Horowitz, J., and Kohlmeier, V. (1967), *Biochim. Biophys. Acta 142*, 208.
Lane, B. G. (1963), *Biochim. Biophys. Acta 72*, 110.
Leloir, L. F., and Cardini, E. E. (1957), *Methods Enzymol. 3*, 840.
Lindahl, T., Adams, A., and Fresco, J. R. (1967), *J. Biol. Chem. 242*, 3129.
Lindahl, T., Henley, D. D., and Fresco, J. R. (1965), *J. Am. Chem. Soc. 87*, 4961.
Lowrie, R. J., and Bergquist, P. L. (1968), *Biochemistry 7*, 1761.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193*, 265.
Muench, K. H., and Berg, P. (1966), *in* Procedures in Nucleic Acid Research, Cantoni, G. L., and Davies, D. R., Ed., New York, N. Y., Harper & Row, p 375.
Munyon, W., and Salzman, N. P. (1962), *Virology 18*, 95.
Nakada, D., and Magasanik, B. (1964), *J. Mol. Biol. 8*, 105.
Rosen, B. (1965), *J. Mol. Biol. 11*, 845.
Rubin, I. B., Kelmers, A. D., and Goldstein, G. (1967), *Anal. Biochem. 20*, 533.
Schleich, T., and Goldstein, J. (1964), *Proc. Natl. Acad. Sci. U. S. 52*, 744.
Soffer, R. L. (1964), *Biochim. Biophys. Acta 87*, 416.
Volkin, E., and Cohn, W. E. (1954), *Methods Biochem. Anal. 1*, 287.
Wahba, A. J., Gardner, R. S., Basilio, C., Miller, R. S., Sperer, J. F., and Lengyel, P. (1963), *Proc. Natl. Acad. Sci. U. S. 49*, 116.
Zubay, G. (1966), *in* Procedures in Nucleic Acid Research, Cantoni, G. L., and Davies, D. R., Ed., New York, N. Y., Harper & Row, p 455.

On the Reaction of Guanine with Glyoxal, Pyruvaldehyde, and Kethoxal, and the Structure of the Acylguanines. A New Synthesis of N^2-Alkylguanines*

Robert Shapiro,† Bertram I. Cohen,‡ Shian-Jan Shiuey, and Hans Maurer

ABSTRACT: The adducts of guanine with glyoxal, pyruvaldehyde, and kethoxal (β-ethoxy-α-ketobutyraldehyde) were prepared. The structure of the glyoxal–guanine adduct (Ib) is similar to that established for the analogous compound of glyoxal and guanosine (Shapiro, R., and Hachmann, J. (1966), *Biochemistry 5*, 2799). Cleavage of Ib by periodate yielded N^2-formylguanine (Va). The structures of the pyruvaldehyde–guanine adduct (IIa) and kethoxal–guanine adduct (IIb) were determined by periodate cleavage to N^2-acetylguanine (Vb) and N^2-α-ethoxypropionylguanine (Vc). The acylguanines Va and Vb were reduced by lithium aluminum hydride to the known alkylguanines: N^2-methylguanine (VIIa) and N^2-ethylguanine (VIIb). This established the point of attachment of the acyl group to guanine in the acylguanines. The above reduction constitutes a new synthetic procedure for N^2-alkylguanines. This was applied to the synthesis of N^2-benzylguanine (VIIc) and N^2-β,β,β-trifluoroethylguanine (VIId) from N^2-benzoylguanine (Vd) and N^2-trifluoroacetylguanine (Ve).

It was first reported by Staehelin (1959) that glyoxal and kethoxal[1] (β-ethoxy-α-ketobutyraldehyde) can react with and inactivate the RNA of tobacco mosaic virus. This was ascribed to a specific reaction of these α-ketoaldehydes with the guanine residues of the RNA. The product of reaction of guanosine with glyoxal was subsequently isolated in pure form in this laboratory, and the structure established as Ia (Shapiro and Hachmann, 1966). This adduct was found to be quite labile, and readily decomposed into its components if kept at a pH above 7 in the absence of glyoxal. A number of additional applications of glyoxal (Kochetkov *et al.*, 1967; Nakaya *et al.*, 1968) and kethoxal (Litt and Hancock, 1967) to the specific modification of nucleic acids have been reported recently. The cancerostatic activity of ketoaldehydes in general (Szent-Györgi *et al.*, 1967) and of pyruvaldehyde in particular (Együd and Szent-Györgi, 1968) have been stressed. The antiviral activity of these compounds had earlier been noted (Tiffany *et al.*, 1957).

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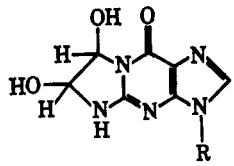
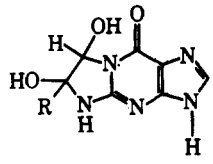
[1] Kethoxal is the registered trademark of the Upjohn Co., Kalamazoo, Mich.

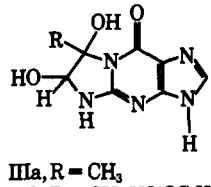


Ia, R = β-D-ribofuranosyl
b, R = H

One object of this investigation was the determination of the structures of the reaction products of guanine derivatives with kethoxal and pyruvaldehyde. While only one orientation (I) is possible in the reaction with glyoxal, two possible modes of addition, II or III, exist for the addition of an unsymmetrically substituted glyoxal to guanine. While both structures II and III have been written for these products (Staehelin, 1959, 1960; Litt and Hancock, 1967), no evidence exists on this



IIa, R = CH_3
b, R = $CH_3CH(OC_2H_5)$



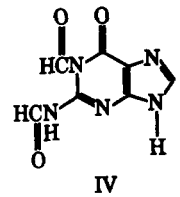
IIIa, R = CH_3
b, R = $CH_3CH(OC_2H_5)$

point. We wish to present proof here that the actual structures are represented by II. In the course of this work we have also developed a transformation of these substances, suitable for application to nucleic acids, a proof of the structures of the long-known acylguanines, and a new synthesis of N^2-alkylguanines.

