360. *The Constitution of the Purine Nucleosides. Part II.*

By J. Masson Gulland, Ensor R. Holiday, and Thomas F. Macrae.

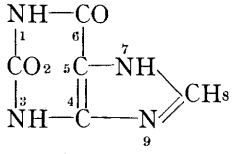
In Part I * an outline was given of the evidence on which the carbohydrate radical of the purine nucleosides has hitherto been assigned to position 7 or 9 of the purine ring. Experiments which were designed to distinguish between these alternatives failed to do so, however, because the glycoside linkage was too labile and fission of the nucleosides occurred.

* "The Constitution of the Purine Nucleosides," Gulland and Macrae, J., 1933, 662, is to be regarded as Part I.

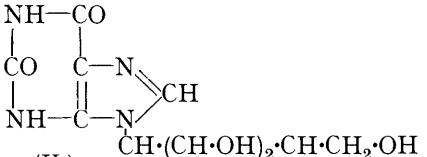
During the course of further work, the scope of the problem became wider for the reasons which are described below. It was desirable to obtain, in addition to xanthosine, a second xanthine nucleoside derived essentially from natural sources, and many attempts were made to prepare 1 : 3-dimethylxanthosine by methylating xanthosine under a variety of conditions. Treatment of xanthosine with methyl sulphate and caustic soda and with methyl iodide and silver oxide, and the action of methyl iodide on xanthosine silver, all yielded products consisting of methylated derivatives of xanthine and methylriboside, showing that the glycosidic linkage had again suffered fission. Levene (*J. Biol. Chem.*, 1923, **55**, 437) mentions similar difficulties, but claims to have obtained dimethylxanthosine by the action of diazomethane. His product, an amorphous hygroscopic powder, had approximately the correct analytical composition and when hydrolysed gave a very small yield of theophylline. Levene and Sobotka (*ibid.*, 1925, **65**, 463) stated that this product was identical with crystalline synthetic theophylline riboside (1 : 3-dimethylxanthine riboside) on the grounds of an agreement between specific rotations and rates of hydrolysis. Unfortunately this identity is now untenable for two reasons. In the first place, the triacetyl ribosidyl bromide used in the synthesis of theophylline riboside has probably a pyranoside structure; in fact, Pryde and Williams (J., 1933, 640) have proved that the analogous synthetic theophylline arabinoside is an arabopyranoside. Levene and Tipson (*J. Biol. Chem.*, 1932, **97**, 491) on the other hand have shown that natural guanosine is a ribofuranoside. Secondly, it will be demonstrated below that the carbohydrate radicals of xanthosine and of synthetic theophylline nucleosides occupy different positions of the purine molecules.

On repeating the methylation of xanthosine with diazomethane no difficulty was experienced in obtaining a product resembling that of Levene in properties, analytical composition and rotation. It was, however, an undoubted mixture of methylated purines and methylriboside, and no evidence was obtained that any purine riboside was present as such. When hydrolysed, this material yielded ribose (as furfuraldehyde), 1 : 7-dimethylxanthine, 3-methylxanthine, and probably 1 : 7 : 9-trimethyluric acid; no trace of theophylline was found.

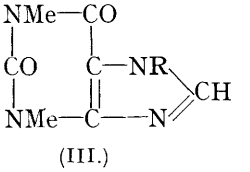
The sole positive evidence for the allocation of the carbohydrate radicals in position 7 or 9 of natural purine nucleosides thus became unreliable, and further, so far as xanthosine, guanosine, and guanylic and xanthylic acids were concerned, positions 1 and 3 could not be definitely excluded, although the latter was improbable. The question of the position of the carbohydrate thus became reopened, the alternatives being positions 1, 3, 7, and 9 of the xanthine molecule (I).



(I.)



(II.)



(III.)

