1009. *The Methylation of Guanosine and Related Compounds with Diazomethane.*

By J. A. Haines, C. B. Reese, and Lord Todd.

Guanosine, deoxyguanosine, and their derivatives are methylated by diazomethane at position 7. The chemistry of 7-methylguanosine (II; R = OH, R′ = H) has been investigated. The decomposition of derivatives of deoxy-7-methylguanosine (II; R = R′ = H) in aqueous solution proceeds with glycosidic fission at low pH and with cleavage of the imidazole ring at high pH. The relevance of this to the mutagenic action of alkylating agents is considered.

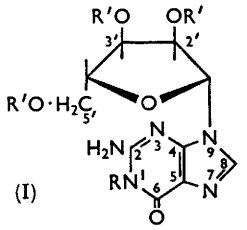
For a number of reasons we have been for some time engaged on a systematic study of the methylation of nucleic acid derivatives. First, the observation that methylating and ethylating agents brought about mutagenesis in certain bacteriophage species [1] has prompted a closer investigation of the action of these reagents on simple nucleosides and nucleotides, in an attempt to correlate chemical change with mutagenesis. Secondly, several *N*-methyl derivatives of adenine and guanine have recently been isolated [2] in small quantities, especially from ribonucleic acids (RNA); whether such bases arise by methylation of the macromolecule or by incorporation of *N*-methylated mononucleotides is unknown. Thirdly, the selective alteration of certain bases in a polynucleotide could inhibit the action of hydrolytic enzymes at these sites and provide a useful tool for determination of the base sequence; for example,[3] the hydrolytic action of pancreatic ribonuclease at a particular uridine residue is inhibited by methylation at N-1. Finally, removal of hydrogen-bonding protons by methylation would have a disruptive effect on the secondary structure postulated in the Watson–Crick model [4] of deoxyribonucleic acid (DNA).

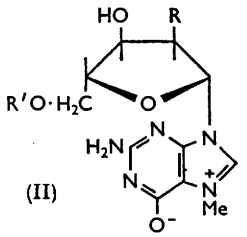
The methylation of other nucleosides, nucleotides, and polynucleotides will be discussed in later papers but the behaviour of guanine nucleosides differs sufficiently from that of the others to warrant separate discussion at this stage. Of the two different types of powerful methylating agents available—methyl esters of strong acids (*e.g.*, dimethyl sulphate) and diazomethane—we were especially interested in the less thoroughly investigated action of the latter. During the course of this work, two other groups [5,6] published findings which are in accord with some of our observations.

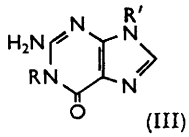
About fifteen years ago, Bredereck and his co-workers published two papers on the methylation of nucleosides with diazomethane [7] and with dimethyl sulphate,[8] respectively. In the first paper,[7] it was reported that, when 2′,3′,5′-tri-*O*-acetylguanosine (I; R = H, R′ = Ac) in methanol–acetone reacted with ethereal diazomethane, a precipitate of 1-methylguanosine (I; R = Me, R′ = H) was obtained. They showed later [9] that the unexpected deacetylation which accompanied the methylation was an example of a general exchange reaction of esters in alcoholic solution, which was catalysed by diazomethane. The structure of the methylguanosine rested on the claim that it was degraded by acid to

[1] Loveless, *Proc. Roy. Soc.*, 1959, *B*, **150**, 497; Bautz and Freese, *Proc. Nat. Acad. Sci., U.S.A.*, 1960, **46**, 1585.
[2] Dunn and Smith, *Nature*, 1955, **175**, 336; *Biochem. J.*, 1958, **68**, 627; Littlefield and Dunn, *ibid.*, 8p; Smith and Dunn, *ibid.*, 1959, **72**, 294; Adler, Weissmann, and Gutman, *J. Biol. Chem.*, 1958, **230**, 717; Dunn, *Biochim. Biophys. Acta*, 1961, **46**, 198.
[3] Szer and Shugar, *Acta Biochim. Polon.*, 1960, **7**, 491.
[4] Watson and Crick, *Nature*, 1953, **171**, 737.
[5] Brookes and Lawley, *J.*, 1961, 3923.
[6] Pfleiderer, *Annalen*, 1961, **647**, 167.
[7] Bredereck, *Chem. Ber.*, 1947, **80**, 401.
[8] Bredereck, Haas, and Martini, *Chem. Ber.*, 1948, **81**, 307.
[9] Bredereck, Sieber, and Kamphenkel, *Chem. Ber.*, 1956, **89**, 1169.

