580. *Chemical Studies in the Biosynthesis of Purine Nucleotides. Part III.*[1] *The Synthesis of 5-Amino-1-(β-D-ribofuranosyl)glyoxaline-4-carboxyamide and 4-Amino-1-(β-D-ribofuranosyl)glyoxaline-5-carboxyamide.*

By J. BADDILEY, J. G. BUCHANAN, F. E. HARDY, and J. STEWART.

The ribosyl derivative (III) of 5-aminoglyoxaline-4-carboxyamide, indistinguishable from the natural material, has been synthesised by condensing the silver or chloromercury salt of methyl 5-nitroglyoxaline-4-carboxylate (VII) with tri-*O*-benzoylribofuranosyl chloride, treatment with ammonia, separation of the resulting isomers (XIV and XVI; R = ribofuranosyl), and hydrogenation.

The isomers were oriented by comparison of their spectra with authentic methylated nitro-amides (XIV and XVI; R = Me) and amino-amides (XVII and XVIII; R = Me). An unusual rearrangement was noted during this work.

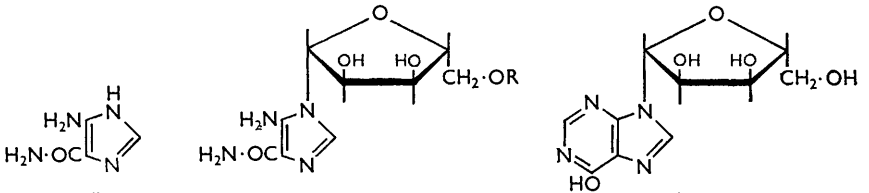
The preparation and properties of glucosyl derivatives of 4-nitro-5-styrylglyoxaline (V) were investigated. A preliminary account of this work has been published.[2]

IT was observed by Stetten and Fox [3] that a base, subsequently identified [4] as 5-aminoglyoxaline-4-carboxyamide (I), accumulated during sulphonamide-inhibition of *Escherichia coli*. It was suggested [4] that the base was an intermediate in purine biosynthesis in *E. coli*. Later work showed that the carboxyamide (I) was not on the direct pathway,[5] and that the ribose phosphate derivative [6] (II) was the true precursor. Greenberg and Spilman [7] isolated the ribofuranosyl derivative (III) from sulphonamide-inhibited *E. coli*, and examined its structure. Acid-hydrolysis gave the base (I) and ribose, while formylation and ring-closure gave inosine (IV). A chemical synthesis of (III) was clearly of interest.

We envisaged a synthesis by reaction of the mercury or silver salt of a suitable glyoxaline with a tri-*O*-acylribofuranosyl halide. The carboxyamide (I) has been synthesised by a number of methods [8-10] and is readily available. It is, however, unstable to heat and to

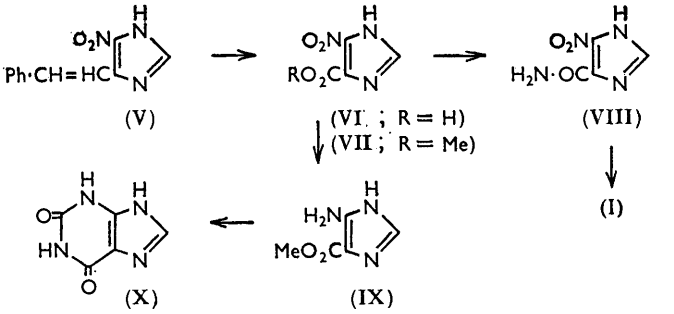
[1] Part II, Baddiley, Buchanan, Hodges, and Prescott, *J.*, 1957, 4769.
[2] Baddiley, Buchanan, and Stewart, *Proc. Chem. Soc.*, 1957, 149.
[3] Fox, *Proc. Soc. Expt. Biol. Med.*, 1942, **51**, 102; Stetten and Fox, *J. Biol. Chem.*, 1945, **161**, 333.
[4] Shive, Ackermann, Gordon, Getzendaner, and Eakin, *J. Amer. Chem. Soc.*, 1947, **69**, 725.
[5] Cf. Baddiley and Buchanan, *Ann. Reports*, 1957, **54**, 329.
[6] Gots, *Nature*, 1953, **172**, 256.
[7] Greenberg and Spilman, *J. Biol. Chem.*, 1956, **219**, 411.
[8] Windaus and Langenbeck, *Ber.*, 1923, **56**, 683.
[9] Shaw and Woolley, *J. Biol. Chem.*, 1949, **181**, 89.
[10] Cook, Heilbron, and Smith, *J.*, 1949, 1440.