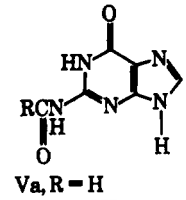
Results and Discussion

Structures of the Adducts of Guanine with Pyruvaldehyde and Kethoxal. The key to this proof of structure was the cleavage reaction of the adducts with periodate. This was first studied with the simpler glyoxal adduct. The already prepared glyoxal–guanosine adduct (Ia) was unsuitable for this study as the glycol group in the ribose unit would also react with periodate, so the preparation of the glyoxal–guanine adduct, Ib, was undertaken. Fortunately, this adduct separated readily from aqueous solution and could be isolated without the use of the tedious procedure employed for Ia. Its spectroscopic and chemical properties were quite similar to those of Ia. It reverted to glyoxal and guanine in ammonia solution and was deaminated to xanthine by nitrous acid. One stereochemical point is worthy of note. The nuclear magnetic resonance spectra of Ia and Ib, after exchange of the active hydrogens for deuterium, show sharp singlets for the protons on the carbon atoms derived from glyoxal. This absence of coupling is consistent with a dihedral angle of approximately 90° between these protons and a *trans* relationship of them to one another on the five-membered ring (Dyer, 1965).

Oxidation of the adduct Ib by periodate proceeded smoothly, and led to the formation of a single product in good yield. Although the formation of the diformylguanine, IV, was expected, the product obtained analyzed as a monoformylguanine. It was shown to have the structure Va, N^2-formylguanine. The method by

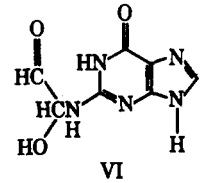


IV



Va, R = H
b, R = CH_3
c, R = $CH_3CH(OC_2H_5)$
d, R = C_6H_5
e, R = CF_3

which the point of attachment to guanine of the acyl group in this and other acylguanines was determined is described in the next section. Two reasonable suggestions can be made to explain the formation of a monoformylguanine in the periodate reaction. It may be that the attachment of a formyl group to the 1 position of guanine is an unstable one, and hydrolyzes quickly in aqueous solution. Alternatively, the reaction of Ib with periodate may be slow, because of the *trans* relation of the hydroxyl groups, and the oxidation may proceed through the open form, VI, present to a small extent in equilibrium with Ib.



VI

Attention was now turned to the reaction of pyruvaldehyde with guanine. In our previous paper (Shapiro and Hachmann, 1966), we reported that we had failed to detect any reaction between pyruvaldehyde and guanosine. In those studies, however, the pH of the reaction mixture was invariably below 5, either because of the deliberate addition of acetic acid or because of air oxidation of a part of the aldehyde to the corresponding carboxylic acid. In the present study it was found that the reactions went considerably faster at pH 7 than at acidic pH. For example, guanosine had required 6 days at 25° for conversion into the adduct Ia, but this was now found to occur within 15 min at pH 7. Pyruvaldehyde was similarly seen to convert guanosine rapidly into a new product at pH 7. A larger scale reaction of pyruvaldehyde with guanine went more slowly, due to the limited solubility of guanine at pH 7, but nonetheless went to completion. Unlike the glyoxal–guanine adduct, however, this product remained in solution. Attempts to isolate it by a number of methods involving crystallization, extraction, or ion-exchange chromatography led to its decomposition, or at best to the isolation of partly purified material contaminated with pyruvaldehyde self-condensation products. The behavior and ultraviolet spectrum of the pyruvaldehyde adduct resembled those of the glyoxal adduct, Ib, and it was assumed to

have either structure IIa or IIIa. The pyruvaldehyde adduct was cleaved *in situ* in the reaction mixture with excess periodate. Thin-layer chromatography indicated the formation of a single cleavage product. This was isolated and shown to be N^2-acetylguanine, Vb. No trace was seen of N^2-formylguanine, which might be expected from cleavage of IIIa by periodate. This established the structure of the pyruvaldehyde–guanine adduct as IIa.

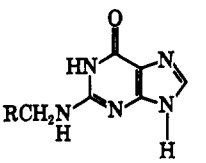
The adduct of kethoxal and guanine separated readily from aqueous solution. Its properties were analogous to those of the glyoxal adduct (Ib). It was cleaved by periodate to give a single product, N^2-α-ethoxypropionylguanine (Vc), in good yield. This adduct was assigned the structure IIb. An attempt was also made to obtain a reaction product of phenylglyoxal with guanine and guanosine at pH 7. After weeks of reaction, however, the guanine or guanosine was largely unaffected.

The formation of the structures II in preference to III in the reaction of guanine with pyruvaldehyde and kethoxal can be rationalized if it is assumed that the more reactive carbonyl group (the aldehyde) reacts first with the more reactive site on guanine. This is followed by a slower cyclization reaction between the ketone group and the less reactive site on guanine. In recent studies on the reaction of formaldehyde with nucleic acid components, imino groups (corresponding to N-1 of guanine) have been found to react more rapidly with formaldehyde than amino groups (Eyring and Ofengand, 1967).

In studies on the modification of nucleic acids with α-ketoaldehydes, difficulties have arisen because of the lability of the adducts in neutral and alkaline solution (Litt and Hancock, 1967; Kochetkov *et al.*, 1967). The periodate cleavage reaction of these adducts suggests a way of relieving this problem. Thus, treatment of a nucleic acid with an α-ketoaldehyde, followed by periodate, should lead to the specific acylation of the guanine residues of the nucleic acid. The identity of the acyl group would depend upon the α-ketoaldehyde used. Experiments are now in progress to test this hypothesis.

