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N^2,3-Ethenoguanosine and IA'-Metamorphosine: ^{15}N NMR Spectroscopy and Elucidation of Physico-chemical Properties by Kinetic and Equilibrium Measurements

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Key Words: Hypermodified nucleosides; ^{15}N NMR spectroscopy; protonation; hydrolysis; base-stacking

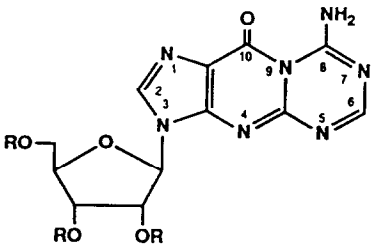
Abstract: Tautomerism, protonation and electronic properties of the base moieties of IA'-metamorphosine (1a), N^2,3-ethenoguanosine (2a) and O^6-benzyl-N^2,3-ethenoguanosine (3a) were investigated by ^{15}N NMR spectroscopy. pK_a values of the same compounds were determined spectrophotometrically, and hydrolytic stability of the N-glycosidic bond was studied at various hydronium ion concentrations. The base stacking ability and metal ion complexation of N^2,3-ethenoguanosine and its 9-ethyl counterpart (7) were elucidated by phase distribution and potentiometric measurements.

INTRODUCTION

As part of our study on tricyclic nucleosides derived from guanosine[1-3], we have investigated the electronic properties of 8,9-dihydro-9-oxo-3-ß-D-ribofuranosylimidazo[2,1-b]purine(N^2,3-ethenoguanosine) and 8-amino-9,10-dihydro-10-oxo-3-ß-D- ribofuranosyl-3H-1,3,5-triazino[1,2-a]purine (IA'-metamorphosine) by ^{15}N NMR spectroscopy. IA'-Metamorphosine[4,5] (**1a**) formed by reaction of guanosine with methyl-N-cyanomethanimidate is a laterally extended adenosine analogue which is fluorescent. Angular tricyclic N^2,3-ethenoguanosine (**2a**) formed by the condensation of guanosine with chloroacetaldehyde[6] is also fluorescent and has a structure resembling that of wyosine[3] (**4a**). The fluorescent nature of these heterotricyclic systems due to the formation of an additional five- or six-membered fused ring on guanine moiety suggests their structural similarities. It

is therefore of interest to understand the electronic implication of an additional ring as in **1a**, **2a** and **3a**, and how their π-electrons are delocalized compared to the guanine skeleton in the corresponding ß-D-nucleoside. We report herein our studies on **1b**, **2b** and **3b** and their comparison with **4b** by ^{15}N NMR spectroscopy. These 2',3',5'-tri-O-acetyl derivatives were used instead of unprotected nucleosides owing to inherent instability of the parent compounds in acidic medium and their poor solubility even in DMSO.

The physico-chemical properties of **1a** and **2a** are also relevant in attempting to understand the biochemical consequences that their formation may result in. For example, it has been recently shown[7,8] that the DNA of rodents exposed to vinyl chloride contains, among other modified nucleobases, N^2,3- ethenoguanine, the formation of which may increase mispairing and thus the frequency of mutagenic events[9]. To characterize the physico-chemical behavior of N^2,3-ethenoguanosine in more detail, its protolytic equilibria, base-stacking efficiency, metal ion complexation and hydrolytic stability were studied.



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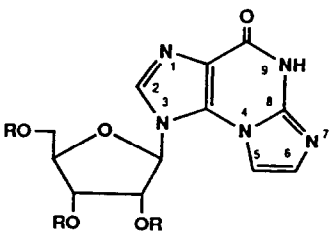
1b: R = Ac



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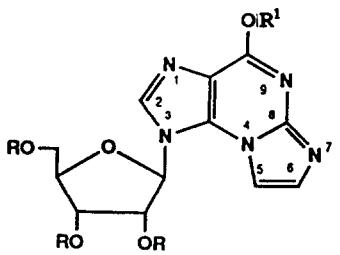
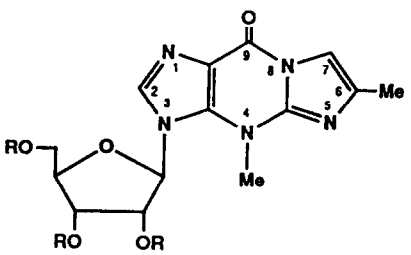
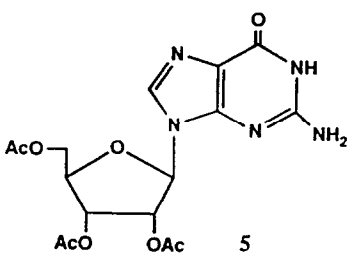


3a: R = H, R^1 = Bn
3b: R = Ac, R^1 = Bn
3c: R = H, R^1 = Et



4a: R = H
4b: R = Ac



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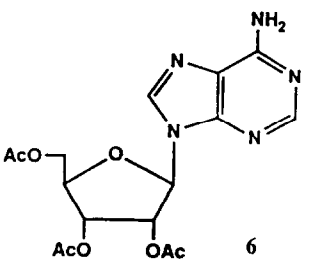

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RESULTS AND DISCUSSION

^{15}N NMR Spectroscopy

Assignment of resonances. The detailed ^{15}N chemical shifts and coupling constants for compounds **1b**, **2b** and **3b** are given in Tables 1 and 2.

Table 1. ^{15}N Chemical Shifts (ppm) of Tricyclic Compounds **1b**, **2b** and **3b** in Neutral and Acidic DMSO and CH_2Cl_2 Solutions.

