Synthesis, Crystal Structure, and Spectroscopic Properties of 8-(8-Guanosyl)guanosine

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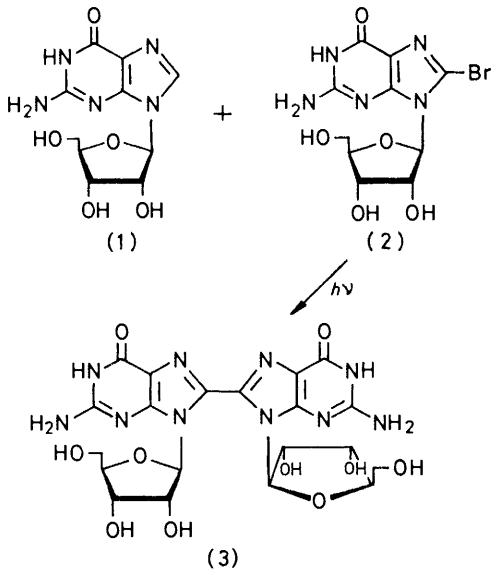
The title compound (3) has been prepared by u.v. irradiation of a mixture of guanosine and 8-bromoguanosine. Its tetrahydrate, $C_{20}H_{24}N_{10}O_{10}\cdot 4H_2O$, crystallises as monoclinic needles with a = 26.69(1), b = 8.425(5), c = 12.286(7) Å, β = 104.9(2)°, Z = 4, space group $C2$. Its structure was solved by direct methods and refined to R 0.068 for 1 071 diffractometer reflections. Two independent molecules (A) and (B) are found in the crystal with a crystallographic two-fold axis bisecting the C(8)–C(8) bonds. The conformation about the glycosidic bonds is *syn* for both molecules with the torsion angles χ_{CN} [O(1′)–C(1′)–N(9)–C(4)] equal to 35.7° in molecule (A) and 47.6° in molecule (B). The orientation about the C(4′)–C(5′) bonds is gauche–gauche in both molecules. The puckering of the ribose rings is C(3′)-*endo* for molecule (A) and C(2′)-*endo* for molecule (B). Owing to steric crowding, the ribose moieties in 8-(8-guanosyl)guanosine must adopt the *syn*-orientation in solution as well as in the solid state; its u.v., ^{1}H n.m.r., and c.d. spectra are discussed in the context of its molecular geometry. On excitation at 330 nm, (3) exhibits intense fluorescence emission in the region of 405 nm.

PURINE bases and nucleosides are generally resistant to photochemical alteration. They can, however, be substituted at C(8) by a variety of free-radical species generated by u.v. irradiation of simple alcohols, amines, and ethers.[1] We have recently shown [2] that guanosine (1) can be substituted in a similar fashion by the free radicals produced on photolysis of 8-bromopurine nucleosides to give (8→8) coupled purine nucleosides. Thus, u.v. irradiation of equimolar mixtures of guanosine and 8-bromoguanosine (2), in aqueous solution, affords 8-(8-guanosyl)guanosine (3).



The (8→8) coupled purine nucleosides constitute a new class of nucleoside derivatives which may be important in the photochemistry and radiation chemistry of nucleic acids. Owing to their extended conjugation and the steric crowding of their ribose moieties, these compounds exhibit unusual spectroscopic and conformational features. We give here details of the preparation, crystal structure, and spectroscopic properties of 8-(8-guanosyl)guanosine.

EXPERIMENTAL

U.v. spectra were recorded with a Cary 118 spectrophotometer, the uncorrected fluorescence spectra with a Perkin-Elmer MPF 2A spectrofluorimeter, and the c.d. spectrum with a Cary 61 instrument. ^{1}H N.m.r. spectra were recorded, at 22 °C, with a Bruker WH 90 spectrometer for solutions in [$^2H_6$]dimethyl sulphoxide with tetramethylsilane as internal standard.

Guanosine and 8-bromoguanosine (Sigma Chemical Co.) were used without further purification.

Synthesis of 8-(8-Guanosyl)guanosine (3).—A solution containing guanosine (100 mg, 0.35 mmol) and 8-bromoguanosine (125 mg, 0.35 mmol) dissolved in 30% aqueous acetone (1 l) was irradiated for 30 h under nitrogen in a Hanovia 1 l photochemical reactor with a 100 W medium pressure u.v. lamp through a Pyrex glass filter. The progress of the reaction was followed by monitoring the fluorescence emission intensity of the solution at 405 nm, on excitation at 330 nm. The irradiation products were fractionated by dry column chromatography.[3] 3 l of irradiated solution were combined, concentrated under reduced pressure to 10 ml, and then evaporated to dryness in the presence of 10 g silica gel M.F.C. (Hopkin and Williams) on a rotary evaporator. The resulting mixture was placed on top of a column (2.5 cm diam.) of silica gel M.F.C. (150 g, deactivated with 15 ml water) packed under dry conditions. The column was eluted with a solvent mixture of ethyl acetate–water–propanol (4 : 3 : 1, upper phase). Fractions containing 8-(8-guanosyl)guanosine, which was eluted after unchanged 8-bromoguanosine and guanosine, were detected by their absorbance at 322 nm. These fractions were pooled and evaporated to dryness to give crude product in a spectroscopic yield of *ca.* 7%. Subsequent recrystallisation from water gave colourless needles (22 mg, 4%) which darkened above 260 °C but did not melt below 300 °C. A sample for elemental analysis was dried at 80 °C *in vacuo* for 24 h (Found: C, 42.15; H, 4.45; N, 24.70. Calc. for $C_{20}H_{24}N_{10}O_{10}$: C, 42.55; H, 4.28; N, 24.81%); δ 10.42 [2 H, br s, exchangeable, H–N(1)], 6.52 [4 H, br s, exchangeable, H_2N–C(2)], 6.21 [2 H, d, $J_{1'2'}$

