Multiple Base-pairing Mode of 9-Ethyl-8-hydroxyguanine in Three Different Crystal Phases

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Three different crystals of 9-ethyl-8-hydroxyguanine have been analysed by X-ray diffraction. Extensive 'cyclic' hydrogen bonds were formed among the bases related by pseudo-2-fold symmetry. The bonding parameters suggested that the 8-hydroxyguanine base consists of a predominant 2-amino-6,8-dioxo isomer (diketo form) including some tautomers.

It has been shown that the C(8) position of guanine base can be easily hydroxylated by agents which produce oxygen radicals *in vitro* and *in vivo*.[1] The DNA template containing the modified base(s) is replicated erroneously by polymerases, and the replicated product is a probable cause of carcinogenesis. Recently, this possibility was demonstrated using synthetic DNA templates.[2] The replication did not terminate at the hydroxylated guanosine residue, and the product contained bases mismatched at the modified position. The DNA mutation by 8-hydroxyguanosine directed the insertion of all four bases, and further caused misreading of the amino acid sequence at the neighbouring bases. This misreading on the DNA template can be attributed to the base-pairing diversity of 8-hydroxyguanine, which would be substantiated by the base tautomerism. Therefore, it is interesting to examine the structure of 8-hydroxyguanine base and the possible base pairing mode. We report here three different crystal structures of a model compound, 9-ethyl-8-hydroxyguanine (^{8}oG).

Table 1 Crystal data for three different crystals of ^{8}oG

	Crystal I	Crystal II	Crystal III
Formula	$C_7H_9N_5O_2$	$C_7H_9N_5O_2{\cdot}H_2O$	$C_7H_9H_5O_2{\cdot}H_2O$
M	195.18	213.20	213.20
Crystal system	monoclinic	triclinic	triclinic
Space group	$P2_1$	$P\bar{1}$	$P\bar{1}$
Cell dimensions:			
a (Å)	8.947(2)	10.239(5)	9.731(1)
b (Å)	5.611(1)	10.594(4)	18.180(4)
c (Å)	16.743(4)	9.920(4)	5.294(1)
α (°)	90.0	92.48(4)	98.01(1)
β (°)	97.61(2)	114.22(4)	99.97(1)
γ (°)	90.0	69.35(2)	84.87(2)
V (Å^3)	833.1(3)	911.7(7)	911.5(2)
Z	4	4	4
μ(Cu-Kα) (cm^{-1})	9.66	10.12	10.12
F(000)	408	448	448
No. of reflections	1448	2306	2259
R	0.059	0.052	0.068
R_w	0.031	0.066	0.036

Results and Discussion

Data for the crystals of ^{8}oG are summarized in Table 1, and the fractional co-ordinates obtained by X-ray analysis are indicated in Table 2 with the atomic numbering scheme. The three different crystals are denoted as Crystals I, II and III (see Experimental section). Crystal I was composed of the ^{8}oG molecule alone, though there was a water molecule of crystallization in Crystals II and III. The unit-cell contents of Crystals II were identical with those of Crystal III (polymorphism). There were two crystallographically independent molecules in Crystal I, four molecules in Crystals II and III, and these independent molecules are denoted as molecules A–D in this paper.

Stacking Interactions.—Fig. 1 shows three kinds of stacking modes observed in the crystal structure, along with the mean interplanar spacings. In each stacking, the adjacent ^{8}oG bases are almost parallel to each other, and the dihedral angles between them are all within 5°. In Crystal I, molecules A and B overlap over their imidazole moieties, and the polar O(8) atom is located over the pyrimidine ring as shown in Fig. 1(a). Since two overlapped molecules are related by a pseudo-2-fold symmetry, their dipole moments are almost antiparallel to each other. Furthermore, the interplanar spacing is somewhat shorter than the van der Waals separation distance (3.4 Å). Therefore, it could be expected that the π–π charge transfer and dipole–dipole coupling forces function in this ring-stacking interaction.

In Crystal II [Fig. 1(b)], the pair of molecules C · · · D (base pairing is discussed in next section) is located over the pair A · · · B with an interplanar distance of *ca.* 3.4 Å. Although the N(2) atom superimposes over the adjacent base, the overlap area is not so significant compared with that of Crystal I. The parallel base stacking is, therefore, mainly stabilized by van der Waals forces. A similar stacking mode is observed in Crystal III [Fig. 1(c)], although the molecular arrangement is different from that in Crystal II. The pair of hydrogen-bonded molecules A · · · B (or C · · · D) is located over the same pair translated by one unit cell. Crystals II and III, which are polymorphs could be essentially distinguished by the difference in their molecular arrangements.

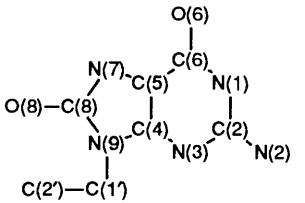
Hydrogen Bonds in the Crystal Packing.—The crystals of ^{8}oG exhibited further extensive base pairing modes *via* hydrogen bonds in addition to the aforementioned vertical stacking interactions. Hydrogen bonds in the crystal packing are shown in Figs. 2, 3 and 4, and are summarized in Table 3. In Crystal I, two independent molecules A and B related by a pseudo-2-fold symmetry are linked to each other by two kinds of 'cyclic' hydrogen bond, between N(2) · · · N(3) and N(7) · · · O(8). These base pairings form an infinite plane along the (110) direction. The ^{8}oG molecules translated by the crystallographic diad symmetry further form hydrogen-bonding pairs for N(1) · · · O(6) and N(2) · · · O(6), which are almost at right angles to each other.