1-methylguanine (III; R = Me, R′ = H). In the second paper,[8] it was reported that treatment of guanosine with dimethyl sulphate at pH 4 yielded the same product (I; R = Me, R′ = H). Later, both Lawley[10] and Reiner and Zamenhof[11] noted that 7-methylguanine (IV) was a product of methylation of both deoxyguanylic acid and


(I)


(II)


(III)

deoxyribonucleic acid (DNA) with dimethyl sulphate in neutral aqueous solution. Lawley and Wallick[12] suggested that guanosine reacted with dimethyl sulphate to yield the betaine (II; R = OH, R′ = H), which was irreversibly destroyed by alkali.

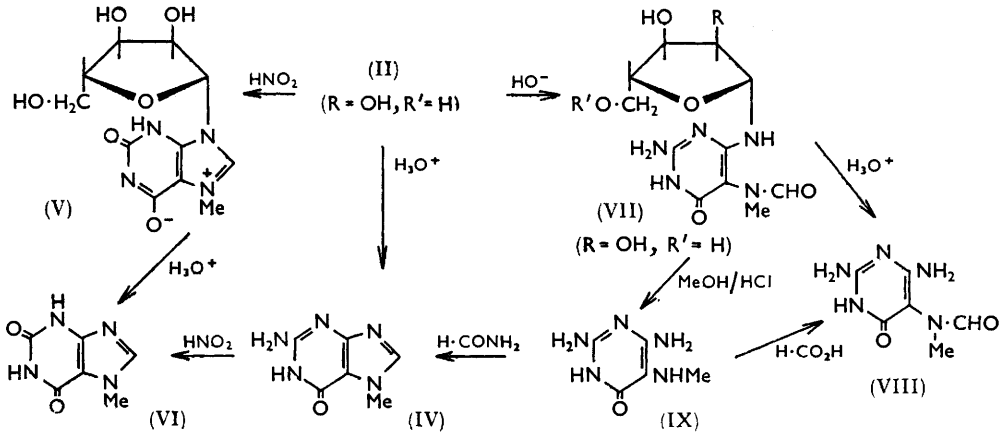
At about the same time, Ackermann and List[13] isolated herbipoline, a dimethylguanine, from Mediterranean sponges. This substance, which fluoresced in ultraviolet light, was later synthesised[14] by treating either 7- (IV) or 9-methylguanine (III; R = H, R′ = Me) with methyl toluene-*p*-sulphonate and thus shown to be 7,9-dimethylguanine. It was found[6] to be a strong base with pK_a >7. Very recently,[5] the corresponding 7,9-bis-2′-hydroxyethylguanine was shown to be attacked at position 8 by alkali with resultant cleavage of the imidazole ring.

We repeated Bredereck's reaction[7] between diazomethane and 2′,3′,5′-tri-*O*-acetylguanosine (I; R = H, R′ = Ac) and obtained a precipitate which crystallised from aqueous methanol in colourless plates. This compound fluoresced in ultraviolet light and was a strong base with pK_a 7·0; it gave analyses for methylguanosine. It consumed periodate and its ultraviolet absorption spectrum corresponded with that of 7,9-dimethylguanine. By the series of reactions described below, we have proved conclusively that the compound is the betaine, 7-methylguanosine, one of the mesomeric forms of which is represented by (II; R = OH, R′ = H) and has recently been shown to be one of the minor constituent nucleosides present in RNA.[15]

The glycosidic bond of compound (II; R = OH, R′ = H) was readily cleaved by 0·1N-hydrochloric acid on a steam-bath, yielding a methylguanine of which the infrared spectrum, the ultraviolet spectrum in neutral, acidic, and alkaline solutions, and the R_F's in several solvent systems were identical with those of authentic 7-methylguanine[6] (IV), and distinct from those of 1-methylguanine[16] (III; R = Me, R′ = H). An aqueous solution of 7-methylguanosine (II; R = OH, R′ = H) at pH 11 rapidly decomposed at room temperature into a single, non-fluorescent, ultraviolet-absorbing compound to which the structure (VII; R = OH, R′ = H) was allotted. This compound was a weak base which consumed periodate; it was readily cleaved by aqueous hydrochloric acid to yield its aglycone (VIII). The conversion of the guanosine (II; R = OH, R′ = H) into the opened compound (VII; R = OH, R′ = H) is analogous to the postulated ring opening of the 1,3-dimethylbenzimidazolinium ion with alkali.[17] Even more does it resemble the ring opening[5] of 7,9-bis-2′-hydroxyethylguanine. However, we found that the imidazole ring in the nucleoside (II; R = OH, R′ = H) was opened under much milder conditions