6.2 Hz, H–C(1′)], 4.92 [2 H, m, $J_{2'3'} \approx 5.4$ Hz, H–C(2′)], 4.20 [2 H, m, $J_{3'4'} \approx 3.8$ Hz, H–C(3′)], 3.9—3.4 (multiplets due to other ribose protons); $\lambda_{max.}$ (pH 7.0) 278 (ε 24 400) and 322 (ε 21 500); (0.1M HCl) 278 (ε 24 100) and 321 (ε 21 800); (0.1M NaOH) 275sh (ε 13 400) and 325 (ε 20 700); fluorescence spectra at pH 7.0: $\lambda_{max.}$ (excitation) 330, $\lambda_{max.}$ (emission) 405 nm. The c.d. spectrum of (3), at pH 7.0, is shown in Figure 4 (see later).

Crystal Data.—$C_{20}H_{24}N_{10}O_{10}\cdot 4H_2O$, $M = 636$. Monoclinic, $a = 26.69(1)$, $b = 8.425(5)$, $c = 12.286(7)$ Å, $\beta = 104.9(2)$, $D_m = 1.577(5)$ (flotation), $Z = 4$, $D_c = 1.582$ g cm^{-3}, $F(000) = 1\,336$. Space group $C2$ from systematic absences and considerations of symmetry and chirality of the molecule. Mo-K_α radiation, $\lambda = 0.710\,7$ Å; μ(Mo-K_α) = 1.45 cm^{-1}.

Crystallographic Measurements.—A single, extremely fine colourless needle-like crystal (1.0 × 0.1 × 0.1 mm) elongated along b was mounted in a Lindemann capillary containing mother-liquor. Cell parameters were obtained from a least-squares analysis of the settings of 25 reflections measured on a Philips PW 1100 four-circle diffractometer with graphite-monochromated Mo-K_α radiation, ω—2θ scan mode (scanwidth 1.2° θ, scan speed 0.04° θ s^{-1}). Of 1 740 unique reflections collected in the range $3° < \theta < 24°$, 1 071 having $I_{(rel)} > 2\sigma I_{(rel)}$ were considered observed. The intensities of three standard reflections measured every hour remained constant to within ±2.2% of their mean

TABLE 1

Fractional atomic co-ordinates (× 10^4) and isotropic temperature factors for non-hydrogen atoms (Å^2 × 10^3)

	x	*y*	*z*	*U*
Molecule (A)				
N(1)	1 968(4)	77(19)	5 929(9)	32(3)
C(2)	1 937(5)	844(22)	6 902(11)	33(4)
N(2)	2 390(4)	1 006(21)	7 718(10)	51(3)
N(3)	1 489(4)	1 269(19)	7 109(8)	23(3)
C(4)	1 071(4)	908(21)	6 235(9)	15(3)
C(5)	1 079(4)	166(21)	5 246(10)	19(3)
C(6)	1 560(5)	−260(21)	5 027(11)	25(3)
O(6)	1 648(3)	−826(16)	4 175(7)	36(2)
N(7)	581(4)	3(19)	4 569(8)	25(3)
C(8)	280(4)	597	5 164(10)	21(3)
N(9)	559(3)	1 192(18)	6 212(8)	24(3)
O(1′)	649(3)	3 011(16)	7 700(6)	26(2)
C(1′)	317(5)	1 817(21)	7 053(11)	25(3)
C(2′)	241(4)	505(21)	7 872(10)	24(3)
O(2′)	−241(3)	859(17)	8 129(7)	34(2)
C(3′)	684(5)	844(22)	8 888(11)	30(3)
O(3′)	600(3)	259(16)	9 941(8)	32(2)
C(4′)	735(5)	2 648(21)	8 907(12)	32(4)
C(5′)	1 241(5)	3 344(23)	9 565(11)	39(4)
O(5′)	1 665(3)	2 787(17)	9 157(7)	37(2)
Molecule (B)				
N(1)	1 835(4)	6 318(19)	7 563(9)	27(3)
C(2)	1 940(5)	5 725(21)	6 595(10)	29(3)
N(2)	2 445(4)	5 344(19)	6 736(9)	34(3)
N(3)	1 602(4)	5 544(17)	5 628(8)	22(2)
C(4)	1 109(4)	5 976(22)	5 688(10)	20(3)
C(5)	966(4)	6 530(20)	6 599(10)	17(3)
C(6)	1 349(5)	6 750(21)	7 601(11)	26(3)
O(6)	1 286(3)	7 264(17)	8 540(8)	37(2)
N(7)	437(4)	6 801(18)	6 353(8)	23(3)
C(8)	260(4)	6 367(21)	5 310(10)	20(3)
N(9)	669(3)	5 881(18)	4 842(8)	20(2)
O(1′)	886(3)	3 846(16)	3 766(7)	26(2)
C(1′)	608(5)	5 328(21)	3 695(10)	27(3)
C(2′)	863(5)	6 386(22)	2 978(10)	28(3)
O(2′)	545(3)	7 643(17)	2 432(8)	40(2)
C(3′)	985(5)	5 217(21)	2 129(10)	26(3)
O(3′)	536(3)	4 935(17)	1 224(7)	36(2)
C(4′)	1 110(5)	3 667(21)	2 805(11)	26(3)
C(5′)	1 684(5)	3 348(21)	3 270(11)	36(4)
O(5′)	1 962(3)	4 679(17)	3 818(7)	32(2)
Water molecules				
OW(1)	435(3)	7 137(17)	9 372(8)	35(2)
OW(2)	1 450(3)	−149(19)	11 885(8)	46(3)
OW(3)	2 650(5)	5 939(21)	9 371(10)	71(3)
OW(4)	3 059(4)	2 957(21)	9 326(9)	63(3)

