44. *The Constitution of Purine Nucleosides. Part X. A New Synthesis of Xanthine and Attempted Syntheses of Xanthine Glucosides from Glyoxalines.*

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A new synthesis of xanthine is described, starting from a glyoxaline derivative. Experiments are recorded in which it was sought to utilise this or a similar method for the synthesis of purine nucleosides with the glycosidic radical in the position occupied in the naturally occurring nucleosides. These experiments failed, because it was not found possible to introduce the glycosidyl radical into the glyoxaline molecule, but successful parallel research using methyl iodide instead of the acetylated bromo-sugar indicated which glyoxalines would have yielded glycosidyl derivatives correctly, or incorrectly, oriented for nucleoside synthesis.

Adenine-9-glucoside and guanine- and hypoxanthine-glucosides, all prepared from the same precursor, are the only known synthetic purine glycosides in which the sugar occupies the same position as do the glycosidic radicals of the naturally occurring nucleosides. The determination of the structure of adenine-9-glucoside (Gulland and Story, J., 1938, 259) depends on the comparison of ultra-violet absorption spectra, and although no question arose of the efficacy of this method in this and other cases it was desirable if possible to confirm its results by an independent means, such as an unequivocal synthesis. For this the starting material must contain either a pyrimidine or a glyoxaline ring, but not both, so that the need for decision between the tautomeric 7- and 9-positions of the purine as the point of entry of the glycosidic radical does not arise. The former alternative was investigated by Gulland and Macrae (J., 1933, 662), who tried unsuccessfully to obtain theophylline-9-glucoside by condensing 1 : 3-dimethyluramil and tetra-acetylglucose *iso*thiocyanate. The present communication records experiments following the latter alternative.

Two syntheses of purines from glyoxalines are known, both yielding 7-substituted derivatives. Sarasin and Wegmann (*Helv. Chim. Acta*, 1924, **7**, 713) converted 4-amino-1-methylglyoxaline-5-carboxyamide into 7-methylxanthine (heteroxanthine) by heating with ethyl carbonate, and Montequi (*Chem. Zentr.*, 1927 ii, 1351) condensed 4-amino-5-cyano-2-methyl-1-ethylglyoxaline with urethane and hydrolysed the product to 8-methyl-7-ethylxanthine. Fargher and Pyman (J., 1919, **115**, 217) and Windaus and Langenbeck (*Ber.*, 1923, **56**, 683) attempted to synthesise xanthine from 4(5)-aminoglyoxaline-5(4)-carboxylic acid, but were obliged to abandon the scheme owing to experimental difficulties.

We have now converted the hydrochloride of the methyl ester of this aminoglyoxalinecarboxylic acid into *methyl* 4(5)-*ureidoglyoxaline*-5(4)-*carboxylate* by means of potassium cyanate, and the corresponding *acid* (I), obtained by hydrolysis, yielded xanthine when heated with hydrochloric acid.

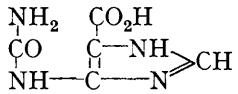
Glycosidic radicals can be substituted for the tautomeric hydrogen atom of a glyoxaline ring by inter-

action of the silver salt with the acetobromo-sugar, and the point of entry may be determined by methylation, fission of the glycosidyl-glyoxaline methiodide by hydrolysis, and identification of the methylated glyoxaline (Gulland and Macrae, *loc. cit.*).

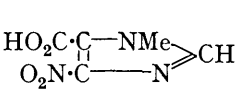
Experiments were made to unite the foregoing synthesis of xanthine with this method of introducing the glycosidyl radical into a suitable glyoxaline in a subsequently determined position. These failed because tetra-acetobromoglucose did not react with the silver salt of the methyl ester of 4(5)-nitroglyoxaline-5(4)-carboxylic acid or of 4(5)-nitro-5(4)-styrylglyoxaline, from which the preceding acid is obtained by oxidation. It was then shown that, even if the condensation had succeeded in the case of the ester, the product would ultimately have led to 7-glucosidylxanthine, because in a comparable reaction methyl iodide transformed the silver salt of the ester into *methyl 4-nitro-1-methylglyoxaline-5-carboxylate*, of which the constitution was ascertained by hydrolysis to the acid (II), obtained by oxidation of *4-nitro-5-styryl-1-methylglyoxaline*, which in turn was prepared from 4-nitro-1: 5-dimethylglyoxaline and benzaldehyde. The orientation here is such as to lead to heteroxanthine.

