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The Structures of Adducts from the Reaction between Guanosine and Glycidaldehyde (Oxiranecarbaldehyde): a ^{15}N Labelling Study

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Reaction of guanosine (**1a**) with racemic glycidaldehyde (**2**) in aqueous medium at pH 10 gives 5,9-dihydro-9-oxo-3-β-D-ribofuranosylimidazo[1,2-*a*]purine (1,N^2-ethenoguanosine) (**3c**), its 7-hydroxymethyl derivative (**3a**), and methylene-7,7′-bis-5,9-dihydro-9-oxo-3-β-D-ribofuranosylimidazo[1,2-*a*]purine (**5**); the structures of adducts (**3a**) and (**5**) were deduced from ^{13}C n.m.r. analysis of ^{15}N labelled adducts derived from [1-^{15}N]guanosine (**1b**) and [$^{15}NH_2$]guanosine (**1c**).

The reaction between guanosine (**1a**) and glycidaldehyde (**2**) in aqueous solution at pH 10 was reported to yield a 1:1 adduct, for which alternative structures (**3a**) and (**3b**) were proposed.[1] A recent re-investigation of this reaction led to the conclusion that structure (**3b**) is correct.[2] We have also re-investigated this problem and report evidence based on analyses of the ^{13}C n.m.r. spectra of adducts derived from ^{15}N-enriched guanosines [(**1b**) and (**1c**), respectively] that structure (**3a**) is correct.

Using both h.p.l.c. and high-field ^1H n.m.r. spectroscopy to monitor reactions between guanosine and glycidaldehyde, we have found that besides the 1:1 adduct (**3a**) there are formed four diastereoisomeric intermediates [(**4a**)—(**4d**)], 1,N^2-ethenoguanosine (**3c**), and a 2:2 adduct (**5**); formaldehyde can be detected in the reaction mixture (*cf.* ref. 3). Two pairs of intermediates [(**4a**) and (**4b**), (**4c**) and (**4d**)] arise because the guanosine used was optically pure, whereas the glycidaldehyde was racemic.† The same intermediates and products were observed over the pH range 7—11, although the reactions were much faster at higher pH.

† Prepared by epoxidation (2 mol. equiv. of *m*-chloroperbenzoic acid in CH_2Cl_2 for 5.5 h at 20 °C) of racemic but-1-ene-3,4-diol, followed by periodate cleavage of the resulting (1,2-dihydroxyethyl)oxirane.

The $^{13}C\{^1H\}$ n.m.r. spectrum of the 1:1 adduct [in $(CD_3)_2SO$] shows resonances for the heteroaromatic system at δ 113.8, 116.0, 124.7, 137.4, 146.7, 150.2, and 153.7 assigned to C-6, C-9a, C-7, C-2, C-4a, C-3a, and C-9, respectively [see structure (**3a**)]. These assignments are aided by analysis of the corresponding ^{13}C spectrum in which, for example, C-6 appears as a double triplet (*J* 196 and 4.5 Hz) and C-7 as a double triplet (*J* 10 and 5 Hz). However, neither these spectra nor the ^1H n.m.r. spectrum of the 1:1 adduct exclude the alternative structure (**3b**).

By heating with [^{15}N]ammonia (1.1 mol. equiv.) in dimethyl sulphoxide (10 days; 100 °C), the amide (**6**)[4] was converted into [^{15}N]guanosine (≥90 atom % of ^{15}N) consisting of *ca.* 75% of [1-^{15}N]guanosine (**1b**) and 15—20% of [$^{15}NH_2$]guanosine (**1c**) [analysis by 30.42 MHz ^{15}N n.m.r. spectroscopy which showed resonances at δ (with respect to external $^{15}NO_3^-$) −302.6 (t, NH_2, J_{N-H} 90.5 Hz) and −228 (d, NH J_{N-H} 87 Hz)].[5] Reaction of this mixture of ^{15}N-labelled guanosines with racemic glycidaldehyde (1.1 mol. equiv.) in aqueous solution at pH 10.0 gave the corresponding labelled adducts (**3a**) and (**5**). The ^1H n.m.r. spectrum of one sample of adduct (**3a**) showed it to be contaminated with ^{15}N-labelled (**3c**), which exhibited signals for H-6 and H-7 that were both double doublets (*J* 3.0 Hz) showing near equality of their