Compd.	Molar eqv. of TFA	N-1	N-3	N-4	N-5	N-7	N-9	NH_2
1b[a]	0	-136.9	-213.1	-188.0	-150.8	-178.2	-208.2	-278.0
	1	-144.8	-210.1	-189.0	-169.4	-179.8	-209.5	-276.6
1b[b]	0	-129.9	-212.8	-187.5	-149.1	-175.3	-206.1	-278.8
	1	-130.5	-212.3	-188.9	-159.4	-175.0	-207.8	-275.4
2b[a]	0	-128.9	-216.9	-232.6		-177.1	-242.1	
	0.4	-129.8	-215.9	-232.5		-195.1	c	
2b[b]	0	-127.4	-214.9	-231.2		-169.6	-238.1	
	1	-129.5	-213.8	-227.9		-206.7	c	
3b[a]	0	-135.6	-218.4	-220.5		-157.0	-173.2	
	1	-135.4	-214.2	-221.5		-227.5	-182.6	

[a]In CH_2Cl_2. [b]In DMSO [c]Could not be observed.

Table 2. ^{15}N,^{1}H Spin Coupling Constants[a] (Hz) in DMSO.

Compd.	^1J	^2J	^3J	^4J
1b	91(N^8,8-NH_2)	11.9(N1,H2)	3.1(N7,8-NH_2)	1.2(N4,H2)
		8.7(N3,H2)	4.0(N9,8-NH_2)	1.5(N9,H6)
		2.5(N3,H1')		
		15.5(N5,H6)		
		16.0(N7,H6)		
2b		11.6(N1,H2)	4.1(N4,H6)	
		9.6(N3,H2)	1.8(N7,H5)	
		9.3(N4,H5)		
		11.1(N7,H6)		

[a]The coupling constants are given in absolute values.

2',3',5'-Tri-O-acetyl-IA'-metamorphosine (**1b**). Five different kinds of nitrogen atoms are present, the unambiguous assignments of which may be based on the observation of long range coupling constants and

selective proton decoupling experiments (Fig.1). The most downfield signal at -129.9 ppm (in DMSO) is attributed to the "azine-like" N-1 nitrogen. The "pyrrole-like" imidazole nitrogen, N-3, resonates at -212.8 ppm. These two nitrogens have chemical shifts and two bond coupling with the H-2 comparable to those in common purine nucleosides.[10,11] The N-4, N-5 and N-7 are "pyridine-like" nitrogens. The absorption at -187.5 ppm exhibits only a long range coupling of 1.2 Hz with the H-2 proton, as found from selective decoupling, and was assigned to the N-4 nitrogen. The N-5 and N-7 nitrogens absorb at -149.1 ppm and - 175.3 ppm, respectively. The distinction between these two nitrogen atoms may be based on the fact that while both nitrogens show two bond coupling with H-6, the N-7 is additionally coupled with the exocyclic NH_2 protons [$^3J(N7,8\text{-}NH_2) = 3.1$ Hz]. The N-9 atom is similar to an "amide-like" nitrogen but it resonates at higher field than a usual amide nitrogen, since it is located at the junction of two fused pyrimidine rings. The resonance at - 278.8 ppm is attributed to the nitrogen of the exocyclic amino group. Compared to the NH_2 group of adenosine, this amino resonance in **1b** is shifted downfield by 20 ppm. In addition, the N-7 resonance is shielded by 30 ppm compared to N-1 in adenosine, while the N-5 resonance is less affected. A small increase of the $^1J(N^6,8\text{-}NH_2)$ coupling by 1.1 Hz (Table 2) compared to that of adenosine suggest a decrease of the sp^3 character, i. e. a more effective delocalization of the exocyclic nitrogen lone pair into pyrimidine ring, which results in an increase of the partial double character of the C8-N^6 bond in **1b**. It has been suggested[4] that the exocyclic amino group at C-8 is hydrogen bonded to the pericarbonyl (C-10) group. This would promote delocalization of the nitrogen lone pair electrons into the aromatic system compared to that of adenosine, a planar structure being most favorable for conjugation. Changing the solvent from DMSO to CH_2Cl_2 results in an increase of the $^1J(N^6,8\text{-}NH_2)$ coupling by 3 Hz and a slight downfield shift of the NH_2 resonance. Possibly, the intramolecular hydrogen bond between the carbonyl and amino group is stronger in CH_2Cl_2 and the delocalization of N^6 electrons into the pyrimidine ring is thus enhanced.

2',3',5'-Tri-O-acetyl-N^2,3-ethenoguanosine (**2b**) *and its O^6- benzyl derivative* (**3b**). The assignment of ^{15}N resonances in **2b** (Fig. 2) and **3b** is straightforward on the basis of the $^{15}N,^1H$ coupling constants and by comparison with purine nucleosides[10-13] and wyosine nucleosides (Table 3). In N^2,3-ethenoguanosine, **2b**, the resonances at 127.4 and 214.9 ppm are assigned to N-1 and N-3, respectively. They have chemical shift close to those of an isolated imidazole[14,15] and are comparable to the N-7 and N-9 shifts of guanosine. The resonance at 238.1 ppm is readily assigned to the N-9 since it is the only one which does not give any long range coupling with protons. The spread in chemical shifts between the N-4 and N-7 nitrogens of the second imidazole ring is much reduced compared to that in the purine imidazole moiety. This may indicate a closer similarity of the valence state of the two nitrogens in the former imidazole ring (N-4, N- 7) compared to those in the latter (N-1, N-3) and a stronger conjugation with the purine moiety. For O^6-benzyl derivative, **3b**, the N-1 resonance is shielded by 6.7 ppm as compared to N-1 in **2b** (in CH_2Cl_2). The upfield shift is explained by a better delocalization of the charge from the imidazole to the pyrimidine system which results in a shift toward higher frequency. The enhanced aromatic character of the pyrimidine ring in **3b** promotes a more effective delocalization of the π-electrons from the imidazole moiety, and hence the N-1 resonance is shifted upfield.

The fact that the N-9 resonance of **2b** exhibits a chemical shift approximately equal to that of N-1 of guanosine triacetate (**5**) indicates that N^2,3-ethenoguanosine is an oxo tautomer analogous to guanosine. The N-9 shift of **3b**, a model of a fixed hydroxy tautomer, is less negative by 70 ppm.