The Structure of the Acylguanines. Three monoacylguanines were produced by the cleavage reactions discussed above. Their similar spectroscopic properties strongly suggested that they were substituted at the same point in the guanine nucleus. Two of these, formylguanine and α-ethoxypropionylguanine, were new compounds, but the third, acetylguanine, was found to be identical with the product of reaction of guanine and hot acetic anhydride (Wulff, 1893). Although this compound had been known for over 70 years, and had been used for the synthesis of guanine nucleosides (Shabarova, *et al.*, 1959; Jenkins *et al.*, 1965), its structure had not been determined. The synthesis from it of 7- and 9-substituted guanines, which retained the acetyl group (Jenkins *et al.*, 1965), eliminated those sites as the point of attachment of the group. Acetylguanine was found to resist nitrous acid, which suggested substitution of the amino group. This structure, Vb, was confirmed by the discovery that acetylguanine could be reduced by lithium aluminum hydride in good yield to N^2-ethylguanine (VIIb) identical with a sample prepared by an alternative synthetic route (Elion *et al.*, 1956).

Lithium aluminum hydride has been used by several groups of workers to reduce acyladenines to alkyladenines (Baizer *et al.*, 1956; Bullock *et al.*, 1957; Lettré and Ballweg, 1958). The success of this reaction in the guanine series is somewhat more surprising because of the presence of a carbonyl group on the guanine ring. The only side product identified in the reduction was guanine. It is possible that the carbonyl group in the guanine nucleus was protected from reduction by the formation of the anion, VIII, during the reaction.

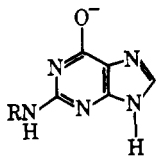


VIIa, R = H
b, R = CH_3
c, R = C_6H_5
d. R = CF_3

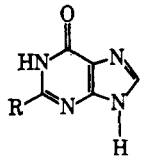
The application of the reduction reaction to formylguanine lead to the formation of the known (Elion *et al.*, 1956) N^2-methylguanine (VIIa), but in considerably less yield than for N^2-ethylguanine. The principal product of the reaction was guanine. Formylguanine is much more sensitive to alkaline hydrolysis than the acetyl compound. It is likely that the presence of traces of moisture in the reaction mixture catalyzed its hydrolysis to guanine. This reduction established the structure of formylguanine as Va. The structure of α-ethoxypropionylguanine was assigned as Vc on the basis of the similarity of this compound to formylguanine and acetylguanine. Its reduction was not attempted because of the limited supplies of kethoxal available and because the corresponding alkylguanine was unknown.

The acetyl group has been used as a protecting group for guanine nucleosides and nucleotides during oligonucleotide syntheses (Ralph *et al.*, 1963). No definite conclusion was arrived at about the location of the group. Because the ultraviolet spectral data reported for those compounds resemble the data determined here for N^2-acetylguanine, it is probable that in the acetylguanine nucleotides it is also the amino group that is acetylated.

A New Synthesis of N^2-Alkylguanines. The lithium aluminum hydride reduction reaction discussed above represents a new and facile synthetic route to N^2-alkylguanines. In the Experimental Section of this paper, an improved procedure for the preparation of N^2-acetylguanine from guanine is given. N^2-Ethylguanine can thus be prepared in two steps from guanine in 57% over-all yield. This compound was previously produced in 30% yield from the reaction of ethylamine with 2-methylmercaptopurin-6-(1H)-one (IXa), which itself was prepared *via* a lengthy synthetic route (Elion *et al.*, 1956). To test the usefulness of this synthetic route, two new alkylguanines were prepared. Reaction of guanine with hot benzoic anhydride was known to give a benzoylguanine (Wulff, 1893) to which we assign structure Vd. An improved preparation of this compound is



VIII



IXa, R = SCH_3
b, R = Cl

given in the Experimental Section. Lithium aluminum hydride reduction of N^2-benzoylguanine afforded N^2-benzylguanine (VIIc) in 35% yield. This compound was also synthesized from benzylamine and 2-chloropurin-6-(1*H*)-one (IXb) as a proof of structure. Reaction of guanine with trifluoroacetic anhydride yielded a moisture-sensitive acyl derivative assumed to be Ve. Reduction of this produced N^2-β,β,β-trifluoroethylguanine (VIId) in 38% yield.

Experimental Section

Ultraviolet spectra were obtained using a Perkin-Elmer 202 spectrophotometer. The spectra were taken in aqueous solution, in buffers of the indicated pH. Infrared spectra were run in KBr with a Perkin-Elmer Infracord spectrophotometer. Only prominent bands in the areas 2.8–4.0, 5.5–6.5, and 12–15 μ are reported. Nuclear magnetic resonance spectra were determined using a Varian A-60 instrument and are reported on the τ scale with tetramethylsilane (τ 10.00) as standard. When trifluoroacetic acid was used as the solvent, an external standard was employed. Melting points were obtained on a Thomas-Hoover capillary apparatus and are uncorrected. Thin-layer chromatography was carried out on Avicel microcrystalline cellulose (American Viscose Co., Marcus Hook, Pa.). After development of the chromatograms, ultraviolet-absorbing materials were located with the aid of an ultraviolet lamp equipped with a short-wavelength filter. The solvent systems employed were: solvent 1, 1-butanol–water (86:14); solvent 2, 2-propanol–water (7:3); solvent 3, isobutyric acid–ammonia–water (66:1:33); solvent 4, isoamyl alcohol–5% (w/v) aqueous Na_2HPO_4 (1:1); solvent 5, 1-butanol–30% aqueous acetic acid (2:1); solvent 6, 1-butanol saturated with 0.2 N aqueous ammonia; and solvent 7, 1-propanol–water (3:1). Reference R_F values for guanine are: solvent 1, 0.28; solvent 2, 0.58; solvent 3, 0.79; and solvent 4, 0.40. Microanalyses were performed either by Mr. George L. Robertson, Jr., Florham Park, N. J., or on an automatic CHN analyzer (F & M Scientific Corp., Model 185) at New York University, or in the analysis department of the Institute of Organic Chemistry, University of Heidelberg.

