

766. *The Alkylation of Guanosine and Guanylic Acid.*

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Alkylation of guanosine with diethyl sulphate, 1,4-dimethanesulphonyloxybutane, ethylene oxide, and butadiene dioxide, and of guanylic acid with di-(2-chloroethyl)methylamine, gave products which yielded 7-alkylguanines on acid hydrolysis. The difunctional alkylating agents gave also di(guanin-7-yl) derivatives.

Further alkylation of 7-2'-hydroxyethylguanine with ethylene oxide gave 7,9-di-(2-hydroxyethyl)guanine. This was unstable in alkali, undergoing fission of the imidazole ring to yield a substituted 2,4,5-triamino-6-hydroxypyrimidine.

METHYLATION of guanosine and guanylic acid occurs at N₍₇₎ since the products yield 7-methylguanine on acid hydrolysis; ¹⁻³ and di-(2-chloroethyl) sulphide effects 7-alkylation of guanine residues in nucleic acids *in vitro* and *in vivo*.⁴ We wished to prepare other 7-alkylguanines in order to identify the products expected from alkylation of nucleic acids by other reagents. It has also been proposed ⁵ that difunctional alkylating agents yield di(guanin-7-yl) derivatives on reaction with nucleic acids, but no compound of this type was obtained pure: their preparation was therefore also attempted.

Alkylated derivatives of guanosine and guanylic acid are unstable in alkali,¹ but the nature of the products has not been established. It has now been shown that further alkylation of a 7-alkylguanine yields a 7,9-dialkylguanine, the structure of which has been established by a study of its reaction in alkali and by formation of an identical substance on alkylation of the 9-alkylguanine.

The general method for preparation of 7-alkylguanines was to heat guanosine with an

¹ Lawley and Wallick, *Chem. and Ind.*, 1957, 633.

² Lawley, *Proc. Chem. Soc.*, 1957, 290.

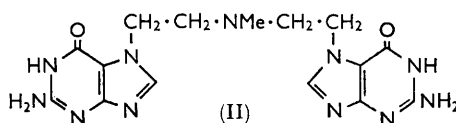
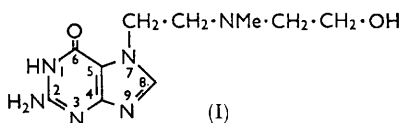
³ Reiner and Zamenhof, *J. Biol. Chem.*, 1957, **228**, 475.

⁴ Brookes and Lawley, *Biochem. J.*, 1960, **77**, 478.

⁵ Brookes and Lawley, *Biochem. J.*, 1961, **78**, 2P.

excess of alkylating agent in dimethylformamide (for 1,4-dimethanesulphonyloxybutane) or acetic acid [for ethylene oxide, butadiene dioxide, or di-(2-chloroethyl) sulphide ⁴] or in absence of solvent (for diethyl sulphate). Di-(2-chloroethyl)methylamine is unstable as the free base and the hydrochloride was used in reaction with the sodium salt of guanylic acid in neutral aqueous solution. Complete reaction of guanosine was achieved only with ethylene oxide.

Acid hydrolysis of the alkylated guanosine or guanylic acid yielded the 7-alkyl-guanines (with unchanged guanine). Hydrolysis of our 7-alkyldeoxyguanylic acids occurred in neutral aqueous solution, as previously found ² for 7-methyldeoxyguanylic acid. The hydrolysis products were separated by chromatography on a cation-exchange resin, the components being detected by ultraviolet absorption spectroscopy. The general order of elution was guanine, 7-alkylguanine, and di-(guanine-7-yl) derivative (if present); but the 7-alkylguanines from ethylene oxide and butadiene dioxide were eluted before guanine. The products were generally purified as bases which were sparingly soluble in water. Paper chromatography proved valuable for identifying them, especially when difunctional alkylating agents were used. Monoguanine derivatives, *e.g.*, (I), had R_F values in both acid and alkaline solvents greater than those of guanine; diguaninyl



compounds, *e.g.*, (II), had $R_F \sim 0$ in all solvents (see Table). All the difunctional alkylating agents used gave diguaninyl compounds; those from di-(2-chloroethyl)methylamine and butadiene dioxide were obtained pure. However, the 7-alkylguanine (I) could not be purified owing to its high solubility in water. 1,4-Di(guanine-7-yl)butane-2,3-diol was a microcrystalline solid, the analysis of which suggested a molecular complex with 1.5 mol. of erythritol, the latter presumably derived by hydrolysis of the excess of butadiene dioxide used. The ultraviolet absorption spectra gave the values of ϵ_{\max} , expected for a compound of molecular weight equal to that of this complex and containing two 7-alkyl-guanine residues. The strong binding of the polyhydric alcohol, although unexpected, is in line with the general tenacious binding of water by compounds of this series.