Crystal II is composed of four crystallographically different molecules A · · · B [Fig. 3(a)] and C · · · D [Fig. 3(b)]. Two kinds of infinite planes are observed in this crystal, which are parallel to each other and alternately located. Three types of 'cyclic' hydrogen bond, for N(2) · · · N(3), N(7) · · · O(8), and N(1) · · · O(6) are commonly observed between the partner

Table 2 Fractional atomic co-ordinates ($\times 10^4$) of ^{8}oG in three different crystals, with esds in parentheses

	Molecule A			Molecule B			Molecule C			Molecule D		
Atom	x	y	z	x	y	z	x	y	z	x	y	z
Crystal I												
N(1)	4 839(3)	3 788(6)	4 075(1)	10 237(3)	−10 299(6)	958(1)						
C(2)	4 027(4)	4 936(7)	3 392(2)	11 001(4)	−11 437(8)	1 567(2)						
N(2)	3 197(3)	6 858(6)	3 581(2)	11 842(3)	−13 234(6)	1 395(2)						
N(3)	4 050(4)	4 362(7)	2 643(1)	10 956(4)	−10 653(7)	2 321(1)						
C(4)	4 940(4)	2 313(7)	2 602(2)	10 107(4)	−8 872(7)	2 388(2)						
C(5)	5 733(4)	1 157(7)	3 206(2)	9 261(4)	−7 553(8)	1 781(2)						
C(6)	5 699(4)	1 820(8)	3 998(2)	9 303(4)	−8 251(8)	989(2)						
O(6)	6 279(3)	835(5)	4 637(1)	8 623(3)	−7 354(5)	366(1)						
N(7)	6 496(3)	−714(6)	2 867(1)	8 527(3)	−5 668(6)	2 114(1)						
C(8)	6 171(4)	−602(9)	2 063(1)	8 891(4)	−5 793(8)	2 921(2)						
O(8)	6 591(3)	−1 857(6)	1 546(1)	8 391(3)	−4 487(5)	3 440(1)						
N(9)	5 170(4)	1 302(8)	1 894(2)	9 842(3)	−7 754(7)	3 125(2)						
C(1′)	4 086(5)	1 363(10)	1 075(2)	9 974(5)	−9 210(10)	3 946(2)						
C(2′)	4 859(5)	3 079(11)	727(3)	11 367(5)	−8 370(10)	4 353(3)						
Crystal II												
N(1)	11 056(4)	4 613(4)	5 939(5)	5 340(5)	14 363(4)	8 783(4)	11 086(5)	−395(4)	5 952(4)	5 306(4)	9 360(4)	8 775(4)
C(2)	9 941(5)	4 603(5)	4 628(5)	6 481(5)	14 384(5)	10 188(5)	9 953(5)	−413(5)	4 639(5)	6 453(5)	9 366(4)	10 162(6)
N(2)	10 318(5)	3 433(4)	3 940(5)	6 202(5)	15 406(4)	10 903(5)	10 323(6)	−1 579(4)	3 944(5)	6 201(4)	10 394(4)	10 906(5)
N(3)	8 599(5)	5 591(4)	3 993(4)	7 815(4)	13 355(4)	10 783(4)	8 581(4)	594(4)	3 995(5)	7 822(4)	8 328(4)	10 796(4)
C(4)	8 475(5)	6 597(4)	4 803(5)	7 981(5)	12 313(5)	9 917(5)	8 467(5)	1 604(4)	4 822(5)	8 009(5)	7 294(5)	9 924(5)
C(5)	9 516(5)	6 701(5)	6 229(5)	6 912(6)	12 282(5)	8 554(5)	9 518(5)	1 713(4)	6 246(5)	6 904(5)	7 264(5)	8 587(5)
C(6)	10 965(6)	5 667(6)	6 865(5)	5 483(5)	13 318(5)	7 900(5)	10 949(6)	663(5)	6 866(5)	5 476(6)	8 313(5)	7 908(5)
O(6)	12 075(4)	5 606(4)	8 093(4)	4 406(4)	13 424(4)	6 710(4)	12 060(4)	606(4)	8 092(4)	4 386(4)	8 426(4)	6 706(4)
N(7)	8 897(5)	7 942(5)	6 690(5)	7 566(5)	11 019(4)	8 094(5)	8 887(5)	2 943(4)	6 686(5)	7 555(4)	6 021(4)	8 125(4)
