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DETERMINATION OF THE STRUCTURE OF THE ADDUCT FROM GUANOSINE AND GLYCIDALDEHYDE

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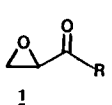
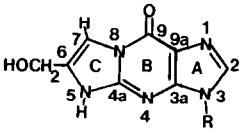
Abstract: The complete structure of the 1:1 adduct between guanosine and glycidaldehyde has been unequivocally determined by high-field NMR data and by synthesis of related model compounds.

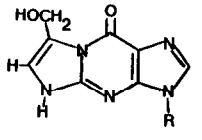
The mode of formation and the detailed structures of adducts between carbonyl compounds and nucleic acid bases have been of considerable interest in studies of the constitution and mechanism of action of nucleic acids. In relation to some work in our Laboratory associated with the synthesis of compounds related to the "Y" bases,[1] we examined the reaction of glycidaldehyde (1, R = H) with guanosine. Glycidaldehyde is known to be reactive towards nucleic acids and appears to be highly specific for guanine components.[2] Despite its importance as a probe in the study of the mode of action of alkylating carcinogens,[3] the structural details of its modification of guanine residues have not been fully determined. The suggestion has been made that guanine is modified by glycidaldehyde into a tricyclic base. Two structures 2 and 4 were proposed as possible products.[4] However, the data presented did not distinguish between these possibilities. On structural and mechanistic grounds it is logical to assume that the loci of reaction on guanine includes the 2-NH_2 and one endocyclic nitrogen of the base.[5] Therefore, two other structures 6 and 7 are possible. We now report on an unambiguous assignment of the structure of this adduct.



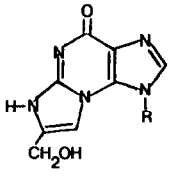
1



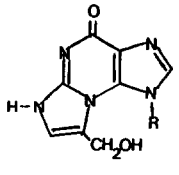
2 R= Ribosyl
3 R= $-C_2H_5$



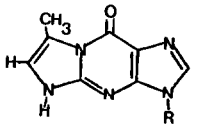
4 R = Ribosyl
5 R = $-C_2H_5$



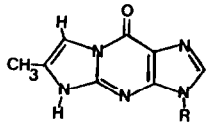
6 R= Ribosyl
R= $-C_2H_5$



7 R= Ribosyl
R= $-C_2H_5$



8 R=Ribosyl



9 R=Ribosyl
10 R=C_2H_5

Glycidaldehyde (1, R = H)[6] was prepared from acrolein by oxidation with sodium hypochlorite by modification of a method developed for the synthesis of 3,4-epoxybutanone (1, R = CH_3) by White and his coworkers.[7] When guanosine was treated with glycidaldehyde at pH 10 and 25°C for 1.5 h and the reaction mixture neutralized to pH 7 and cooled to 5°C, a white crystalline product, $C_{13}H_{15}N_5O_6$, m.p. >300° (decomp.), precipitated out of the reaction mixture (59.0% yield). Its ultraviolet spectral data shown in Figure 1 indicate that the compound is a linear adduct. This conclusion is based on the work of Leonard and his coworkers[8] who showed that tricyclic heteroaromatic systems of the linear type exhibit lower energy electronic transitions than their angular isomers. The fluorescence spectrum of the guanosine-glycidaldehyde adduct in 0.1N NaOH showed an intense emission maximum at 420 nm with irradiation near 320 nm confirming the presence of the tricyclic base. Its infrared spectrum (Nujol) indicated the retention of the carbonyl (1700 cm^{-1}).

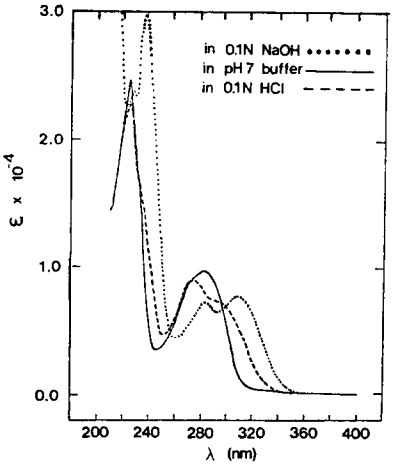


Fig. 1. The UV spectra of the adduct of guanosine and glycidaldehyde at selected pH values.

Although the UV data provide strong evidence that the product is not 6 or 7 (or some tautomeric form of these structures), but more likely a linear modified guanine derivative, they do not distinguish between the linear possibilities 2 and 4.[9] As the reaction appears to be regiospecific, the high-field NMR data of the single isomer produced cannot be used to distinguish unequivocally between the two possible structures. As the difference between 2 and 4 resides in the position of the substituents on carbons 6 and 7, evidence to differentiate between these structures may be found in the chemical shift difference between H_6 and H_7. A hydrogen at C_7 would be expected to be deshielded by the carbonyl at C_9. However, in the absence of both isomers 2 and 4, it is not clear if the resonance at δ 7.23 (Table 1) should be assigned to H_6 in 4 or H_7 in 2. We resorted to related model compounds to solve this problem. It was envisaged that an alkylation-condensation reaction of appropriate α-bromo carbonyl compounds with guanosine would provide model systems with a hydrogen at C_6 or C_7 depending on the nature of the reactant. Guanosine is known to undergo alkylation at N_1 when treated with alkyl halides under conditions of base catalysis.[10,11] Thus, when the preformed anion of guanosine (K_2CO_3 in DMSO) was treated with α-bromopropionaldehyde (prepared from bromine and the silyl enol ether of propionaldehyde[12,13]) and the reaction mixture stirred at 25°C for 15 h, the tricyclic compound 8 was isolated. It was purified by HPLC on Amberlite XAD-4-resin (45-50 µm) with 80% water/ethanol as the eluting solvent (18.0% yield), m.p. 204-206°. Similarly, when the anion of guanosine

