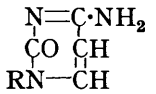
170. *The Constitution of the Purine Nucleosides.*

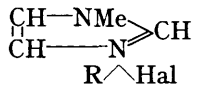
By J. M. Gulland and T. F. Macrae.

It is generally accepted that the molecules of animal and plant nucleic acids are composed of four nucleotides (I) united by ester or ether linkages involving the phosphoric acid and carbohydrate groups. Mild hydrolysis liberates the nucleotides, two containing purine residues and two pyrimidine residues, and further hydrolysis with ammonia causes fission of the nucleotides and liberation of phosphoric acid. It is also recognised that the resulting nucleosides are purine- and pyrimidine-ribosides, examples of the two types being guanosine (II) and cytidine (III), in which R represents the ribose radical (for literature, see Levene and Bass, " Nucleic Acids," 1931, New York, Chemical Catalog Co.).

(I.) Phosphoric acid–pentose–pyrimidine or purine



(III.)



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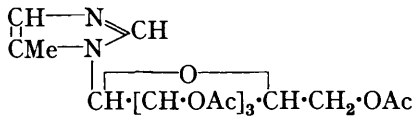

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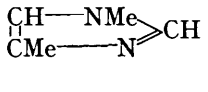
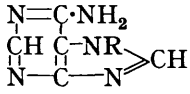


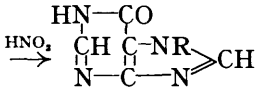
(VI.)

The chemistry of the pyrimidine nucleosides shows that the pentose residue must be attached to the nitrogen in position 3, but the position of the carbohydrate group in the molecule of the purine nucleosides is as yet undecided. This communication describes experiments designed to elucidate this problem.

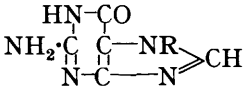
Four purine nucleosides are derived from nucleic acids, and these compounds and the parent purines form two groups, as shown below. It is convenient to discuss the case of the ribosides, rather than that of the deoxyribosides.



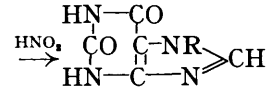
Adenine, R = H. Adenosine, R = Ribose.



Hypoxanthine, R = H. Inosine, R = Ribose.



(II.) Guanine, R = H. Guanosine, R = Ribose.



(VII.) Xanthine, R = H. Xanthosine, R = Ribose.

It may readily be deduced that the pentose radical is not attached to certain positions of these nucleosides. Levene (*J. Biol. Chem.*, 1923, **55**, 437) transformed xanthosine into its 1 : 3-dimethyl derivative, theophylline riboside, which was synthesised from acetobromoribose and silver theophylline (Levene and Sobotka, *J. Biol. Chem.*, 1925, **65**, 463). It follows, therefore, that in guanosine and in xanthosine the pentose is attached to position 7 or 9. In adenosine and inosine, positions 1, 3 and 6 are obviously impossible, and, since the four nucleosides are hydrolysed rapidly and with equal ease by dilute mineral acids, it has been assumed (Levene, *loc. cit.*) that the pentose molecules are not attached to carbon (positions 2 and 8 thus being excluded) and that the four nucleosides are similarly constituted. The pentose groups therefore occupy either position 7 or 9 in the glyoxaline ring of the purine.

In view of the virtual tautomerism in the amidine group of the glyoxaline nucleus, the synthesis of a purine glycoside through the replacement of the tautomeric hydrogen by a sugar residue does not permit a decision to be made regarding the point of entry of the sugar.

Rung and Behrend (*Annalen*, 1892, **271**, 34) and Pinner and Schwarz (*Ber.*, 1902, **35**, 2444, 2457) observed that 1-methylglyoxaline is converted into 1-methylglyoxalinium 3-alkyl halides (IV) by the action of alkyl halides. It is thus recognised that the addition of an alkyl halide to an *N*-alkylglyoxaline takes place at that nitrogen atom which does not carry the original alkyl group, and this fact seemed to offer a method of ascertaining the position of the pentose residue in the nucleosides. If, for example, xanthosine could be combined with methyl iodide, and the resulting xanthosine methiodide hydrolysed by acids,

the product would be either 7- or 9-methylxanthine hydriodide, the methyl group being attached to the nitrogen atom which was not previously united to the pentose. The identification of the methylated xanthine would offer no difficulty.