Site of Protonation. Addition of one equivalent of trifluoroacetic acid (TFA) into a DMSO solution of **1b** results in an upfield shift of the N-5 resonance by 9 ppm indicating that protonation takes place at this site (Table 1). The other nitrogen resonances are very little affected by addition of TFA. It is worthwhile to note

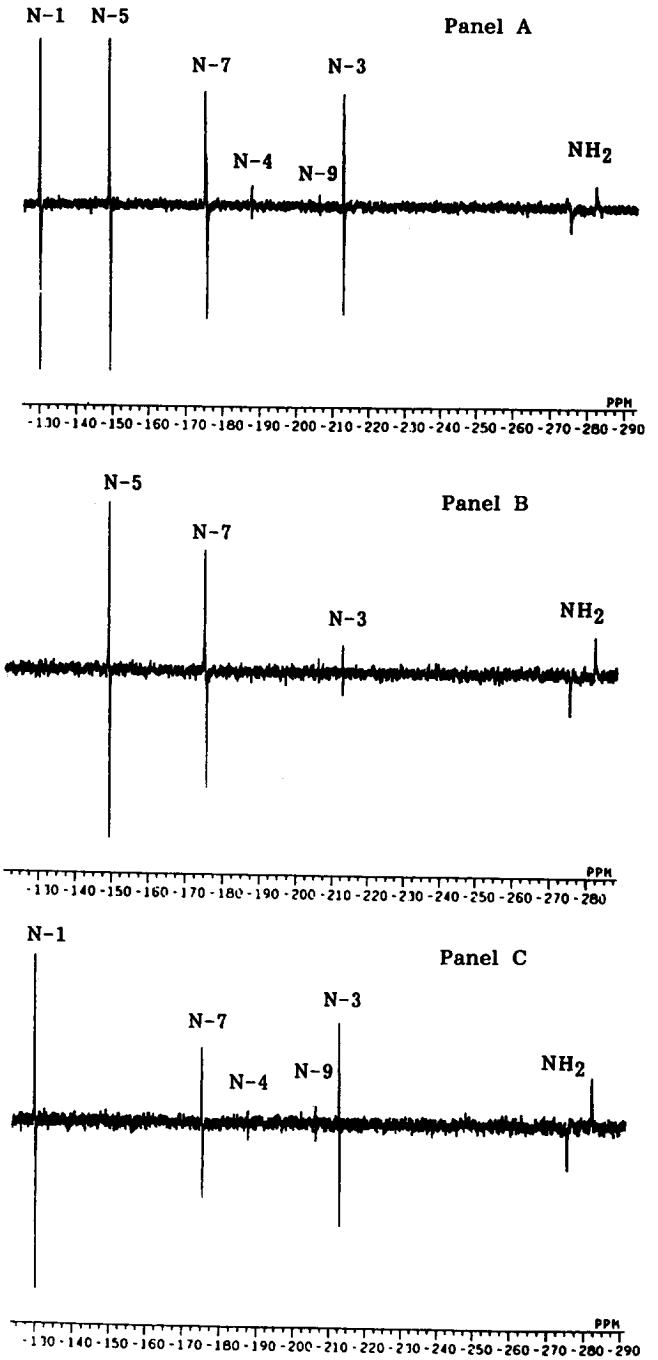


Fig. 1. ^{15}N NMR of compound **1b**. Panel A: INEPT spectrum. Panel B: INEPT spectrum with selective decoupling of H-2. Panel C: INEPT spectrum with selective decoupling of H-6.

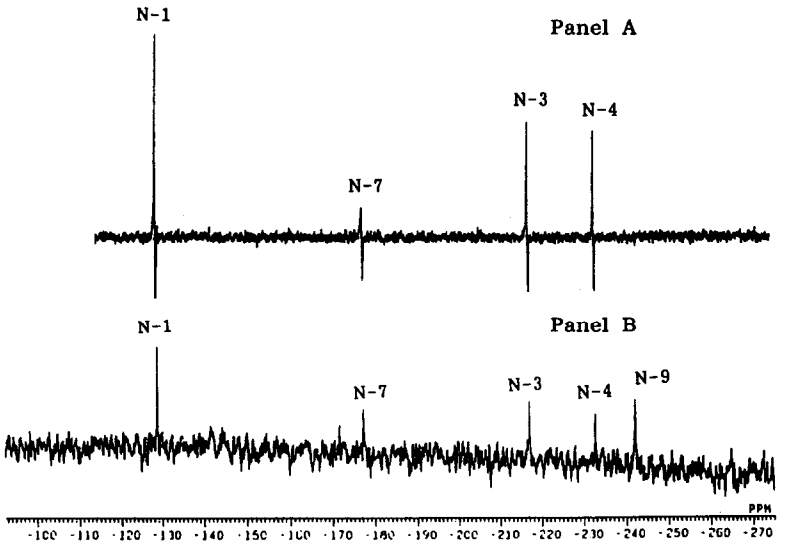


Fig. 2. ^{15}N NMR of compound **2b**. Panel A: INEPT spectrum. Panel B: ^{1}H decoupled ^{15}N NMR spectrum without NOE.

Table 3. ^{15}N Chemical Shifts (ppm) of 2',3',5'-Tri-O-acetates of Guanosine (**5**), Adenosine (**6**) and Wyosine (**4b**) in DMSO.

Compd.	Molar equiv. of TFA	N-1	N-3	N-7	N-9	NH_2
5[a]	0	-233.5	-216.1	-131.5	-215.5	-307.0
6[b]	0	-144.4	-157.5	-139.1	-215.6	-299.3
		N-1	N-3	N-4	N-5	N-8
4b[c]	0	-129.6	-218.0	-287.4	-156.6	-191.2
	1	-129.8	-217.4	-286.7	-168.0	-191.5

[a]From *Ref.* 2. [b]From *Ref.* 20. [c]From *Ref.* 3.

that in guanosine, it is the N-7 site which is protonated while in adenosine, the protonation occurs at the N-1 nitrogen.[16] Ab initio SCF calculations[17] and semiemperical quantum-chemical calculations with MINDO/3[18] and AM1[19] have, in turn, suggested that in adenine and adenosine, the N-3 nitrogen is a potential site of protonation, although it is energetically less favourable than the N-1 site.