The position ($N_{(7)}$) of the alkyl groups was shown by comparison of the ultraviolet absorption spectra with those of 7-methylguanine (Table). The only significant difference was that for di-(2-guanine-7'-ylethyl)methylamine (II) stronger acid was required to change the spectrum from the free-base to the cation type. The spectrum of the cation was significantly different in shape from that of other 7-alkylguanines, presumably owing to the presence of a strongly basic alkylamino-group in proximity to the basic group of the guanine.

7-Alkylation of 9-substituted guanines being established, it was of interest to determine the position of alkylation of the 7-substituted guanines. 7-2'-Hydroxyethylguanine was obtained from guanosine and ethylene oxide in good yield and further alkylation gave a di-(2-hydroxyethyl)guanine (III), again in good yield. This was stable in acid but was converted by alkali into a single product (IV), as shown by paper chromatography and by the occurrence of an isosbestic point as the ultraviolet absorption spectrum changed with time.

The ultraviolet absorption spectrum of compound (III) was unchanged over the pH range 7—1, showing the absence of a basic pK_a in this region. This spectrum and that of the product of the alkaline reaction were similar to those of 7-alkylguanylic acid ² and its alkaline reaction product.^{1,2} These facts are inconsistent with formulation of compound (III) as a 1,7-dialkyl- or a 7-alkyl-2-alkylamino-guanine since both these would have a basic pK_a of ~ 3 (similar to that of guanine), the spectrum of the former would be unchanged in

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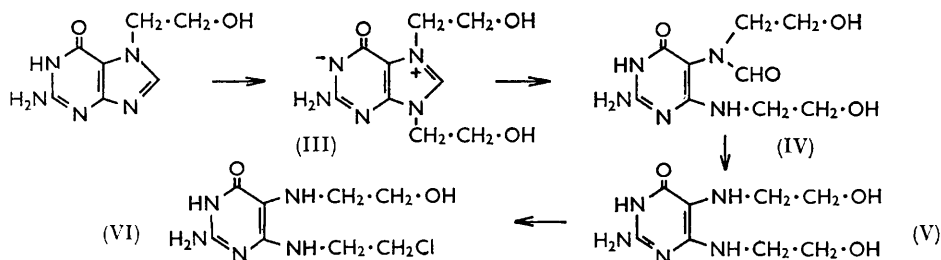
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Absorption spectra and R_F values of guanine and pyrimidine derivatives.

