Studies on the Alkylation of Guanine. 4*. Use of ^{13}C NMR Spectroscopy for Establishing the Structure of Tetraacylated Glyoxal-Guanine Adducts

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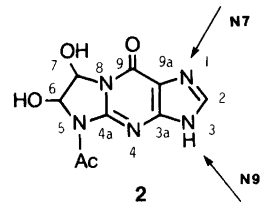
The synthesis and the structure of tri- and tetraacylated glyoxal-guanine adducts are described. Glyoxal reacted with N^2-acetylguanine to form a tricyclic compound which was further acylated with acetic or isobutyric anhydride. The constitution, in particular the position of the fourth acyl group, was established by use of ^{13}C NMR and UV spectroscopy. Acylation gave, in contrast to alkylation, the N9-substituted product.

Buciclovir (9-[(*R*)-3,4-dihydroxybutyl]guanine) shows potent antiviral activity *in vivo* and *in vitro*.[1] We have studied different approaches to the synthesis of buciclovir and related compounds, in which alkylation of the derivatized purine with an acyclic side chain, appropriately protected, was the crucial step. In this paper, we describe the synthesis of the tetraacylated glyoxal-guanine adducts **3** and **4**, which are interesting new compounds with potential application in nucleoside and nucleotide chemistry.

The compounds **3** and **4** were synthesized by acylation of N^1, N^2-glyoxal-N^2-acetylguanine adduct (**2**) (Scheme 1).

Acid-catalyzed reaction of guanine with glyoxal in water afforded an adduct,[2] which could be acetylated by a large excess of acetic anhydride in pyridine to give a mixture of products.[3] However, the reaction proceeds more smoothly if performed on N^2-acetylguanine (**1**) in dry pyridine. Acylation of the glyoxal-guanine adduct **2** with acetic anhydride or isobutyric anhydride in pyridine gave the tetraacylated compounds **3** and **4**, which after monodeacylation in aqueous ethanol afforded compounds **5** and **6**, respectively, in about 35 % overall yield.

Compounds **3**, **5** and **6** were obtained as homogeneous products and were characterized by their NMR, UV and MS spectroscopic data.[4] An analytically pure sample of **4** could not be isolated. The fourth acyl group of compounds **3** and **4** is relatively labile, and on recording the NMR spectrum of **4** in deuterated dimethyl sulfoxide some



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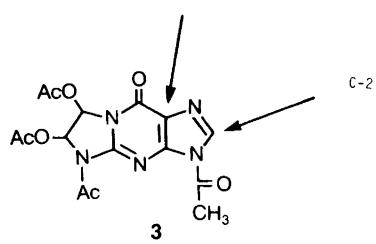

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*Part 3, see Ref. 22.

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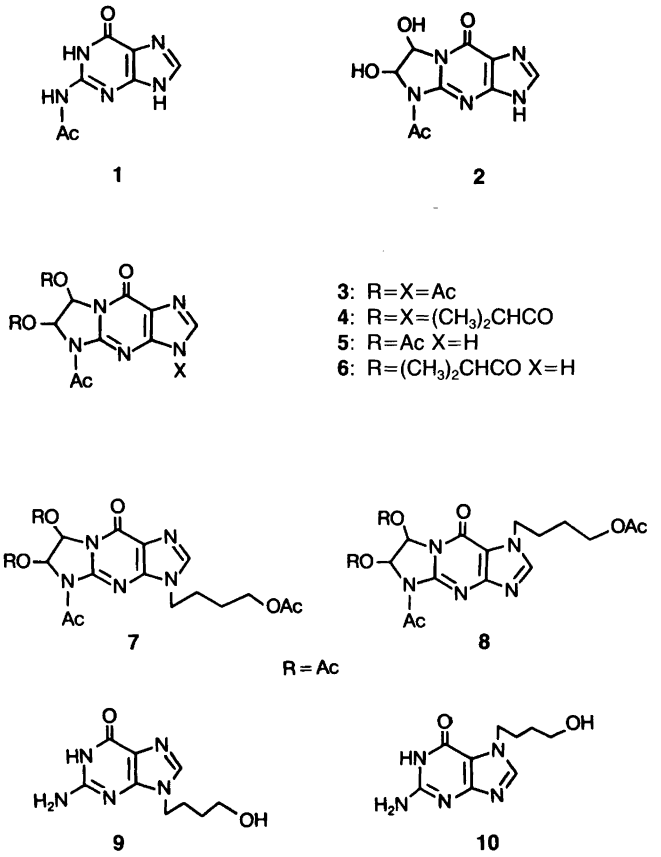

Scheme 1.

deacylation to **6** occurred. The labile acyl group would be expected to occupy either the N7 or the N9 position in the imidazole moiety of the ring system.