[10] Lawley, *Proc. Chem. Soc.*, 1957, 290.
[11] Reiner and Zamenhof, *J. Biol. Chem.*, 1957, **228**, 475.
[12] Lawley and Wallick, *Chem. and Ind.*, 1957, 633.
[13] Ackermann and List, *Z. physiol. Chem.*, 1957, **308**, 270; **309**, 286.
[14] Bredereck, Christmann, and Koser, *Chem. Ber.*, 1960, **93**, 1206.
[15] Dunn, personal communication.
[16] Traube and Dudley, *Ber.*, 1913, **46**, 3839.
[17] Smith, Rasmussen, and Ballard, *J. Amer. Chem. Soc.*, 1949, **71**, 1082.

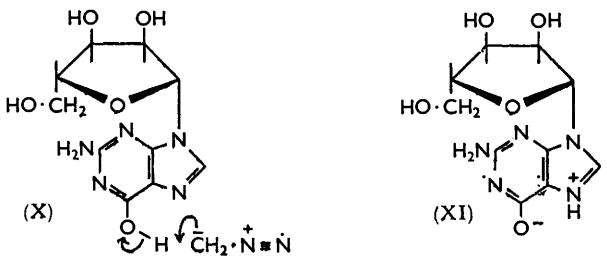
than it was in 7,9-dimethylguanine. When the nucleoside (VII; R = OH, R′ = H) was treated with methanolic hydrogen chloride, the de-formylated aglycone (IX) was obtained, and the pure aglycone (VIII) was obtained by the treatment of this base with formic acid.



This supports the formulation of (VIII) as an $N_{(5)}$-formyl compound, as 2,4,5-triamino-6-hydroxypyrimidine is formylated on N-5 by formic acid.[16] The expected[18] 7-methylguanine (IV) was obtained by heating compound (IX) in formamide solution.

7-Methylguanosine (II; R = OH, R′ = H) was converted by nitrous acid into another betaine, 7-methylxanthosine (V), which was readily hydrolysed by acid to 7-methylxanthine (VI) and, like (II; R = OH, R′ = H), was labile in alkaline solution.[19]

As it was our intention to investigate the action of diazomethane on nucleotides and nucleic acids, a technique for methylating aqueous solutions of these substances was developed. Such methylations were effected by shaking aqueous solutions of substrates with an excess of ethereal diazomethane. This proved to be a satisfactory general technique and was applied to guanosine with success. When a 10—25 molar excess of diazomethane was used, the only product other than starting material had the same electrophoretic mobility, the same R_F in several solvent systems, and the same ultraviolet absorption spectrum at several pH's as 7-methylguanosine (II; R = OH, R′ = H) described above. Further, the product was degraded by alkali in the same way. When a 50-molar excess of diazomethane was used, a second product, which so far has not been characterised, was indicated by paper chromatography. A greater than 100-molar excess of diazomethane led to a complex mixture of products. We found that guanosine reacted with dimethyl sulphate in aqueous buffer at pH 7 or pH 4, to yield 7-methylguanosine (II; R = OH, R′ = H).



As 9-methylguanine (III; R = H, R′ = Me) has been shown by spectroscopic measurements [6] to protonate on N-7, it was not unexpected that this was the most nucleophilic

[18] Robins, Dille, Willits, and Christensen, *J. Amer. Chem. Soc.*, 1953, **75**, 263.
[19] Pfleiderer and Nübel, *Annalen*, 1961, **647**, 155; Pfleiderer, *ibid.*, p. 161.

centre of guanosine in its reaction with dimethyl sulphate. However, in view of the observation that diazomethane methylated both uridine and thymidine [20] at position 1 and that it reacted with inosine [21] to yield a mixture of 1-methylinosine and 6-methoxypurine-riboside, 1-methylguanosine (I; R = Me, R′ = H) might have been the expected product from its reaction with guanosine. Eistert [22] suggested a mechanism for the methylation of active-hydrogen compounds by diazomethane whereby the latter abstracted the active proton to produce a methanediazonium cation which, in turn, methylated the resultant anion. This mechanism may be adapted to account for methylation of guanosine at position 7. By analogy with 2-pyridone [23] and to some extent inosine,[21] it may be assumed that the reaction proceeds by attack of diazomethane on one of the more acidic, less stable tautomers of guanosine. Proton abstraction by diazomethane from such a tautomer, indicated in (X), would produce methanediazonium ion in the vicinity of N-7 as well as of O-6. Solvation of this cation by N-7 would lead to the observed product (II; R = OH, R′ = H). Alternatively, 7-methylguanosine would be the expected product from the reaction between tautomer (XI) and diazomethane.