	pH	$\lambda_{\max.}$ (m μ)	$10^{-3} \epsilon$	$\lambda_{\min.}$ (m μ)	$\frac{\epsilon_{280}}{\epsilon_{260}}$	R_F † in solvent			
						1	2	3	4
<i>Guanine derivatives</i>									
7-Me	1	270,* 250	6.9, 10.6	228	0.79	0.27	0.22	0.12	0.24
	7	283, 248	7.4, 5.7	261, 235	1.8				
	12	280.5	7.3	257	1.9				
7-Et	1	274, 250	7.0, 11.1	268, 228	0.81	0.33	0.33	0.30	0.45
	7	284, 245	7.7, 5.9	261, 237	1.9				
	12	280	7.4	256	1.8				
7-CH ₂ ·CH ₂ ·OH	1.5	250	9.7	229	0.77	0.36	0.29	0.13	0.27
	7	284, 245 *	7.2, 5.5	257	1.9				
	12	281	6.9	261	1.9				
7-[CH ₂] ₄ ·OH	1	249	11.0	228	0.75	0.40	0.40	0.20	0.45
	7	284	7.8	261	1.8				
	12	280	7.3	258	1.7				
7-CH ₂ ·[CH(OH)] ₃ ·H	1	269,* 250	7.1, 11.9	229	0.74	0.26	0.30	0.03	0.22
	7	284	7.8	260.5	1.9				
	12	281	7.5	257.5	2.0				
1,4-Di(guanin-7-yl)butane-2,3-diol	1	274,* 252.5	14.3, 21.2	230	0.74	0.04	0.04	0.0	0.0
	7	283.5, 250	14.7, 13.9	266, 237	1.3				
	12	281	14.6	257	1.4				
(II)	-0.4	252	20.7	228	0.55	0.05	0.05	0.0	0.0
	7	284	12.5	261	1.8				
	12	281	14.2	257	1.8				
(III)	1.5	281, 254	7.1, 10.9	271, 229	0.70	0.52	0.52	0.05	0.31
	7	281, 254	7.2, 11.1	271, 229	0.71				
	12	282.5, 252	7.8, 5.6	262, 244	1.5				
<i>2-Amino-6-hydroxypyrimidine derivatives</i>									
(IV)	1.5	269	18.1	240	0.90	0.60	0.60	0.17	0.50
	12	265	11.8	242	0.22				
(V)	-0.2	267	12.4	231	0.53	0.36	0.46	Decomp.	
	7	282	10.1	242	2.4				
	11.5	277, 248 *	7.6, 4.1	236	1.6				
4,5-(NAc·[CH ₂] ₂ ·OAc)	1.5	274	6.9	250	1.2	0.91			0.81
	7	274	6.9	250	1.2				
	11.5	265	7.8	254	0.74				
(VI)	-0.2	262	15.1	232	0.06	0.40		Decomp.	
	7	279.5	8.8	236	1.7				
	11.5	279	7.2	237	1.7				
4,5-(NH ₂) ₂	-0.2	262	19.1	225	0.08				
	7	288	10.5	229	1.7				
	11.5	282, 240	8.1, 5.7	257, 229	1.6				
4-(NH·[CH ₂] ₃ ·OH)-5-(NH·CHO)	2	271	15.9	238	1.03				
	13	265.5	10.7	237	0.34				

* Infection. † For solvents see p. 3926.

alkali owing to absence of an acidic group, and the spectrum of the latter in alkali would be changed reversibly (as for guanine). The remaining possibilities are a 3,7- or a 7,9-di-alkylguanine. No 3-substituted guanines are known, suggesting that there is little



tendency for alkylation to occur on the pyrimidine ring in the guanine series. Methylation of the pyrimidine ring of adenine at N₍₁₎ has been shown ⁶ to weaken this ring, so that it is

⁶ Brookes and Lawley, *J.*, 1960, 539.

opened by acid, yielding a substituted imidazole, while alkali causes a net migration of the alkyl group from the ring-nitrogen atom to the extranuclear amino-group, possibly by ring fission and reclosure. Compound (III) was stable to acid and the product of its alkaline reaction appeared from its absorption spectrum to be a substituted pyrimidine rather than an imidazole or a purine.

The formulation of compound (III) as 7,9-di-(2-hydroxyethyl)guanine is therefore consistent with its lack of a basic pK_a , with the similarity of its absorption spectra to those for 7-alkylguanylic acids, and with the effect of alkali (which would be expected for both compounds to result in fission of the imidazole ring). This structure was confirmed by alkylation, with ethylene oxide, of 9-2'-hydroxyethylguanine (kindly supplied by Dr. C. L. Leese of this Institute) which gave the same product (III).

The ultraviolet absorption spectra of compound (IV) [which was obtained from (III) by the action of alkali] were similar to those of 2,4-diamino-5-formamido-6-hydroxy-⁷ and 2-amino-5-formamido-6-hydroxy-4-2'-hydroxyethylamino-pyrimidine, suggesting that it was 2-amino-6-hydroxy-4-2'-hydroxyethylamino-5-2'-hydroxyethylformamidopyrimidine. Compound (IV) did not crystallise. Attempted removal of the formyl group by mild acid yielded a mixture which was resolved by cation-exchange chromatography. The principal component had absorption spectra similar to those of 2,4,5-triamino-6-hydroxypyrimidine (Table), suggesting that loss of the formyl group had given 2-amino-6-hydroxy-4,5-di-(2'-hydroxyethylamino)pyrimidine (V); this compound was characterised as its tetraacetyl derivative. The minor component (VI) had ultraviolet absorption spectra of a substituted triaminopyrimidine; it was the sole ultraviolet-absorbing product of more vigorous acid treatment, so that compound (V) has limited stability in acid. Analysis of compound (VI) suggested esterification of one of the 2-hydroxyethylamino-groups by hydrochloric acid; this is analogous to the esterification of 5-amino-6-dimethylamino-4-2'-hydroxyethylaminopyrimidine by hydrochloric acid observed by Lister,⁸ which suggests that the 4- rather than the 5-2'-hydroxyethylamino-group had reacted in the present case.

