125. *Constitution of the Purine Nucleosides. Part VII. Guanosine and Guanine Deoxyriboside.*

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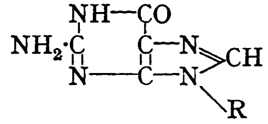
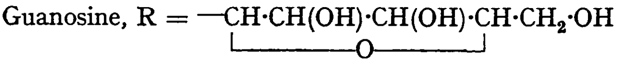
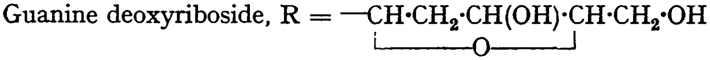
The method of determining the position of the carbohydrate radical in purine nucleosides (J., 1934, 1639; 1936, 765; 1937, 1912; this vol., p. 259) by comparing their ultra-violet absorption spectra with those of the corresponding 7- and 9-methylated purine derivatives has now been applied to guanosine, prepared from yeast nucleic acid, and to guanine deoxyriboside, obtained from thymus nucleic acid (Klein, *Z. physiol. Chem.*, 1933, **218**, 173). In both compounds (I), the carbohydrate radical has been shown to occupy position 9 for the reasons outlined below.

(I.)





Guanosine, $R = -\underbrace{CH\cdot CH(OH)\cdot CH(OH)\cdot CH}_{O}\cdot CH_2\cdot OH$



Guanine deoxyriboside, $R = -\underbrace{CH\cdot CH_2\cdot CH(OH)\cdot CH}_{O}\cdot CH_2\cdot OH$

THE spectra of 7-methylguanine (Fischer, *Ber.*, 1897, **30**, 2400) (Fig. 2) and of 9-*methylguanine* (Fig. 1) are widely different in neutral and in alkaline solutions. The spectra of guanosine (Fig. 3) are scarcely to be distinguished from those of 9-methylguanine. This result confirms the placing of the carbohydrate radical in the 9-position by Gulland, Holiday, and Macrae (J., 1934, 1639) from a comparison of the spectra of xanthosine, prepared from guanosine, with those of 7- and 9-methylxanthines.

The spectra of guanine deoxyriboside in neutral and in alkaline solutions (Fig. 4) resemble those of 9-methylguanine and guanosine, but not those of 7-methylguanine. The spectrum of guanine deoxyriboside in acid solution has not been determined on account of the sensitivity of this substance to acid; Levene and Bass ("Nucleic Acids," p. 182, Chemical Catalog Co., New York) state that it is completely hydrolysed by 0·01N-sulphuric acid in 5 minutes.

EXPERIMENTAL.

We are greatly indebted to Dr. W. Klein for a specimen of guanine deoxyriboside prepared from thymus nucleic acid by fission with nucleophosphatase from calf's intestinal mucosa.

Guanosine was prepared from guanylic acid, *ex* yeast nucleic acid, by hydrolysis with bone phosphatase (Gulland and Macrae, J., 1933, 662).