By the same technique of methylation in a two-phase system, diazomethane was shown to attack 2′-deoxyguanosine primarily at N-7, to yield the same product (II; R = R′ = H) as was obtained with dimethyl sulphate. Lawley's observation [10] that this compound (II; R = R′ = H) spontaneously yielded its aglycone, 7-methylguanine (IV), in neutral buffer at room temperature, was confirmed. However, the glycosidic bond was not broken at higher pH but, as with 7-methylguanosine (II; R = OH, R′ = H), the imidazole ring was opened to give the pyrimidine nucleoside (VII; R = R′ = H). As it was thought that the properties of deoxy-7-methylguanosine (II; R = R′ = H) might be important in the study of the mechanism of the mutagenic action of alkylating agents, its stability, or rather that of its 5′-phosphate monomethyl ester [24] [II; R = H, R′ = PO(OH)·OMe], was investigated. Aqueous solutions of this ester at various pH's were kept at room temperature and the products were examined by paper-chromatographic, paper-electrophoretic, and spectroscopic methods at suitable intervals. It was found that the glycosidic link of the ester was extremely labile at low pH and even moderately labile at pH 7 at room temperature. At pH 9, the opening of the imidazole ring to produce compound [VII; R = H, R′ = PO(OH)·OMe] competed with the deglycosidation which produced 7-methylguanine (IV). At higher pH's fission of the imidazole ring was the exclusive reaction and no 7-methylguanine was obtained. Therefore it seems likely that mutagenesis may result simply from dissociation [25] of the $N_{(1)}$-proton of the deoxyguanosine residue after the guanosine has been methylated, or else by cleavage of the glycosidic bond and loss of the purine base. The nucleotide derivative [II; R = H, R′ = PO(OH)·OMe] was used in this experiment rather than the simple nucleoside as the former was a dialkyl phosphate and thus more nearly resembled DNA, and also because it simplified electrophoretic analysis of the products.

Experimental

Ultraviolet absorption spectra were measured on a Cary recording spectrophotometer, model 14M-50. Paper electrophoresis on Whatman No. 1 paper was conducted in a CCl_4-cooled apparatus (33 v/cm.) with the following buffers: 0·2M-acetate, pH 3·5; 0·2M-acetate, pH 4·3; 0·05M-phosphate, pH 6·5; 0·05M-triethylammonium hydrogen carbonate, pH 8; 0·05M-borate, pH 9·1; 0·05M-borate, pH 10. Whatman No. 1 paper was used for ascending chromatography in all systems except D and E (below), for which Whatman No. 4 paper was used. The constitutions of the chromatographic systems were: A, propan-2-ol–ammonia (*d* 0·88)–water (7,1,2); B, butan-1-ol saturated with water; C, butan-1-ol–acetic

[20] Miles, *Biochim. Biophys. Acta*, 1956, **22**, 247; *J. Amer. Chem. Soc.*, 1957, **79**, 2565.
[21] Miles, *J. Org. Chem.*, 1961, **26**, 4761.
[22] Eistert, *Angew. Chem.*, 1941, **54**, 99.
[23] von Pechmann, *Ber.*, 1895, **28**, 1624.
[24] Khorana, *J. Amer. Chem. Soc.*, 1959, **81**, 4657.
[25] Lawley and Brookes, *Nature*, 1961, **192**, 1081; *J. Mol. Biol.*, 1962, **4**, 216.

acid–water (5,2,3); D, saturated aqueous ammonium sulphate–propan-2-ol–0·1M-phosphate buffer (pH 7·2) (79,2,19); E, saturated aqueous ammonium sulphate–propan-2-ol–water (79,2,19); F, butan-1-ol–85% formic acid–water (77,10,13); G, isobutyric acid–2N-aqueous ammonia (66,34); H, propan-2-ol–concentrated hydrochloric acid–water (65,16·7,18·3); I, butan-1-ol–0·6N-aqueous ammonia (6,1); J, 0·05M-borate buffer (pH 9); K, 0·05M-phosphate buffer (pH 7·2); L, butan-1-ol–acetic acid–water (4,1,1).