Preparation of the Glyoxal–Guanine Adduct (Ib). To 1020 ml of water were added 8.71 g (180 mmoles) of glyoxal monohydrate (British Drug Houses), 1.55 g (10 mmoles) of guanine, and 5 ml of glacial acetic acid. (In subsequent smaller scale runs, it was found that smaller glyoxal to guanine molar ratios could be used, and that the acetic acid was unnecessary.) The suspension was stirred for 21 hr at 60°. Analysis of the reaction mixture by thin-layer chromatography in solvents 2 and 3 indicated that the guanine had been completely converted into another product. The suspension was kept at 5° for 2 days and filtered, and the filtered product was washed with water and dried at 80° under vacuum. The yield was 1.86 g (88.5%) of the adduct Ib, as a white powder, which decomposed when heated to 220–235°, without melting: infrared absorption at 2.96, 3.20, 3.35, 3.68, 5.86, 6.21, 6.35, 12.85, 13.15, and 14.05 μ; ultraviolet maxima (pH 5) at 246 mμ (ϵ 10,600) and 279 mμ (ϵ 6300), minima at 230 and 263 mμ; nuclear magnetic resonance spectrum (CD_3SOCD_3), τ 1.78, broad (NH); 2.28, singlet (guanine H_8); 2.90, broad (OH); 3.56, broad (OH); 4.28, broad (CH); 5.10, broad (CH); on addition of D_2O, the peaks at τ 1.78, 2.90, and 3.56 disappeared and the peaks at 4.28 and 5.10 became sharp singlets; thin-layer chromatography, R_F 0.40 in solvent 1; R_F 0.73 in solvent 2; R_F 0.59 in solvent 3; and R_F 0.62 in water.

Anal. Calcd for $C_7H_7N_5O_3$: C, 40.20; H, 3.37; N, 33.48. Found: C, 40.33; H, 3.50; N, 33.25.

Reversion of Ib to Guanine. Samples of Ib were dissolved in buffer solutions of pH 2.3 (dilute HCl), 4.2 (sodium acetate), 6.2 (sodium phosphate), 8.1 and 9.0 (sodium borate), and 11.0 (NH_4OH). The reversion of Ib to guanine was followed by means of thin-layer chromatography in solvent 3. The reversion of the sample at pH 11 was complete within 75 min. The others were found to be stable at room temperature for 48 hr.

Reaction of Ib with Nitrous Acid. To 8 mg of Ib dissolved in 8 ml of sodium acetate buffer (pH 4.0) was added 2 ml of 1 N sodium nitrite solution. The solution was allowed to stand at room temperature overnight. The precipitate that formed was collected and dried at 80°. Its infrared spectrum and R_F on thin-layer chromatography in solvent 4 were identical with those of xanthine.

Preparation of N^2-Formylguanine (Va). To a suspension of 491 mg (2.3 mmoles) of Ib in 250 ml of water was added 5.00 g (23 mmoles) of sodium metaperiodate. The reaction mixture was stirred at room temperature for 18 hr. The white solid present was separated by filtration and washed with distilled water until the washings gave a negative test to starch–iodide paper. The solid was dried at 80° under vacuum for 24 hr to yield 322 mg (86%) of N^2-formylguanine (Va) as a white powder which did not melt below 300°; infrared absorption at 3.21, 3.45, 3.92, 5.80, 5.95, 6.35, 12.70, 13.54, 14.28, and 14.50 μ; ultraviolet maximum (pH 1) at 258 mμ, minimum at 228 mμ; maximum (pH 7) at 261 mμ (ϵ 15,000), minimum at 237 mμ; nuclear magnetic resonance spectrum (CF_3CO_2H), τ −0.65, broad (NH); 0.72, singlet (HCO); and 1.15, singlet (guanine H_8); thin-layer chromatography, R_F 0.33 in solvent 1; R_F 0.54 in solvent 2; R_F 0.72 in solvent 3; R_F 0.39 in solvent 4; and R_F 0.37 in 1-butanol–formic acid–water (77:10:13).

Anal. Calcd for $C_6H_5N_5O_2$: C, 40.23; H, 2.81; N, 39.03. Found: C, 40.30; H, 2.92; N, 39.15.

Stability to Hydrolysis of N^2-Formylguanine. Dilute solutions of N^2-formylguanine (Va) were prepared in buffer solutions of the following pH values: 1.0 (HCl), 3.8 (sodium acetate), 6.9 (sodium phosphate), 9.0 and

9.9 (sodium borate), and 12.6 (NaOH). Aliquots of the reaction mixtures were withdrawn from time to time and adjusted to pH 7 with sodium phosphate buffer. The hydrolysis to guanine was followed by noting the changes in the ultraviolet spectra with time. The samples at pH values from 3.8 to 9.0 were unaffected after 4 days at room temperature. The sample at pH 9.9 showed little decomposition after 23 hr, but was largely converted to guanine after 4 days. The samples at pH 1.0 and 12.0 had partially hydrolyzed to guanine within 45 min.