TABLE 2

Bond lengths (Å)

	Molecule (A)	Molecule (B)
C(2)–N(1)	1.38(2)	1.38(2)
C(6)–N(1)	1.37(2)	1.36(2)
N(2)–C(2)	1.36(2)	1.35(2)
N(3)–C(2)	1.33(2)	1.30(1)
C(4)–N(3)	1.37(2)	1.39(2)
C(5)–C(4)	1.37(2)	1.35(2)
N(9)–C(4)	1.38(1)	1.36(1)
C(6)–C(5)	1.42(2)	1.40(2)
N(7)–C(5)	1.38(1)	1.39(2)
O(6)–C(6)	1.23(1)	1.28(2)
C(8)–N(7)	1.32(1)	1.30(1)
N(9)–C(8)	1.40(1)	1.42(2)
C(1′)–N(9)	1.45(2)	1.45(2)
C(4′)–O(1′)	1.47(2)	1.46(1)
O(1′)–C(1′)	1.44(2)	1.44(2)
C(2′)–C(1′)	1.54(2)	1.53(2)
O(2′)–C(2′)	1.44(1)	1.41(2)
C(3′)–C(2′)	1.51(2)	1.53(2)
O(3′)–C(3′)	1.45(2)	1.43(1)
C(4′)–C(3′)	1.53(2)	1.54(2)
C(5′)–C(4′)	1.50(2)	1.52(2)
O(5′)–C(5′)	1.43(2)	1.42(2)
C(8)–C(8^{III})	1.45(2)	1.40(2)

For atom numbering system refer to Figure 1

TABLE 3

Bond angles (°)

	Molecule (A)	Molecule (B)
C(6)–N(1)–C(2)	126(1)	122(1)
N(2)–C(2)–N(1)	116(1)	113(1)
N(3)–C(2)–N(1)	123(1)	125(1)
N(3)–C(2)–N(2)	120(1)	122(1)
C(4)–N(3)–C(2)	112(1)	111(1)
C(5)–C(4)–N(3)	127(1)	128(1)
N(9)–C(4)–N(3)	126(1)	126(1)
N(9)–C(4)–C(5)	108(1)	106(1)
C(6)–C(5)–C(4)	120(1)	118(1)
N(7)–C(5)–C(4)	110(1)	112(1)
N(7)–C(5)–C(6)	130(1)	130(1)
C(5)–C(6)–N(1)	111(1)	115(1)
O(6)–C(6)–N(1)	119(1)	118(1)
O(6)–C(6)–C(5)	130(1)	127(1)
C(8)–N(7)–C(5)	105(1)	105(1)
N(9)–C(8)–N(7)	113(1)	111(1)
C(8)–N(9)–C(4)	104(1)	106(1)
C(1′)–N(9)–C(4)	132(1)	128(1)
C(1′)–N(9)–C(8)	124(1)	126(1)
C(4′)–O(1′)–C(1′)	109(1)	111(1)
O(1′)–C(1′)–N(9)	109(1)	107(1)
C(2′)–C(1′)–N(9)	111(1)	115(1)
C(2′)–C(1′)–O(1′)	108(1)	104(1)
O(2′)–C(2′)–C(1′)	106(1)	114(1)
C(3′)–C(2′)–C(1′)	101(1)	103(1)
C(3′)–C(2′)–O(2′)	110(1)	112(1)
O(3′)–C(3′)–C(2′)	114(1)	111(1)
C(4′)–C(3′)–C(2′)	104(1)	104(1)
C(4′)–C(3′)–O(3′)	111(1)	108(1)
C(3′)–C(4′)–O(1′)	102(1)	106(1)
C(5′)–C(4′)–O(1′)	110(1)	107(1)
C(5′)–C(4′)–C(3′)	118(1)	114(1)
O(5′)–C(5′)–C(4′)	112(1)	113(1)
N(7)–C(8)–C(8^{III})	125(1)	125(1)
C(8^{III})–C(8)–N(9)	122(1)	123(1)

values. Lorentz polarisation corrections were applied. No absorption correction was made