4(5)-Nitro-5(4)-styrylglyoxaline, on the other hand, would have been a potential starting point, because methyl iodide transformed its silver salt into *5-nitro-4-styryl-1-methylglyoxaline*, also obtained from 5-nitro-1 : 4-dimethylglyoxaline and benzaldehyde. This was oxidised by permanganate to *5-nitro-1-methylglyoxaline-4-carboxylic acid* (III), in which the orientation is such as to lead to 9-methylxanthine.

In view of the lack of success in these reactions with tetra-acetobromoglucose, attention was directed to Sarasin and Wegmann's purine synthesis, and its applicability to the production of a 9-substituted xanthine was tested with positive results by methylation of the silver salt of 4(5)-nitroglyoxaline-5(4)-carboxyamide into *5-nitro-1-methylglyoxaline-4-carboxyamide*, which was hydrolysed to the corresponding acid (III) by Bouveault's method. Unfortunately all attempts failed to effect interaction of the silver salt of the amide with tetra-acetobromoglucose. It is hoped to continue these experiments if opportunity arises.



(I.)



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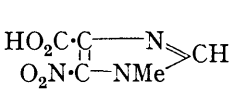

(III.)

Experimental.

4(5)-*Nitro*-5(4)-*methylglyoxaline*.—Methylglyoxaline was prepared from methylglyoxal essentially as described by Gulland and Macrae (*loc. cit.*), but in the larger scale reactions of the present research the purification of the crude base through its silver derivative was omitted, distillation under reduced pressure providing adequate refinement.

Methylglyoxaline was slowly dissolved in nitric acid (*d* 1·42; 2 parts) cooled in a freezing mixture, and concentrated sulphuric acid was gradually added to the cooled solution. The mixture was heated in a boiling brine bath for 2 hours, then boiled in an oil-bath for ½ hour, cooled, and poured on ice. Fargher and Pyman (*loc. cit.*) record that the nitro-derivative separated at this stage, but we found it necessary to neutralise (cooling) with concentrated sodium hydroxide solution. Nitromethylglyoxaline then separated; it crystallised from water in colourless prisms, m. p. 248° as recorded by Fargher and Pyman.

In the hope of avoiding the tedious decomposition of the zinc salt of methylglyoxaline with hydrogen sulphide, followed by the lengthy evaporation of the resulting solution (see Gulland and Macrae, *loc. cit.*), an attempt was made to nitrate the zinc salt directly by the procedure just outlined. Crude nitromethylglyoxaline, m. p. 215°, was produced, but the difficulties encountered in its adequate purification rendered the proposed short-cut uneconomical.

4(5)-*Nitro*-5(4)-*styrylglyoxaline*.—A mixture of nitromethylglyoxaline (20 g.), freshly distilled benzaldehyde (40 g.), and piperidine (2 c.c.) was refluxed for 2 hours at 150—160°. When cold, the resulting golden-yellow mass was extracted with a little hot alcohol to remove tarry by-products, extracted repeatedly with boiling water, in which it was insoluble, crystallised from glacial acetic acid, and washed with acetone. Nitrostyrylglyoxaline (18 g.) formed golden-yellow needles, m. p. 303° (decomp.) (Found: N, 19·5. Calc. for $C_{11}H_9O_2N_3$: N, 19·5%). Windaus and Langenbeck (*loc. cit.*) state that the compound decomposes from 220° onwards.

The aqueous extracts (above) deposited, on cooling, crystalline nitromethylglyoxaline, m. p. 244° (Found: N, 33·1. Calc. for $C_4H_5O_2N_3$: N, 33·1%), which was used in another preparation. It was preferable both here and in the later cases to work under the conditions stated and to recover unchanged material than to raise the temperature; under such conditions the reaction was more complete and little or no nitromethylglyoxaline was recovered, but a large amount of tar was produced and purification of the product was difficult.