Preparation of the Kethoxal–Guanine Adduct (IIb). To 35 ml of water was added 2 ml of a 28.3% solution (565 mg, 4.35 mmoles) of kethoxal (the Upjohn Co., Kalamazoo, Mich.)and 200 mg(1.32 mmole)of guanine. The reaction mixture was stirred at 60° and the progress of the reaction followed by thin-layer chromatography in solvents 1 and 2. After 18 hr, the guanine had been converted completely to one new product. The suspension was cooled to 5°, kept at that temperature for 2 days, and filtered. The white solid obtained was washed with 25 ml of water and dried at 80° under vacuum to give 284 mg (77%) of the adduct IIb, which when heated decomposed without melting at 230–250°; infrared absorption at 3.07, 3.17, 3.36, 3.61, 5.94, 6.25, 6.34, 12.46, 12.74, 13.33, and 13.92 μ; ultraviolet maxima (pH 7) at 246 mμ (ϵ 10,000) and 279 mμ (ϵ 6100), minima at 236 and 265 mμ; nuclear magnetic resonance spectrum (CF_3CO_2H, −10°), τ 1.38, singlet (guanine H_8); 1.76, broad (NH); 4.09, singlet (*H*COH); 6.20–6.90, multiplet, two protons (CH_2O); and 8.80–9.40, multiplet, six protons (CH_3C); thin-layer chromatography, R_F 0.53 in solvent 1; R_F 0.82 in solvent 2; R_F 0.91 in solvent 3; and R_F 0.79 in solvent 4.

Anal. Calcd for $C_{11}H_{15}N_5O_4$: C, 46.97; H, 5.38; N, 24.91. Found: C, 46.60; H, 5.43; N, 25.52.

Preparation of N^2-α-Ethoxypropionylguanine (Vc). To 35 ml of water was added 100 mg (0.36 mmole) of IIb and 452 mg (2.1 mmoles) of sodium metaperiodate. The suspension was stirred at room temperature for 18 hr and gradually became a clear solution. Thin-layer chromatography (solvent 1) indicated that IIb had been completely converted into a new substance. The solution was evaporated to dryness under vacuum and the residue was extracted twice with 35-ml portions of 2-propanol. The extracts were filtered while hot to yield 81 mg (90% crude) of the product, Vc. An analytical sample (mp 234–235° dec) was prepared by recrystallization from ethanol–chloroform (1:1) and dried at 56° under vacuum for 14 hr; infrared absorption at 3.20, 3.39, 5.83 (sh), 5.93, 6.20, 6.37, 12.75, and 13.95 μ; ultraviolet maximum (pH 1) at 260 mμ (ϵ 19,100), minimum at 225 mμ; maximum (pH 7) at 259 mμ (ϵ 13,800), shoulder at 280 mμ, minimum at 231 mμ; maximum (pH 10.8) at 267 mμ, minimum at 232 mμ; nuclear magnetic resonance spectrum (CF_3CO_2H), τ 0.84, singlet (guanine H_8); 5.54, quartet, J = 7 Hz, one proton ($HCOCH_2CH_3$); 6.07, quartet, J = 7 Hz, two protons (CH_2O); 8.32, doublet, J = 7 Hz, three protons ($CH_3CHC{=}O$); and 8.52, doublet, J = 7 Hz, three protons (CH_3CH_2); thin-layer chromatography, R_F 0.85 in solvent 1; R_F 0.94 in solvent 2; and R_F 0.98 in solvent 3.

Anal. Calcd for $C_{10}H_{13}N_5O_3 \cdot H_2O$: C, 44.61; H, 5.62; N, 26.01. Found: C, 44.77; H, 5.51; N, 25.56.

Reaction of Guanosine with Pyruvaldehyde. To 20 ml of 0.043 M NaH_2PO_4 buffer solution (pH 7) was added 50 mg (0.18 mmole) of guanosine and 170 mg (approximately 2.2 mmoles) of pyruvaldehyde (J. T. Baker, distilled under vacuum). The progress of the reaction was followed by ultraviolet spectroscopy (as compared to a control reaction lacking guanosine). After 5 min at room temperature the reaction appeared to be complete. The spectrum resembled the corresponding adduct of glyoxal and guanosine (Shapiro and Hachmann, 1966). Its maximum was at 252 mμ with a shoulder at 277 mμ ($\epsilon_{277}/\epsilon_{252}$ 0.49). The R_F of the product in solvent 2 was 0.86.

Reaction of Guanine with Pyruvaldehyde. Preparation of N^2-Acetylguanine by Periodate Cleavage of the Adduct. To 75 ml of 0.043 M sodium phosphate buffer (pH 7.0) was added 250 mg (1.65 mmoles) of guanine and 4.00 g (about 52 mmoles) of pyruvaldehyde (J. T. Baker, distilled under vacuum). The reaction mixture was heated for 18 hr at 65° and all of the guanine was seen to dissolve. Thin-layer chromatography indicated that the guanine had been completely converted into a single new product (IIa): R_F in solvent 1, 0.40; R_F in solvent 2, 0.90; R_F in solvent 3, 0.84. The ultraviolet spectrum of the reaction mixture showed maxima at 249 and 277 mμ, minima at 230 and 265 mμ.

To this reaction mixture, cooled in a bath at 5°, was added 20 g (94 mmoles) of sodium metaperiodate. The solution was diluted with 50 ml of water and stirred at 25° for 18 hr. It was then evaporated to dryness under vacuum and the residue extracted with a hot mixture of 2-propanol–water (9:1). The mixture was filtered and the solids were washed with additional hot solvent. The filtrate was evaporated to dryness and the residue stirred with 50 ml of cold water. The aqueous extract was decanted and discarded. The water-insoluble solids were extracted with warm (70°) dimethyl sulfoxide and the cooled, filtered solution was diluted with 30 ml of water. This was kept at 5° and the precipitate that formed was collected. It was recrystallized from 2-propanol–water (50:50) and dried under vacuum at 56° for 18 hr. This yielded 26 mg (10%) of N^2-acetylguanine (Vb) identical in its infrared spectrum, ultraviolet spectra, and thin-layer chromatography R_F in solvent 1 with the product produced by acetylation of guanine.