EXPERIMENTAL

M. p.s were observed on a microscope hot-stage. Absorption spectra were measured with a Unicam S.P. 500 spectrophotometer for aqueous solutions. Paper chromatography was carried out on Whatman No. 1 filter paper, the following solvents being used: (1) methanol-concentrated hydrochloric acid-water (7:2:1); (2) methanol-ethanol-concentrated hydrochloric acid-water (50:25:6:19); (3) butan-1-ol saturated with water-aqueous ammonia (d 0.88) (100:1); (4) ethanol-water-aqueous ammonia (d 0.88) (80:18:2). Descending chromatography was used for solvent (3) and ascending for solvents (1), (2), and (4).

7-Ethylguanine.—Guanosine (1 g.) and diethyl sulphate (1 c.c., ~ 2 equiv.) were heated in a sealed tube at 100° for 2 hr., then extracted with *N*-hydrochloric acid (25 c.c.), refluxed for 1 hr., and applied to a column (20 \times 3 cm.) of Dowex-50 (H^+ form, equilibrated with *N*-hydrochloric acid). The column was developed with *N*-hydrochloric acid. Fractions (50 c.c.) were collected and the optical densities at 260 and 280 $m\mu$ measured, with dilution where necessary with *N*-hydrochloric acid. Fractions 85–115, containing the 7-ethylguanine, were bulked and evaporated to dryness and most of the acid was removed by addition of water and re-evaporation. A solution of the residue in water (10 c.c.) was made neutral with concentrated ammonia solution. The resulting precipitate was recrystallised from water, giving prisms which sublimed above 250° (Found: C, 46.4; H, 4.9; N, 38.8. $C_7H_9N_5O$ requires C, 46.9; H, 5.0; N, 39.1%). The yield obtained by summation of the optical densities of the appropriate fractions was 27%; the yield of recrystallised material was 15%.

7-2'-Hydroxyethylguanine.—Guanosine (2.8 g., 0.01 mole) was heated in glacial acetic acid (20 c.c.) with ethylene oxide (4.4 g., 0.1 mole) at 100° for 30 min. The clear solution was evaporated and the residue heated in *N*-hydrochloric acid (10 c.c.) at 100° for 1 hr. The solution

⁷ Hems, *Nature*, 1958, **181**, 1721.

⁸ Lister, *J.*, 1960, 899.

was made neutral with potassium hydroxide and the resulting precipitate washed with cold water and dried (yield 1.23 g., 65%). Recrystallisation from water gave 7-2'-hydroxyethyl-guanine monohydrate as prisms which did not melt below 325° (Found: C, 39.4; H, 5.1; N, 32.7. $C_7H_9N_5O_3 \cdot H_2O$ requires C, 39.6; H, 5.2; N, 32.9%). The material was unchanged (analysis) after being heated at 100° *in vacuo*.

7,9-Di-(2-hydroxyethyl)guanine.—7-2'-Hydroxyethylguanine (2.3 g.) was heated in glacial acetic acid with ethylene oxide (5.2 g., 10 equiv.) at 100° for 1 hr. The solvent was removed and the residue dissolved in water (5 c.c.) and neutralised. The resulting precipitate was washed and dried, yielding 7,9-di-(2-hydroxyethyl)guanine sesquihydrate (1.8 g.) which recrystallised from water as needles, m. p. 320° (Found: C, 40.8; H, 6.2; N, 26.6. $C_9H_{13}N_5O_3 \cdot 1.5H_2O$ requires C, 40.7; H, 6.0; N, 26.3%). This analysis was obtained after drying at 100° *in vacuo*. 9-2'-Hydroxyethylguanine (0.2 g.) was heated in glacial acetic acid (5 c.c.) with ethylene oxide (0.44 g., 10 equiv.) at 100° for 1 hr. The product was isolated as described above and shown to be identical with that from 7-2'-hydroxyethylguanine by ultraviolet and infrared absorption spectra and R_F in 3 solvent systems.