In **3b** the addition of TFA results in an upfield shift of the N-7 resonance by 70 ppm (Table 1). The N-9 nitrogen moves upfield by 9 ppm while the chemical shift of N-1 is not affected. The primary site of protonation is therefore the N-7 nitrogen. The upfield shift observed for N-9 could be either due to a small amount of protonation or to electronic effect through the conjugated system. For N^2,3-ethenoguanosine (**2b**) in CH_2Cl_2, it was not possible to determine the ^{15}N shifts after addition of 0.6 equivalent of acid due to the

solubility problem. In DMSO, the N-7 resonance is shifted upfield by 40 ppm and N-1 by 2.1 ppm upon addition of 1 equivalent of TFA, which indicate that the N-7 is the first site of protonation. It may be noted that O^6-benzyl derivative, **3a**, is three orders of magnitude more basic than N^2,3- ethenoguanosine, **2a**, (Table 4) and this basicity difference is reflected in the δ ^{15}N shift of the nitrogen atom undergoing protonation. In wyosine triacetate, **4b**, the N-5 atom (corresponding to N-7 in **2b**) is also the first site of protonation, while the N-1 is only protonated to a small extent.

^1H NMR shift studies on protonation of **2a** and **3a** in DMSO-d_6 lend some additional support to the suggested N-7 protonation. With **2a** the H-2, H-5 and H-6 signals were shifted downfield by 0.21, 0.25 and 0.47 ppm, respectively, on addition of 3 equivalents of TFA. With **3a** the corresponding changes in shifts were: 0.29 (H-2), 0.27 (H-5) and 0.59 (H-6). In other words, the signal of the proton adjacent to the N-7 site undergoes the largest downfield shift upon protonation.

In conclusion, the creation of a second five- or six-membered fused ring in guanosine modifies considerably the electronic distribution in the resultant heterocyclic system. This is clearly reflected in the altered basicity and nucleophilicity of the nitrogen atoms. N^2,3-Ethenoguanosine shows electronic features which are quite closely similar to those of wyosine: in both compounds, the site of protonation is the "azine-like" nitrogen of the second imidazole ring, while the imidazole ring of the parent guanine moiety is more electron deficient. For IA'- metamorphosine, the protonation also takes place in the new formed pyrimidine ring, the structure of which is the same as in adenosine. It is, however, the N-5 nitrogen which is protonated (corresponding to N-3 in adenosine), in contrast to adenosine in which the main site of protonation is N-1.

Protolytic Equilibria.

Table 4 records the pK_a values obtained spectrophotometrically for IA'-metamorphosine (**1a**) and several derivatives of N^2,3- ethenoguanine. The pK_a value of the monocation of N^2,3- ethenoguanosine (**2a**) is 0.6 pH

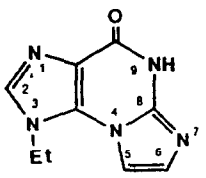
Table 4. pK_a Values of IA'-Metamorphosine and Substituted N^2,3- ethenoguanines at 298.2 K.[a]

Compd.	$pK_a(LH_3^{2+})$[b]	$pK_a(LH_2^{+})$[c]	$pK_a(LH)$[d]
1a		1.14	
2a	e	2.11	8.45
3a	e	5.20	
3c	e	5.21	
7	-1[f]		
8	-1[f]	2.17	

[a]Ionic strength adjusted to 0.1 mol dm^{-3} with sodium chloride at pH > 1. [b]Thermodynamic acidity constant of the dication based on the acidity function H_o.[21] [c]Concentration acidity constant of the monocation. [d]Concentration acidity constant of the neutral compound. [e]Could not be determined owing to rapid hydrolysis. [f]An approximate value.

units lower than that reported[3] for wyosine (2.1 and 2.7, respectively), while IA'-metamorphosine is one order of magnitude less basic than **2a** (pK_a 1.1). The data in Table 4 support the view that the predominant tautomer of **2a** is the oxo form where the labile proton is attached at N-9. Firstly, the pK_a value of the monocation of 2a (2.1) is almost equal to that of monoprotonated **8** (2.2), which is a fixed oxo tautomer. Secondly, the pK_a

value of neutral **2a** is about one log unit lower than that of the parent nucleoside, guanosine[16], which is known to bear the labile proton at N1 (corresponding N-9 in **2a**). The acidity difference of this magnitude may well be attributed to electron- withdrawal by the neighboring etheno group. Thirdly, the ethyl analogue of **2a** (**7**) and its fixed oxo tautomer (**8**) are both converted to a dicationic form at H_o = -1. In striking contrast, the fixed hydroxy tautomers, **3a** and **3c**, are much more basic than **2a**, the pK_a value of the monocation being 5.2.



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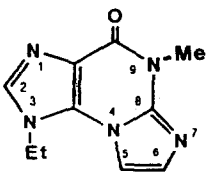

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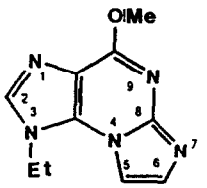

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Hydrolytic Stability.

Fig. 3 shows the pH-rate profiles for cleavage of the N-glycosidic bond of N^2,3-ethenoguanosine (**2a**) and its O-alkyl derivatives, **3a** and **3c**. As with purine nucleosides[22] and wyosine[3,23,24], the rate is linearly related to hydronium ion concentration over the whole acidity range studied, even on passing the acidity constant of the monocation. In other words, the reaction must proceed by both mono- and diprotonated substrates, the former reaction predominating at pH > pK_{a1} and the latter under more acidic conditions. In all likelihood, the mechanism accepted for purine nucleosides[22], their isosteric analogues[25-27] and wyosine[2,23,24] may also be applied to the hydrolysis of N^2,3-ethenoguanosine. Accordingly, the N-protonated mono- and di-cations of the starting material undergo a unimolecular rate-limiting heterolysis to the free base and a resonance stabilized cyclic oxocarbenium ion derived from the sugar moiety. With **2a** the first protonation takes place at N-7 and the second at N-1.