Before proceeding to the alkylation of the purine nucleosides themselves, the proposed procedure was examined by studying a more simple example. Accordingly, 4-methylglyoxaline in the form of its silver derivative was converted into *3-tetra-aceto-β-glucosido-4-methylglyoxaline* (V), the constitution of which was ascertained as described below. The yield was poor, the greater part of the product being an uncrystallisable oil which may have contained the isomeric glucoside. This material decomposed (acid reaction) and was abandoned. The methochloride of (V), prepared from the readily accessible *methiodide*, was exceptionally resistant to acid hydrolysis. Only the acetyl groups were attacked by 5% hydrochloric acid at 100°, the customary conditions for the hydrolysis of nucleosides, and *3-β-glucosido-4-methylglyoxaline methochloroaurate* was isolated. Complete hydrolysis was effected with concentrated hydrochloric acid at 150°, however, and 1 : 4-dimethylglyoxaline (VI) was isolated as the characteristic chloroaurate (Pyman, J., 1910, **97**, 1814).

The 4-methylglyoxaline for these experiments was prepared by the formaldehyde–zinc chloride–ammonia method from glucose (Windaus and Knoop, *Ber.*, 1905, **38**, 1166) and from methylglyoxal (Bernhauer, *Z. physiol. Chem.*, 1929, **183**, 67). The last author states that the picrate and oxalate of methylglyoxaline only could be isolated. In addition to 4-methylglyoxaline, we have isolated as by-products of the reaction 2 : 4-dimethylglyoxaline and a *base*, $C_5H_7N_2Cl$. The molecular formula and properties of this substance are those of a chlorodimethylglyoxaline or pyrazole, but it is not one of the known members of these groups; possibly it is 5-chloro-2 : 4-dimethylglyoxaline.

The applicability of the proposed method having thus been established, attention was turned to the purine nucleosides. The early experiments were carried out with theophylline tetra-aceto-β-glucoside (Fischer and Helferich, *Ber.*, 1914, **47**, 210), partly in the hope of devising a general method with readily accessible material, and partly in order to avoid complications arising from the methylation of tertiary nitrogen atoms other than those in positions 7 and 9 (as in hypoxanthine) or of imino-groups (as in xanthine). In spite of many varied attempts, it was impossible to prepare theophylline tetra-acetoglucoside exactly as described by Fischer, but this difficulty was ultimately ascribed to the inactivity of the surface of the silver theophylline used, and a successful modification is described below. When the glucoside was heated with methyl iodide at 150°, some charring occurred, indicating the liberation of glucose, and the sole product of the reaction was the periodide of caffeine methiodide, which was identified by analysis and conversion into caffeine (1 : 3 : 7-trimethylxanthine) and caffeine methohydroxide. In the hope that fission of the glucoside might be avoided at a lower temperature, methylation was carried out at 125° for a longer period, but here again caffeine methiodide periodide was formed, accompanied by much unchanged theophylline tetra-acetoglucoside. Presumably the quaternary ammonium salt was first formed, and the cation then suffered decomposition by elimination, as halide, of the less firmly bound radical—in this case tetra-acetoglucose, the iodide of which would naturally char under the conditions of the experiment. The *iso*caffeine, or caffeine, thus formed then underwent methylation to caffeine methiodide, a reaction which occurs at 130° in the case of caffeine.

The action of boiling benzyl chloride in presence of potassium carbonate (to neutralise hydrochloric acid) was equally unsuccessful. In this case, however, the alkylation of the purine did not occur, but the acetyl groups were removed and converted into benzyl acetate, theophylline-*d*-glucoside (Fischer and Helferich, *loc. cit.*) being the sole nitrogenous product.

Attention was then directed to the alkylation of xanthosine (VII), prepared by the action of nitrous acid on guanosine obtained from yeast nucleic acid. Some modifications have been introduced into the procedure of Levene and Jacobs (*Ber.*, 1910, **43**, 3150) for performing this reaction. In addition, the conversion of the nucleotide guanylic acid into guanosine has been accomplished by means of bone phosphatase at p_H 8·6 (Martland and Robison, *Biochem. J.*, 1929, **23**, 237), the product being identical with that prepared by ammoniacal hydrolysis. The dephosphorylation of nucleotides by liver and intestinal nucleotidases is

known, but their fission by bone phosphatase has not hitherto been recorded, and is of special interest in view of the formation of blood corpuscles and the loss of nuclei by nucleated erythrocytes which occurs in bone marrow.

During the experiments described below, we have again been impressed by the utility of palladous chloride for the purification of small quantities of purine compounds (cf. Gulland and Macrae, J., 1932, 2231).