Action of Alkali on 7,9-Di-(2-hydroxyethyl)guanine.—7,9-Di-(2-hydroxyethyl)guanine (1 g.) was heated in saturated barium hydroxide solution (30 c.c.) at 100° for 2 hr. by which time the reaction was complete, as indicated by the change in ultraviolet absorption spectrum. The solution was made slightly acid with sulphuric acid and then neutralised by an excess of barium carbonate. The precipitate was removed by centrifugation and the clear solution on evaporation gave the formamidopyrimidine (IV), as a solid which did not crystallise. A solution of this (0.5 g.) in *N*-hydrochloric acid (10 c.c.) was heated at 100° for 4 hr., the change in ultraviolet absorption (a fall in D_{280}/D_{260} from 0.82 to 0.6) showing the reaction to be then complete. The acid solution was applied to a column (18 × 2 cm.) of Dowex-50 (H^+ form, equilibrated with *N*-hydrochloric acid). The column was developed with *N*-acid (2.5 l.) which removed small amounts of minor products, and then with 1.6*N*-acid. The optical density of the fractions showed two products following closely one on the other, the first in fractions 68—82 amounting to approx. 80% of the total. Evaporation of these fractions gave the aminopyrimidine (V) as a yellow deliquescent hydrochloride (0.18 g.) which did not crystallise (nor did the free base, sulphate, or picrate). Absorption spectra (see Table) were obtained for the dried sulphate. The hydrochloride was refluxed for 6 hr. in acetic acid and acetic anhydride (~3:1); evaporation gave a solid which recrystallised from chloroform-ether as prisms of 4,5-di-(*N*-2'-acetoxyethylacetamido)-2-amino-6-hydroxypyrimidine, m. p. 176—178° (Found: C, 48.8; H, 5.5; N, 17.4. $C_{16}H_{23}N_5O_7$ requires C, 48.4; H, 5.8; N, 17.6%).

Compound (IV) (0.5 g.) was refluxed with 6*N*-hydrochloric acid (10 c.c.) for 4 hr., D_{280}/D_{260} falling to 0.27 and D_{280} decreasing to 40% of its initial value. The solution was diluted to 60 c.c. and chromatographed on Dowex-50 (H^+ -form) as described above. 1.6*N*-Acid gave as the only ultraviolet-absorbing component a substance (VI) identical with the minor product of the *N*-acid treatment. This compound was isolated and recrystallised from methanol-ethyl acetate as needles of 2-amino-4-2'-chloroethylamino-6-hydroxy-5-2'-hydroxyethylaminopyrimidine hydrochloride, m. p. 178—183° (Found: C, 34.0; H, 4.7; N, 23.1. $C_8H_{14}ClN_5O_2 \cdot HCl$ requires C, 33.8; H, 5.3; N, 24.6%).

7-4'-Hydroxybutylguanine.—Guanosine (2 g.) and 1,4-dimethanesulphonyloxybutane (2 g.) were added to dimethylformamide (20 c.c.) and heated at 110° for 4 hr. After cooling, the unchanged guanosine was filtered off and the clear solution evaporated to dryness; the residue was heated in *N*-hydrochloric acid for 1 hr. at 100°, then chromatographed on Dowex-50 (H^+ -form) (26 × 3.8 cm.) in the usual way. Elution with *N*-acid removed guanine in fractions 90—140, and then 2*N*-acid gave a product in fractions 192—210 (8% yield) with ultraviolet absorption spectra characteristic of a 7-alkylguanine. The second product was isolated as the free base in the usual way and recrystallised from water as plates of 7-4'-hydroxybutylguanine, sublimes ~280°, melts finally at 308—315° (Found: C, 47.9; H, 6.0; N, 33.4. $C_9H_{13}N_5O_2$ requires C, 48.2; H, 5.8; N, 31.4%).

Reaction of Guanosine with Butadiene Dioxide.—Guanosine (850 mg.) and butadiene dioxide (400 mg., 1.5 equiv.) were heated in glacial acetic acid (10 c.c.) at 100° for 1 hr. The resulting clear solution was evaporated and the residue heated in *N*-hydrochloric acid (10 c.c.) at 100° for 1 hr., cooled, diluted with 3 vol. of water, and chromatographed on Dowex-50 (H^+ -form; 15 × 3 cm.; equilibrated with 0.25*N*-hydrochloric acid). Elution with 0.25*N*-hydrochloric acid yielded in fractions 6—10 a product (~3% yield) with $\epsilon_{280}/\epsilon_{260} = 1.7$ which was destroyed

on evaporation; in fractions 20—30 a product (~10% yield) with $\epsilon_{280}/\epsilon_{260} = 0.68$; and in fractions 52—74 a product (~30% yield) with $\epsilon_{280}/\epsilon_{260} = 0.74$. Elution was continued with *N*-hydrochloric acid and, after removal of guanine in fractions 90—100, with 2*N*-hydrochloric acid, which gave a product (~15% yield) with $\epsilon_{280}/\epsilon_{260} = 0.68$ in fractions 180—210.