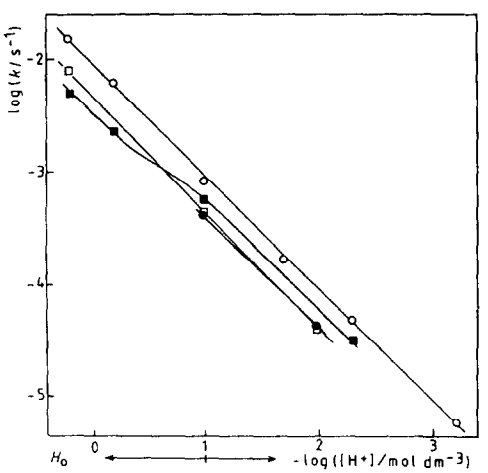

Fig. 3. pH-Rate profile for hydrolysis of N^2,3-ethenoguanosine (**2a**, open circles), its O^6-ethyl (**3c**, open squares) and O^6-benzyl (**3a**, filled circles) derivatives, and the 5'-monophosphate of **2a** (filled squares) at 298.2 K (I = 0.1 mol dm^{-3} with NaCl).

N^2,3-Ethenoguanosine (**2a**) is hydrolyzed, like other N3-alkylated guanosines, several orders of magnitude faster than its parent nucleoside. The relative rate constants reported for guanosine[28], **2a** (this work), 3-methylguanosine[23] and wyosine[23] are 1, $6.5x10^3$, $6.8x10^4$ and $3.1x10^4$, respectively. The exceptionally rapid hydrolysis of the latter two compounds was originally attributed to a steric repulsion between the ribose and N-alkylated heterocyclic base moiety[23,29], but this explanation was later critisized,[30] and the reason for the hydrolytic instability was suggested to be an electronic one. However, no attempt was made to describe the nature of this electronic acceleration that the N3-alkylation results in. Recent measurements with 4- and 7-methyl derivatives of 1-(2-deoxy-β-D-*erythro*-pentofuranosyl)benzotriazole nucleosides also strongly suggest that the exceptional hydrolytic instability of N3 alkylated purine nucleosides cannot be of steric origin.[27] The monocation of the 7- methyl derivative is heterolyzed only 15 times as fast as its 4- methyl counterpart. In other words, introduction of a methyl group at a position equivalent to N3 of purine nucleosides decreases the hydrolytic stability of N-glycosidic bond by only one order of magnitude.

Although **3a** and **3c** are 3 orders of magnitude more basic than **2a**, they are hydrolyzed slightly less readily. Evidently the O^6- substitution, which changes the predominant tautomeric structure from oxo to hydroxy, results in opposing effects on the pre-equilibrium protonation and heterolysis of the protonated substrate. The monocation formation is facilitated, but this rate-enhancing effect is overcompensated by retardation of the rate-limiting bond rupture. As shown previously[3,22,31], it is quite normal that the influences of base moiety substituents on basicity and heterolysis cancel each other almost completely.

The hydrolysis rate of N^2,3-ethenoguanosine 5'-monophosphate was observed to be 36 % of that of the parent nucleoside under conditions where the phosphate group is fully protonated ($pH << 1$), and 63 % under conditions where the phosphate group is present as a monoanion ($pH >> 1$). The influence of the 5'-phosphate group is thus very similar to that observed with 2'-deoxyadenosine and its 5'- monophosphate[32], in consistence with the assumed mechanistic similarity of the hydrolysis reactions.

IA'-Metamorphosine did not undergo cleavage of the N-glycosidic bond under acidic conditions, but was decomposed to two unidentified products both of which were subsequently converted to guanosine. The first-order rate constant for the disappearance of the starting material was $1.34x10^{-2}$ s^{-1} at $[H^+] = 0.10$ mol dm^{-3} (T = 333.2 K).

Base-stacking Properties of N^2,3-Ethenoguanosine.

Table 5 records the equilibrium constants, $K_{ass.}$, obtained by a phase distribution method[33] for association of substituted N^2,3-ethenoguanines, **7** - **9**, with free purine base. As seen, the fixed, **9**, stacks twice as

Table 5. Equilibrium Constants, $K_{ass.}$, for Association of Substituted N^2,3-Ethenoguanines with Purine at 298.2 K.[a]

Compd.	$K_{ass.}$/dm^3 mol^{-1}	b	r
7	8.7 ± 0.4	0.9 ± 0.1	0.996
8	12.8 0.7	0.9 0.1	0.996
9	23.2 0.6	0.7 0.1	0.998

[a]Formation of 1:1 adducts assumed. b and r are the intercept and correlation coefficient of eqn. (2).

efficiently as the fixed oxo tautomer, **8**. The base-stacking ability of N^2-etheno-9-ethylguanine, **7**, closely resembles that of **8**, as expected on the basis of their similar tautomeric structures. Comparison with the data reported in literature reveals that **7** and **8** associate with purine approximately as readily as 9-methyladenine.[33] Since the affinities of adenine and guanine derivatives to purine are known to be of the same order of magnitude,[34] one may conclude that creation of an additional imidazole ring in guanine base has only a moderate effect on its base-stacking ability, as long as association with bases exhibiting an intermediate π-electron polarizability is concerned.

Metal Ion Complexation of N^2,3-Ethenoguanosine.