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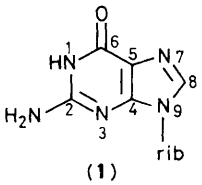
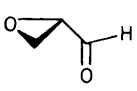
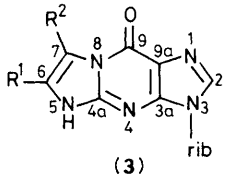


(1)

a; unlabelled
b; ^{15}N at N-1
c; ^{15}N at NH_2

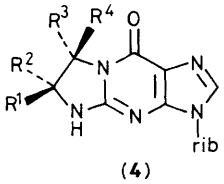


(2)



(3)

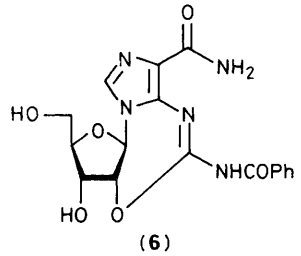
a; $R^1 = H$, $R^2 = CH_2OH$
b; $R^1 = CH_2OH$, $R^2 = H$
c; $R^1 = H$, $R^2 = H$



(4)

a; $R^1 = OH$, $R^2 = R^3 = H$, $R^4 = CH_2OH$
b; $R^1 = R^4 = H$, $R^2 = OH$, $R^3 = CH_2OH$
c; $R^1 = OH$, $R^2 = R^4 = H$, $R^3 = CH_2OH$
d; $R^1 = R^3 = H$, $R^2 = OH$, $R^4 = CH_2OH$

(5) = 2 molecules of **(3c)** linked at C-7 by CH_2



(6)

coupling to each other and ^{15}N-8 (^2J and ^3J). Therefore, the ^{15}N–H coupling of *ca.* 3.0 Hz observed in the ^1H n.m.r. spectrum of the 1 : 1 adduct does not define the position of the CH_2OH group although it does prove that the structure of the 1 : 1 adduct is linear [as in (**3a**)] as opposed to non-linear (*i.e.* etheno bridge linking N-4 and N-5). The non-linear structures were previously excluded on u.v. spectroscopic evidence.[2] However, the $^{13}C\{^1H\}$ n.m.r. spectrum of the 1 : 1 adduct does enable the position of the CH_2OH group to be defined as at C-7. This is because only four of the carbon resonances show significant coupling to ^{15}N: δ 116.0 (C-9a, $^2J_{CN}$ 10 Hz), 124.7 (C-7, $^1J_{CN}$ 9 Hz), 146.7 (C-4a, $^1J_{CN}$ 13 Hz), and 153.7 (C-9, $^1J_{CN}$ 10 Hz) (see Figure 1a—d). The outer lines of the triplets for C-9a, C-7, and C-9 arise from significant coupling (*ca.* 10 Hz) between ^{15}N-8 (*ca.* 75% of the molecules contain ^{15}N at this position) and these carbon atoms. The central line of each triplet derives from a carbon in a molecule labelled at N-5 (15—20% of molecules) or not labelled at all. The outer lines of the pentuplet for C-4a arise from ^1J coupling between this carbon and ^{15}N-5, whilst the two intense inner lines arise from ^1J coupling with ^{15}N-8. The central line is a measure of the molecules lacking ^{15}N at either N-5 or N-8. Data for a large number of nitrogen heterocycles show that $^1J(^{13}C–^{15}N)$ is always significant (*ca.* 10 Hz) when the nitrogen lone pair resides in a p orbital (*e.g.* in pyrrole or N-1 of *N*-methylimidazole) whereas it is small (*ca.* 1 Hz) when the lone pair is in an sp^2 orbital (*e.g.* as in pyridine).[6] It is assumed that the N-8 lone pair in structure (**3a**) has high p character because of sp^2 hybridisation at this nitrogen. The substantial ^2J coupling (10