Data for some characteristic ^{1}H and ^{13}C NMR signals are given in Table 1, together with corresponding data for the alkylated guanine derivatives **7** and **8**.[4]

Differently substituted purines exhibit characteristic shift differences in their ^{1}H, ^{13}C and ^{15}N NMR spectra.[5–9] The presence of one peak for the proton at C-2 indicated that **3** and **4** are not mixtures of acylated derivatives. As can be seen from Table 1, the ^{13}C shifts for the N^{9}-alkylated compound **7** are shifted downfield for C-9a and upfield for C-2 relative to the shifts of the non-

Table 1. Selected ^{1}H and ^{13}C shifts relative to TMS.

Shifts	5	3	6	4	7	8	Solvent
H-2	8.03	8.40	8.11	8.38	7.69	7.79	$CDCl_3$
H-2	8.15	8.56	8.13				DMSO-d_6
C-9a	–	124.0	112.8	124.1	120.1	111.5	$CDCl_3$
C-9a	115	124.0					DMSO-d_6
C-2	–	137.9	142.0	138.6	139.3	143.5	$CDCl_3$
C-2	141.2	138.4					DMSO-d_6

alkylated compounds **5** and **6**. For the N7-alkylated compound **8**, the corresponding ^{13}C resonances are shifted in the reverse direction, i.e. upfield for C-9a and downfield for C-2 relative to those of compounds **5** and **6**. The tetraacylated compounds **3** and **4** have downfield shifts for C-9a and upfield shifts for C-2, relative to **5** and **6**, and thus the imidazole acyl group of **3** and **4** should occupy the N9 (3) position. This is in analogy with the conclusion reached by Nachman[10] regarding the position of the acetate group in N^9-acetyl-1-methylisoguanine.

Our assumption is further supported by the UV spectrum of **3**, recorded in ethanol so as to avoid the monodeacetylation which occurs in water, which closely resembles those of N9-alkylated guanine derivatives.[11,12] The UV spectra of 9-(4-hydroxybutyl)guanine and 7-(4-hydroxybutyl)guanine are shown in Fig. 1. The use of ultraviolet spectra as a tool for distinguishing N7- and N9-substituted purine derivatives has been intensively studied.[12]

The presence of the N^2, N^9-diacetylguanine moiety[13-16] is also supported by the magnitude of the ^{13}C shift for C-8 (δ 137.4). Due to the low solubility of diacetylguanine we could not detect the shifts for the other ring carbons with certainty.

Acylation of compound **2** gave the N9-acylated regioisomer (3-substituted) as the only isolated product, in contrast to the alkylation of compound **5** which gave a predominance of the N^7-regioisomer.[4] The acetylation of **2** possibly is a reversible process which leads to the thermodynamically most stable product. Ogilvie *et al.* have shown that N^7-[2-benzyloxy-1-(benzyloxymethyl) ethoxy]methyl-6-chloroguanine rearranges to the more stable N^9-isomer in strongly alkaline solution.[17] Analogous rearrangement of 6-chloro-7-hexylguanine to 9-hexylguanine has not been observed.[18] Other similar isomerizations have been reported.[19,20,21] This would indicate that the preferential N7 alkylation described by Kjellberg and Johansson[4] is kinetically controlled (cf. Ref. 22).

Experimental

The NMR spectra were recorded on a Jeol NJM-FX 200 instrument. NMR signals for water-exchangeable protons were not observed. Melting points were measured on a Büchi 510 apparatus and are uncorrected. The mass spectra were recorded on an LKB 9000 mass spectrometer (70 eV). Elemental analyses were performed by Novo Microanalytical Laboratory, Novo Allé, DK-2880 Bagsværd, Denmark and by Kemicen-