4(5)-*Nitro*-5(4)-3′ : 4′-*methylenedioxystyrylglyoxaline* (2·5 g.), prepared from nitromethylglyoxaline (3 g.) and piperonal (8·4 g.) under analogous conditions to those described above, formed yellow-brown plates, m. p. 288° (decomp.) (Found: N, 16·1. $C_{12}H_9O_4N_3$ requires N, 16·2%).

4(5)-*Nitroglyoxaline*-5(4)-*carboxylic Acid and its Methyl Ester*.—Powdered (80 mesh sieve) potassium permanganate (40 g.) was added gradually to a mechanically stirred solution of nitrostyrylglyoxaline (20 g.) in

acetone (200 c.c.) cooled in ice-water. The manganese dioxide sludge was collected and thoroughly extracted with hot water, and the solution, rendered just acid to Congo-red with hydrochloric acid, was evaporated to dryness under reduced pressure. The residue was extracted with carbon tetrachloride in a Soxhlet apparatus for about 30 minutes to remove benzoic acid; ether was found to dissolve some of the nitroglyoxalinecarboxylic acid, and removal of benzoic acid by sublimation was accompanied by partial decomposition of the desired acid.

The nitroglyoxalinecarboxylic acid, left undissolved by carbon tetrachloride, had m. p. 302—303° (decomp.) when crystallised from water (yield, 40%) (Found : N, 26·7. Calc. for $C_4H_3O_4N_3$: N, 26·7%). A small further quantity could sometimes be isolated from the acetone fraction of the oxidation. Windaus and Langenbeck (*loc. cit.*) claim theoretical yields by oxidising nitrostyrylglyoxaline in water, but we have never succeeded in repeating those, and the above method was found more convenient.

Several experiments were carried out in the hope of condensing *p*-nitrosodimethylaniline with nitromethylglyoxaline, since the resulting Schiff's base could be expected to yield nitroglyoxaline aldehyde by acid hydrolysis, and this would readily undergo oxidation to give the acid. Some interaction probably took place under the influence of piperidine or of zinc chloride, but since no pure product could be isolated and not inconsiderable amounts of nitromethylglyoxaline were recovered, the attempt was abandoned.

The methyl ester, colourless plates, m. p. 212—213°, from alcohol (Found : N, 24·6. Calc. for $C_5H_5O_4N_3$: N, 24·6%), was readily obtained from the pure acid by esterification for 1 hour with methyl alcohol (20 parts) containing 5% of dry hydrogen chloride (Windaus and Langenbeck, *loc. cit.*) and partial concentration of the solution. When working on a larger scale, however, it was found convenient to esterify directly the mixture of acid and potassium chloride left from the Soxhlet extraction with carbon tetrachloride, concentrating to dryness the methyl-alcoholic hydrogen chloride solution after the esterification, and adding water, in which the ester was insoluble, to dissolve potassium chloride. In the attempted synthetical experiments the silver salt of the ester was prepared in aqueous alcoholic solution with ammonia and silver nitrate, in order to facilitate its being dried. All attempts failed to condense the dried silver salt with tetra-acetobromoglucose in boiling xylene (Gulland and Macrae, *loc. cit.*; Fischer and Helferich, *Ber.*, 1914, **47**, 210), and the use of the ester itself in acetone in presence of alkali or silver carbonate (Robertson and Robinson, J., 1926, 1715) was equally unsuccessful.

Reduction of the ester to the hydrochloride of methyl 4(5)-aminoglyoxaline-5(4)-carboxylate, m. p. 215° (Found : N, 23·3. Calc. for $C_5H_7O_2N_3$,HCl : N, 23·7%), was effected by hydrogen and palladised charcoal (Windaus and Langenbeck, *loc. cit.*).