Reduction of N^2-Formylguanine (VA) to N^2-Methylguanine (VIIA). This reaction was run in a drybox under a nitrogen atmosphere. To 200 mg (1.11 mmoles) of N^2-formylguanine (dried at 78° under vacuum, and finely pulverized) and 900 mg of well-pulverized lithium aluminum hydride (Metal Hydrides, Inc., Beverly, Mass.) was added 100 ml of absolute ether (dried by refluxing with lithium aluminum hydride for 45 min). The reaction mixture was stirred at room temperature for 7 days. The reaction flask was removed from the drybox and cooled to 0°, and 35 ml of ethanol was added to it drop by drop, to decompose the excess lithium aluminum hydride. The solvents were removed by evaporation under a stream of nitrogen and the discolored residue was dried in an oven at 90°. This residue, consisting largely of hydrated ethoxides of lithium and

aluminum, weighed 6.32 g. A 50-mg aliquot was dissolved in 1 N HCl and worked up by preparative thin-layer chromatography, using 2-mm-thick plates. The plates were developed by running three times in solvent 1. Two principal mobile bands were observed which corresponded in R_F to N^2-methylguanine and guanine. The yields of each were determined by eluting the bands with hot water and determining the amount of each substance from the absorption shown at its ultraviolet maximum (read against a suitable blank derived from an empty cellulose plate). The calculated yields were: guanine, 76%; N^2-methylguanine, 12%. The guanine was identified further by its ultraviolet spectra at pH 1, 7, 11, and 14. For the definite identification of N^2-methylguanine, ten thin-layer plates were run. The combined eluates of N^2-methylguanine were evaporated to dryness and the light yellow solid obtained was recrystallized from ammonia solution, washed with warm water, and dried. The sample was identical in the following properties with an authentic sample of N^2-methylguanine (Elion *et al.*, 1956) recrystallized in the same manner; infrared absorption at 3.14, 3.30, 3.41, 3.60, 3.80, 5.94, 6.20, 12.80, and 13.85 μ; ultraviolet spectra at pH 1.2, 6.8, 10.8, and 13.5 (these have been tabulated (Shapiro, 1968)); thin-layer chromatography, R_F 0.53 in solvent 5; R_F 0.40 in solvent 6; R_F 0.38 in 2-propanol–6 N HCl (68:32); and R_F 0.17 (as hydrochloride) in 2-propanol–H_2O (95:5).

Preparation of N^2-Acetylguanine from Guanine. To 2.0 g (133 mmoles) of dried, finely pulverized guanine was added 30 ml of acetic anhydride and the reaction mixture was heated at reflux for 20 hr. The mixture bumped initially, but this subsided as the guanine went into solution. Crystals began to separate from the solution after 12-hr heating. After cooling, the excess acetic anhydride was removed by extracting the mixture three times with a total of 200 ml of ether. The filtered, ether-insoluble residue was recrystallized from 500 ml of water, and then twice from ethanol–water (7:3), using charcoal the first time. A yield of 2.2 g (86%) of N^2-acetylguanine was obtained, as white needles that did not melt below 350°; infrared absorption at 3.15, 5.90 (sh), 6.05, 6.24 (sh), 12.15, 12.50, 12.75, 13.70, and 13.90 μ; ultraviolet maximum (pH 1) at 260 mμ minimum at 224 mμ; maximum (pH 7) at 260 mμ (ϵ 14,200), shoulder at 275 mμ, minimum at 230 mμ; maximum (pH 11) at 265 mμ, minimum at 241 mμ; maximum (pH 14) at 268 mμ, minimum at 245 mμ; nuclear magnetic resonance spectrum (CD_3SOCD_3), τ 1.94, singlet (guanine H_8); 7.81, singlet, three protons ($CH_3C{=}O$); in CF_3CO_2H, these protons appeared at τ 1.34 and 7.88, the NH protons were not observed; thin-layer chromatography, R_F 0.45 in solvent 1; R_F 0.44 in solvent 2; and R_F 0.80 in solvent 3; paper chromatography, R_F 0.55 in solvent 7.

Anal. Calcd for $C_7H_7N_5O_2$: C, 43.52; H, 3.65; N, 36.25. Found: C, 43.35; H, 3.79; N, 35.96.

Reduction of N^2-Acetylguanine (VB) to N^2-Ethylguanine (VIIB). To a suspension of 657 mg (3.40 mmoles) of finely pulverized, dry N^2-acetylguanine in 250 ml of dry (refluxed over lithium aluminum hydride and distilled) tetrahydrofuran was added 1.4 g of finely powdered lithium aluminum hydride (Metal Hydrides, Inc., Beverly, Mass.). The reaction mixture, protected from moisture by a drying tube, was heated at reflux, with stirring, for 48 hr. It was then cooled to 0°, 40 ml of absolute ethanol was added slowly, and the solvents were distilled. The residue was pulverized and heated with 200 ml of 5% ammonia for 1 hr, and the suspension was filtered. The filtrate was concentrated under vacuum to 75 ml, and 2 N HCl was added dropwise from a buret. As the pH fell to 11.2 (10.4 ml of HCl added), a precipitate of aluminum hydroxide appeared. It was quickly separated by filtration and the addition of HCl to the solution was continued. When the pH fell to 10.6 (an additional 6.2 ml of HCl had been added), crystallization of N^2-ethylguanine began. After 30 min, the crystals were collected by filtration. By concentration and cooling of the filtrate, additional crops of crystals were obtained. The combined crops weighed 532 mg (87% crude yield). Thin-layer chromatography (solvent 1) showed the product to be slightly contaminated with guanine. By repeated recrystallization from water, 404 mg (66%) of pure N^2-ethylguanine was obtained, identical in its infrared spectrum, ultraviolet spectra at pH 1, 7, 11, and 13.5, and thin-layer chromatography in the solvents listed below with an authentic sample of N^2-ethylguanine (Elion, *et al.*, 1956) purified by recrystallization; melting point, none below 360°; infrared absorption at 3.10, 3.25, 3.40, 3.60, 5.92, 6.20, 12.68, and 14.13 μ; ultraviolet maxima (pH 1) at 251 and 280 mμ, minima at 227 and 272 mμ; maxima (pH 7) at 244 and 278 mμ, minima at 224 and 264 mμ; maxima (pH 11) at 244 and 275 mμ, minima at 235 and 261 mμ; maximum (pH 14) at 275 mμ, shoulder at 254 mμ, minimum at 241 mμ; nuclear magnetic resonance spectrum (CF_3CO_2H), τ 1.12, singlet (guanine H_8); 6.27, quartet, J = 7 Hz, two protons (CH_2); and 8.52, triplet, J = 7 Hz, three protons (CH_3); thin-layer chromatography, R_F 0.54 in solvent 1; R_F 0.56 in solvent 5; R_F 0.96 in 2-propanol–water (9:1); and R_F 0.39 in saturated aqueous ammonium bicarbonate.