The stability constants obtained by phase distribution[35] for the 1:1 complexes of Ni(II) ion with neutral forms of substituted N^2,3-ethenoguanines, **7** - **9**, are listed in Table 6. These values are useful in attempting to

Table 6. Stability constants, $K(LHM^{2+})$, for 1:1 Ni(II) complexes of the neutral forms of substituted N^2,3-ethenoguanines at 298.2 K.[a]

Compd.	$\log[K(LHM^{2+})/dm^3\ mol^{-1}]$	b	r
7	1.30 ± 0.05	1.4 ± 0.3	0.98
8	0.84 0.02	0.94 0.04	0.998
9	2.18 0.01	1.1 0.1	0.9997

[a]Ionic strength was adjusted to 1.0 mol dm^{-3} with potassium nitrate. b and r are the intercept and correlation coefficient of eqn. (3).

distinguish between the two potential coordination sites in N^2,3-ethenoguanosine, viz. N-1 and N-7 (corresponding to N-7 and N^2 in guanosine, respectively). For the following reasons N-7 binding appears more attractive. Firstly, replacing the N-9 proton in **7** with a methyl group considerably destabilizes the Ni(II) complex, the logarithmic stability constants of the complexes of **7** and **8** being 1.3 and 0.8, respectively. Evidently the methyl group forms a steric hindrance for attachment of Ni(II) ion at N-7. If N-1 were the main coordination site no such destabilization could be expected. For comparison, the corresponding methylation in inosine (methylation of N-1) has practically no effect on metal ion binding of the imidazole moiety.[36] Secondly, the Ni(II) complex of **9** is almost one order of magnitude more stable than that of **7**, which may be accounted for by chelate formation between N-7 and N-9 sites of **9**. With **7** the corresponding two dentate binding is impeded, since the proton at N-9 cannot be displaced by a metal ion under acidic conditions.

Table 7 records the stability constants obtained potentiometrically for various metal ion complexes of both neutral and monoanionic forms of N^2,3-ethenoguanosine. The stabilities of the neutral ligand complexes are very similar to those reported previously[36] for the corresponding complexes of guanosine. The latter complexes are known to be N-7 coordinated (corresponding to N-1 binding in **2a**).[16] Accordingly, this binding mode (N-1 binding) may also contribute to the observed stability constants, although N-7 coordination is suggested to prevail. The stability constants, $K(LHM^{2+})$, in Table 7 should thus be regarded as macroscopic constants. As with purine nucleosides, the stabilities of the 3d transition metal complexes follow the Irving-Williams order.[16] Soft metal ions, such as Hg(II), and particularly (dien)Pd(II), complex more firmly. The predominant binding mode of these ions cannot be concluded on the basis of the available data.

Table 7. Stability Constants of Various Metal Ion Complexes of Neutral (LH) and Anionic (L^-) N^2,3-Ethenoguanosine at 298.2 K.[a]

Metal Ion	$\log[K(LHM^{2+})/dm^3\ mol^{-1}]$	$\log[K(LM^+)/dm^3\ mol^{-1}]$
Co(II)	1.2 ± 0.1	3.4
Ni(II)	1.4 0.1	4.0
Cu(II)	1.8 0.1	5.9
Zn(II)	0.9 0.2	3.6
Hg(II)	2.0 0.1	
(dien)Pd(II)	4.2 0.2	

[a]Ionic strength was adjusted to 1.0 mol dm^{-3} with potassium nitrate. The metal ions listed were added as nitrates.

The stability constants, $K(LM^+)$, of the anionic ligand undoubtedly refer to binding at deprotonated N-9, analogously to anionic complexes of inosine and guanosine[16]. The observed stabilities are, however, one order of magnitude higher than those reported for guanosine[37] and inosine[36] monoanion. Obviously, chelate formation via simultaneous binding to N-7 and N-9 stabilizes the complexes of **2a**.

Effect of (dien)Pd(II) Ion on Hydrolytic Stability of N^2,3- Ethenoguanosine.

Fig. 4 shows the effect of (dien)Pd(II) ion on the hydrolysis rate of N^2,3-ethenoguanosine at two hydronium ion concentrations. The hydrolysis rate initially decreases with increasing concentration of (dien)Pd(II) ion, and levels off to a constant value at $[(dien)Pd^{2+}] > 0{,}01$ mol dm^{-3}. The rate retarding effect of (dien)Pd(II) ion is considerably larger at $[H^+] = 0{,}01$ mol dm^{-3} than at $[H^+] = 2.6\times10^{-3}$ mol dm^{-3}. In fact the plateau values are in both cases almost equal, suggesting that the hydrolysis reaction is not acid catalyzed at high (dien)Pd(II) concentrations but proceeds by spontaneous cleavage of a N-1, N-7 dicoordinated species. For comparison, the corresponding dicoordinated complexes of 2'-deoxyadenosine and 2'-deoxyinosine have been shown[38,39] to hydrolyze spontaneously.

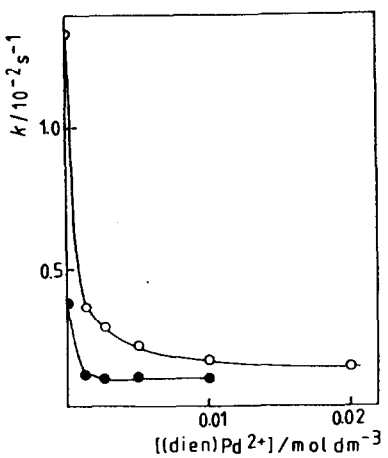

Fig. 4 Effect of (dien)Pd(II) ion on acid-catalyzed hydrolysis of N^2,3-ethenoguanosine (**2a**) at 343.2 K (I = 0.1 mol dm^{-3} with $NaNO_3$).

EXPERIMENTAL

Materials.

General. Analytical TLC separations were carried out on 0.25 mm thick Silica gel 60 F_{254} plates (Merck), and preparative separations on 20x20 cm plates prepared from 20 g of Silica gel 60 PF_{254} (Merck). The eluents employed were: chloroform/methanol 8:2 (system I), chloroform/2-propanol 8:2 (system II), 2-propanol/conc. aq. ammonia/water 7:1:1 (system III).

Preparation of N^2,3-ethenoguanosine (**2a**), its 5'- monophosphate, and O^6-ethyl (**3c**) and O^6-benzyl (**3a**) derivatives has been reported previously.[6] The other compounds employed were obtained as follows.