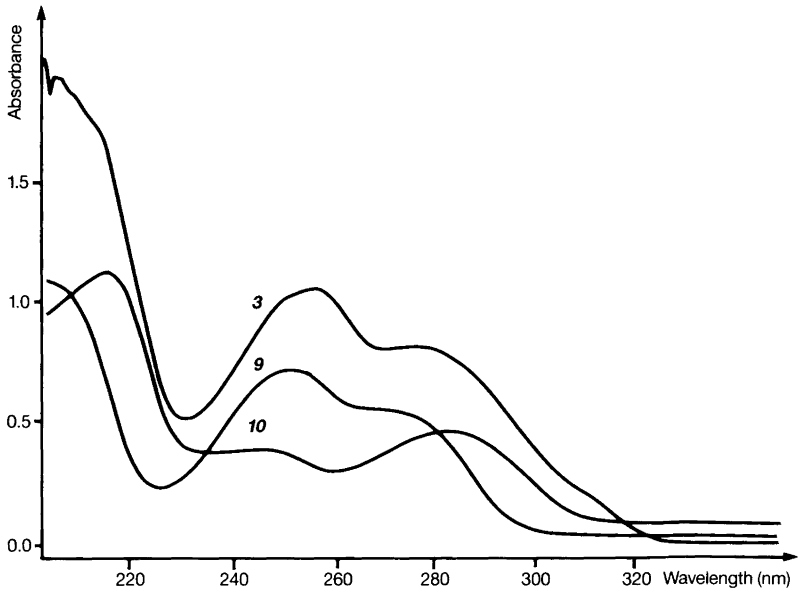

Fig. 1. Ultraviolet spectra of compounds **3** (ethanol), **9** (pH 7), and **10** (pH 7).

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Thin–layer chromatography (TLC) was performed on pre-coated plates of silica gel 60 F_{254} (Merck). Ultraviolet spectra were recorded on a Hewlett Packard 8450 A UV/VIS spectrophotometer. All solvents and starting materials were of the highest available purity.

N^2-Acetylguanine (**1**) was prepared by slightly modifying the procedure of Hřebabecký and Farkaš.[23] Acetic anhydride (50 ml) was added to a stirred suspension of finely ground guanine (32 g) in 1-methyl-2-pyrrolidone (300 ml). The mixture was heated (~145 °C) for 3 h, during which it turned brown. Acetic anhydride (2 ml) was added and the remaining undissolved guanine was filtered off. The resulting solution was kept for 18 h at room temperature. White crystals precipitated and were collected by filtration. The crude product was washed with ethyl acetate and recrystallized from 50 % (*v/v*) aqueous ethanol to give *N^2*-acetylguanine (28 g, 68 %). ^{1}H NMR (DMSO-d_6): δ 2.16 (3H, s, CH_3), 8.03 (1H, s, H-8). UV λ_{max} (nm): 204, 260 (aq. pH 1); λ_{max}: 222, 270 (aq. pH 13).

5-Acetyl-5,6,7,9-tetrahydro-9-oxo-(3H-imidazo[1,2-a]purine)-6,7-diol (**2**). *N^2*-acetylguanine (4.8 g) and glyoxal polymer (30 g) were suspended in dry pyridine (200 ml). The suspension was stirred overnight at room temperature. The solvent was evaporated under reduced pressure. The residue was dissolved in water (300 ml) and washed with chloroform (2 × 150 ml). The aqueous solution was kept overnight at 4 °C, whereupon adduct **2** precipitated as white crystals. The yield after filtration and drying was 28 g (74 %). Found: C 40.2; H 4.14; N 25.2; O 30.0. Calc. for $C_9H_9N_5O_4 \cdot 1\ H_2O$: C 40.2; H 4.12; N 26.0; O 29.7. ^{1}H NMR (DMSO-d_6): δ 2.62 (3H, s, CH_3), 5.54 (2H, d, CHCH), 8.08 (1H, s, H-8). ^{13}C NMR (DMSO-d_6): δ 25.0 (CH_3), 81.1, 84.0 (CHCH), C5 not detected, 140.6 (C8), 147.7 (C4), C2 and C6 not detected, 168.9 (CO). UV λ_{max} (nm): 258 (aq. pH 1); λ_{max}: 270 (aq. pH 13).

3,5-Diacetyl-6,7-diacetoxy-5,6,7,9-tetrahydro-9-oxo(3H-imidazo[1,2-a]-purine) (**3**). Acetic anhydride (1.8 g) was added to a solution of **2** (1.25 g) in dry pyridine (150 ml). On stirring for 2 h at room temperature a pale red solution was produced. The solvent was evaporated under reduced pressure and the residue was washed with ethyl acetate and ether. The tetraacylated product **3** was isolated as white crystals (1.31 g, 70 %). Anal. $C_{15}H_{15}N_5O_7$: C, H, N. ^{1}H NMR ($CDCl_3$): δ 2.12 (6H, 2s, 2 $OCOCH_3$), 2.73 (3H, s, $NCOCH_3$), 2.87 (3H, s, $NCOCH_3$), 6.86 (2H, CHCH), 8.39 (1H, s, H-8). ^{13}C NMR ($CDCl_3$): δ 20.5, 20.6 (2 $COCH_3$), 25.2 ($NCOCH_3$), 25.3 ($NCOCH_3$), 78.3, 81.5 (CHCH), 124.0 (C5), 137.9 (C8), 147.4 (C4), 149.4 (C2), 153.2 (C6), 166.8 (CO), 167.2 (CO), 167.8 (CO), 168.0 (CO). UV λ_{max} (nm): 258, 279 (ethanol). MS (*m/e*): 377. M.p. 208–211 °C.