Methyl 4(5)-*Ureidoglyoxaline*-5(4)-*carboxylate*.—Solutions of the amino-ester hydrochloride (2 g.) and potassium cyanate (1·8 g.; 2 mols.), each in water (20 c.c.), were mixed, heated on the water-bath for 15 minutes, and cooled. The resulting curdy solid was collected, and a further amount was thrown down by acidifying the filtrate with acetic acid. The combined products were purified by solution in dilute aqueous ammonia (charcoal) and reprecipitation with acetic acid, or by crystallisation from a large volume of water. The *ester* formed a microcrystalline (polarising microscope) powder, m. p. 213° (Found : N, 30·2. $C_6H_8O_3N_4$ requires N, 30·4%). An ammoniacal solution of the ester yielded a curdy white precipitate of the silver salt with ammoniacal silver nitrate solution.

A warm solution of the ester in dilute hydrochloric acid deposited, on cooling, the *hydrochloride*, m. p. 208°, as a microcrystalline powder (Found : N, 25·6. $C_6H_8O_3N_4$,HCl requires N, 25·4%), which tended to lose hydrogen chloride when heated. The picrate, m. p. 208°, separated when aqueous solutions of the ester and picric acid were mixed.

4(5)-*Ureidoglyoxaline*-5(4)-*carboxylic Acid*.—The ester was not hydrolysed by concentrated hydrochloric acid at 100° in 1 hour. A solution of the ester (0·3 g.) in N/2-sodium hydroxide (20 c.c.) was heated at 100° for 1½ hours and neutralised to methyl-orange with hydrochloric acid; the solid which separated was purified by reprecipitation from ammonia solution with acetic acid. The *acid* decomposed at a high temperature without melting (Found : N, 33·1. $C_5H_6O_3N_4$ requires N, 32·9%), and dissolved readily in sodium carbonate solution.

Xanthine.—Ureidoglyoxalinecarboxylic acid was heated with boiling hydrochloric acid (equal parts of acid and water) for 15 minutes. The liquid was made alkaline with ammonia, then acid with acetic acid; xanthine separated as a cream-coloured powder. It was purified by boiling a solution in a slight excess of dilute aqueous ammonia; xanthine then separated in very sparingly soluble flakes (Found in material dried at 110° : N, 36·7. Calc. for $C_5H_4O_2N_4$: N, 36·8%), which gave a strong murexide reaction. The identity of this material was confirmed in two ways. (i) It acted as a substrate in the anærobic system methylene-blue–xanthine dehydrogenase of milk, the times required for decolourisation being the same as those when authentic xanthine was used in similar conditions. (ii) The perchlorates of the synthetic and the authentic xanthine, prepared by dissolving these in hot 30% perchloric acid and cooling the solutions, had the same microscopic appearance and both sintered and darkened at 260° (Biltz and Beck, *J. pr. Chem.*, 1928, **118**, 166).

4-*Nitro*-1 : 5-*dimethylglyoxaline and* 5-*Nitro*-1 : 4-*dimethylglyoxaline*.—Pyman (J., 1922, **121**, 2616) has described these compounds and their preparation from the dimethylglyoxalines (J., 1910, **97**, 1814). The following are additional useful methods, nitromethylglyoxaline being used as starting material.

(i) The silver salt of 4(5)-nitro-5(4)-methylglyoxaline, prepared with aqueous alcoholic ammonia and silver nitrate, was freed from alcohol and water by means of the pump and then by distilling benzene from its suspension in that solvent. Methyl iodide (1·25 mols.) was added, and the mixture boiled for 8 hours; a further

quantity of methyl iodide was added, and the heating repeated. The benzene was distilled from the silver precipitates, which were thoroughly extracted with hot water, and the combined extracts were made alkaline and extracted with chloroform. Distillation of this solvent yielded 4-nitro-1 : 5-dimethylglyoxaline, m. p. 160° after crystallisation from water (Found : N, 29·6. Calc. for $C_5H_7O_2N_3$: N, 29·8%); Pyman (*loc. cit.*) records m. p. 160—161° (corr.).