R = ·CH·(CH·OH)$_3$·CH$_2$ (O-bridge) or ·CH·(CH·OH)$_3$·CH·CH$_2$·OH (O-bridge)

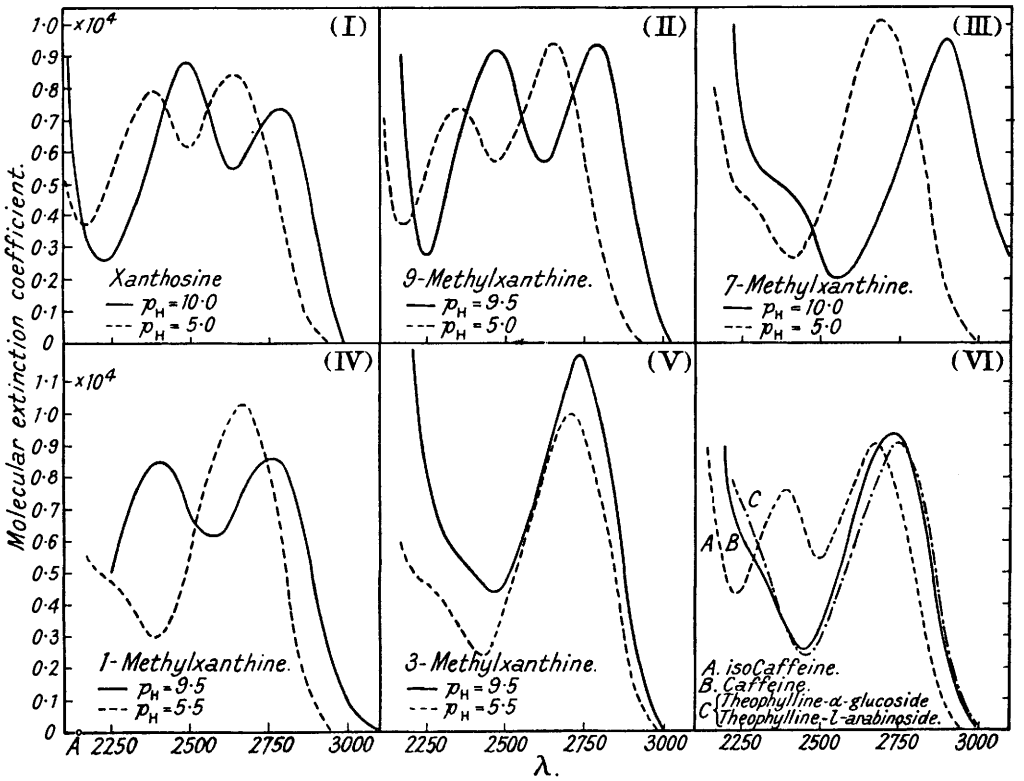
The lability of the glycosidic linkage suggested that success might best be achieved by means of a physical method such as the comparison of the ultra-violet absorption spectra of nucleosides and methylated derivatives of xanthine. A preliminary report has already been published (Gulland and Holiday, *Nature*, 1933, **748**), and the interpretation of the differences in absorption of xanthine and its methylated derivatives and of other purines under various conditions will form a later communication. There is justification for comparing in this way the spectra of compounds in which a methyl group replaces a glycosidic radical, since it may be concluded from the work of Goos, Schlubach, and Schröter (*Z. physiol. Chem.*, 1930, **186**, 148) that simple carbohydrate groups would exert little or no effect on such spectra; our experiments, also, confirm this view, although such

a conclusion is, of course, a reversal of the present line of argument on the absorption spectra of nucleosides.

Xanthine, from which the compounds discussed below are derived, is customarily regarded as (I); substitution of the hydrogen of the iminazole nucleus gives rise potentially to two isomerides (at 7 and 9) and consequently stabilises the double-bond at C_8–N_9 and C_8–N_7 respectively.

The absorption spectra now recorded were measured in aqueous media at p_H 5—5·5 and at p_H 9·5—10. Dilutions were uniformly $M/5000$. The results are summarised in Table I.

The spectrum of xanthosine exhibits two bands at p_H 5 and at p_H 10 (Fig. 1). 9-Methylxanthine also shows two bands in the same solvents (Fig. 2), the wave-lengths of the heads being the same as those of the xanthosine spectra.