Anal. Calcd for $C_7H_9N_5O$: C, 46.91; H, 5.06; N, 39.08. Found: C, 47.00; H, 5.05; N, 39.40.

Preparation of N^2-Benzoylguanine. Well-dried guanine (5.0 g, 33 mmoles) and benzoic anhydride (25 g, 110 mmoles) were heated together for 16 hr at 130°, with stirring and exclusion of moisture. After cooling, the green melt was pulverized and extracted several times with ether to remove excess benzoic anhydride. The ether-insoluble residue was heated for 10 min with 700 ml of water, and the suspension was filtered. The filtrate was cooled to 0° and deposited yellowish crystals of N^2-benzoylguanine. These were collected by filtration and the filtrate was used for another extraction of the ether-insoluble residue. This cycle was repeated ten times. The combined crops of product were recrystallized several times from ethanol–water (1:1) to give 2.97 g (33%) of N^2-benzoylguanine monohydrate as white needles which melted with decomposition between 315 and 340°; infrared absorption at 3.01, 3.27, 3.42, 3.62, 5.91, 6.02, 6.20, 6.36, 12.82, 13.88, 14.37, and 14.70 μ; ultraviolet maximum (pH 1) at 267 mμ, shoulder at 247 mμ, minimum at 222 mμ; maxima (pH 5)

at 236 and 265 mμ, shoulder at 280 mμ, minima at 222 and 246 mμ; maxima (pH 10) at 233 and 268 mμ, shoulder at 310 mμ, minimum at 254 mμ; maxima (pH 12) at 235, 276, and 316 mμ, minima at 248 and 300 mμ; thin-layer chromatography, R_F 0.37 in solvent 6; paper chromatography, R_F 0.61 in solvent 1; R_F 0.72 in solvent 7.

Anal. Calcd for $C_{12}H_9N_5O_2 \cdot H_2O$: C, 52.75; H, 4.06; N, 25.63. Found: C, 52.76; H, 4.07; N, 26.05.

By recrystallization of the monohydrate three times from glacial acetic acid, with exclusion of moisture, followed by drying the product at 130° over KOH under vacuum, a product free of water was obtained.

Anal. Calcd for $C_{12}H_9N_5O_2$: C, 56.46; H, 3.55; N, 27.44. Found: C, 56.40; H, 3.63; N, 27.45.

Reduction of N^2-Benzoylguanine (VD) to N^2-Benzylguanine (VIIC). To 350 mg (1.28 mmoles) of finely pulverized, dry (100°, 2 days under vacuum over P_2O_5) benzoylguanine monohydrate and 800 mg of lithium aluminum hydride was added 250 ml of tetrahydrofuran (dried by refluxing 45 min with lithium aluminum hydride and distilling). The reaction mixture was heated at reflux for 48 hr with stirring and exclusion of moisture, then cooled to 0°. The excess lithium aluminum hydride was decomposed by the slow addition of 7 ml of H_2O. The solvents were distilled and the residue was pulverized and dried at 100° for 2 hr. The powder was heated at reflux with 80 ml of absolute ethanol for 30 min, and the suspension was filtered. This procedure was repeated with the residue and an additional 80 ml of ethanol. The combined filtrates were evaporated to dryness on the steam bath and 50 ml of water was added to the residue. The precipitate observed (aluminum hydroxide) was removed by filtration and to the filtrate (pH 11.2) 2 N HCl was slowly added. Precipitation began when the pH had fallen to 9.6, and the HCl addition was continued until the pH reached 9.3. The suspension was cooled to 0° and filtered to separate the product (N^2-benzylguanine). By concentration and cooling of the filtrate, additional crops of product were obtained. The crude yield (132 mg) was purified by recrystallization to give 108 mg (35%) of N^2-benzylguanine as white needles; melting point 275–276° dec; infrared absorption at 3.20, 3.38, 3.55, 5.94, 6.22, 6.30 (sh), 12.79, 13.37, and 14.15 μ; ultraviolet maxima (pH 1) at 252 and 280 mμ, minima at 230 and 274 mμ; maxima (pH 7) at 247 and 280 mμ, minima at 227 and 267 mμ; maxima (pH 11) at 246.5 and 277 mμ, minima at 240 and 266 mμ; maximum (pH 13.5) at 278 mμ, shoulder at 257 mμ, minimum at 247 mμ; nuclear magnetic resonance spectrum (CF_3CO_2H), τ 1.56, singlet (guanine H_8); 3.04, singlet, five protons (benzene H); and 5.71, singlet, two protons (CH_2); thin-layer chromatography, R_F 0.78 in solvent 6; paper chromatography, R_F 0.84 in solvent 1 and R_F 0.89 in solvent 7.

Anal. Calcd for $C_{12}H_{11}N_5O$: C, 59.73; H, 4.59; N, 29.03. Found: C, 59.88; H, 4.72; N, 28.77.

Preparation of N^2-Benzylguanine (VIIC) from 2-Chloropurin-6(1H)-one (IXB). The starting material (IXb) had been prepared by Montgomery and Holum (1957). As the use of their procedure gave a mixture of products, it was altered to the following procedure. 2,6-Dichloropurine was heated at 100° with a three molar excess of 1 N KOH for 3 hr. The solution was neutralized with HCl and cooled at 0° for 1 hr. The amorphous precipitate was recrystallized from water.