C(8)	7 455(5)	8 605(5)	5 574(5)	9 006(6)	10 328(5)	9 147(6)	7 457(5)	3 598(5)	5 603(5)	8 993(6)	5 311(5)	9 177(6)
O(8)	6 540(4)	9 734(3)	5 616(4)	9 936(4)	9 204(4)	9 179(4)	6 543(4)	4 707(3)	5 629(4)	9 939(4)	4 197(4)	9 173(4)
N(9)	7 171(4)	7 842(4)	4 440(4)	9 205(5)	11 155(4)	10 299(4)	7 172(4)	2 809(4)	4 456(4)	9 227(4)	6 133(4)	10 327(4)
C(1′)	5 775(6)	8 203(5)	3 109(6)	10 666(6)	10 738(5)	11 702(6)	5 801(6)	3 176(5)	3 118(5)	10 659(6)	5 726(5)	11 706(6)
C(2′)	4 582(6)	7 719(6)	3 207(6)	11 821(7)	11 210(7)	11 560(6)	4 604(6)	2 733(6)	3 242(7)	11 819(7)	6 186(6)	11 588(7)
Water*	13 195(5)	1 324(4)	5 525(6)	3 273(5)	17 633(4)	9 294(6)	13 183(5)	−3 662(4)	5 536(6)	3 268(5)	12 625(5)	9 312(5)
Crystal III												
N(1)	10 554(6)	1 352(3)	−2 048(12)	3 386(6)	2 774(3)	11 737(12)	5 545(6)	6 345(3)	−2 055(13)	−1 598(6)	7 761(3)	11 697(12)
C(2)	10 057(7)	678(4)	−3 234(13)	3 817(7)	3 451(4)	12 867(14)	5 083(6)	5 665(3)	−3 213(13)	−1 172(7)	8 438(3)	12 846(13)
N(2)	10 719(7)	338(4)	−5 127(13)	3 121(7)	3 808(3)	14 703(13)	5 732(6)	5 322(3)	−5 121(12)	−1 865(7)	8 817(3)	14 714(13)
N(3)	9 048(6)	353(3)	−2 497(11)	4 885(6)	3 756(3)	12 239(11)	4 062(6)	5 361(3)	−2 497(11)	−113(5)	8 760(3)	12 211(11)
C(4)	8 476(7)	790(4)	−665(12)	5 440(6)	3 336(4)	10 283(13)	3 489(6)	5 778(3)	−585(12)	457(7)	8 328(4)	10 289(15)
C(5)	8 822(7)	1 455(4)	550(16)	5 063(7)	2 640(3)	9 097(12)	3 843(7)	6 470(4)	580(14)	70(6)	7 625(3)	9 088(12)
C(6)	9 946(6)	1 800(3)	−52(14)	3 965(7)	2 320(4)	9 777(14)	4 945(8)	6 799(4)	−34(16)	−1 054(7)	7 326(4)	9 792(15)
O(6)	10 424(5)	2 414(3)	837(11)	3 459(5)	1 716(3)	8 784(11)	5 430(6)	7 418(3)	797(11)	−1 567(5)	6 725(3)	8 788(11)
N(7)	7 994(6)	1 713(3)	2 497(11)	5 950(6)	2 436(3)	7 305(11)	2 992(6)	6 723(4)	2 462(12)	959(6)	7 419(3)	7 302(12)
C(8)	7 092(6)	1 204(3)	2 422(12)	6 855(7)	2 996(4)	7 428(15)	2 091(7)	6 205(4)	2 427(15)	1 820(6)	7 980(3)	7 414(13)
O(8)	6 199(5)	1 193(3)	3 727(10)	7 766(5)	2 974(3)	5 891(10)	1 177(5)	6 165(3)	3 725(11)	2 849(4)	7 956(2)	6 199(8)
N(9)	7 425(6)	584(3)	502(12)	6 535(6)	3 517(3)	9 199(10)	2 417(6)	5 579(3)	503(11)	1 524(5)	8 513(3)	9 191(10)
C(1′)	6 643(6)	−46(3)	−23(13)	7 264(8)	4 217(4)	9 872(15)	1 683(7)	4 895(5)	−105(14)	2 227(7)	9 233(4)	9 877(14)
C(2′)	5 507(7)	−8(4)	−2 364(16)	8 566(9)	4 152(5)	11 831(26)	494(8)	4 985(4)	−2 382(16)	3 572(8)	9 162(4)	11 829(14)
Water*	12 880(7)	1 130(4)	−6 504(13)	1 076(6)	3 016(4)	16 177(14)	7 889(7)	6 137(4)	−6 486(13)	−3 938(6)	8 023(4)	16 200(13)