Methylation of xanthosine with methyl iodide at 140—150° gave a good yield of 3 : 9-dimethylxanthine hydriodide and the corresponding periodide. Here again the sugar residue had been eliminated during the reaction, and in the hope of deciding whether this occurred before or after the formation of the quaternary ammonium salt, theophylline was methylated under the same conditions. Caffeine hydriodide was readily obtained, and it appeared at first as if the comparison of these results offered proof of the attachment of the pentose molecule to position 7. This became dubious, however, when methylation of xanthine under the same conditions gave 3 : 9-dimethylxanthine hydriodide, but in this experiment the yield was only about 20%, the remainder of the xanthine being recovered unchanged. Finally, xanthosine was methylated in presence of barium carbonate, with the intention of preventing the elimination of the pentose molecule if this should be due to the formation of free acid during the reaction. Theobromine, its methiodide, and barium iodide were produced, this being the only case in which the purine base, and not the hydriodide, was obtained.

Summarising these reactions carried out under the same conditions:

(A) Xanthosine ⟶ 3 : 9-dimethylxanthine hydriodide in good yield;
(B) xanthine ⟶ 3 : 9-dimethylxanthine hydriodide in poor yield;
(C) theophylline ⟶ 1 : 3 : 7-trimethylxanthine hydriodide;
(D) xanthosine + barium carbonate ⟶ 3 : 7-dimethylxanthine and methiodide.

The balance of evidence favours the attachment of the pentose molecule to position 7 of xanthosine, and therefore of guanosine, but the facts are not decisive and further work is in progress. If the customary lines of reasoning be justified, the pentose would occupy position 7 of adenosine and inosine also, and this has a bearing on the constitution of adenylpyrophosphate, recognised by Lohmann as the co-enzyme of zymase (yeast) and myozymase (muscle). The two alternative possibilities are (i) that methylation follows, and (ii) that it precedes, elimination of the pentose molecule. The first alternative offers no explanation of the pronounced difference in the results of reactions A and B. In the second case, the production of the 9-methyl derivative in reaction A indicates that the pentose is in position 7, whereas the formation of a 7-methyl derivative in reaction D favours the 9-position for the sugar. These conclusions are incompatible, but reaction D might receive an explanation through the following series of changes:

xanthosine ⟶ 7-ribose-3 : 9-dimethylxanthine iodide ⟶ 3 : 9-dimethylxanthine by elimination of ribose iodide ⟶ 3 : 7 : 9-trimethylxanthine iodide ⟶ 3 : 7 : 9-trimethylxanthine carbonate by slow reaction with barium carbonate ⟶ 3 : 7-dimethylxanthine by elimination of methyl carbonate.

Quaternary carbonates decompose more readily than do methiodides, and it is necessary to postulate the formation of a methocarbonate, since the experiments with theophylline tetra-acetoglucoside show that caffeine methiodide is stable under the conditions of these reactions. Caffeine methiodide decomposes at 190° into caffeine and methyl iodide (Schmidt and Schilling, *Annalen*, 1885, **228**, 142), proving that the methyl group in position 7 is more firmly bound than that in 9.

Reaction C has no direct bearing on the present problem, but when considered in conjunction with reaction B, indicates that during reaction with methyl iodide the imino-groups in the glyoxaline rings of xanthine and theophylline occupy different positions.

In 1922, Benedict, Davis, and Newton (*J. Biol. Chem.*, **54**, 595, 601, 603) isolated a uric acid riboside from the erythrocytes of beef blood, and showed that it was widely distributed, being also present in human, horse, sheep, pig, dog and chicken bloods. It is possible that this substance is formed by the oxidation of nucleosides, just as uric acid

results from the oxidation of xanthine and hypoxanthine by the dehydrogenase of liver and of milk. If this enzymic oxidation could be established *in vitro*, the methylation of the uric acid riboside to a trimethyl derivative and subsequent hydrolysis to a trimethyluric acid would offer strong presumptive evidence on the position of the ribose in the nucleosides. Since xanthine dehydrogenase of liver is accompanied by nucleosidases which hydrolyse the glucosidic linkage (Levene and Medigreceanu, *J. Biol. Chem.*, 1911, **9**, 65; Levene, Yamagawa, and Weber, *ibid.*, 1924, **60**, 693, 707, 717), xanthine dehydrogenase was prepared from cream (Wieland and Rosenfeld, *Annalen*, 1929, **477**, 32), from which it may be obtained apparently free from nucleosidase. Unfortunately, this preparation failed completely to oxidise xanthosine or guanosine, but was quite active towards xanthine, methylene-blue being used as hydrogen acceptor under anærobic conditions (Thunberg, *Skand. Arch. Physiol.*, 1920, **40**, 1) in the apparatus of Wieland and Rosenfeld. It is hoped to determine the constitution of the uric acid riboside by methylation.

Experimental.

Tetra-acetoglucosidomethylglyoxaline.