Table 1. 360 MHz ^{1}H NMR Data for Tricyclic Adducts in DMSO-d_6

Compd	H_2	H_5	H_6	H_7	6-$\underline{CH_2}$OH	6-$CH_2\underline{OH}$	6-CH_3	7-CH_3	H_{others}
2	8.14 (s)	12.36 (s,br)	-	7.23 (s)	4.84 (d)	4.98 (t)	-	-	3.53, 3.65 (m,m, $H_{5'}$), 3.91 (m, $H_{4'}$), 4.12 (m, $H_{3'}$), 4.45 (m, $H_{2'}$), 5.05 (t, 5'-CH_2OH), 5.16, 5.42 (d,d, 2'-OH, 3'-OH), 5.81 (d, J = 5.9, $H_{1'}$)
3	7.93 (s)	12.26 (s,br)	-	7.22 (s)	4.84 (d)	4.98 (d)	-	-	1.38 (t, CH_3), 4.08 (q, CH_2)
8	7.90 (s)	12.34 (s,br)	6.84 (s)	-	-	-	-	2.63 (s)	3.55, 3.63 (m,m, $H_{5'}$), 3.91 (m, $H_{4'}$), 4.11 (m, $H_{3'}$), 4.57 (m, $H_{2'}$), 5.06 (t, 5'-CH_2OH), 5.16, 5.41 (d,d, 2'-OH, 3'-OH), 5.71 (d, J = 6.1, $H_{1'}$)
9	8.13 (s)	12.34 (s,br)	-	7.36 (s)	-	-	2.26 (s)	-	3.54, 3.65 (m,m, $H_{5'}$), 3.92 (m, $H_{4'}$), 4.13 (m, $H_{3'}$), 4.49 (m, $H_{2'}$), 5.06 (s, br, 5'-CH_2OH), 5.16, 5.41 (d,d, 2'-OH, 3'-OH), 5.81 (d, J = 5.4, $H_{1'}$)
10	7.90 (s)	12.30 (s,br)	-	7.33 (s)	-	-	2.25 (s)	-	1.38 (t, CH_3), 4.08 (q, CH_2)

Table 2. 90.57 MHz ^{13}C NMR Data for Tricyclic Adducts in DMSO-d_6

Compd	C_2	C_6	C_7	C_9	C_{9a}	C_{3a}	C_{4a}	6-CH_2OH	6-CH_3	7-CH_3	C_{others}
2	137.2	113.7	124.6	153.6	115.9	146.7	150.2	55.0	-	-	61.2 ($C_{5'}$), 70.2 ($C_{3'}$), 73.6 ($C_{2'}$), 85.1 ($C_{4'}$), 86.8 ($C_{1'}$)
3	138.7	113.5	124.4	153.7	115.9	146.8	150.1	55.0	-	-	15.0 (CH_3), 37.9 (CH_2)
8	136.4	118.4	119.4	155.1	114.8	149.2	150.0	-	-	13.0	61.7 ($C_{5'}$), 70.6 ($C_{3'}$), 73.0 ($C_{2'}$), 85.5 ($C_{4'}$), 87.5 ($C_{1'}$)
9	137.1	103.3	126.1	151.1	115.5	145.5	149.8	-	10.4	-	61.3 ($C_{5'}$), 70.3 ($C_{3'}$), 73.6 ($C_{2'}$), 85.2 ($C_{4'}$), 86.9 ($C_{1'}$)
10	138.2	103.0	125.7	151.1	115.4	145.7	149.7	-	10.4	-	15.0 (CH_3), 37.6 (CH_2)

(NaH in DMF) was treated with α-bromoacetone, compound 9[14] was isolated and was subsequently purified by HPLC (39.0% yield), m.p. 199-202°.

A comparison of the high-field ^{1}H NMR spectra of 8 and 9 with that of the adduct of glycidaldehyde and guanosine shown in Table 1 clearly suggests that 2 is the correct assignment of the structure of the original adduct. The chemical shift for H_7 in 2 is at δ 7.23, at least 0.5 ppm downfield from what would be expected for H_6 in structure 4. That the carbonyl group at C_9 is indeed involved in the deshielding of H_7 is supported by the observation that the methyl group resonance in 8 occurs as a singlet at δ 2.63 and that in 9 appears as a singlet at δ 2.26. The ^{13}C NMR data (Table 2) supported these conclusions. Further confirmation of the structural assignment came from the conversion of 3 (via its tosylate) to 10 (m.p. 273-275°), an authentic sample of which was prepared from 9-ethylguanine and α-bromoacetone.

The formation of the tricyclic adduct 2 can best be appreciated by considering the regiochemistry and a plausible mechanism for the reaction. Under basic conditions (pH 10), guanosine forms an anion at N_1 preferentially (pKa = 9.2). If the anion cleaves the epoxide ring in an S_N2 manner at the more electrophilic carbon, then the intermediate formed can undergo cyclization and subsequent dehydration to give 4. If the N_1 anion attacks the carbonyl carbon and ring cleavage of the epoxide is brought about by the 2-amino group, then compound 2 is the product. Our experimental results support the regiospecific formation of 2 and the latter mechanism. Additional evidence for this mechanism was provided by the observation that the reaction of 3,4-epoxybutanone (1, R = CH_3) with guanosine at pH 10 was extremely sluggish.

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