Structure Solution and Refinement.—The structure was solved by multisolution tangent refinement with the program SHELX. A starting set of eight reflections generated 256 permutations. The calculated E maps were ranked by the reliability index R_A (ref. 4) and one such map (R_A 0.093) yielded the positions of 33 of the 44 non-hydrogen atoms. Subsequent cycles of least-squares refinement followed by difference-Fourier syntheses revealed the positions of all the remaining heavy atoms and 19 of the 32 hydrogen atoms. The final refinement was carried out with all heavy atoms treated isotropically. Hydrogen atoms bonded directly to the ribose carbon atoms were constrained at 1.08 Å, their positions being dictated by the geometry of the molecule. All the remaining hydrogen atoms were constrained to ride on their corresponding oxygen and nitrogen atoms with bond lengths of 1.00 ± 0.01 Å. In addition, as an aid to the refinement, the amino-hydrogen atoms were constrained to within 2.05 ± 0.01 Å of C(2) in the purine ring and the hydroxy-hydrogen atom at O(5′) to within 1.90 ± 0.05 Å of N(3) [molecule (B)]. Hydrogen atoms not located on a difference map were placed with due consideration of the expected geometries of the molecules and the hydrogen-bonding network. The isotropic temperature factors of the hydrogen atoms were refined as several parameters, some for groups and some for individual atoms; their values ranged between 0.025 and 0.101 Å^2. This technique of constrained least-squares refinement, with rigid groups, bond-length constraints, and location and refinement of hydrogen atoms constitutes part of the program SHELX. The refinement converged to $R' = \Sigma w^{\frac{1}{2}}|F_o - F_c|/\Sigma w^{\frac{1}{2}}|F_o| = 0.058$ and R 0.068 with $w = 1/\sigma^2$. A difference map was computed and had no peaks >0.15 eÅ^{-3}. Positional and thermal parameters and principal bond lengths and angles are listed in Tables 1—3.

All calculations were carried out on a Univac 1106 computer at the University of Cape Town. Tables of observed and calculated structure factors, fractional co-ordinates of the hydrogen atoms, and isotropic temperature factors are listed in Supplementary Publication No. SUP 22511 (10 pp., 1 microfiche).*

RESULTS AND DISCUSSION

Synthesis.—The formation of 8-(8-guanosyl)guanosine involves the substitution of a guanosine molecule at C(8) by a guanosine free-radical generated by photolytic fission of the C(8)–Br bond of 8-bromoguanosine. This reaction parallels the well documented substitution of guanosine and its nucleosides at C(8) by free radicals derived from alcohols, amines, and ethers.[1] Although (3) is produced on irradiation of equimolar mixtures of guanosine and 8-bromoguanosine in aqueous solution at 254 nm with a low-pressure mercury lamp, higher yields and fewer side-products are obtained by use of acetone as a photosensitiser and by irradiating at wavelengths >290 nm with a medium-pressure mercury lamp through a Pyrex filter. The yield achieved under these conditions did not exceed 10% but no attempt was made to improve it. In view of the low concentration of reactants, and the likelihood of the guanosine free-radicals being quenched by hydrogen abstraction from the solvent, the yield is surprisingly high. This probably reflects the ability of purine nucleosides to associate in aqueous solution *via* stacking interactions.[5] The formation of stacked arrays containing adjacent guanosine and 8-bromoguanosine molecules should promote their efficient coupling. Irradiation of 8-bromoguanosine alone also gives rise to (3) but in this case, where direct coupling of two guanosine free radicals may occur, the yields are much lower than when a mixture with guanosine is used.

Crystal Structure.—Examination of a molecular model shows that steric interactions between the two linked nucleoside moieties in (3) impose severe constraints on rotation about both the glycosidic [N(9)–C(1′)] bonds and about the C(8)–C(8) bond. In consequence, the two ribosyl groups are forced to adopt the *syn*-orientation in relation to their respective guanine bases.† Recently much attention has been focused on the conformational analysis of purine nucleosides in the solid state [6-8] especially with regard to their conformation about the glycosidic bond and the puckering of the furanose ring. Simple purine nucleosides are found predominantly in the *anti*-conformation in the crystal, whereas those bearing bulky substituents at C(8) adopt a *syn*-conformation.[7-9] In this context, the crystal structure of (3) is of considerable interest, not only because the individual molecules comprise two identical C(8)-substituted purine nucleoside moieties, but also because these moieties are restricted to the *syn*-conformation about their glycosidic bonds in both the solid state and in solution.