To 200 mg (1.17 mmoles) of IXb was added 15 ml (137 mmoles) of benzylamine, and the reaction mixture was heated at 120° for 2 hr. After cooling, the excess benzylamine was removed by extraction with ether. The ether-insoluble residue was recrystallized two times from water–ethanol (7:3) to yield 260 mg (91%) of N^2-benzylguanine as white needles. The infrared and ultraviolet spectra and R_F values of this product were identical with that of the product of lithium aluminum hydride reduction of N^2-benzoylguanine.

Preparation of N^2-Trifluoroacetylguanine (VE) and Reduction of It to N^2-β,β,β-Trifluoroethylguanine (VIID). To 1 g (6.6 mmoles) of guanine was added 10 ml of trifluoroacetic anhydride and the reaction mixture was heated at reflux for 6 hr. The guanine gradually dissolved, and thick prisms separated from the solution. The excess anhydride was removed by distillation, and the residue was dried under vacuum to give a white, amorphous powder. This was recrystallized several times from absolute (dried over sodium) tetrahydrofuran to give 1.12 g (68.5%) of VIId as needles which did not melt below 360°; ultraviolet maxima (ethanol) at 237 and 261 mμ, shoulders at 268 and 291 mμ, minima at 234 and 239 mμ. The compound was rapidly hydrolyzed to guanine by moisture, even during the time required for analysis. The presence of water indicated by the analysis may be due to partial hydrolysis.

Anal. Calcd for $C_7H_4N_5O_2F_3 \cdot 0.5H_2O$: C, 32.83; H, 1.97; N, 27.30. Found: C, 32.99; H, 2.13; N, 26.78.

To a suspension of 750 mg (3.0 mmoles) of N^2-trifluoroacetylguanine in 10 ml of dry tetrahydrofuran was added 60 ml of 0.5 M solution of lithium aluminum hydride in tetrahydrofuran. After the vigorous reaction had subsided, the reaction mixture was heated for 3 hr at reflux and the excess lithium aluminum hydride decomposed with water. The solvents were distilled and the residue was extracted with 200 ml of hot ethanol. The alcohol solution was evaporated to dryness. This residue was dissolved in a small amount of water and the resulting alkaline solution slowly neutralized by addition of 2 N HCl. As the pH fell to 10, a precipitate of product mixed with aluminum hydroxide appeared. This was removed, and on continued neutralization a precipitate of almost pure N^2-β,β,β-trifluoroethylguanine (VIId) was obtained. Recrystallization from water yielded 270 mg (38%) of product as needles which decomposed when heated above 300°; ultraviolet maxima (pH 1) at 249 and 275 mμ, minima at 224 and 268 mμ; maxima (pH 11) at 242 and 274 mμ, minima at 234 and 258 mμ; paper chromatography, R_F 0.79 in solvent 1 and R_F 0.86 in solvent 7.

Anal. Calcd for $C_7H_6N_5OF_3$: C, 36.05; H, 2.59; N, 30.03. Found: C, 35.93; H, 2.79; N, 30.31.

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References

Baizer, M. M., Clark, J. R., Dub, M., and Loter, A. (1956), *J. Org. Chem. 21*, 1276.

Bullock, M. W., Hand, J. J., and Stokstad, E. L. R. (1957), *J. Org. Chem. 22*, 568.

Dyer, J. D. (1965), Applications of Absorption Spectroscopy of Organic Compounds, Englewood Cliffs, N. J., Prentice-Hall, p 117.

Együd, L. G., and Szent-Györgi, A. (1968), *Science 160*, 1140.

Elion, G. B., Lange, W. H., and Hitchings, G. H. (1956), *J. Am. Chem. Soc., 78*, 217.

Eyring, E. J., and Ofengand, J. (1967), *Biochemistry 6*, 2500.

Jenkins, S. R., Holly, F. W., and Walton, E. (1965), *J. Org. Chem. 30*, 2851.

Kochetkov, N. K., Budowsky, E. I., Broude, N. E., and Klebanova, L. M. (1967), *Biochim. Biophys. Acta 134*, 492.

Lettré, H., and Ballweg, H. (1958), *Chem. Ber. 649*, 114.

Litt, M., and Hancock, V. (1967), *Biochemistry 6*, 1848.

Montgomery, J. A., and Holum, L. B. (1957), *J. Am. Chem. Soc. 79*, 2185.

Nakaya, K., Takenaka, O., Horinishi, H., and Shibata, K. (1968), *Biochim. Biophys. Acta 161*, 23.

Ralph, R. K., Conners, N. J., Schaller, H., and Khorana, H. G., (1963), *J. Am. Chem. Soc. 85*, 1983.

Shabarova, Z. A., Polyakova, Z. P., and Prokof'ev, M. A. (1959), *Z. Obshch. Khim. 29*, 215; *J. Gen. Chem. USSR 29*, 218.

Shapiro, R. (1968), *Progr. Nucleic Acid Res. Mol. Biol. 8*, 73.

Shapiro, R., and Hachmann, J. (1966), *Biochemistry 5*, 2799.

Staehelin, M. (1959), *Biochim. Biophys. Acta 31*, 448.

Staehelin, M. (1960), *Experentia 16*, 473.

Szent-Györgi, A., Együd, L. G., and McLaughlin, J. A. (1967), *Science 155*, 539.

Tiffany, B. D., Wright, J. B., Moffett, R. B., Heinzelman, R. V., Strube, R. E., Aspergen, B. D., Lincoln, E. H., and White, J. L. (1957), *J. Am. Chem. Soc. 79*, 1682.

Wulff, C. (1893), *Z. Physiol. Chem. 17*, 469.