*4-*Methylglyoxaline and the Base* $C_5H_7N_2Cl$.—The methylglyoxal was in 50% aq. solution, prepared from acetone by means of SeO_2 (Riley, Morley, and Friend, J., 1932, 1875). Mr. K. F. Armstrong kindly supplied us with this material and informed us that the concn. of the solution calc. from the carbon percentage found by combustion was the same as that determined by the iodine titration method of Fischler and Boettner (*Z. anal. Chem.*, 1928, **74**, 28). Consequently, the methylglyoxal was probably pure.

Methylglyoxal solution (80 c.c.) was added to a mixture of formalin (41 g.) and $Zn(OH)_2$–NH_3 aq. (800 c.c.; Windaus and Knoop, *Ber.*, 1905, **38**, 1166). Reversal of the order of addition decreased the yield. The mixture was heated for 4 hr. at 100°, the solid collected, washed with H_2O, and decomposed with H_2S. The filtrate from ZnS was acidified (p_H 2) with HCl aq., concentrated to small vol. in vac., and saturated with K_2CO_3. The oil (32 g.) which separated was extracted with Et_2O, dried, and freed from solvent. It contained 4-methylglyoxaline, 2 : 4-dimethylglyoxaline, and the chloro-base described below. A picrate, which did not crystallise readily, melted at 144°, and two oxalates were isolated, m. p. 205—206° and 170—178°. 4-Methylglyoxaline picrate melts at 159—160° (Windaus and Knoop, *loc. cit.*) and its oxalate at 206° (Bernhauer, *loc. cit.*). 2 : 4-Dimethylglyoxaline picrate melts at 142—143°, and is less sol. than methylglyoxaline oxalate. Its oxalate, mixed with methylglyoxaline oxalate, melts at 172—178° (Windaus, *Ber.*, 1906, **39**, 3886).

An aq. solution of the crude oil (above) was poured into excess of ammoniacal $AgNO_3$, and the silver salt collected (Z), washed, and worked up for methylglyoxaline by treatment with HCl aq., liberation of the base, conversion into and purification as the oxalate, m. p. 207°, and distillation of the base, m. p. 54° (Jowett and Potter, J., 1903, **83**, 464, give 55°).

Ag was removed from the filtrate (Z) by an excess of HCl aq., and the acid solution was concentrated and made strongly alkaline with K_2CO_3. The colourless solid obtained crystallised from H_2O in needles, m. p. 207° (Found : C, 45·9, 45·6; H, 5·3, 5·3; N, 20·7; Cl, 27·3; *M*, by Rast, 135. $C_5H_7N_2Cl$ requires C, 46·0; H, 5·4; N, 21·5; Cl, 27·2%; *M*, 131). This *substance* dissolved readily in EtOH, acetone, and dil. mineral acids, but was sparingly sol. in H_2O, Et_2O, C_6H_6, and $CHCl_3$. It was insol. in cold 2*N*-NaOH, but dissolved on warming, or in cold 15% NaOH aq. It gave no AgCl with $AgNO_3$ in HNO_3. A yellow colour was developed with diazobenzenesulphonic acid in Na_2CO_3 aq. An oil which separated when a solution of the base in 15% Na_2CO_3 aq. was shaken with PhCOCl was taken up in Et_2O. Crystals, m. p. about 195—197°, were obtained by crystn. from light petroleum, but recrystn. of these from H_2O yielded the base, m. p. 207° (alone or mixed with fresh material). Evidently the benzoyl derivative was very readily hydrolysed. The base yielded a colourless ppt. of a silver salt with ammoniacal $AgNO_3$, but this must be appreciably sol. in view of the method of isolation of the base. The properties described above are those which might be predicted for a chlorodimethylglyoxaline, but lack of material prevented further investigation except by synthesis.

The oxalate, prepared in Et_2O solution and cryst. from acetone, had m. p. 148°.

3-*Tetra-aceto-β-glucosido-4-methylglyoxaline* (V).—Pure silver 4-methylglyoxaline (20 g.) was dried for 30 min. in vac. in a 250 c.c. Geissler flask at 120° in an oil-bath. Tetra-aceto-

* Mr. H. V. Ansell collaborated with us in experiments marked *.

bromoglucose * (43 g.; Fischer, *Ber.*, 1916, **49**, 584) in dry S-free xylene (120 c.c.) was added, the condenser fitted, and the mixture heated for 15 min. at 170°. The hot solution was decanted from AgBr, cooled, and diluted with ligroin until no further ppt. formed. The liquid was decanted, and the residue dissolved in warm Et_2O, from which crystals (5 g.), m. p. 157—167°, were obtained by careful concn. The Et_2O filtrate contained a more sol. gum (see below). 3-*Tetra-aceto-β-glucosido-4-methylglyoxaline* crystallised from H_2O in colourless needles, m. p. 176° (Found: C, 52·3; H, 5·8; N, 6·9. $C_{18}H_{24}O_9N_2$ requires C, 52·3; H, 5·9; N, 6·8%), readily sol. in EtOH and acetone but only sparingly in H_2O and Et_2O.