Two independent molecules, (A) and (B), are found in the crystal structure of 8-(8-guanosyl)guanosine; they are shown in perspective in Figure 1. There are two independent half-molecules in the asymmetric unit, with the opposite halves generated by a two-fold axis which runs through the centre of the bond joining the C(8) atoms of the purine rings. The C(6)–O(6) bond lengths of 1.23 and 1.28 Å and the C(2)–N(3) lengths of 1.33 and 1.30 Å correspond to C–O and C–N double bonds respectively for molecules (A) and (B). In addition, protonation at N(1) (hydrogen atoms being found in a difference map for both molecules), confirms that the guanine bases are in the lactam form.

The conformation about the glycosidic bonds is *syn* for both molecules and is stabilised, in each case, by intramolecular O(5′)–H · · · N(3) hydrogen bonds. The torsion angle χ_{CN} [O(1′)–C(1′)–N(9)–C(4)] is 35.7° for molecule (A) and 47.6° for molecule (B). The two independent molecules differ considerably in the puckering of their ribose rings. In molecule (A) they show a C(3′)-*endo*-puckering distorted towards a C(4′)-*exo*-con-

* See note about Supplementary Publications in Notice to Authors, No. 7 in *J.C.S. Perkin II*, 1978, Index issue.

† The terminology used in this article to describe the conformational features of nucleosides has gained widespread acceptance and is currently under consideration by a IUPAC–IUB Commission formulating standard conventions and nomenclature for the description of the conformation of polynucleotide chains. Definitions of specific terms, such as *syn* and *anti*, may be found in W. Saenger, *Angew. Chem. Internat. Edn.*, 1973, **12**, 591, and in refs. 7 and 8.

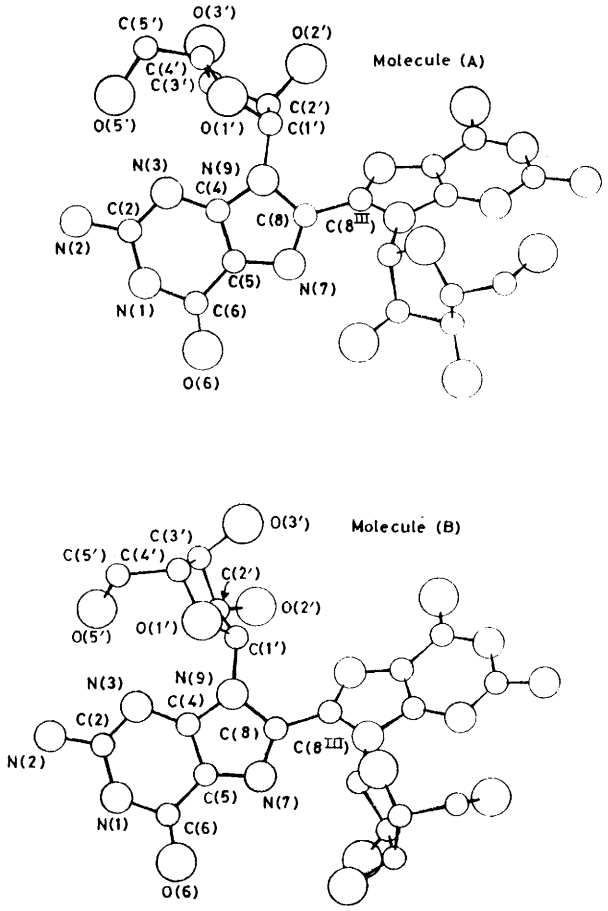


FIGURE 1 Perspective view of molecules (A) and (B) showing atom numbering system

formation with the pseudorotation parameter [6] P 28.6° ($^3T_4$). In molecule (B), however, the ribose rings show C(2')-*endo*-puckering distorted towards a C(1')-*exo*-conformation, with P 156.2° ($^2T_1$). For both molecules the conformation about the C(4')–C(5') bonds is gauche–gauche which facilitates hydrogen bonding between the 5'-hydroxy-group and N(3) of the guanine base. The torsion angles O(5')–C(5')–C(4')–C(3') and O(5')–C(5')–C(4')–O(1') are 59.0 and −56.8° for molecule (A) and 47.6 and −69.3° for molecule (B).