Numbering scheme



* The water molecules (oxygen atoms) of molecules A, B, C and D in crystals II and III correspond to W1, W2, W3 and W4, respectively.

Table 3 List of possible hydrogen bonds* (Å)

Crystal I							
N(1)A–O(6)A	2.746	N(2)A–N(3)B	3.049	N(1)B–O(6)B	2.807	N(2)B–N(3)A	2.999
N(2)A–O(6)A	3.014	N(7)A–O(8)B	2.801	N(2)B–O(6)B	2.964	N(7)B–O(8)A	2.836
Crystal II							
N(1)A–O(6)B	2.970	N(1)B–O(6)A	2.911	N(1)C–O(6)D	2.927	N(1)D–O(6)C	2.896
N(2)A–N(3)B	3.151	N(2)B–N(3)A	3.094	N(2)C–N(3)D	3.147	N(2)D–N(3)C	3.091
N(7)A–O(8)B	2.753	N(7)B–O(8)A	2.758	N(7)C–O(8)D	2.748	N(7)D–O(8)C	2.785
N(2)A–W(1)	2.829	N(2)B–W(2)	2.918	N(2)C–W(3)	2.809	N(2)D–W(4)	2.919
W(1)–O(8)A	3.205	W(2)–O(6)A	2.824	W(3)–O(6)B	2.974	W(4)–O(6)A	3.054
W(1)–O(6)B	2.897	W(2)–O(8)B	3.185	W(3)–O(8)C	3.223	W(4)–O(6)C	2.825
W(1)–O(6)D	2.964	W(2)–O(6)C	3.038	W(3)–O(6)D	2.878	W(4)–O(8)D	3.169
Crystal III							
N(1)A–O(6)B	2.907	N(1)B–O(6)A	2.956	N(1)C–O(6)D	2.899	N(1)D–O(6)C	2.958
N(2)A–N(3)D	3.126	N(2)D–N(3)A	3.099	N(2)C–N(3)B	3.106	N(2)B–N(3)C	3.136
N(7)A–O(8)B	2.762	N(7)B–O(8)A	2.726	N(7)C–O(8)D	2.786	N(7)C–O(8)D	2.786
N(2)A–W(1)	2.897	N(2)B–W(2)	2.829	N(2)C–W(3)	2.916	N(2)D–W(4)	2.856
W(1)–O(6)B	3.002	W(2)–O(6)A	2.870	W(3)–O(6)D	2.998	W(4)–O(6)C	2.899
W(1)–O(6)B	2.832	W(2)–O(6)A	3.018	W(3)–O(6)D	2.284	W(4)–O(6)C	2.983
				W(3)–O(8)C	3.187	W(4)–O(8)D	3.139

* The standard deviations of the lengths are 0.004, 0.006 and 0.009 Å for Crystals I, II and III, respectively.

Table 4 Selected bond lengths* (Å) and populations† (%) of [imino]/[amino] and [enol]/[keto] in parentheses

	Crystal I		Crystal II				Crystal III			
Bond	A	B	A	B	C	D	A	B	C	D
C(2)–N(2)	1.370	1.312	1.398	1.281	1.398	1.299	1.344	1.340	1.335	1.366
	(0)	(35)	(0)	(63)	(0)	(46)	(6)	(9)	(14)	(0)
C(6)–O(6)	1.253	1.244	1.265	1.216	1.265	1.228	1.246	1.254	1.250	1.253
	(18)	(11)	(27)	(0)	(27)	(0)	(12)	(19)	(15)	(18)
C(8)–O(8)	1.213	1.263	1.243	1.226	1.225	1.236	1.202	1.298	1.226	1.275
	(0)	(25)	(10)	(0)	(0)	(5)	(0)	(52)	(0)	(35)

* The standard deviations of the bond lengths are 0.005, 0.007 and 0.009 Å for Crystal I, II and III, respectively.
† Populations were calculated from $100(D - d2)/(d2 - d1)$, where D, $d1$ and $d2$ are the bond, standard C=O (1.23 A) [or C–NH_2 (1.35 Å)] and C–OH (1.36 Å) [or C=NH (1.24 Å)] lengths, respectively. A negative value is represented by (0).