The spectrum of 7-methylxanthine, on the other hand, is one-banded at p_H 5 and p_H 10, a shift towards longer wave-length taking place in the alkaline solvent (Fig. 3). A deep trough occurs at the wave-length of the short-wave bands of xanthosine and 9-methylxanthine.

The spectrum of 1-methylxanthine (Fig. 4) shows one band at p_H 5, the wave-length of the head and the coefficient of extinction being the same as those of the corresponding band of 7-methylxanthine. At p_H 9·5 1-methylxanthine shows two bands.

3-Methylxanthine exhibits one-banded spectra at p_H 5 and p_H 10, the wave-length of the head remaining practically unchanged (Fig. 5). This uniformity in acid and alkali is characteristic of certain 3-methyl derivatives of xanthine.

Thus the spectra of xanthosine resemble those of 9-methylxanthine and are unlike those of 1-, 3-, and 7-methylxanthines. This is strong evidence that xanthosine is xanthine-9-riboside, and since Levene and Tipson (*loc. cit.*) have shown that guanosine is a ribofuranoside, as also are adenosine and inosine (Levene and Tipson, *J. Biol. Chem.*,

1932, **94**, 809; Bredereck, *Ber.*, 1933, **66**, 198), it follows that xanthosine is xanthine-9-ribofuranoside (II), and that guanosine and guanylic and xanthylic acids also contain their carbohydrate radicals in position 9.*

Synthetic theophylline-*d*-glucoside (Fischer and Helferich, *Ber.*, 1914, **47**, 210) and theophylline-*l*-arabinoside (Pryde and Williams, *loc. cit.*) at p_H 5—10 exhibit spectra (Fig. 6) which are mutually indistinguishable and closely resemble the one-banded spectra of caffeine (1 : 3 : 7-trimethylxanthine) (Fig. 6). They are unlike the two-banded spectra of *iso*caffeine (1 : 3 : 9-trimethylxanthine) (Fig. 6) under the same conditions. These synthetic nucleosides are therefore 7-substituted xanthines (III), a conclusion which was almost foregone, since they are formed from silver theophylline and tetra-acetyl glucosidyl and triacetyl arabinosidyl bromides respectively under the conditions in which silver theophylline and methyl iodide yield caffeine (Kossel, *Ber.*, 1888, **21**, 2164; *Z. physiol. Chem.*, 1889, **13**, 298).

Experimental.

Methylation of Xanthosine by Diazomethane.—Successive batches of diazomethane were distilled in ether into a mechanically-stirred suspension of powdered anhydrous xanthosine in dry methyl alcohol protected from moisture. When all the xanthosine had dissolved, and the solution still contained diazomethane after several hours, it was evaporated to dryness under reduced pressure. The residue was dissolved twice in absolute alcohol and freed from solvent by distillation under reduced pressure. This process was repeated. The solid thus obtained was dissolved in hot dry alcohol, and the solution poured into much anhydrous ether. Next day the precipitate was collected, washed with dry ether, and treated further as described below (P). The ethereal alcoholic filtrate was concentrated and yielded first needles of pure caffeine and finally a dark brown gum, consisting largely of carbohydrate material giving a strong pentose reaction with phloroglucinol and hydrochloric acid.

The solid (P) was dissolved in hot absolute alcohol, and the solution was cooled and filtered into dry ether. This treatment was repeated twice, and the product, a colourless hygroscopic powder, was dried at 80° under reduced pressure. It melted at about 130° after beginning to shrink at 100°, and remained unchanged in m. p. and weight when dried over phosphoric oxide at 90°/10 mm. for 2 hours [Found : C, 45·3; H, 5·6; N, 16·8. Calc. for $C_{12}H_{16}O_6N_4$ (dimethylxanthosine) : C, 46·1; H, 5·2; N, 17·9%]. It showed $[\alpha]^{20°}_{5461} = -20°$ in water, and did not reduce hot Fehling's solution but gave a strongly positive pentose reaction. The analytical composition of Levene's material was C, 45·71, 45·66; H, 5·45, 5·48; N, 17·72, 17·42; Levene and Sobotka recorded $[\alpha]^{25°}_{D} = -23°$ in alcohol.