The *methiodide*, prepared by heating the base (0·38 g.) with MeI (3 c.c.) at 120—130° for 3 hr., separated as yellow crystals, which, recryst. from EtOH, formed elongated rectangular tablets (0·40 g.), m. p. 232° (decomp.) (Found: C, 41·4; H, 5·0. $C_{19}H_{27}O_9N_2I$ requires C, 41·1; H, 4·9%).

The gum obtained from the Et_2O soln. (see above) was sparingly sol. in hot H_2O, and was therefore not recovered methylglyoxaline. It yielded large amounts of a cryst. picrolonate, which was sparingly sol. in EtOH. When re-examined after 3 months, the gum had become strongly acid in reaction, and AcOH could be detected by smell. Very little picrolonate was then formed in EtOH solution. These facts suggest that the gum may have contained considerable amounts of 1-tetra-acetoglucosido-4-methylglyoxaline.

3-β-*Glucosido-4-methylglyoxaline Methochloroaurate.*—An aq. solution (2 c.c.) of the methochloride of (V) prepared from the methiodide (0·4 g.) by means of AgCl was heated with HCl aq. (20 c.c. of 5%) for 1·5 hr. at 100° and evaporated to dryness in a desiccator. The residue was taken up in H_2O, mixed with chloroauric acid (3·5 c.c. containing 175 mg. Au), and evaporated in a desiccator to 2 c.c. The *methochloroaurate* (0·26 g.) separated in pale yellow, coarse tablets, m. p. 144—145° (Found: C, 21·9; H, 3·2; N, 4·4; Au, 33·1. $C_{11}H_{19}O_5N_2Cl_4Au$ requires C, 22·1; H, 3·2; N, 4·7; Au, 33·1%).

Isolation of 1 : 4-*Dimethylglyoxaline.*—3-Glucosido-4-methylglyoxaline methochloroaurate was recovered unchanged (m. p. 145°) after being heated with boiling 21% HCl aq. for 1·5 hr. The methochloroaurate (0·20 g.) was heated with HCl aq. (5 c.c. of 38%) in a sealed tube for 2 hr. at 150°. The solution was evaporated to dryness, and when H_2O (5 c.c.) was added, 1 : 4-dimethylglyoxaline chloroaurate separated in sheaves of prismatic needles, m. p. 138°, which dissolved readily in cold EtOH (Found: Au, 45·9. Calc. for $C_5H_8N_2,HAuCl_4$: Au, 45·2%). 1 : 4-Dimethylglyoxaline chloroaurate melts at 137—138° and is readily sol. in EtOH, whereas 1 : 5-dimethylglyoxaline chloroaurate melts at 218—219° and is sparingly sol. in EtOH (Pyman J., 1910, **97**, 1814).

Tetra-aceto-β-glucosidotheophylline.

Action of Methyl Iodide.—Tetra-acetobromoglucose failed to react with silver theophylline under Fischer's conditions (*loc. cit.*) after the silver salt had been dried at 140° for 2 hr. Small quantities of theophylline only could be isolated. The following modification was adopted. Silver theophylline (6·5 g., dried at 100° for 30 min.) and S-free xylene (100 c.c.) were heated at 180° so that the H_2O and half the xylene distilled away. Acetobromoglucose (9·4 g.) was added to the residue, and the mixture was boiled vigorously for 5 min., filtered hot, cooled to 70°, and filtered again to remove a small quantity of theophylline. The cooled filtrate was mixed with light petroleum (260 c.c.), and the glucoside collected, washed with light petroleum, and crystallised from H_2O; m. p. 163—164°; yield, 6·1 g.

(i) The glucoside (2 g.) and MeI (15 c.c.) were heated in a sealed tube at 145—150° for 6 hr. (liberation of I and some charring). After evaporation of the MeI, the residue was dissolved in acetone (100 c.c.) and filtered from traces of C, the acetone evaporated, and the solid dissolved in boiling EtOH (100 c.c.). On cooling, the periodide of caffeine methiodide separated in dark red-brown columns, m. p. 159—160°, and when I was added to the alc. mother-liquor a further quantity of the periodide separated. This periodide decomposed slowly when heated with boiling H_2O, and when the liberation of I was finished, the resulting solution of caffeine methiodide was evaporated to dryness, the solid dissolved in hot MeOH (10 c.c.), and hot EtOAc (10 c.c.) added. The methiodide (1·2 g.) separated, and crystallised from abs. EtOH in columns, m. p. 190—191° (decomp.) (Found in material dried at 80°: C, 30·8; H, 4·2. Calc. for $C_9H_{13}O_2N_4I,H_2O$: C, 30·5; H, 4·2%). When it was heated at 190°, MeI and H_2O were evolved, and the residue consisted of caffeine, m. p. 233°, which was further identified by the formation of the palladochloride, which crystallised in pale yellow, irregular leaflets with staggered edges

* Crystn. from Et_2O is simpler and more effective than from amyl alcohol as recommended by Fischer.