(ii) A mixture of 4(5)-nitro-5(4)-methylglyoxaline (20 g.), benzene (100 c.c.), and pure methyl sulphate (1·25 mols.; 19 c.c.) was refluxed for 3 hours. When cold, the benzene was decanted, and the residual pasty mass stirred with acetone; the solid was collected, washed with acetone, and crystallised from alcohol. The *methosulphate* formed prisms or fern-like needles, m. p. 143—144° (Found : N, 16·5; S, 12·5. $C_6H_{11}O_6N_3S$ requires N, 16·6; S, 12·6%). An aqueous solution of the methosulphate was made strongly alkaline with sodium hydroxide and extracted with chloroform; distillation of the dried extract yielded 5-nitro-1 : 4-dimethylglyoxaline, m. p. 55—56°, or 57—58° when recrystallised from light petroleum (Found : N, 30·0. Calc. for $C_5H_7O_2N_3$: N, 29·8%). Pyman (*loc. cit.*) records m. p. 57—58° (corr.).

4-Nitro-5-styryl-1-methylglyoxaline.—A mixture of 4-nitro-1 : 5-dimethylglyoxaline (2 g.), freshly distilled benzaldehyde (3·6 g.), and piperidine (0·3 c.c.) was refluxed at 150—160° for 2 hours; when cold, the product was stirred with alcohol to remove tar and extracted with hot water (see below). The residue, the *styryl* compound, crystallised from ethyl acetate in yellow plates, m. p. 150—151° (Found : N, 18·0. $C_{12}H_{11}O_2N_3$ requires N, 18·3%), which were sparingly soluble in hot water and alcohol. The yield was 40—50%, but the aqueous extract (see above) yielded unchanged nitrodimethylglyoxaline, which was used for subsequent preparations.

Methyl 4-Nitro-1-methylglyoxaline-5-carboxylate.—The silver salt of methyl 4(5)-nitroglyoxaline-5(4)-carboxylate, largely freed from water by being stirred with alcohol, was dried by distillation of much of the benzene from its suspension in sulphur-free benzene. Methyl iodide (1·5 mols.) was added, and the mixture refluxed for 8 hours; methyl iodide (1 mol.) was added, and boiling continued for 8 hours. The benzene was removed by distillation, and the residue extracted with hot alcohol. Evaporation of the alcohol left a solid, which was stirred with dilute sodium carbonate solution in order to remove traces of unmethylated ester; the insoluble *methyl 4-nitro-1-methylglyoxaline-5-carboxylate* crystallised from alcohol in prisms or in fern-like needles, m. p. 128—129° (Found : N, 22·7. $C_6H_7O_4N_3$ requires N, 22·7%).

4-Nitro-1-methylglyoxaline-5-carboxylic Acid.—(i) Hydrolysis of the methyl ester by 10% sodium hydroxide solution for 5 minutes at 50° and acidification with acetic acid yielded large prisms, m. p. 160°, which were identified as 4-nitro-1-methylglyoxaline-5-carboxylic acid (Sarasin and Wegmann, *loc. cit.*, give m. p. 160°) by mixed m. p. with a sample prepared from the styryl compound by oxidation, and also by conversion into 4-nitro-1-methylglyoxaline, m. p. 135°, through elimination of carbon dioxide at 160°.

(ii) 4-Nitro-5-styryl-1-methylglyoxaline was oxidised in acetone solution with powdered potassium permanganate exactly as described for 4(5)-nitro-5(4)-styrylglyoxaline. The product formed prisms, m. p. 160°. The yields were not good and the method (i) above is to be regarded as preferable.

4-Nitro-1-methylglyoxaline.—A slight excess of alcoholic silver nitrate solution was added slowly to a stirred solution of 4(5)-nitroglyoxaline (Fargher and Pyman, *loc. cit.*) (2·2 g.) in aqueous alcoholic ammonia. The gelatinous precipitate of silver salt soon became granular; it was collected, washed with alcohol, and freed from alcohol and water by distillation of its suspension in benzene. Methyl iodide (2·5 g.) was added, and the mixture refluxed for 8 hours; this treatment was repeated twice. The benzene and methyl iodide were distilled off, and the residue extracted with hot water, from which 4-nitro-1-methylglyoxaline, m. p. 135°, separated in needles after concentration (Hazeldine, Pyman, and Winchester, J., 1924, **125**, 1431, record m. p. 133—134°). None of the isomeric 5-nitro-1-methylglyoxaline was found.