Analysis of the crystal structures of 8-substituted purine nucleosides has established a strong correlation between the *syn*-conformation about the glycosidic bond and C(2')-*endo*-puckering (*S*-type conformation) of the ribose ring.[9] This combination is most frequently associated with the gauche–gauche conformation of the exocyclic hydroxymethyl group and the presence of an intramolecular O(5')–H · · · N(3) hydrogen bond which stabilizes the *syn*-conformation.[7-9] The geometry of molecule (B) in the crystal structure of (3) is perfectly in keeping with this general pattern. Molecule (A), however, is exceptional in that the *syn*-conformation and intramolecular hydrogen bond are found in conjunction with C(3')-*endo*-puckering (*N*-type conformation) of the ribose ring. It appears that for purine nucleosides in the *syn*-conformation, *S*- as opposed to *N*-type conformations of the ribose ring are preferred on energetic grounds.[10] In the case of molecule (A) the packing forces in the crystal are presumably sufficient to compensate for the difference in free energy between the two types of ribose ring conformers. The purine analogue 2-methylformycin [11] is the only other example reported of a *syn*-nucleoside with C(3')-*endo*-puckering and an intramolecular sugar–base hydrogen bond. Both 6-chloropurine riboside [12] and 8-bromoinosine [13] crystallise in the *syn*-conformation with C(3')-*endo*-ribose pucker but in these cases the glycosidic torsion angles (χ_{CN}) are such that no intramolecular hydrogen bond is possible. The pseudorotation angle for molecule (A) (28.6°) is somewhat outside the range (3—23°) commonly encountered [6] for purine nucleosides and nucleotides with *N*-type furanose conformations, although a similar value (28.9°) applies to one of the two independent molecules in the 8-bromoinosine crystal.[13]

Figure 2 shows the stacking of alternate molecules down a two-fold axis. The distances between successive molecules measured at the C(8)–C(8) bonds are 4.86 and 3.56 Å. The large difference between these two distances is due to the bulky ribose rings lying predominantly

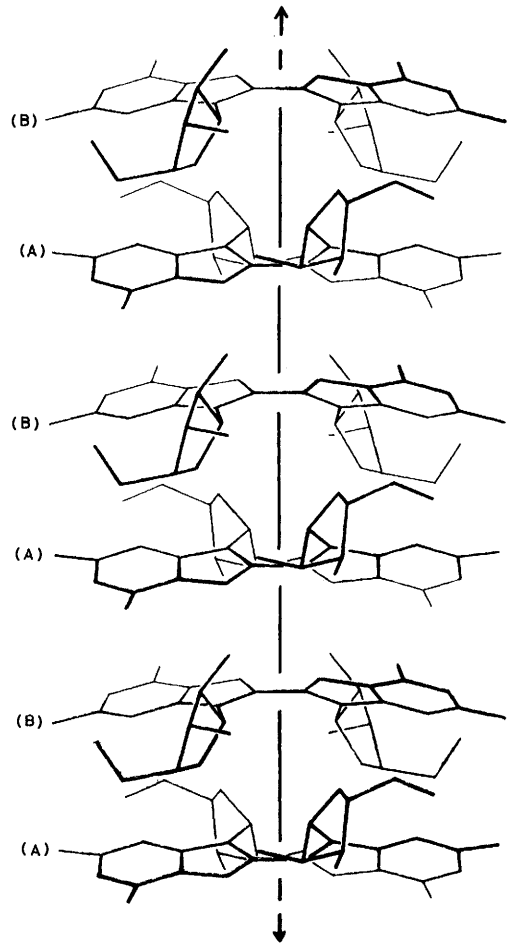


FIGURE 2 The stacking of the molecules about the crystallographic two-fold axis

either above or below the base planes in both molecules (Figure 2). The stacks of base planes lie nearly parallel to each other [the angle subtended between the mean planes of bases (A) and (B) is 8.8°]. The packing (Figures 2 and 3) of the purine bases is unusual, differing from all those quoted [14] in a review. This is probably due to the symmetry of the molecule [a C_2 axis through the C(8)–C(8) bond] and the subsequent space group imposing a stacking pattern not found for other nucleosides. The bases within the individual molecules are not coplanar but are twisted about their C(8)–C(8) bonds. The angles between the normals to the mean planes of the separate bases, which are essentially planar, are 53.3 [molecule (A)] and 41.8° [molecule (B)]. In a similar type of molecule, 8,8′-biquinolyl,[15] the normals to the two halves subtend an angle of 83.2° between them.

The hydrogen bonding in the structure (Figure 3) is extensive, there being nineteen individual hydrogen

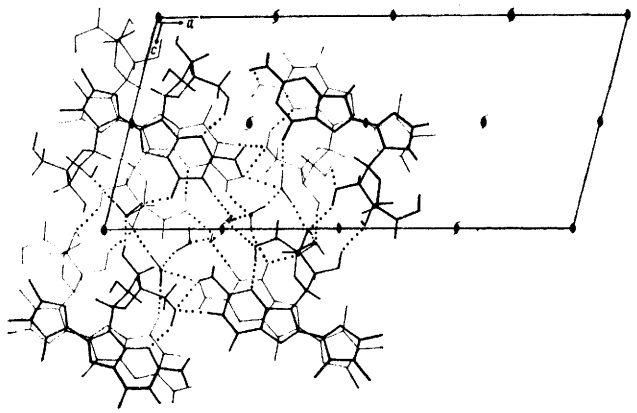


FIGURE 3 An [010] projection showing the hydrogen bonding (dotted lines)

bonds in the asymmetric unit (Table 4). Both intra- and inter-molecular hydrogen bonds form a network concentrated mainly about alternate two-fold screw axes. Each of the four water molecules is involved in three or four hydrogen bonds, both to other water molecules, and to the main molecules.