(Gulland and Macrae, J., 1932, 2231). An aq. solution of the methiodide, when shaken with AgOH and evaporated (Schmidt and Schilling, *loc. cit.*), yielded caffeine methohydroxide, m. p. 85—87°, and 132—134° after being dried at 100° (Found in material dried in a vac.: C, 44·9; H, 6·6; N, 22·9. Calc. for $C_9H_{14}O_3N_4,H_2O$: C, 44·3; H, 6·6; N, 22·9%).

(ii) An unsuccessful attempt was made to avoid fission of the glucoside by methylating the substance at a lower temp. The glucoside (3 g.) and MeI (15 c.c.) were heated at 125° for 36 hr., and acetone (150 c.c.) was added (A); an *iodide* (40 mg.) remained undissolved as pale yellow, elongated plates with rounded ends, m. p. 208° (decomp.) (Found: C, 20·4; H, 2·5; N, 9·1; I, 46·8. $C_5H_7O_4N_2I$ requires C, 21·0; H, 2·4; N, 9·8; I, 44·5%). We are unable to assign a formula to this substance unless it be 4-keto-5-hydroxy-1(3)-methyl-4 : 5-dihydroglyoxaline-5-carboxylic acid hydriodide, but the analytical figures indicate that decomp. of the purine ring systems has occurred.

The acetone solution (A) contained a small amount of caffeine methiodide periodide and much unchanged glucoside. When C_6H_6 was substituted for acetone at stage (A), a mixture consisting only of periodide and the substance, m. p. 208°, remained undissolved, and the C_6H_6 contained only unchanged glucoside. No other substances were produced in the reaction.

An attempt to methylate the glucoside with MeCl (MeI and AgCl) at 130° yielded only unchanged material.

Action of Benzyl Chloride.—The glucoside (1 g.), anhyd. K_2CO_3 (0·5 g.), and neutral $PhCH_2Cl$ (10 c.c.), protected from moisture, were heated under reflux at 190° for 3 hr. The $PhCH_2Cl$ was distilled in vac. and the last traces were removed with light petroleum. The odour of benzyl acetate was noticeable. The residue was extracted with hot abs. EtOH, from which theophylline-*d*-glucoside separated in colourless leaflets, m. p. 267—269° (Found in material dried at 110°/10 mm.: C, 45·5; H, 5·3; N, 16·2. Calc. for $C_{13}H_{18}O_7N_4$: C, 45·6; H, 5·3; N, 16·4%).

Other Experiments.—(i) By heating 8-chlorocaffeine with NaOMe, Fischer (*Ber.*, 1884, **17**, 1785) obtained 8-methoxycaffeine, which Wislicenus and Körber (*Ber.*, 1902, **35**, 1991) converted into tetramethyluric acid. An attempt to conduct an analogous series of reactions with the glucoside yielded no cryst. material, and was abandoned.

(ii) Boehringer und Söhne (*Chem. Zentr.*, 1901, i, 1219) prepared 9-alkylxanthines by the interaction of uramil with alkyl *iso*thiocyanates. Unsuccessful attempts were made to carry out analogous expts. with dimethyluramil and tetra-acetoglucose *iso*thiocyanate (Fischer, *Ber.*, 1914, **47**, 1377) in anisole, xylene, H_2O, and $NaHCO_3$ solutions. In each case the *iso*thiocyanate was recovered unchanged, and identified by conversion into the thiourethane (Fischer, *loc. cit.*).

Xanthosine.

Enzymatic Preparation of Guanosine from Guanylic Acid.—An aq. solution of guanylic acid, prepared from yeast nucleic acid (50 g.) (Buell and Perkins, *J. Biol. Chem.*, 1927, **72**, 21), was brought to p_H 8·6 with NaOH (final vol., 200 c.c.) and mixed with a filtered aq. solution of a bone phosphatase prepn. (250 mg. of $A/W = 0·23$, dispersed by shaking in 100 c.c. of H_2O; Martland and Robison, *Biochem. J.*, 1929, **23**, 237). The mixture was incubated at 37°, and the progress of the hydrolysis followed by estimating colorimetrically the free and the total phosphate.

