f 0.8; glucose, R_f 0.5–0.7) was eluted with 1:1 water–EtOH. The solvent was evaporated, and the syrupy residue was diluted with a small amount of water and filtered for preparative HPLC (system 2a). The product eluting after 16 min was

collected. The freeze-dried substance was recrystallized from MeOH; yield 19.6 mg (1.1%); mp 238–240°C (dec). ^{1}H NMR (400 MHz, D_2O): δ ⟨ppm⟩ 7.91 (s, 1 H, Guo), 5.86 (d, 1 H, $J_{1,2}$ 5.1 Hz, H-1 Rib), 5.20 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1 Glc), 4.80 (t, 1 H, J 5.1 Hz, H-2 Rib), 4.34 (t, 1 H, J 5.0 Hz, H-3 Rib), 4.10 (m, 1 H, H-4 Rib), 3.6–3.8 (m, 4 H, H-5a,5b Rib, 6a,6b Glc), 3.50–3.60, (m, 2 H, H-3,5 Glc), 3.43 (t, 1 H, J 8.8 Hz, H-4 Glc), and 3.41 (t, J 9.1 Hz, H-2 Glc); ^{13}C NMR: 171.1 (s, C-6 Guo), 158.9 (s, C-2 Guo), 148.1 (s, C-4 Guo), 138.9 (d, C-8 Guo), 117.7 (s, C-5 Guo), 88.3 (d, C-1 Rib), 84.8 (d, C-4 Rib), 81.4 (d, C-1 Glc), 77.4 (d, C-5 Glc), 76.6 (d, C-3 Glc), 73.2 (d, C-2 Rib), 72.2 (d, C-2 Glc), 70.4 (d, C-3 Rib), 69.3 (d, C-4 Glc), 61.4 (t, C-5 Rib), 60.6 (t, C-6 Glc). UV (H_2O, pH 7): λ_{max} ⟨nm⟩ 255; FABMS (Xe, 7 KV, glycerol, LiCl): m/z 452 ($M + Li^+$), 446 ($M + H^+$).

Reactions at lower temperatures.—Analytical samples containing glucose (180 mg, 1 mmol), dipotassiumhydrogen phosphate (64 mg, 0.37 mmol), potassiumdihydrogen phosphate (50 mg, 0.37 mmol), guanosine (28 mg, 0.1 mmol), and 125 mg H_2O were kept at 70 or 40°C, respectively. From the first experiment daily samples were taken, from the latter weekly, and analysed by HPLC.

Reaction of GMP.—Glucose (180 mg, 1 mmol), dipotassiumhydrogen phosphate (64 mg, 0.37 mmol), potassiumdihydrogen phosphate (50 mg, 0.37 mmol), GMP (44 mg, 0.1 mmol, Fluka 51090) and, 125 mg H_2O were stored at 40°C and weekly samples were taken and analysed by HPLC (system 1a). After 5 weeks ca. 10% of GMP had been converted to a product with a shorter retention time and an UV spectrum similar to **1**. In another experiment glucose (180 mg, 0.1 mmol) and GMP (44 mg, 0.1 mmol) in 200 μL 1 M Tris HCl buffer (pH 8.1) were heated to 100°C for 4 h. A 20-μL sample of the mixture containing ca. 5 μmol phosphate ester was incubated with 10 μL alkaline phosphatase suspension (Boehringer 108162) for 1 h at 25°C. The solution was diluted, filtered, and chromatographed (system 1a, Fig. 3).

2-(β-D-Glucopyranosylamino)-1,9-dihydro-6-purinone (**6**).—2′-Deoxyguanosine monohydrate (570 mg, 2 mmol, Fluka 31070) and glucose (2.8 g, 15.6 mmol) in 2 mL H_2O were refluxed for 6 h. The hot solution was filtered and diluted with 10 mL H_2O. The brown solution with pH 4.5 was neutralized with 6 N NH_3. Portions of 200 μL were used for preparative HPLC (system 2a). The product eluting after 14.2 min was collected; yield 11 mg (1.6%); mp 128–130°C; ^{1}H NMR (400 MHz, D_2O): δ ⟨ppm⟩ 7.91 (s, 1 H, Guo), 4.98 (d, 1 H, J 9.1 Hz, H-1), 3.68 (dd, 1 H, J_{gem} 12.0, $J_{6,5}$ 2.2 Hz, H-6a), 3.54 (dd, 1 H, J_{gem} 12.0, $J_{6,5}$ 5.0 Hz, H-6b), 3.43 (m, 1 H, H-5), 3.39 (t, 1 H, J 9.2 Hz, H-3), 3.30 (t, 1 H, J 8.8 Hz, H-2), 3.26 (t, 1 H, J 8.7 Hz, H-4). UV (H_2O, pH 7): λ_{max} ⟨nm⟩ 249.8; 271.1; FABMS (Xe, 7 KV, glycerol, LiCl): m/z 320 ($M + Li^+$), 314 ($M + H^+$).