O^6-Methylguanine. 2-Amino-6-chloropurine (6 mmol, 1.02 g) was heated with sodium methoxide (16 mmol) in methanol (10 cm^3) for 36 h at 105 °C. The cooled reaction mixture was poured into water (20 cm^3), and pH was adjusted to 6 with aqueous hydrogen chloride. The resulting solution was decolorized with charcoal, evaporated to dryness, and the residue was crystallized from a mixture of water and methanol (25 cm^3, 4:1). The product obtained (4.7 mmol, 78 %) was chromatographically pure (system I R_F 0.44) and melted above 360 °C.

9-Ethyl-O^6-methylguanine. O^6-Methylguanine (4.5 mmol, 0.75 g) was treated (12h, 37 °C) with ethyl iodide (12.5 mmol, 1 cm^3) in DMF (25 cm^3) in the presence of potassium carbonate (10 mmol, 1.38 g). The product mixture was fractionated on preparative TLC plates (system I), and the appropriate band, identified on the basis UV spectral similarity with O^6-methylguanosine,[40] was eluted to give 9-ethyl-O^6-methylguanine (2.0 mmol, 45 %). The compound recrystallized from a mixture of benzene and cyclohexane exhibited a m.p. of 137-139 °C, R_F values of 0.73 (system I) and 0.59 (system II), and UV maxima at 251 and 281 nm (water).

N^2,3-Etheno-9-ethyl-O^6-methylguanine (**9**). 9-Ethyl-O^6- methylguanine (3 mmol) was dissolved in a 2:1 mixture of acetic acid/sodium acetate buffer (20 cm^3, 1 mol dm^{-3}, pH 5.5) and ethanol. 3 portions of bromoacetaldehyde (5 cm^3, 1.3 mol dm^{-3}, *Ref.* 6) were added at 24 h intervals, and the mixture incubated at 37 °C. The starting material disappeared in 3 d with concomitant formation of the N^2,3-etheno derivative as the main product. The mixture was evaporated to half of its original volume, extracted with chloroform (3x50 cm^3), and the organic phase evaporated. The residue was fractionated on preparative TLC plates (system II). The main band contained chromatographically pure **9** (R_F 0.62, 0.27 and 0.68 in systems I, II and III, respectively). The sample recrystallized from 2-propanol was UV spectroscopically similar to N^2,3-etheno-O^6-methylguanosine.[6]

N^2,3-Etheno-9-ethylguanine (**7**). Treatment of **9** (1.5 mmol) with aqueous potassium hydroxide (2.5 cm^3, 1 mol dm^{-3}) for 1 h at 100 °C gave **7** as the main product (R_F 0.19 and 0.29 in systems I and II, respectively). The mixture was passed through Dowex 1x4 in HCO_3^- form (2x22 cm, 200/400 mesh) using a linear gradient of water (0.6 dm^3) and triethylammonium bicarbonate (0.6 dm^3, 0.8 mol dm^{-3}). Fractions of 15 cm^3 were collected at a flow rate of 2.5 cm^3 min^{-1}. The main peak (fractions 34-50) was evaporated, and the residue crystallized from boiling methanol (5 cm^3) containing a few drops of water. The crystalline product (0.48 mmol, 32 %) was UV spectroscopically similar to **2a**[6]. The mother liquor contained 0.35 mmol (23 %) of chromatographically pure **7**, which was used for further reactions.

9-Ethyl-1-methylguanine. Alkaline hydrolysis of 9-ethyl-O^6- methylguanine gave 9-ethylguanine. The reaction and separation of the product was carried out as described above for **7**. Methylation with methyl iodide in DMF in the presence of potassium carbonate yielded 9-ethyl-1-methylguanine, which was separated on preparative TLC plates. The product was UV spectroscopically similar to 1-methylguanosine.[40]

N^2,3-Etheno-9-ethyl-1-methylguanine (**8**). 9-Ethyl-1-methylguanine (0.05 mmol) was treated in acetic

acid/sodium acetate buffer (0.5 cm^3, 1 mol dm^{-3}, pH 4.5) with excess of bromoacetaldehyde (10:1) at 37 °C, and the course of reaction was followed by TLC. Disappearance of the starting material (R_F 0.45 and 0.55 in systems I and III, respectively) was accompanied with formation **8** as the main product (R_F 0.50 and 0.40 in systems I and III, respectively). After 10 d about half of the starting material was reacted. The separated product was UV spectroscopically and chromatographically identical with that obtained *via* methylation of **7**, as described below.

7 (0.5 mmol) was treated with methyl iodide (0.8 mmol) in DMF (3 cm^3) in the presence of potassium carbonate (1 mmol). The reaction was completed in 18 h at room temperature.The product (0.2 mmol, 40 %) was, after separation on preparative TLC plates (system I) and crystallization from boiling aqueous ethanol (95 %), UV spectroscopically identical with **8**[6].

NMR spectroscopic measurements.

The ^{15}N NMR spectra were recorded at 27.4 MHz on a Jeol JNM- GX 270 spectrometer using a 10 mm probehead. The ^{15}N chemical shifts were determined from proton decoupled spectra (without NOE) and were referenced against an external solution of $CH_3{}^{15}NO_2$ in $C^2H_3NO_2$. The data were acquired with a 45 pulse angle (13 μs pulse width), an acquisition time of 0.9 s for 16K data points and a relaxation delay of 15 s. INEPT was used to acquire fully ^1H coupled spectra. Selective decoupling were achieved by an inept where the irradiation gate remained open and the level of irradiation was adjusted to 9 W during the ^1H pulse or was at 1.5 W otherwise. For INEPT experiment, a pulse delay of 1 s and an acquisition time of 0.9 s for 16K data point zero filled to 64K before Fourier transformation was used. For a spectral width of 9000 Hz, the digital resolution was 0.3 Hz. A negative value for the chemical shifts denotes an upfield shift. The concentrations used for ^{15}N NMR measurements falled between 0.35 and 0.5 mol dm^{-3}.