7-Methylguanosine (II; R = OH, R′ = H).—Bredereck's method [7] was used. To a solution of 2′,3′,5′-tri-*O*-acetylguanosine [7] (0·7 g.) in methanol (20 c.c.) and acetone (3 c.c.), cooled to 0°, ethereal diazomethane [26] (33 c.c., prepared from 3,3 g. of *N*-nitrosomethylurea) was added. Nitrogen was evolved and after about 5 min., 7-methylguanosine (II; R = OH, R′ = H) began to be precipitated. Dry ether (23 c.c.) was added to the mixture, which was then set aside at 0° overnight. The precipitate (0·27 g., 53%) was collected and washed with dry ether. The material crystallised from aqueous methanol in colourless plates, having a potentiometrically determined pK_a 7·0 (Found, in material dried over P_2O_5 *in vacuo* at 100° for 5 hr.: C, 44·2; H, 5·0; N, 23·2%; equiv., 298. $C_{11}H_{15}N_5O_5$ requires C, 44·4; H, 5·1; N, 23·6%; equiv., 297). *7-Methylguanosine* shows a strong blue fluorescence in ultraviolet light and has R_F 0·69 (system E). Paper chromatography of the filtrate from the above reaction mixture indicated the presence of several minor products.

Acid Hydrolysis of 7-Methylguanosine.—7-Methylguanosine (0·06 g.) was heated in 0·1N-hydrochloric acid (30 c.c.) at 100° for 1½ hr. 7-Methylguanine hydrochloride (0·03 g., 74%), precipitated from the concentrated (to 2 c.c.) solution, was collected by filtration. The free base (IV) was obtained by neutralising a concentrated aqueous solution of the hydrochloride with 0·1N-sodium hydroxide. It crystallised from water in fine needles (Found, in material dried over P_2O_5 *in vacuo* at 120° for 6 hr.: C, 43·7; H, 4·3; N, 42·2. Calc. for $C_6H_7N_5O$: C, 43·6; H, 4·3; N, 42·2%). Paper chromatography (systems A, D, F, G, I, J, K) and infrared and ultraviolet spectroscopy showed it to be 7-methylguanine [10] and not the 1-methyl isomer as previously suggested.[7] It had R_F 0·30 (system G).

Deamination of 7-Methylguanine (IV).—Sodium nitrite (0·5 g.) was added to a solution of 7-methylguanine (0·104 g.) in N-hydrochloric acid (20 c.c.). The solution was heated at 80° for 1 hr. and left for several days at room temperature, until deamination was complete (as indicated by paper chromatography, system D). A pale yellow precipitate of 7-methylxanthine (VI) (0·053 g., 50%) was obtained from the neutralised solution; it was recrystallised from water (charcoal) (Found, in material dried over P_2O_5 *in vacuo* at 120° for 4 hr.: C, 42·9; H, 4·1; N, 33·8. Calc. for $C_6H_6N_4O_2$: C, 43·4; H, 3·6; N, 33·7%). It was identical in its ultraviolet absorption (several pH's) and paper chromatographic [systems D (R_F 0·25), F, I, J, L] properties with 7-methylxanthine.

Deamination of 7-Methylguanosine (II; R = OH, R′ = H).—Aqueous nitrous acid [from barium nitrite (2·294 g.) and sulphuric acid] at 0° was added to a solution of 7-methylguanosine (0·15 g.) in water (25 c.c.). The mixture was kept at 0° for 30 days. Further small quantities of nitrous acid were added to it from time to time until paper chromatography (system E) and paper electrophoresis (pH 4·3) indicated that all the 7-methylguanosine had been consumed. The mixture was filtered and the filtrate lyophilised, to yield a chromatographically homogeneous (systems B, E, F) colourless oil. This material was assumed to be 7-methylxanthosine (V). It had R_F 0·55 (system E), showed a blue fluorescence in ultraviolet light, and had at pH 1 $\lambda_{max.}$ 262, $\lambda_{min.}$ 237 mμ ($\varepsilon_{max.}/\varepsilon_{min.}$, 1·55), at pH 7 $\lambda_{max.}$ 258, 286, $\lambda_{min.}$ 234, 267 mμ, and at pH 13 $\lambda_{max.}$ 254, 284, $\lambda_{min.}$ 233, 263 mμ initially but after 16 hr. $\lambda_{max.}$ 270, $\lambda_{min.}$ 244 mμ, which indicates alkali-promoted fission of the imidazole ring.

A small quantity of the material was dissolved in N-sulphuric acid and left at room temperature for several days, then neutralised with aqueous sodium hydroxide. The precipitate obtained was recrystallised from water. It showed the same paper chromatographic (systems A, D, F) and ultraviolet spectroscopic properties (several pH's) as 7-methylxanthine (VI).