Hydrolysis (i). The methylation product (0·2 g.) was hydrolysed for 6 hours on the water-bath with 3*N*-hydrochloric acid (10 c.c.). A small amount of black precipitate was discarded, and the brown solution evaporated to dryness under reduced pressure; the distillate contained furfuraldehyde. The residue was dissolved in water, freed from chloride with silver nitrate, made just alkaline with ammonia, and mixed with silver nitrate solution. The buff precipitate blackened owing to reduction by furfuraldehyde, ribose, or a methylated uric acid. The silver precipitate was centrifuged, washed with water, suspended in hot water, and decomposed with hydrogen sulphide. The hot filtrate from silver sulphide was evaporated to dryness, the residue dissolved in boiling alcohol, and the solution concentrated in stages, three fractions being collected : A, columns separating in the liquid, darkened at 270° and melted at about 350°; B, chiefly columns separating in the liquid, softened at 270° and melted at 350°; C, needles adhering to the glass walls, m. p. 270°.

Fraction C separated from alcohol in hexagonal leaflets, m. p. 290° (uncorr.), identical with the characteristic crystals of 1 : 7-dimethylxanthine [Found : $N{\cdot}CH_3$, 31·0. Calc. for $C_5H_2O_2N_2(N{\cdot}CH_3)_2$: $N{\cdot}CH_3$, 32·2%]. The m. p. of a mixture with authentic 1 : 7-dimethylxanthine was not depressed, whereas a mixture with theophylline melted at 230°. This substance was therefore 1 : 7-dimethylxanthine.

Fraction A was recrystallised from water, and melted with decomposition at about 350°. It yielded a silver salt, soluble in excess of ammonia, which did not reduce hot ammoniacal silver nitrate. In view of these properties we regarded this substance as being in all probability 1 : 7 : 9-trimethyl uric acid (Fischer and Ach, *Ber.*, 1899, **32**, 256).

* In Part I it was stated that the balance of evidence favoured position 7, but that the facts were not decisive. We now adopt the views expressed in this communication.—J. M. G. and T. F. M.

Hydrolysis (ii). A further batch of xanthosine was methylated by diazomethane exactly as described by Levene (*J. Biol. Chem.*, 1923, **55**, 437), the methylation being stopped while unchanged xanthosine remained; the product was worked up as before. The hygroscopic powder (0·7 g.) was hydrolysed by being distilled for 2·5 hours with 20% hydrochloric acid (50 c.c., renewed as required) until the distillate was free from furfuraldehyde, as was shown by the absence of colour with aniline and sodium acetate (Hoffman, *J. Biol. Chem.*, 1927, **73**, 15). Finally the liquid was filtered from a trace of carbonaceous material and evaporated under reduced pressure. The brown gummy residue was dissolved in very dilute nitric acid, freed from chloride by excess of silver nitrate, and treated with ammonia till just alkaline. The buff precipitate of silver salt, part of which readily dissolved in excess of ammonia, was collected and the filtrate was mixed with 20% lead acetate solution and ammonia until no further precipitate of lead salt separated on addition of either reagent. The silver and lead salts were decomposed separately with hydrogen sulphide, and the residues obtained by evaporating the filtrates to dryness were dissolved as far as possible in hot alcohol, the small insoluble portions consisting of high-melting xanthine derivatives which were not theophylline. The alcoholic solutions were mixed and evaporated to dryness, and the residues were dissolved in water and converted into the palladochloride (Gulland and Macrae, J., 1932, 2231). This resembled **3**-methylxanthine palladochloride, and when decomposed in the usual way with silver yielded colourless needles (2—**3** mg.) which darkened at 280° and decomposed at a high temperature without melting. This substance, which was unsubstituted in the iminazole nucleus, since it gave an orange-red coloration with diazotised sulphanilic acid, was evidently **3**-methylxanthine. The amount of palladochloride which was obtained above corresponded to only a part of the solid from which it was prepared, the remainder of the organic material remaining in solution. This was not examined further, since any theophylline would have been precipitated as palladochloride.