The ^1H NMR spectra were recorded on a Jeol GX-400 spectrometer.

Spectrophotometric measurements.

pK_a values were determined spectrophotometrically (Cary 17D) by recording the UV spectra of substrates at various hydronium ion concentrations adjusted with hydrogen chloride (pH < 3), or with acetic acid/sodium acetate (pH 3-6) or triethanolammonium chloride/triethanolamine buffers (pH 7-9). Hydronium ion concentrations of the buffer solutions were calculated from the acidity constants reported for the buffer acids under the experimental conditions.[41,42] pK_a values smaller than unity were based on the H_o-scale.[21] Nonlinear least-squares regression[43] was applied to fit the data with eqn. (1). Here A_H is the absorbance

$$A_H = (K_a A_1 + A_2[H^+])/(K_a + [H^+]) \quad (1)$$

observed at hydronium ion concentration $[H^+]$, and A_1 and A_2 are the absorbances at pH = pK_a + 3 and pH = pK_a - 3, respectively. Usually only one of the quantities A_1 and A_2 could be determined experimentally, and the other one was determined as an adjustable fitting parameter.

Kinetic measurements.

First-order rate constants for the cleavage of N-glycosidic bond were obtained by the HPLC technique described earlier.[22] With **2a**, **3a** and **3c** the separations were carried out on a Spherisorb RP-18 column (4x250 mm, 5 μm) using a mixture of acetonitrile and acetic acid/sodium acetate buffer (0.025 mol dm^{-3}, pH 4.3) as eluent. The content of acetonitrile was 13, 80 and 75 %, respectively. Aliquots of the 5'-monophosphate

of **2a** were analyzed on a Hypersil RP-18 column (4x250 mm, 5 μm), the eluent being a 8:92 (v/v) mixture of acetonitrile and aqueous ammonium acetate (0.1 mol dm^{-3}).

Phase distribution measurements.

Association of **7** - **9** with purine was studied by distributing these compounds between dichloromethane and aqueous solutions of purine, the concentrations of which were varied form 0 to 0.3 mol dm^{-3}. The experimental procedure was similar to that described previously.[33] With **7** the distribution coefficient between the organic and aqueous phase was, however, too small to allow a direct determination of the solute concentration from the organic layer. Accordingly, with this compound the following modification was applied. The volume of dichloromethane was 10 times as large as that of the aqueous solution (20/2 cm^3). Before chromatographic analysis, **7** was extracted from the separated dichlorometane phase with 2 cm^3 of water. All distributions were performed at 298.2 K. Chromatographic separations were carried out on a Serva RP-18 column (4x250 mm, 5 μm), using aqueous methanol (20-35 %, v/v) which contained sodium perchlorate 0.05 mol dm^{-3} as eluent. The association constants, $K_{ass.}$, for formation of 1:1 adducts between purine and **7** - **9** were obtained as slopes of eqn.(2),[33] where K_P and K_d are the distribution coefficients of

$$K_P/K_d = K_{ass.}\,[P] + 1 \qquad (2)$$

7 - **9** between aqueous and organic phases in the presence and in the absence of purine, respectively. [P] denotes the concentration of purine in the aqueous phase.

The phase distribution method was also applied to study complexing of **7** - **9** with nickel(II) ion. The concentration of aqueous nickel(II) nitrate solutions was varied from 0 to 0.2 mol dm^{-3}, and their ionic strength was kept constant (1.0 mol dm^{-3}) with potassium nitrate. pH was adjusted two units above the pK_a value of substrate monocation. The stability constants, $K(LHM^{2+})$, for the 1:1 complexes were obtained by least-squares fitting *via* eqn. (3),[35] where K_M and K_d are the distribution coefficients of **7** - **9** between

$$K_M/K_d = K(LHM^{2+})\,[M^{2+}] + 1 \qquad (3)$$

the aqueous and organic phases in the presence and in the absence of nickel(II) ion, respectively. $[M^{2+}]$ is the concentration of nickel(II) nitrate in the aqueous phase.

Potentiometric measurements.

Stability constants for 1:1 metal ion complexes of neutral N^2,3-ethenoguanosine (**2a**) were obtained by the potentiostatic method described previously.[44] Apparent acidity constant, K_{app}, for the monocation of **2a** was determined potentiostatically at various metal ion concentrations, and the stability constants, $K(LHM^{2+})$, were calculated by eqn. (4).[44] Here K_a is the acidity constant in the absence of the complexing metal ion and $[M^{2+}]$ is the concentration of free metal ion at the equilibrium. The latter quantity may be calculated by eqn. (5): $n(LH_2^+)$ is equal to the amount of nitric acid consumed in the titration, and $n(LH) = (K_a/[H^+])n(LH_2^+)$. All measurements were carried out at I = 1.0 mol dm^{-3} adjusted with potassium nitrate.

$$K(LHM^{2+}) = (K_{app}/K_a - 1)/[M^{2+}] \qquad (4)$$

$$[M^{2+}] = [M^{2+}(tot)] - [LHM^{2+}] = [M^{2+}(tot)] - \{n(LH,tot) - n(LH) - n(LH_2^+)\}/V \qquad (5)$$

Potentiostatic technique was also applied to study the ability of metal ions to displace a proton from **2a**. The measurements were carried out at pH 5.00 as described previously.[36] Equilibrium constants, $K_{dis.}$, of the displacement reaction were calculated by eqn. (6). In this eqn. [LH] stands for the total concentration of all

$$K_{dis} = ([LM^+]\,[H^+])/([LH]\,[M^{2+}] \qquad (6)$$

those species of **2a**, both complexed and free, which have retained the labile proton of the base moiety. $[LM^+]$ is, in turn, the total concentration of all the species which have donated a proton upon complex formation. Stability constants, $K(LM^+)$, of the complexes of anionic **2a** were then obtained by dividing $K_{dis.}$ by the acidity constant, $K_a(LH)$, of the neutral form of **2a**. The latter value was determined potentiostatically under the same conditions as the values of $K_{dis.}$.

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