Reaction between 7-Methylguanosine (II; R = OH, R′ = H) *and Aqueous Ammonia.*—7-Methylguanosine (0·20 g.) was dissolved in 2N-aqueous ammonia (25 c.c.) and set aside at room temperature for 2 hr. The products were lyophilised to a colourless, chromatographically homogeneous (systems A, D) glass. Paper electrophoresis (borate buffer, pH 10) and positive periodate reaction indicated a *cis*-glycol system. The material was purified by paper electrophoresis. It had at pH 1 $\lambda_{max.}$ 272, $\lambda_{min.}$ 246 mμ ($\varepsilon_{max.}/\varepsilon_{min.}$ 4·15), at pH 7 $\lambda_{max.}$ 273, $\lambda_{min.}$ 247

[26] Arndt, *Org. Synth.*, Collected Vol. II, Wiley, New York, 1943, p. 165.

mμ ($\varepsilon_{max.}/\varepsilon_{min.}$ 4·56), and at pH 13 $\lambda_{max.}$ 266, $\lambda_{min.}$ 244 mμ ($\varepsilon_{max.}/\varepsilon_{min.}$ 2·08). This substance was assigned the structure 2-amino-6-hydroxy-5-*N*-methylformamido-4-(*N*-β-ribofuranosylamino)-pyrimidine (VII). It had R_F 0·58 (system D). The same product was rapidly formed when 7-methylguanosine was allowed to decompose in 0·01N-aqueous sodium hydroxide at room temperature.

Acid Hydrolysis of Compound (VII).—A solution of compound (VII) (0·013 g.) in 85% formic acid (1 c.c.) was kept at room temperature for 4 days, and then neutralised. Paper chromatography (systems A, B, C, F, G) indicated that it had been completely converted into a new product (R_F 0·18 in system F) formulated as 2,4-diamino-6-hydroxy-5-*N*-methylformamido-pyrimidine (VIII). This material, purified by paper electrophoresis, had ultraviolet absorption, at several pH's, identical with that of the pure crystalline material (VIII) described below. Paper electrophoresis (borate buffer) of the products of this acid hydrolysis showed the presence of another non-ultraviolet-absorbent product with the same mobility as ribose.

Preparation of Pure 2,4-*Diamino*-6-*hydroxy*-5-N-*methylformamidopyrimidine* (VIII).—A solution of the nucleoside (VII) (from 0·13 g. of 7-methylguanosine) in methanolic hydrogen chloride (20 c.c.) was kept at 0° overnight. The colourless needles of the *hydrochloride* of 2,4-diamino-6-hydroxy-5-methylaminopyrimidine (IX) which separated were washed with cold methanol and dried (0·073 g., 87%). The products were chromatographically homogeneous (systems C, H) and had at pH 1 $\lambda_{max.}$ 262, $\lambda_{min.}$ 232 ($\varepsilon_{max.}/\varepsilon_{min.}$ 3·12), in 95% ethanol $\lambda_{max.}$ 267, $\lambda_{infl.}$ 236, $\lambda_{min.}$ 243 ($\varepsilon_{max.}/\varepsilon_{min.}$ 5·04), and at pH 13 $\lambda_{max.}$ 275, $\lambda_{infl.}$ 250, $\lambda_{min.}$ 231 ($\varepsilon_{max.}/\varepsilon_{min.}$ 2·16), and R_F 0·30 in system H.

A solution of the above hydrochloride of base (IX) in 98% formic acid (2 c.c.) was heated under reflux for 45 min., then lyophilised to yield a pale brown solid which was dissolved in water; the solution was decolorised with charcoal, concentrated *in vacuo* to small bulk, then carefully neutralised with 0·1N-aqueous sodium hydroxide, and ethanol (4 c.c.) was added. When the solution was concentrated on a water-bath, colourless needles of 2,4-*diamino*-6-*hydroxy*-5-N-*methylformamidopyrimidine* (VIII) separated. This compound recrystallised from aqueous ethanol (charcoal) (Found, in material dried *in vacuo* over P_2O_5 at 80° for 6 hr.: C, 39·0; H, 5·3; N, 38·2. $C_6H_9N_5O_2$ requires C, 39·3; H, 4·95; N, 38·2%). It had a potentiometrically determined pK_a 3·8, and R_F 0·42 in system H.