Methylation of Xanthosine Silver with Methyl Iodide.—Several experiments were carried out in which xanthosine silver (Found : Ag, 27·0. Calc. for $C_{10}H_{11}O_6N_4Ag$, 27·5%) was treated with excess of methyl iodide in cold and in boiling anhydrous methyl alcohol. The products of these reactions were fractionated by crystallisation from methyl alcohol and from water, the progress of the purifications being followed by estimations of methoxyl and methylimino-groups. The components of these fractions were unchanged xanthosine; 7-methylxanthine [Found : $N{\cdot}CH_3$, 18·0. Calc. for $C_5H_3O_2N_3(NCH_3)$, 17·5%], which was identified by conversion into its characteristic, sparingly soluble sodium salt; and ribose derivatives containing methoxyl but no nitrogen.

Methylation of Xanthosine with Silver Oxide and Methyl Iodide and with Methyl Sulphate and Alkali.—The procedures were those customarily used in carbohydrate chemistry. In both cases practically theoretical yields of caffeine were obtained together with methylated derivatives of ribose.

TABLE I.

	λ (Å.).				$\epsilon \times 10^{-4}$.			
p_H	10·0	5·0	10·0	5·0	10·0	5·0	10·0	5·0
Xanthosine	2780		2470		0·74		0·86	
		2640		2380		0·84		0·78
9-Methylxanthine	2780		2470		0·93		0·93	
		2640		2350		0·93		0·73
7-Methylxanthine	2900		—		0·97		—	
		2690		—		0·96		—
1-Methylxanthine	2760		2410		0·85		0·85	
		2660		—		1·02		—
3-Methylxanthine	2730		—		1·2		—	
		2710		—		1·0		—
Caffeine	2730		—		0·97		—	
		2730		—		0·97		—
*iso*Caffeine	2690		2400		0·90		0·76	
		2670		2390		0·90		0·76
Theophylline-*d*-glucoside	2740		—		0·91		—	
		2740		—		0·91		—
Theophylline-*l*-arabinoside	2740		—		0·87		—	
		2740		—		0·87		—

Purification of **3**-*Methylxanthine.*—When prepared from methylurea and cyanoacetic acid (Traube, *Ber.*, 1900, **33**, 3049), this substance is frequently contaminated with an impurity which exhibits an intense blue fluorescence in alkaline solution. This impurity remained after treatment with charcoal in acid and alkaline solutions and conversion of **3**-methylxanthine into the sparingly soluble barium salt, but it was readily removed by dissolving the base in hot nitric acid (*d* 1·16), cooling, collecting the sparingly soluble nitrate, and decomposing it with sodium acetate. The pure base crystallised from water [Found: $N{\cdot}CH_3$, 17·0. Calc. for $C_5H_3O_2N_3(NCH_3)$, 17·5%].

Spectrographic Data.—Measurements were made with a Hilger medium quartz spectrograph and Spekker photometer. The light source was a condensed spark between tungsten–steel electrodes.

Values have been expressed as molecular extinction coefficients (ε), where $\varepsilon = \frac{1}{c \,.\, d} \,.\, \log \frac{I}{I'}$, c being the molar concentration of the solution examined, d the layer thickness, and I/I' the ratio of the intensity of the incident light (monochromatic) to that of the light of the same wave-length transmitted by the solution.

Samples were anhydrous, having been dried in a vacuum at 110° over phosphoric oxide for 3—5 hours. Solutions were made to a strength of $M/5000$ and were examined in a layer thickness of 1 cm. Hydrochloric acid or sodium hydroxide was added to give the required hydrogen-ion concentration, and the solutions were examined immediately against controls.

We are much indebted to Mr. J. Pryde for a gift of synthetic theophylline-*l*-arabinoside.

THE LISTER INSTITUTE, LONDON, S.W. 1.
THE LONDON HOSPITAL, LONDON, E. 1.

[*Received, July 30th*, 1934.]