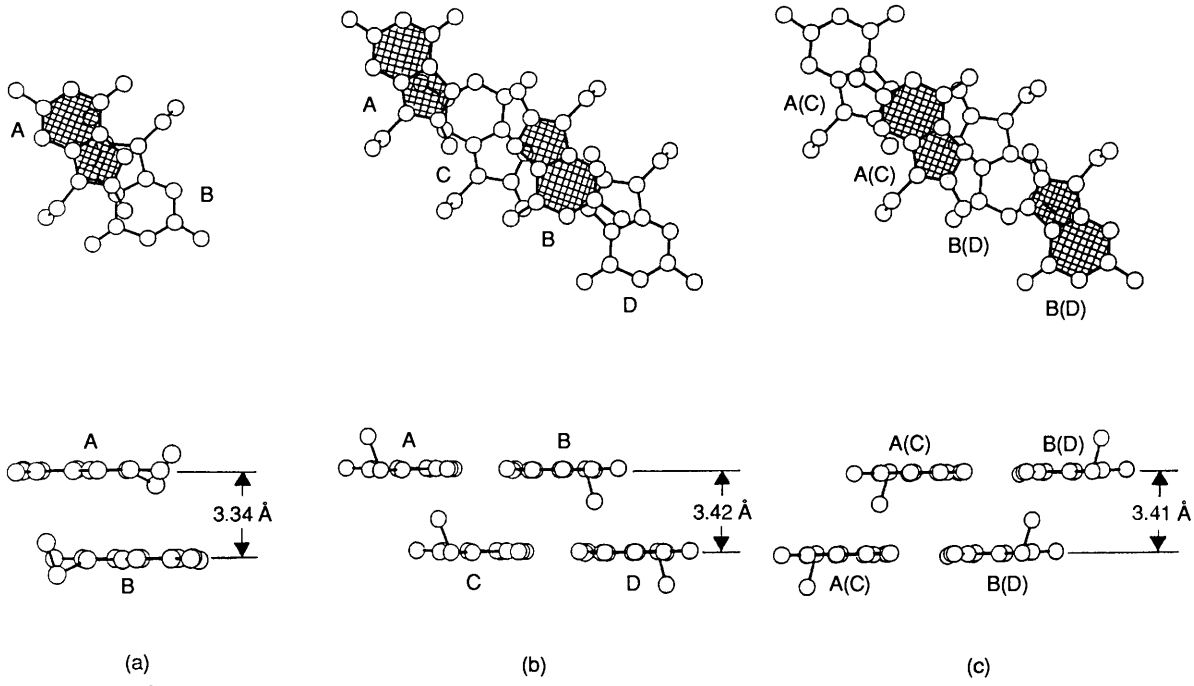


Fig. 1 Stacking mode of ^{8}oGs. The bases of (a) Crystal I, (b) Crystal II and (c) Crystal III are projected perpendicular (upper) and parallel (lower) to the base plane. Hatched bases are located over unhatched bases. The characters A–D represent the crystallographically independent molecules.

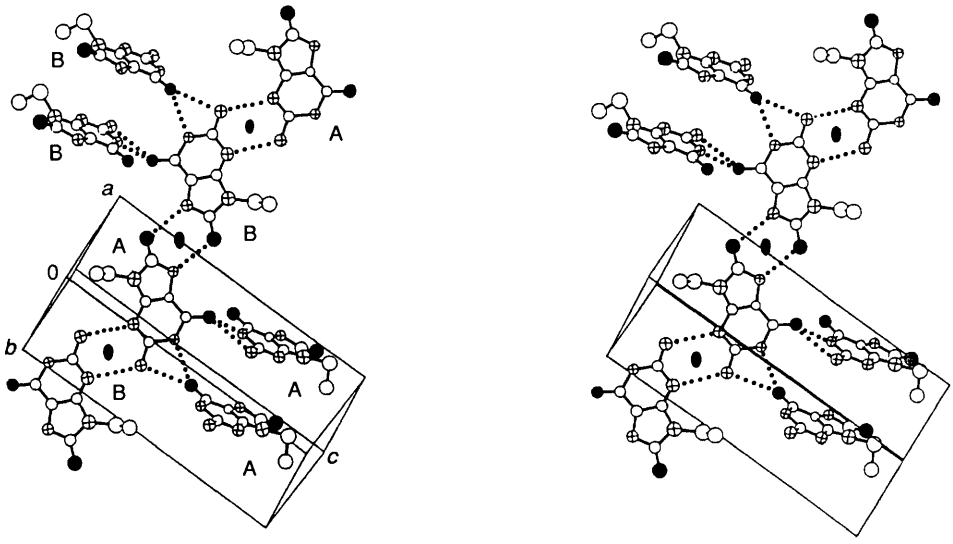


Fig. 2 Stereo drawing of ^{8}oG base pairings in Crystal I. Filled and crossed circles represent oxygen and nitrogen atoms, respectively. Dotted lines represent possible hydrogen bonds. Filled footballs (●) show the pseudo-2-fold axes.

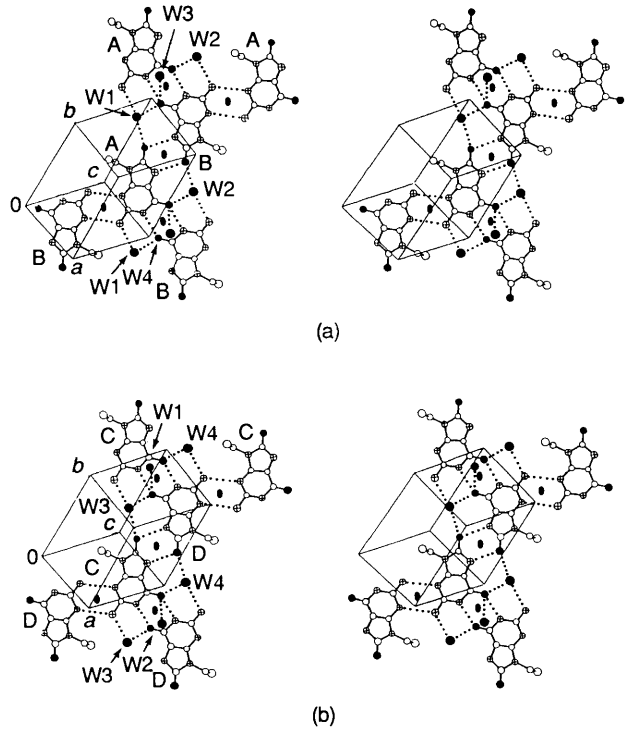


Fig. 3 Stereo drawing of ^{8}oG base pairings in Crystal II. The base-pairing plane is composed of molecules A and B (a), and C and D (b). The letter W represents a water molecule.