Conversion of the Hydrochloride of 2,4-*Diamino*-6-*hydroxy*-5-*methylaminopyrimidine* (IX) *into* 7-*Methylguanine* (IV).—A solution of the hydrochloride of base (IX) (0·047 g.) in formamide (0·5 c.c.) was heated under reflux for 15 min. The cooled mixture was diluted with water (0·5 c.c.) and left at 0° overnight. The brown precipitate was crystallised from water (charcoal) and shown to be chromatographically (systems A, B, C, D, G, H) and spectroscopically (ultraviolet spectra at pH 1, 7, and 13) identical with 7-methylguanine (IV).

Methylation of Guanosine (*in Aqueous Solution*) *with Diazomethane.*—The ethereal diazomethane used in this and subsequent experiments was estimated by titration with standardised benzoic acid. Solutions of guanosine (0·014 g., 5×10^{-5} mole) in water (20 c.c.) and varying amounts of ethereal diazomethane (1 c.c. = 5×10^{-4} mole) were placed in 50-c.c. flasks, which were closed by Bunsen valves. The flasks were shaken rapidly by a mechanical shaker until all the diazomethane was consumed. The aqueous phases were examined by paper chromatography (systems C, E, F). The results (system F) are listed below (w = weak, m = medium, s = strong are used to describe the relative strengths of the chromatographic spots):

Molar ratio, CH_2N_2:Guanosine	R_F (system F) of products
10	0·11(w), 0·17(s)
20	0·11(m), 0·17(s)
50	0·11(s), 0·17(s), 0·51(w)
100	0·11(s), 0·17(s), 0·51(m)

The material with R_F 0·17 was unchanged guanosine. The first methylation product (R_F 0·11) was purified by paper electrophoresis (pH 4·3) and shown to be 7-methylguanosine (II; R′ = H, R = OH) by paper chromatography (systems E, F), ultraviolet spectroscopy (pH 1 and 7), and paper electrophoresis (pH 4·3). The second methylation product (R_F 0·51), produced with a large excess of diazomethane, was not identified.

Methylation of Guanosine with Dimethyl Sulphate.[10]—(*a*) pH 7·2. Dimethyl sulphate (0·4 g.) was added to a stirred solution of guanosine (0·09 g.) in 0·05M-phosphate buffer (pH 7·2; 80 c.c.) during 1 hr. The temperature was kept at 37° and the pH was not allowed to fall

below 7. Barium acetate was added and the precipitate centrifuged. Besides guanosine, the supernatant liquid contained a single product which was electrophoretically (acetate buffer pH 4·3, borate buffer pH 9·1), chromatographically (systems D, E, F), and spectroscopically identical with 7-methylguanosine (II; R = OH, R′ = H).

(*b*) pH 4. Dimethyl sulphate (0·25 g.) was added to a stirred solution of guanosine (0·05 g.) in 0·4M-phosphate buffer (pH 4; 25 c.c.) in the manner described above. The pH was maintained at 4 by occasional addition of aqueous sodium hydroxide. The products were worked up in the same manner with the same result as described in (*a*) above.

Methylation of Deoxyguanosine (in Aqueous Solution) with Diazomethane.—A solution of deoxyguanosine (0·012 g., 1 mol.) in water (15 c.c.) was shaken, as above, with ethereal diazomethane (100 mol.). The products in the aqueous phase were separated by paper chromatography (system D, Whatman No. 3 paper). Besides starting material (R_F 0·32, system D), the main product had R_F 0·64 and showed a blue fluorescence in ultraviolet light. Another fluorescent product, with R_F 0·12, was also present. The material with R_F 0·64 had the same ultraviolet spectrum (pH 7) and chromatographic properties (systems D, E, F, I) as 7-methyldeoxyguanosine, prepared [10] by treating deoxyguanosine with dimethyl sulphate in phosphate buffer at pH 7·2. Also,[10] it decomposed in neutral aqueous solution at room temperature to 7-methylguanine (IV). Deoxy-7-methylguanosine at pH 6 had $\lambda_{max.}$ 257, $\lambda_{infl.}$ 274, $\lambda_{min.}$ 235 ($\varepsilon_{max.}/\varepsilon_{min.}$ 2·10; $\varepsilon_{max.}/\varepsilon_{infl}$ 1·37).

Experiments with smaller quantities of diazomethane showed that the material with R_F 0·64 was the first methylation product of deoxyguanosine.