Spectroscopic Properties.—Viewed as a purine nucleoside derivative, 8-(8-guanosyl)guanosine possesses two structural features of particular significance in relation to its spectroscopic properties. Firstly, its conjugated π-electron system extending over two coupled purine bases constitutes a new type of chromophore whose optical properties have not hitherto been investigated. Secondly, its ^{1}H n.m.r. and c.d. spectra are of special interest because it affords an example of a purine nucleoside system where the conformation about the glycosidic bonds is unequivocally confined to the *syn*-range in solution as well as in the solid state.

Owing to conjugation between the π-electron systems of its two guanine base moieties, the u.v. absorption of 8-(8-guanosyl)guanosine ($\lambda_{max.}^{pH\ 7}$ 278 and 322 nm) extends to much longer wavelengths than that of guanosine ($\lambda_{max.}^{pH\ 7}$ 252 nm). The degree of conjugation

TABLE 4

Hydrogen bond lengths (Å) and angles (°)

O(3′^{I})B · · · OW(1)	2.89
H(O3′^{I})B · · · OW(1)	1.96
O(3′^{I})B–H(O3′^{I})B · · · OW(1)	154
O(3′^{II})A · · · OW(1)	2.73
H(O3′^{II})A · · · OW(1)	1.77
O(3′^{II})A–H(O3′^{II})A · · · OW(1)	158
OW(1) · · · O(2′^{III})B	2.99
HW(11) · · · O(2′^{III})B	2.08
OW(1)–HW(11) · · · O(2′^{III})B	153
OW(1) · · · O(6)B	2.72
HW(12) · · · O(6)B	1.81
OW(1)–HW(12) · · · O(6)B	150
N(2^{IV})B · · · OW(2)	3 03
H(21^{IV})B · · · OW(2)	2.29
N(2^{IV})B–H(21^{IV})B · · · OW(2)	129
OW(2) · · · O(6^{I})A	2.78
HW(21) · · · O(6^{I})A	1.79
OW(2)–HW(21) · · · O(6^{I})A	176
OW(4^{IV}) · · · OW(2)	2.73
HW(42^{IV}) · · · OW(2)	1.95
OW(4^{IV})–HW(42^{IV}) · · · OW(2)	133
OW(2) · · · O(3′)A	2.86
HW(22) · · · O(3′)A	1.92
OW(2)–HW(22) · · · O(3′)A	156
N(1)B · · · OW(3)	2.70
H(1)B · · · OW(3)	1.72
N(1)B–H(1)B · · · OW(3)	166
OW(3) · · · O(5′^{V})A	2.71
HW(31) · · · O(5′^{V})A	2.03
OW(3)–HW(31) · · · O(5′^{V})A	122
OW(3) · · · OW(4)	2.75
HW(32) · · · OW(4)	2.20
OW(3)–HW(32) · · · OW(4)	113
OW(4) · · · O(6^{IV})B	2.81
HW(41) · · · O(6^{IV})B	1.90
OW(4)–HW(41) · · · O(6^{IV})B	149
N(2)A · · · OW(4)	2.83
H(21)A · · · OW(4)	2.06
N(2)A–H(21)A · · · OW(4)	132
N(2)A · · · O(5′^{VI})B	3.08
H(22)A · · · O(5′^{VI})B	2.24
N(2)A–H(22)A · · · O(5′^{VI})B	141
O(5′)A · · · N(3)A	2.75
H(O5′)A · · · N(3)A	1.76
O(5′)A–H(O5′)A · · · N(3)A	173
O(2′)A · · · O(3′^{VII})A	2.77
H(O2′)A · · · O(3′^{VII})A	1.99
O(2′)A–H(O2′)A · · · O(3′^{VII})A	139
O(2′^{VIII})B · · · O(2′)A	2.86
H(O2′^{VIII})B · · · O(2′)A	2.43
O(2′^{VIII})B–H(O2′^{VIII})B · · · O(2′)A	104
N(1)A · · · O(5′^{VI})B	2.81
H(1)A · · · O(5′^{VI})B	1.81
N(1)A–H(1)A · · · O(5′^{VI})B	178
O(5′)B · · · N(3)B	2.74
H(O5′)B · · · N(3)B	1.86
O(5′)B–H(O5′)B · · · N(3)B	145

Roman numeral superscripts denote the following equivalent positions relative to the reference molecule at x, y, z:

I $x, y, 1 + z$	V $\frac{1}{2} - x, \frac{1}{2} + y, 2 - z$
II $x, 1 + y, z$	VI $\frac{1}{2} - x, y - \frac{1}{2}, 1 - z$
III $-x, y, 1 - z$	VII $-x, y, 2 - z$
IV $\frac{1}{2} - x, y - \frac{1}{2}, 2 - z$	VIII $-x, y - 1, 1 - z$

between the bases in (3) will be determined by the torsion angle between their respective planes and in this respect the molecule resembles the dipyrimidine photoadducts [16] in being formally analogous to a biphenyl system. Conjugation will be maximal if the bases are coplanar, minimal if they are mutually perpendicular. The observed large difference between the spectra of (1) and (3) indicates that substantial overlap occurs between

the π-electron systems of the base moieties in (3) and suggests that, in solution, they do not deviate very far from coplanarity.