5-Nitro-4-styryl-1-methylglyoxaline.—(i) A mixture of 5-nitro-1 : 4-dimethylglyoxaline (10 g.), benzaldehyde (20 c.c.), and piperidine (1 c.c.) was refluxed at 150—160° for 2 hours. The cooled product was mixed with light petroleum (b. p. 60—80°; 60 c.c.) to remove the excess of benzaldehyde and left for 12 hours until deposition of yellow prisms had ceased. The light petroleum was decanted, and the residue washed with a little alcohol to remove tar and crystallised from ethyl acetate. The *styryl* compound formed golden-yellow, prismatic needles, m. p. 214—215° (Found : N, 18·1. $C_{12}H_{11}O_2N_3$ requires N, 18·3%), which were more readily soluble in alcohol and acetone than the isomeric 4-nitro-5-styryl-1-methylglyoxaline. The yields did not exceed 25—30%.

(ii) The silver salt of 4(5)-nitro-5(4)-styrylglyoxaline, prepared from aqueous alcoholic ammonia and silver nitrate, was dried by distillation of its suspension in benzene, and then refluxed with methyl iodide as described in other cases. The benzene was distilled off, and the residue extracted with hot ethyl acetate, from which the styryl compound crystallised in good yield after concentration; it was identified by mixed m. p.

5-Nitro-1-methylglyoxaline-4-carboxylic Acid.—(i) Oxidation of the corresponding styryl compound in acetone by powdered potassium permanganate was carried out in the usual way; the *acid* crystallised from water in prisms or hexagonal plates, m. p. 165° (Found in material dried at 110° : N, 24·5. $C_5H_5O_4N_3$ requires N, 24·5%. Found in material dried in a vacuum : N, 21·5; loss at 110°, 11·8. $C_5H_5O_4N_3,1\frac{1}{2}H_2O$ requires N, 21·5; loss, 12·3%).

(ii) 5-Nitro-1-methylglyoxaline-4-carboxyamide withstood hydrolysis by concentrated hydrochloric acid in a sealed tube for 3 hours at 160°, but was successfully hydrolysed by Bouveault's method (*Bull. Soc. chim.*, 1892, **9**, 368). Barium nitrite (220 mg.), dissolved in the minimum quantity of water, was added drop by drop

to a solution of the amide (146 mg.) in 90% sulphuric acid (4 c.c.) cooled in ice. The mixture was left at room temperature for 24 hours and poured into much water, and sulphate ions were exactly removed from the hot solution by means of barium acetate solution. The barium sulphate was centrifuged and the clear, colourless solution, containing neither barium nor sulphate ions, was concentrated under reduced pressure to about 10 c.c. A good yield of the acid separated in hexagonal plates, m. p. 165°.

Specimens of the acid prepared by either method evolved carbon dioxide briskly as they melted, and the residues crystallised at once on cooling and then melted at 54—55°, being thus identified as 5-nitro-1-methylglyoxaline; Hazeldine, Pyman, and Winchester (*loc. cit.*) give m. p. 55°.

5-*Nitro*-1-*methylglyoxaline*-4-*carboxyamide*.—The silver salt of 5(4)-nitroglyoxaline-5(4)-carboxyamide (Windaus and Langenbeck, *loc. cit.*), prepared in aqueous alcoholic ammonia and silver nitrate, was freed from alcohol and water by distillation of its suspension in benzene. Methyl iodide (about 2 mols.) was then added, and the mixture refluxed for 8 hours; more methyl iodide was added, and the boiling continued for 24 hours in all. The benzene was removed by distillation, the residue extracted with hot water, and the extract concentrated under reduced pressure to small volume. The solid which crystallised was stirred with dilute aqueous ammonia to remove traces of unchanged amide, m. p. 291°; the residual 5-*nitro*-1-*methylglyoxaline*-4-*carboxyamide* crystallised from water in both needles and dog-tooth shaped crystals, m. p. 234° (Found: N, 33·1. $C_5H_6O_3N_4$ requires N, 32·9%). The two crystalline forms appeared in the same solutions, and were separated by hand and proved to be identical by mixed m. p. The isomeric 4-nitro-1-methylglyoxaline-5-carboxyamide (Sarasin and Wegmann, *loc. cit.*) has m. p. 257—258°.

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