Preparation and Hydrolysis of Deoxy-7-methylguanosine-5′ Methyl Hydrogen Phosphate [II; R = H, R′ = PO(OH)·OMe].—(*a*) A solution of deoxyguanosine-5′ methyl hydrogen phosphate [24] (0·005 g.) in water (1 c.c.) was treated with ethereal diazomethane as described above. The main product, other than starting material, had chromatographic (system D), electrophoretic, and ultraviolet-spectroscopic properties identical with those of the dimethyl sulphate product described in (*b*) below and used in the hydrolysis experiments.

(*b*) Dimethyl sulphate (0·106 g.) was added during 1 hr. to a stirred solution of deoxyguanosine-5′ methyl hydrogen phosphate (0·023 g.) in 4M-phosphate buffer (pH 7·2; 1 c.c.) maintained at 37°. The pH of the solution was not allowed to fall below 7. The solution was passed through a Dowex 1 × 2 (Cl^-; 4 cm. × 1·7 cm.^{2}) column which was eluted with water. Fractions of 2 c.c. were collected, and the required 7-methyldeoxyguanosine-5′ methyl hydrogen phosphate was found in fractions 2 and 3. This material showed a blue fluorescence in ultraviolet light and had R_F 0·58 (system D) compared with 0·28 for deoxyguanosine-5′ methyl hydrogen phosphate; on paper electrophoresis it migrated towards the anode at half the rate of the latter compound in buffer of pH 8. It had the ultraviolet absorption expected for a 7,9-dialkylguanine at pH 5: $\lambda_{max.}$ 256, $\lambda_{infl.}$ 278, $\lambda_{min.}$ 236 mμ ($\varepsilon_{260}/\varepsilon_{230}$ 0·66).

Portions (1 c.c.) of the above eluate containing deoxy-7-methylguanosine-5′ methyl hydrogen phosphate were adjusted to various pH's, which were measured by a pH meter. The subsequent reactions at these pH's were followed by ultraviolet spectroscopy, paper chromatography (system D), and paper electrophoresis (pH 8). At neutral and acidic pH's the glycosidic bond was cleaved to yield 7-methylguanine (IV). At alkaline pH's, the imidazole ring was cleaved to yield the pyrimidine [VII; R = H, R′ = PO(OH)·OMe] which had $\lambda_{max.}$ 266, $\lambda_{min.}$ 248 ($\varepsilon_{max.}/\varepsilon_{min.}$ 1·45). At pH 9, both reactions occurred as shown in Table 1.

TABLE 1.

pH	Reaction period (hr.)	Starting material (%) [II; R = H, R′ = PO(OH)·OMe]	7-Methylguanine (IV) (%)	Pyrimidine nucleoside [VII; R = H, R′ = PO(OH)·OMe] (%)
1	Very short	0	100	0
7	24	60	40	0
7	48	40	60	0
7	72	25	75	0
9	24	90	0	10
9	48	75	5	20
9	72	60	10	30
10	6	100	0	0
10	20	75	0	25
10	33	50	0	50
10	45	0	0	100
12	0·5	0	0	100

Ultraviolet Spectra.—These are collected in Table 2.

TABLE 2.

Ultraviolet absorption spectra (λ in mμ).

Compound	pH	$\lambda_{max.}$	log $\varepsilon_{max.}$	$\lambda_{min.}$	log $\varepsilon_{min.}$	$\lambda_{infl.}$	log $\varepsilon_{infl.}$
(IV)	1	272	3·86	268	3·86		
		249	4·04	227	3·67		
	7	282	3·87	260	3·59		
		247	3·77	236	3·74		
	13	280	3·89	257	3·61	237	3·69
(III; R = Me, R′ = H)	1	271	3·85	267	3·84		
		250	4·01	227	3·59		
	7	272	3·90	262	3·87		
		248	4·00	227	3·63		
	13	278	3·94	240	3·68	262	3·87
(VI)	0·8	266	3·96	241	3·40		
	4	268	3·97	241	3·40		
	7	268	4·01	240	3·45		
	13	288	3·92	255	3·28		
(VIII)	1	262	4·25	221	3·70		
	7	264	4·14	242	3·66	233	3·69
	13	261	3·99	243	3·76		
(II; R = OH, R′ = H)	2	258	4·00	229	3·34	277	3·84
	7	258	3·93	238	3·66		
		281	3·87	271	3·84		
	9·2	283	3·91	242	3·65	259	3·74
		219	4·24				

One of us (J. A. H.) thanks the Department of Scientific and Industrial Research for the award of a maintenance grant.

UNIVERSITY CHEMICAL LABORATORY, CAMBRIDGE. [*Received, July 10th,* 1962.]