Time, hr.	0	14	18
Free phosphate, mg. P	0	196	211
Total phosphate, mg. P	212	207	214

As the hydrolysis proceeded, the guanosine separated in long colourless needles, m. p. 241°, which were collected after 12 hr. at 0°, and when recryst. from H_2O (yield, 1·7 g.) melted at 242° (decomp.) (Found: N, 21·5. Calc. for $C_{10}H_{13}O_5N_5,2H_2O$: N, 21·9%). This guanosine was identical with a specimen prepared by the ammoniacal hydrolysis of yeast nucleic acid.

Xanthosine from Guanosine.—It has been necessary to modify the procedure of Levene and Jacobs (*Ber.*, 1910, **43**, 3150) as regards the amount of AcOH used and the method of isolation. Guanosine (1 g.) was dissolved in boiling $NaNO_2$ aq. (2·8 g. in 7·5 c.c. of H_2O), and the solution cooled rapidly so that the guanosine separated as a caseous mass. AcOH (1·5 c.c. of glacial acid in 6 c.c. of H_2O) was added, and the mixture simultaneously stirred and warmed gently on the water-bath until the guanosine had completely dissolved. The solution was cooled, and treated with small quantities of neutral lead acetate solution and dil. NH_3 aq., until no further ppt. appeared on the addition of a drop of either reagent. The lead salt was collected, washed with H_2O, suspended in hot H_2O, and decomposed with H_2S. The filtrate from the PbS was

concentrated to 100 c.c. in vac., and xanthosine (0·55 g.) separated in colourless prismatic rods (Found : N, 20·1. Calc. for $C_{10}H_{12}O_6N_4$: N, 19·7%).

Methylation of Xanthosine. 3 : 9-*Dimethylxanthine.*—The following description is typical of a large number of expts. Xanthosine (200 mg.) and MeI (2 c.c.) were heated for 3 hr. at 140—150° in a sealed tube. I was liberated and some charring occurred. After distillation of the excess of MeI, the residue was extracted with cold acetone (50 c.c.). The acetone solution (A) and the insol. residue (B) were examined separately.

(A) The acetone was distilled from the extract, and the dark-coloured periodide decomposed by being heated with boiling H_2O (10 c.c.) until no more I was liberated. The aq. solution was concentrated in a vac. desiccator, and the residual light-brown solid stirred with a little acetone. 3 : 9-*Dimethylxanthine hydriodide* (60 mg.) remained as a pale yellow powder containing traces of free pentose, as shown by the phloroglucinol test (Found : C, 27·0; H, 3·3; I, 40·0. $C_7H_8O_2N_4$,HI requires C, 27·2; H, 2·9; I, 41·3%). When heated very rapidly (electric block), this salt melted at 250°, evolving HI, resolidified, and melted unsharply at about 340°, evolving an alkaline gas. Its aq. solution was strongly acid to Congo-red paper (0·5% soln. had p_H 2).

(B) The solid, dissolved in cold H_2O (10 c.c.), was filtered from C, and the solution evaporated to dryness in a vac. desiccator. 3 : 9-Dimethylxanthine hydriodide (70 mg.) remained as a pale yellow powder, containing traces of pentose (Found : C, 25·9; H, 3·1; I, 41·0%). Its properties were identical with those described above.

The hydriodide (270 mg.) in H_2O (5 c.c.) was converted into the hydrochloride (isolated later, m. p. 290°) by treatment with AgCl (350 mg.), and $PdCl_2$ (9 c.c. of 2% soln.) was added to the filtrate, heated at 100°. The *palladochloride* separated slowly in stellate clusters of yellow elongated tablets (155 mg.) which were very sparingly sol. in boiling H_2O [Found : Cl, 13·6; Pd, 20·4. $(C_7H_8O_2N_4)_2,PdCl_2$ requires Cl, 13·2; Pd, 19·9%]. The palladochloride (130 mg.) and freshly pptd. Ag (250 mg.) were shaken in hot H_2O (10 c.c.) for 1 hr., and the hot filtrate evaporated to 2 c.c. On cooling, pure 3 : 9-dimethylxanthine (75 mg.) separated in rectangular plates, m. p. 364°, with evolution of $MeNH_2$ (Found : C, 46·6; H, 4·4; N, 30·7. Calc. for $C_7H_8O_2N_4$: C, 46·7; H, 4·4; N, 31·1%). The chloroaurate separated in clusters of small needles, m. p. 292—293° (decomp.), when $AuCl_3$ was added to a solution in conc. HCl; Biltz and Strufe (*Annalen*, 1921, **423**, 200) give m. p. 297—300° (corr.).

Methylation of Xanthine.—Xanthine (400 mg.) was heated at 140° for 3 hr. with MeI (5 c.c.). The product was treated with acetone and with H_2O as above, and a small yield of 3 : 9-dimethylxanthine hydriodide was isolated. The remainder of the xanthine was recovered unchanged. The hydriodide was identified by conversion into the free base, the chloroaurate and the palladochloride.