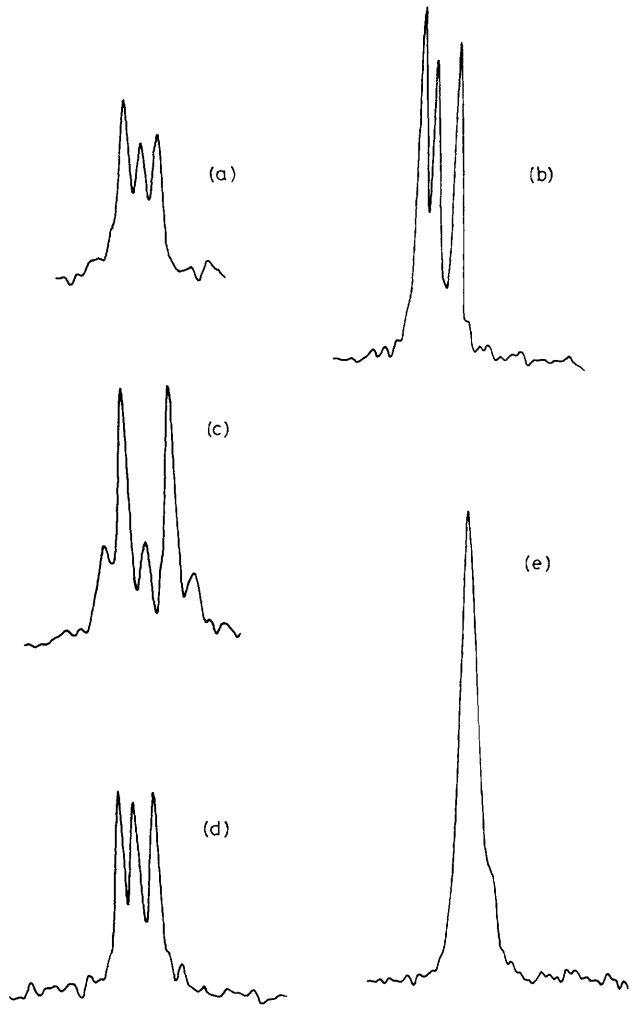


Figure 1. Portions of the 30.42 MHz ^{15}N n.m.r. spectrum of the 1 : 1 adduct (**3a**) derived from [^{15}N]guanosine containing 75% of (**1b**) and 15—20% of (**1c**) [δ 113.8 (e), 116.0 (a), 124.7 (b), 146.7 (c), 153.7 (d)].

Hz) observed between N-8 and C-9 is typical for amides.[6] The observation of the resonance at δ 113.8 as a strong singlet partly concealing a weak doublet (Figure 1e) identifies it as C-6. As expected, a coupling of 9 Hz is observed from ^{15}N-8 to C-7. The ^{13}C spectrum of the 2 : 2 adduct derived from the ^{15}N-labelled guanosine mixture rich in (**1b**) shows very similar $^{13}C–^{15}N$ couplings to those observed for the 1 : 1 adduct (**3a**), and therefore the structure of the 2 : 2 adduct is (**5**).

To confirm the structural conclusions made above, compound (**6**) labelled with ^{15}N in the N(COPh) group was synthesised from [^{15}N]benzoyl isothiocyanate, and was converted into a mixture of [^{15}N]guanosines containing 15—20% of (**1b**) and 75% of (**1c**).[5] Reaction of this [^{15}N]guanosine mixture with glycidaldehyde gave a 1 : 1 adduct, the $^{13}C\{^1H\}$ n.m.r. spectrum of which showed a singlet for C-7 and a doublet for C-6, $^1J_{CN}$ 11 Hz.

There are many possible routes for formation of the adducts (**3a**), (**3c**), and (**5**). An initial reaction may occur between the aldehyde function of glycidaldehyde and the NH_2 group of guanosine to give a carbinolamine. The epoxide ring of this intermediate may be opened by nucleophilic attack from the

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N-1 anion to give one of the intermediates (**4a**)—(**4d**). Dehydration followed by loss of a proton would afford adduct (**3a**), whereas loss of formaldehyde would give compound (**3c**). Reversibility of the latter reaction could lead to adduct (**3a**). The adduct (**5**) could arise from reaction between (**3a**) and (**3c**). Detailed mechanistic studies are in progress. Preliminary studies indicate that following the mixing of guanosine and glycidaldehyde, the intermediates (**4a**)—(**4d**) appear and disappear. Later, compounds (**3a**) and (**3c**) appear, followed by (**5**).

Added in proof: In a recent paper (V. Nair and R. J. Offerman, *J. Org. Chem.*, 1985, **50**, 5627) the structure of the 1 : 1 adduct from guanosine and glycidaldehyde was amended from (**3b**) to (**3a**). However, experimental data to support this conclusion were not presented.

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