5-Acetyl-5,6,7,9-tetrahydro-3-(2-methylpropanoyl)-6-7-bis(2-methylpropanoyloxy)-9-oxo-(3H-imidazo[1,2-a]purine) (**4**). Isobutyric anhydride (4.0 g) was added to a solution of **2** (2.0 g) in dry pyridine (150 ml). The solution was stirred for 24 h at room temperature, during which it turned brown. The solvent was removed *in vacuo*. The residue was dissolved in ethyl acetate (50 ml) and filtered. The solution was washed with water (2 × 50 ml) and 5 % aqueous sodium hydrogen carbonate solution (2 × 50 ml). Ethyl acetate was removed by evaporation and the yellow, foamy residue was dissolved in ethanol (10 ml) and the solution filtered through celite. After evaporation and drying, white crystals of **4** (2.3 g, 67 %) were obtained. ^{1}H NMR ($CDCl_3$): δ 1.14–1.19 (18H, CH_3), 2.58 (3H, m, $3CH_3CH$), 2.80 (3H, s, $NCOCH_3$), 6.78–6.87 (2H, CHCH), 8.11 (1H, s, H-8). ^{13}C NMR ($CDCl_3$): δ 18.5–19.0 (6 CH_3), 25.0 ($NCOCH_3$), 78.6, 81.7 (CHCH), 124.0 (C5), 138.5 (C8), 147.8 (C4), 149.2 (C2), 152.4 (C6), 168.7 (CO, 174.2–174.6 (3 CO). M.p. 167–168 °C.

5-Acetyl-6,7-diacetoxy-5,6,7,9-tetrahydro-9-oxo-(3H-imidazo[1,2-a]-purine) (**5**). The tetraacetylated adduct **3** (1.6 g) was dissolved in 50 % aqueous ethanol (70 ml) and heated at 60 °C for 0.5 h. The clear solution was kept at 4 °C overnight. White needles (1.2 g, 84 %) of compound **5** were collected by filtration. Anal. $C_{13}H_{13}N_5O_6$: C, H, N. ^{1}H NMR (DMSO-d_6): δ 2.08 (3H, s, $OCOCH_3$), 2.12 (3H, s, $OCOCH_3$), 2.68 (3H, s, $NCOCH_3$), 6.78–6.85 (2H, CHCH), 8.15 (1H, s, H-8). ^{13}C NMR (DMSO-d_6): δ 20.6, 20.7 (2 CH_3COOCH), 24.8 ($NCOCH_3$), 77.6, 81.1 (CHCH), 115 (C5) broad, 141.2 (C8), 147.6

(C4), 149.6 (C2), 152.1 (C6), 168.2, 168.4 (3 CO). MS(*m/e*): 335. UV λ_{max} (nm): 256 (aq. pH 1); λ_{max}: 272 (aq. pH 13).

5-Acetyl-6,7-bis(2-methylpropanoyloxy-5,6,7,9-tetrahydro-9-oxo-(3H-imidazo[1,2-a]purine) (**6**). Compound **4** (2 g) was dissolved in 50 % aqueous ethanol (25 ml) and the solution was heated at 75 °C for 15 min. The resulting solution was kept at 4 °C for a week and white crystals of **6** (0.9 g, 53 %) were filtered off. Anal. $C_{17}H_{21}N_5O_6$: C, H, N. ^{1}H NMR (DMSO-d_6): δ 1.06–1.13 (12H, m, CH_3), 2.5–2.7 (2H, m, $2CH_3CH$), 2.68 (3H, s, $NCOCH_3$), 6.68–6.78 (2H, CHCH), 8.16 (1H, s, H-8). ^{13}C NMR (DMSO-d_6): δ 18.6, 18.7, 18.8, 18.9 (4 CH_3CH), 25.3 ($NCOCH_3$), 33.8, 33.9 (2 CH_3CH), 78.1, 81.6 (CHCH), 115 (C5) broad, 142.1 (C8), 147.9 (C4), 149.6 (C2), 152.4 (C6), 168.7 (NCO), 174.1, 174.7 (2 OCO). MS (*m/e*): 391. Uv λ_{max} (nm): 255 (aq. pH 1); λ_{max}: 274 (aq. pH 13). M.p. 169–170 °C.

The syntheses and analysis of the compounds **7, 8, 9** and **10** are described in Refs. 4 and 22.

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