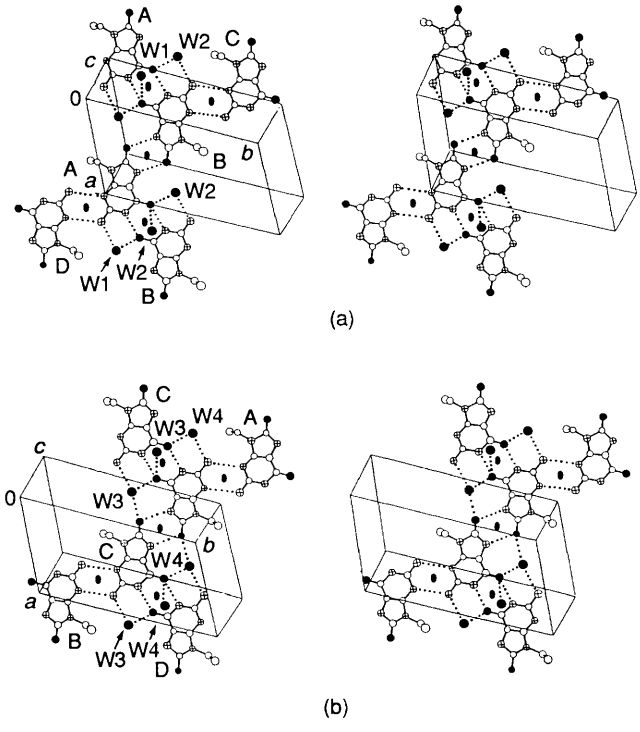


Fig. 4 Stereo drawing of ^{8}oG base pairings in Crystal III. The molecules A, B, C and D are located in the same base-pairing plane. Molecules A···B (a) and molecules C···D (b) pairs are viewed from the centre of the figure.

molecules related by a pseudo-2-fold symmetry; these interactions are also observed in Crystal I. The water molecules of crystallization additionally stabilize the base associations *via* the hydrogen-bonding bridges N(2)···W···O(6) and N(2)···W···O(8). The former bridge mediated by water forms a base-pairing pattern similar to the Watson–Crick G–C pair.

The hydrogen-bonding network and the molecular disposition of Crystal III [Fig. 4(a) and 4(b)] are almost the same as those of Crystal II. However, the molecular arrangements of the ^{8}oG bases are different. All four crystallographically independent molecules are located on a plane, as shown in Fig. 4. This implies different combinations among the partner bases for the formation of base pairing in Crystals II and III. The interactions with the water molecules in Crystal III are also somewhat different from those of Crystal II. The O(8) atoms of molecules A and B are located far from the water molecules of W(1) and W(2), compared with the O(8) atoms of molecules C and D which are hydrogen-bonded to the W(3) and W(4) molecules. This indicates a possibility that the binding states of the O(8) atoms in molecules A and B are different from those in molecules C and D.

Thus, the ^{8}oG molecules are intrinsically associated into a base tetrad by hydrogen-bondings. Such a molecular base aggregation has been observed for the gel formation of guanosine in the presence of an excess of an alkali salt.[3,4] In the guanine-rich DNA, the highly associated strand has also been suggested, termed 'anti/parallel four-strand G4-DNA.'[5–8] The guanine bases in this tetrad structure are almost planar and take