The appropriate fractions were evaporated, aqueous solutions of the resulting hydrochlorides were made alkaline with aqueous ammonia, and the excess of ammonia was boiled off. Fractions 20—30 did not yield an insoluble base, and the hydrochloride was deliquescent and did not crystallise. Fractions 52—74 gave a crystalline base which recrystallised from water as plates of 7-(2,3,4-trihydroxybutyl)guanine monohydrate, m. p. 286—289° (Found: C, 39.3; H, 5.7; N, 25.1. $C_9H_{13}N_5O_4 \cdot H_2O$ requires C, 39.6; H, 5.5; N, 25.6%). Fractions 180—210 gave 1,4-di(guanin-7-yl)butane-2,3-diol which recrystallised from water as a molecular complex with erythritol, m. p. 250—300° (Found: C, 41.9; H, 5.8; N, 24.1. $C_{14}H_{16}N_{10}O_4 \cdot 1.5C_4H_{10}O_4$ requires C, 42.0; H, 5.4; N, 24.5%).

Di-(2-guanin-7'-ylethyl)methylamine.—Di-(2-chloroethyl)methylamine hydrochloride (2 g.) in water (10 c.c.) was added to a solution of disodium guanylate (2 g.) in water (10 c.c.) at 37°. Continued shaking gave a solution of pH 7.5 initially, falling to pH 6.5 after 1 hr. Concentrated hydrochloric acid (2 c.c.) was added and the solution heated at 100° for 1 hr., cooled, and applied to a column (17 × 3.2 cm.) of Dowex-50 (H^+ -form; equilibrated with 2*N*-hydrochloric acid). Development with 2*N*-acid gave guanine in fractions 20—40, then 4*N*-acid gave two products in fractions 54—60 (~4%) and 61—75 (~11%) respectively. These products had identical ultraviolet absorption spectra, corresponding to those of 7-alkylguanines and could not be distinguished by their R_F values [solvent (1) 0.25; (2) 0.25; (3) 0.15; (4) 0.45]. The compounds were isolated but did not crystallise as salts or free bases, the latter being very soluble in water. Further development of the column with 5*N*-acid gave in fractions 100—140 a product (II) which was isolated (~20% yield) as the free base; recrystallisation from water gave di-(2-guanin-7'-ylethyl)methylamine trihydrate as needles, m. p. >330° (Found: C, 41.4; H, 5.6; N, 35.3. $C_{15}H_{19}N_{11}O_2 \cdot 3H_2O$ requires C, 41.0; H, 5.7; N, 35.1%).

Hydrolysis of 7-Alkyldeoxyguanylic Acids in Neutral Aqueous Solution.—Deoxyguanosine-5' phosphate (0.1 millimole) was treated with diethyl sulphate (0.1 millimole) in 0.4*N*-phosphate buffer pH 7.2 (1 c.c.) at 37° for 1 hr. A part (0.3 c.c.) of the solution was chromatographed on Whatman No. 4 paper with saturated aqueous ammonium sulphate–propan-2-ol-0.1*N*-phosphate (79 : 2 : 19) at pH 7.2 as solvent. The component of R_F 0.7 (~14% yield) was eluted from the paper with 0.1*N*-phosphate buffer (pH 7.0), and the ultraviolet absorption of the solution was measured immediately and after 20 hr. and 120 hr. at 37°. The spectrum was initially similar to that for 7-methyldeoxyguanylic acid² and finally to that of 7-ethylguanine, the half-life of the hydrolysis being approx. 20 hr. The remainder of the reaction mixture (0.7 c.c.) was kept at 37° for 120 hr.; a crystalline precipitate had then formed which was shown by paper chromatography and ultraviolet absorption spectroscopy to be 7-ethylguanine.

A similar experiment with deoxyguanylic acid and di-(2-chloroethyl)methylamine hydrochloride yielded a precipitate after 120 hr. which was shown to be di-(2-guanin-7'-ylethyl)-methylamine.

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