Absorption of u.v. light by (3) is accompanied by fluorescence emission in the region of 400 nm. The uncorrected emission spectrum comprised a single unresolved band with a maximum at 405 nm. The emission was sufficiently intense to give a detectable signal at concentrations $<10^{-9}$M.

The simplicity of the low-field ^{1}H n.m.r. spectrum of (3) is in accord with its symmetrical structure, and the chemical shifts of the singlets assigned to the ring NH and exocyclic amino-group protons are very similar to those reported [17] for the corresponding protons in guanosine. The absence of an aromatic proton resonance in the spectrum of (3) confirms that the guanosine moieties are substituted at C(8); C(8)–H in guanosine gives rise to a singlet at δ 7.94. Both the anomeric and C(2′)–H protons in (3) have chemical shifts which are *ca.* 0.5 p.p.m. lower, than those of their counterparts in guanosine. A downfield shift of this magnitude for the C(2′)–H resonance has previously been correlated [5] with the *syn*-conformation in purine nucleosides.

The coupling constants of the ribose ring protons $J_{1'2'}$, $J_{2'3'}$, and $J_{3'4'}$ have been estimated by first-order analysis of the C(1′)–H, C(2′)–H, and C(3′)–H signals observed in the spectrum recorded for (3) in [$^2H_6$]dimethyl sulphoxide containing a drop of deuterium oxide. These coupling constants can be used to define the geometry of the ribose rings in solution according to the analysis of Altona and Sundaralingam.[18] The values of $J_{2'3'}$ (≈ 5.4 Hz) and the sum of $J_{1'2'}$ and $J_{3'4'}$ (≈ 10.0 Hz) are very close to those expected for ribonucleosides with a normal degree of ring puckering.[18] The equilibrium distribution of the ribose rings between *N*- and *S*-type conformations, as deduced from $J_{1'2'}$ (6.2 Hz), is approximately 6 : 4 in favour of the *S*-conformer. While this value is typical for unsubstituted purine ribonucleosides it is somewhat lower than those reported [10] for other *syn*-nucleosides which generally show a marked preference for *S*-type conformations of their ribose rings.

Many attempts [19-21] have been made to relate the c.d. spectra of purine nucleosides to their orientation about the glycosidic bond. These have been only partially successful owing to the complexity of the purine absorption spectra. Nonetheless, in combination with evidence from other physical techniques and comparative data for related molecules, c.d. spectra are potentially useful in defining sugar–base torsion angles. The c.d. spectrum of (3) (Figure 4) is characterised by a strong positive band at long wavelengths. For this molecule, the spectrum can be unambiguously correlated with ribose groups in the *syn*-conformation and it can therefore serve as a reference for other (8→8) coupled purine derivatives.

Concluding Remarks.—The simple photochemical coupling reaction used to prepare (3) gas the advantage that no protection of the nucleoside precursors is necessary. In principle, it should be possible to extend the reaction directly to the synthesis of other (8→8) coupled guanine derivatives such as nucleotides and cyclic nucleotides. Furthermore, the reaction should be applicable to the fluorescent labelling of guanine-containing polynucleotides by irradiation in the presence of 8-bromoguanosine. The fluorescence emission of (3) can be selectively excited by wavelengths which are not absorbed by the normal nucleic acid bases.

The feasibility of coupling guanosine to adenosine and inosine by the same mechanism has been demonstrated.[2] It has yet to be established whether (8→8) coupling of neighbouring purine bases can occur in nucleic acids exposed to agents such as u.v. light or ionising radiation, which promote the formation of free-radical species. If so, this type of reaction may have important biological implications. In this context, it is also important to ascertain whether guanine can be substituted at C(8) by free radicals derived from pyrimidine bases. The 5-methyleneuracil free radical is produced [22] from thymine

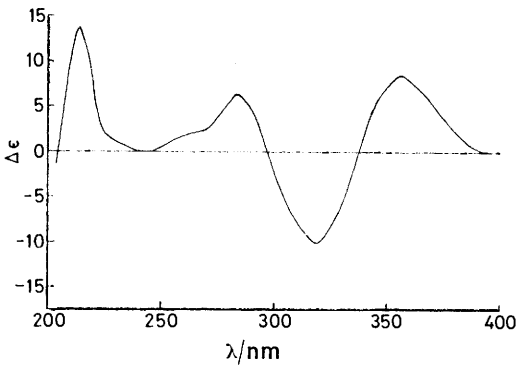


FIGURE 4 C.d. spectrum of 8-(8-guanosyl)guanosine at pH 7.0

when cellular DNA is exposed to near-u.v. light, and u.v. irradiation of polynucleotides substituted with 5-bromouracil is known [23] to generate 5-uracilyl free radicals.

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