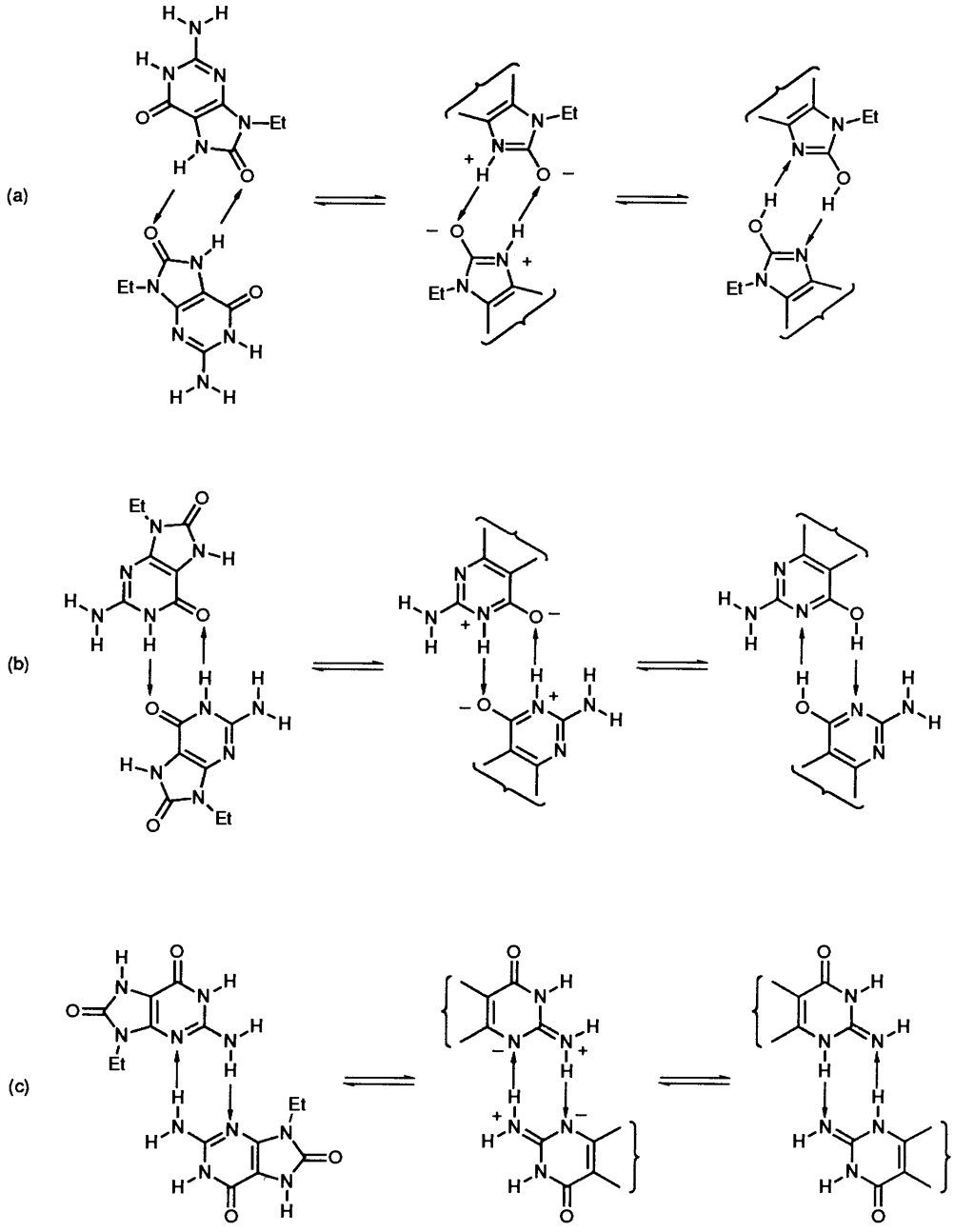
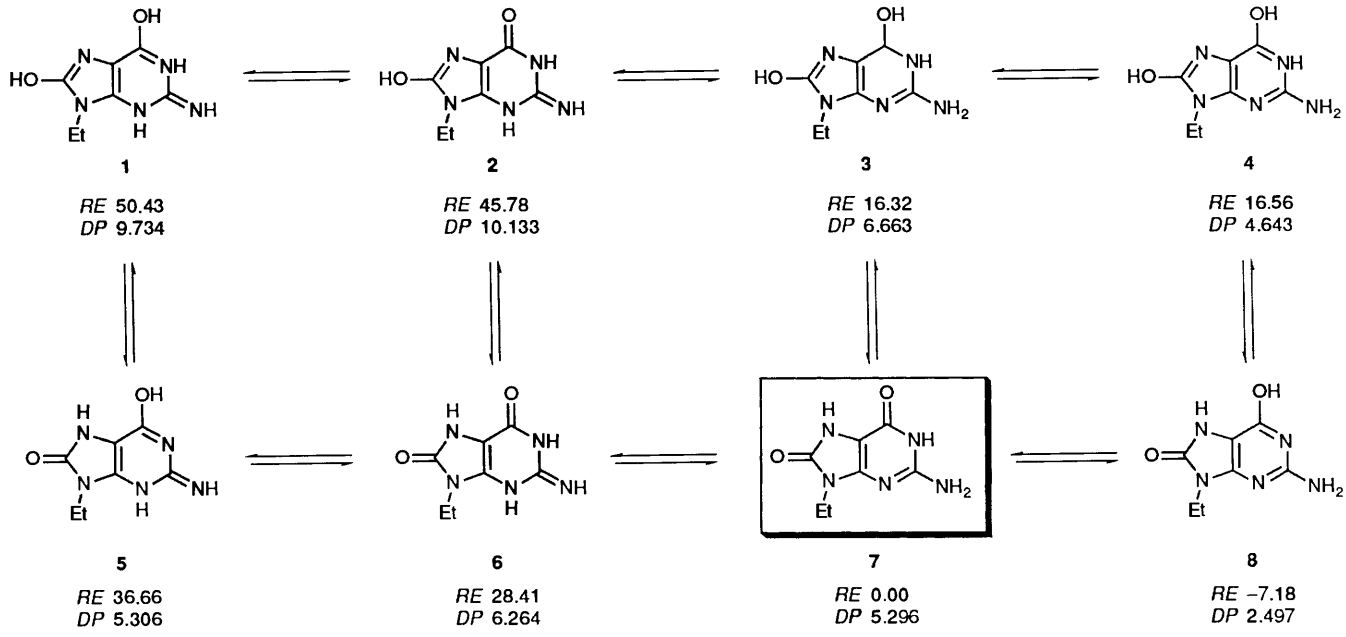


Fig. 5 Three different ^{8}oG base pairings and their isomerizations. The 'cyclic' type hydrogen bond is formed between (a) the N(7) and O(8), between (b) the N(1) and N(2), and between (c) the N(2) and N(3) atoms of the ^{8}oG base. The bold arrows represent the direction from donar to acceptor in each hydrogen bond.

a Hoogsteen-like hydrogen-bonding mode [N(1)···O(6) and N(2)···N(7)].[9] The other guanine–guanine association (base pairing) has been observed in the platinum(II)–[N(7)] guanine complex, with the N(1)···N(1), N(2)···O(6) and O(6)···N(2) pairing modes.[10,11] In contrast, the atomic combinations of the base pairings ('cyclic' hydrogen bonds) are N(1)···O(6), N(2)···N(3) [or N(2)···O(6)], and N(7)···O(8) in the present ^{8}oG crystals, and their aggregation mode is subsequently different from that of guanine.