Methylation of Theophylline.—Theophylline (1 g.) and MeI (5 c.c.) were heated at 130° for 4 hr., and the MeI evaporated. The residue of caffeine hydriodide dissolved readily in H_2O (10 c.c.), forming a solution acid to Congo-red, and was shaken with excess of AgCl to remove I, mixed with a 2% solution of $PdCl_2$ (40 c.c.), and heated at 100° for 1 hr. By decomp. of the palladochloride with Ag in the usual way, caffeine (0·8 g.) was obtained; it was identified by mixed m. p. and cryst. form of the palladochloride (Gulland and Macrae, *loc. cit.*). The filtrate from which the caffeine had separated contained traces of theophylline and caffeine methochloride.

Methylation of Xanthosine in Presence of Barium Carbonate.—Xanthosine (500 mg.), dry $BaCO_3$ (3 g.), and MeI (5 c.c.) were heated for 3 hr. at 140°. After evapn. of the MeI, the residue was extracted with hot acetone (50 c.c.) and the extract (A) and the insol. solid (B) were examined separately.

(A) The acetone was distilled, and an aq. solution (20 c.c.) of the residue was shaken with AgCl to remove I, and evaporated to dryness in a vac. desiccator. When this material was extracted with hot anhyd. EtOH (20 c.c.), $BaCl_2$ remained undissolved, and the filtrate contained theobromine and theobromine methochloride. After concn. to 5 c.c., the alc. solution deposited crystals which were identified as theobromine (50 mg.) by crystn. from EtOH or H_2O in very sparingly sol., colourless, minute, cigar-shaped crystals, m. p. 320—340° (decomp.), and by conversion into the chloroaurate and palladochloride, which formed stellate or frond-like clusters of leaf-shaped needles. These derivatives were identical in cryst. form and/or m. p. with those prepared from authentic theobromine. When the alc. mother-liquors were concentrated to 2 c.c., *theobromine methochloride* (50 mg.) separated in colourless columns, m. p. 320—340° (decomp.), which were very sol. in H_2O (Found in material dried at 80°/10 mm. : C, 39·1; H, 5·3. $C_8H_{11}O_2N_4Cl,H_2O$ requires C, 38·7; H, 5·2%).

The methochloroaurate, prepared by adding $AuCl_3$ to a solution in conc. HCl, formed wool-like yellow needles, m. p. 265° (decomp.) after darkening at 200°.

(B) The residue, consisting chiefly of $BaCO_3$, was extracted with boiling H_2O (20 c.c.), and the solution shaken with AgCl and evaporated to dryness. The residue was extracted with hot EtOH (20 c.c.) to remove insol. $BaCl_2$, and the solution evaporated to dryness, leaving a gum which contained free pentose. An aq. solution of this gum yielded large quantities of theobromine methochloroaurate, m. p. 265°, when mixed with $AuCl_3$ and HCl.

Attempted Oxidation of Xanthosine and Guanosine with Xanthine Dehydrogenase of Milk.—A dry prepn. of xanthine dehydrogenase was prepared from fresh cows' milk, and contained 0·5 enzyme units per mg. (Wieland and Rosenfeld, *Annalen*, 1929, **477**, 32). The procedure adopted was to place the enzyme, H_2O, and buffer solution in a rubber-stoppered Thunberg tube in a thermostat at 37°. In order to drive out air, N, free from O and S, was bubbled through the solution for 3 min.; the tube was then evacuated and again swept out. This sequence was repeated, and the substrate in aq. methylene-blue solution was added from a micro-burette fitted in the rubber stopper. The total vol. was 5 c.c. The time of decolorisation of the methylene-blue was noted. The experimental details are given below.

Enzyme soln., c.c. 1 C.c. = 10 mg.	Substrate, c.c. *M*/100-aq.	Buffer, c.c. *M*/20-phosphate.	Water, c.c.	Methylene-blue, c.c. *N*/1000.	Decolorisation.
1·0	0·2 Xanthine	1·0 p_H 8	1·8	1·0	1·00, 1·05 min.
1·0	0·2 Xanthosine	1·0 p_H 8	2·3	0·5	None in 4 hr.
1·0	2·0 Xanthosine	1·0 p_H 8	0·5	0·5	,,
2·0	0·2 Xanthosine	1·0 p_H 6	1·3	0·5	,,
1·0	0·2 Guanosine	1·0 p_H 8	2·3	0·5	,,
1·0	2·0 Guanosine	1·0 p_H 8	0·5	0·5	,,
2·0	0·2 Guanosine	1·0 p_H 6	1·3	0·5	,,

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