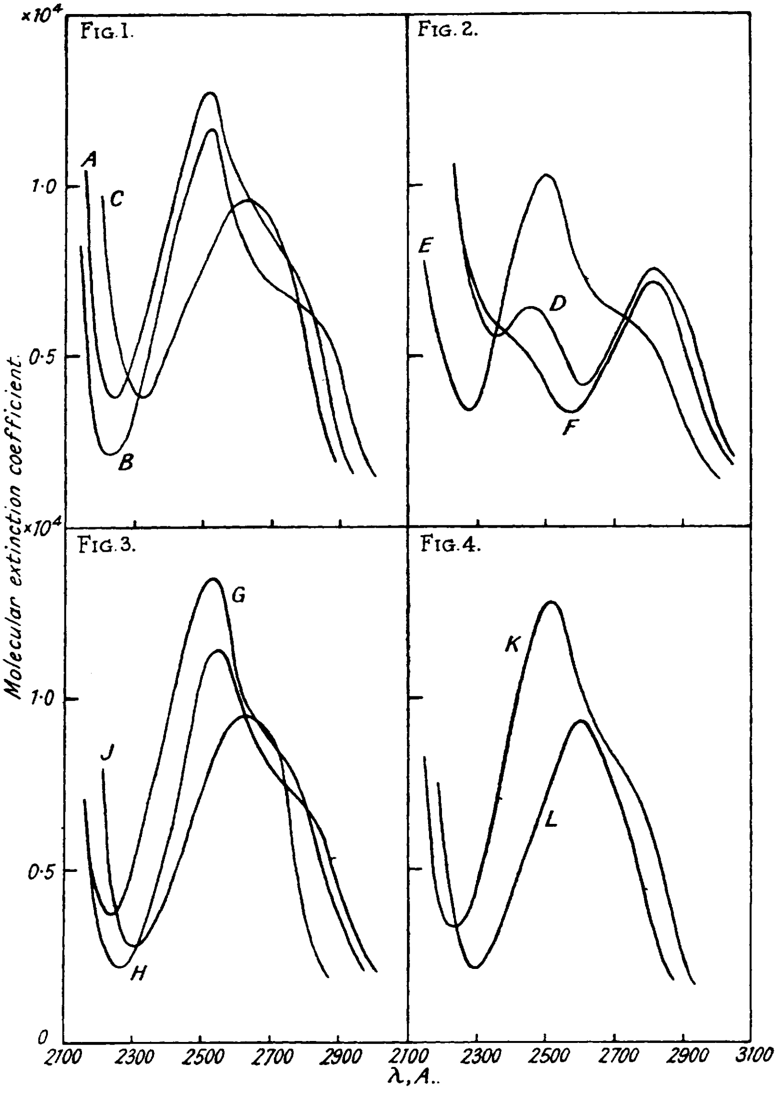


FIG. 1.

9-Methylguanine, M/50,000 : *A, in water*; *B, in* N/20-HCl; *C, in* N/20-NaOH.

FIG. 2.

7-Methylguanine : *D*, M/32,000 *in water*; *E*, M/44,000 *in* N/20-HCl; *F*, M/40,000 *in* N/20-NaOH.

FIG. 3.

Guanosine, M/50,000 : *G, in water*; *H, in* N/20-HCl; *J, in* N/20-NaOH.

FIG. 4.

Guanine deoxyriboside, M/50,000 : *K, in water*; *L, in* N/20-NaOH.

7-Methylguanine was prepared from theobromine through the stages of 2 : 6-dichloro-7-methylpurine and 2-chloro-6-hydroxy-7-methylpurine (Fischer, *Ber.*, 1897, **30**, 2400), and was purified by crystallisation of its hydrochloride from hydrochloric acid (*d* 1·07), decomposition of the hydrochloride by concentrated ammonium acetate, and crystallisation of 7-methylguanine in colourless needles from water (Found : N, 42·4. Calc. for $C_6H_7ON_5$: N, 42·4%).

2 : 6-*Dichloro-9-methylpurine.* Violuric acid, prepared by the action of hydroxylamine on alloxan (Guinchard, *Ber.*, 1899, **32**, 1739), was reduced with hydrogen sulphide, and the resulting uramil was condensed with methyl *iso*thiocyanate to give 9-methylthiouric acid, which was converted into 9-methylxanthine by nitrous acid (Biltz and Strufe, *Annalen*, 1921, **423**, 200). A mixture of 9-methylxanthine (1 g.) and redistilled phosphorus oxychloride (40 g.) was heated at 180—190° for 20 hours in a sealed tube. The phosphorus oxychloride was removed under reduced pressure, ice added to the residue, and some unchanged 9-methylxanthine removed by filtration. When the filtrate was made alkaline with ammonia and then just acid with acetic acid, the crude 2 : 6-*dichloro-9-methylpurine* separated; after being extracted with cold dilute sodium hydroxide solution to remove a little soluble material, it crystallised from hot water in pale yellow, elongated prisms, m. p. 153° (Found : N, 27·6; Cl, 35·9. $C_6H_4N_4Cl_2$ requires N, 27·6; Cl, 35·0%).

9-*Methylguanine.*—The replacement of the chlorine in position 6 and the chlorine in position 2 of 2 : 6-dichloro-9-methylpurine by a hydroxyl and an amino-group respectively through successive treatment with sodium hydroxide and ammonia is inferred by analogy with the formation of 7-methylguanine from 2 : 6-dichloro-7-methylpurine by the same reactions in the same sequence.

Sodium hydroxide (0·25 g.) was added to 2 : 6-dichloro-9-methylpurine (0·6 g.) suspended in hot water (6 ml.), and the solution was shaken for 1 hour and acidified with acetic acid. The resulting 2-chloro-6-hydroxy-9-methylpurine, which crystallised from water in pale yellow prisms (decomp. at high temperature), was heated with 20 parts by vol. of aqueous ammonia (*d* 0·880) for 12 hours at 150—160° in a sealed tube. The solution was evaporated to dryness, and the residue washed with water to remove ammonium chloride, dissolved in dilute aqueous sodium hydroxide (norit), and acidified with acetic acid. Heating on the water-bath caused the formation of a voluminous crystalline mass of 9-methylguanine, which was purified by crystallising the hydrochloride in colourless oblique-ended prisms from hydrochloric acid (*d* 1·07), decomposing this with ammonium acetate, and recrystallising the product from water. 9-*Methylguanine* formed colourless rectangular plates, which decomposed at a high temperature without melting (Found : N, 42·3. $C_6H_7ON_5$ requires N, 42·4%). It dissolved readily in cold dilute hydrochloric acid and sodium hydroxide, was moderately soluble in hot water, slightly soluble in alcohol and chloroform, and insoluble in acetone.

Absorption Spectra.—Measurements were made with a Bellingham and Stanley quartz spectrograph No. 2 and photometer, the light source being a condensed spark between tungsten-steel electrodes. The solutions, prepared from dried materials and made to the strengths recorded on the Figs., were examined immediately in a layer thickness of 4 cm. against controls.

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