Tautomerism of the ^{8}oG *Molecule.*—Although the tautomerism of nucleic acid bases is generally well known,[12] the population of the isomeric mixtures is very low under neutral conditions.[13,14] Its population would be increased by base modification,[15,16] ion co-ordination,[17] or extensive hydrogen-bond formation.[18] For the ^{8}oG molecule, a lot of tautomers can be considered because of the isomerization of the 8-substituted oxygen atom. The possible structures of ^{8}oG tautomers, **1**–**8**, and relative energies from the diketo form **7** are shown in Scheme 1. MNDO calculations[19] show that the 2-amino-6-hydroxy-8-oxo isomer **8** is most energetically stable and its dipole moment is the smallest among those of the possible isomers. The energy calculation also indicates that the transformation of tautomer **8** to 2-amino-6,8-dioxo isomer **7** (diketo form) requires 7.18 kcal mol^{-1},* and this value corresponds to the smallest energy gap compared with those necessary for the other routes. Therefore, it is predictable that the ^{8}oG molecule tends to populate mostly as tautomers **7** and **8**. In the crystal, however, the existence of many intermolecular interactions such as hydrogen bonds would affect the tautomerism of ^{8}oG, and consequently the population of each tautomeric isomer was somewhat different from the results of

* 1 cal = 4.184 J.



Scheme 1 Possible tautomerism of ^{8}oG. The diketo form is shaded. *RE* represents the relative energy (kcal mol^{-1}) (1 cal = 4.184 J) from ^{8}oG diketo form and the dipole moment is indicated by *DP*. Energy calculations were performed by the MNDO method.[19]

the energy calculations. Indeed, the populations of imino and enol isomers, which were calculated on the basis of the C(2)–N(2), C(6)–O(6) and C(8)–O(8) bond lengths (Table 4), suggest the 2-amino-6,8-dioxo isomer **7** as the major tautomer of ^{8}oG in the crystal structure.

Population analysis by the bonding parameters suggested that, in Crystal I, molecule A is the 2-amino-6,8-dioxo isomer **7** predominantly, and that molecule B contains, in addition, the isomer(s) **2** or **3** + **6**; the combination of isomers **3** + **6** would be energetically more advantageous than isomer **2**. In Crystal II, molecules A and C consist mainly of tautomer **7** plus the 6-hydroxy isomer **8**, and molecules B and D contain tautomer **7** and 2-imino isomer **6**. In Crystal III, molecules A and C are occupied by isomer **7**, and molecules B and D contain the 8-hydroxy isomer **3** in addition to tautomer **7**. It is interesting to note that there is a significant difference between the tautomeric populations of molecule B and C in Crystals II and III.

The isomerization of the ^{8}oG base appears to be closely related to its hydrogen-bonding array. The 'cyclic' hydrogen bonds observed for the associated bases can be classified into three kinds of base pairings, as shown in Fig. 5; an antiparallel hydrogen-bonding pair is formed between the N(7)···O(8), N(1)···O(6) and N(2)···N(3) atoms. Each pairing could further consist of three different isomers. Under the elaborate hydrogen-bonding network in the present crystals, the donor–acceptor relationship for the hydrogen bond would be severely restricted. The movement of hydrogens is possible within the pairing bases according to the equilibrium scheme shown in Fig. 5. Such a complementary replacement of hydrogen atoms is able to facilitate tautomerization of the ^{8}oG base, and a similar tautomerization has been considered for tRNA.[18] Since the three kinds of base pairings [Figs. 5(a), 5(b) and 5(c)] are simultaneously responsible for the 'local' isomerization of the ^{8}oG base itself and are independent of each other, we conclude that the crystallographically independent molecules (molecules A–D) result from tautomeric isomerism of ^{8}oG, not from the conformational isomers.

* *Supplementary data* (see section 5.6.3 of Instructions for Authors, in the January issue). Tables of anisotropic thermal parameters, hydrogen atom co-ordinates, bond lengths, bond angles and torsion angles have been deposited at the Cambridge Crystallographic Data Centre.

Experimental

Preparation of 9-Ethyl-8-hydroxyguanine (^{8}oG).—The title compound was synthesized from 2-amino-6-chloropurine according to the described methods.[20–22] Purification was performed by Sephadex LH-20 column chromatography (50% methanol) and HPLC.

Crystallization.—^{8}oG was dissolved in the solvent mixture water–methanol–dimethylformamide (6:4:1). Anhydrous crystals of ^{8}oG (Crystal I) were grown from a solution containing an equimolar amount of guanine. Two different monohydrate crystals were obtained from a solution containing an equimolar quantity of adenine (Crystal II), and from a solution of ^{8}oG alone (Crystal III), respectively. The examination of crystal components by UV spectroscopy and TLC showed that both Crystals I and II consisted of ^{8}oG molecule alone, and the Crystals II and III are polymorphs. Crystal III is likely to be identical with the previously obtained ^{8}oG crystal.[23] Crystal data are summarized in Table 1.

Structure Determinations and Refinement.—Structures were solved by direct methods using the MULTAN87 program,[24] and refined by the block-diagonal least-squares method with anisotropic thermal factors. Hydrogens were calculated on the geometrically ideal positions, and included in the structure-factor calculations with overall isotropic thermal factors, but were not refined. The obtained fractional co-ordinates are listed in Table 2.*

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