N-*(4,6-Dimethyl-2-pyrimidinyl-α-D-glucopyranosylamine* (**4**).—2-Amino-4,6-dimethylpyrimidine **2** (62 mg, 0.5 mmol, Fluka 07850) and glucose (180 mg) were dissolved in 200 μL DMF, kept at 100°C for 16 h, diluted with 1 mL H_2O and portions of 100 μL were used for preparative HPLC (system 2b). The product eluting after 8.0 min was collected; yield 38 mg (27%); mp 118–120°C (EtOH, dec)

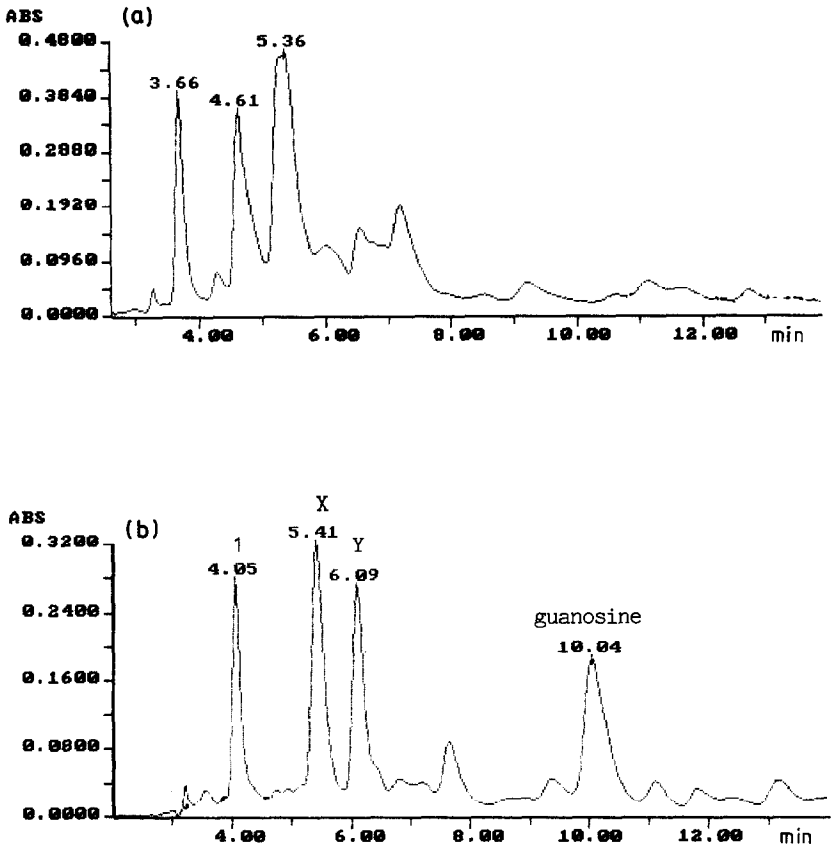


Fig. 3. HPL-chromatogram of the reaction of glucose and GMP (4 h, 100°C, pH 8.1, system 1a; λ, 260 nm). (a) Before and (b) after enzymatic hydrolysis of the phosphate ester. (a) t_R 3.66 min, **1** as phosphate ester; t_R 4.61 min, X as phosphate ester; t_R 5.36 min, mixture of Y as phosphate ester and GMP; (b) t_R 4.05 min, 1; t_R 5.41 min, X; t_R 6.09 min, Y; t_R 10.04 min, guanosine.

^{1}H NMR (400 MHz, CD_3OD): δ ⟨ppm⟩ 6.52 (s, 1 H, H-5), 5.27 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1′), 3.84 (dd, 1 H, J_{gem} 11.8, $J_{6,5}$ 2.0 Hz, H-6′a), 3.69 (dd, 1 H, J_{gem} 12.0, $J_{6,5}$ 5.0 Hz, H-6′b), 3.48 (t, 1 H, *J* 8.8 Hz, H-3′), 3.42 (m, 1 H, H-5′), 3.39 (t, 1 H, *J* 8.8 Hz, H-4′, 3.35 (t, 1 H, *J* 8.8 Hz, H-2′), 2.29 (s, 6 H, CH_3); ^{13}C NMR: 169.3 (s, 2 C, C-4,6), 162.9 (s, C-2), 112.3 (d, C-5), 83.4 (d, C′-1), 79.0 (d, C′-5) 78.9 (d, C′-3) 74.3 (d, C′-2), 71.5 (d, C′-4), 62.7 (t, C′-6), 23.7 (q, 2 C, CH_3). UV (H_2O, pH 7.0): λ_{max} ⟨nm⟩ 230, 286; FABMS (Xe, 7 KV, glycerol + LiCl): *m/z* 292 ($M + Li^+$), 286 ($M + H^+$).

2-(α-D-Glucopyranosylamino)-3H-6-methyl-4-pyrimidinone (**5**).—2-Amino-6-methyl-3*H*-pyrimidin-4-one **3** (125 mg, 1 mmol) and glucose (720 mg, 4 mmol) were dissolved in 2 mL DMF, kept at 100°C for 24 h and diluted with 2 mL H_2O. Portions of 200 μL were used for preparative HPLC (system 2c). The product, which eluted after 8.44 min was collected; yield 10 mg (3.5%); mp 160–162°C (dec).

^{1}H NMR (400 MHz, CD_3OD): δ ⟨ppm⟩ 5.70 (s, 1 H, H-5), 5.06 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1′), 3.80 (dd, 1 H, J_{gem} 12.0, $J_{6,5}$ 2.2 Hz, H-6′a), 3.77 (dd, 1 H, J_{gem} 12.0, $J_{6,5}$ 5.0 Hz, H-6′b), 3.45 (m, 2 H, H-3,5), 3.8 (m, 2 H, H-2′,4′), 2.10 (s, 3 H, CH_3); ^{13}C NMR: 167.7 (s, C-4), 154.9 (s, C-2), 136.0 (s, C-6), 113.6 (d, C-5), 81.1 (d, C′-1), 77.4 (d, C′-5), 76.7 (d, C′-3), 72.4 (d, C′-2), 69.5 (d, C′-4), 60.8 (t, C′-6), 20.3 (q, CH_3). UV (H_2O, pH 7): λ_{max} ⟨nm⟩ 264; FABMS (Xe, 7 KV, glycerol, LiCl): *m/z* 294 ($M + Li^+$) 288 ($M + H^+$).

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