oxidation, and the presence of the amino-group is clearly undesirable in a reaction of this kind. On the other hand, protection of the amino-group by acylation yields derivatives very susceptible to cyclisation, giving xanthine or 2-substituted hypoxanthines under basic conditions.[9] For these reasons we turned to the 4-nitroglyoxalines.



(I) (II ; R = PO_3H_2) (III ; R = H) (IV)

Windaus and Langenbeck [8] prepared 4-nitro-5-styrylglyoxaline (V) and converted it into the nitro-acid (VI), the methyl ester (VII), and the amide (VIII). The ester and amide could be reduced catalytically to the corresponding amino-compounds (IX) and (I) and characterised as hydrochloride or picrate. Allsebrook, Gulland, and Story [11] sought to use some of these intermediates in a synthesis of xanthine and its glucosides. Although they successfully converted the amino-ester (IX) into xanthine (X), they were unsuccessful in attempts to convert any of the glyoxalines (V), (VII), or (VIII) into glucosyl derivatives by the use of silver salts and tetra-*O*-acetyl-α-D-glucopyranosyl bromide. In model experiments using methyl iodide in place of the glucosyl bromide methylated glyoxalines were obtained. The orientation of these products was used to predict which of the nitroglyoxalines might yield glycosyl derivatives correctly oriented for conversion into 9-glycosylpurines. We considered that a reinvestigation of these reactions, using silver or chloromercury salts [12] and tri-*O*-benzoylribofuranosyl chloride,[12,13] might lead to 5-amino-1-(β-D-ribofuranosyl)glyoxaline-4-carboxyamide (III).

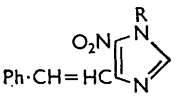


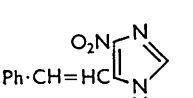
(V) (VI ; R = H) (VII ; R = Me) (VIII) (I) (X) (IX)

The earlier work indicated that the nitro-styrylglyoxaline (V) might give glycosyl derivatives of the correct orientation. It was found, however, that methylation of either the mercury or silver salt gave two products. One was the 1-methyl-5-nitro-derivative (XI; R = Me) as described by Allsebrook *et al.*[11] and the other the 1-methyl-4-nitro-compound (XII), originally made from 1,5-dimethyl-4-nitroglyoxaline.[11] These compounds were separated on alumina and their ultraviolet spectra were sufficiently different for use in the orientation of possible glycosyl compounds by the classical method of Gulland, Holiday, and Macrae.[14] When tri-*O*-acetyl-β-D-xylosyl bromide [15] was condensed with the silver salt of the nitro-compound (V) the triacetate of the xylosyl derivative was isolated

[11] Allsebrook, Gulland, and Storey, *J.*, 1942, 232.
[12] Haynes and Newth, *Adv. Carbohydrate Chem.*, 1955, **10**, 207.
[13] Kissman, Pidacks, and Baker, *J. Amer. Chem. Soc.*, 1955, **77**, 18.
[14] Gulland, Holiday, and Macrae, *J.*, 1934, 1639.
[15] Günther, Helferich, and Pigman, *Ber.*, 1939, **72**, 1953.

in 51% yield. Deacetylation yielded the free xyloside, whose absorption spectrum showed it to be the 5-nitro-1-xylosyl derivative (XI; R = β-D-xylopyranosyl) (see Table 1). The


(XI)


(XII)

xyloside is very labile towards acid, owing no doubt to the presence of the nitro-group, and the spectrum at an acid pH had to be measured rapidly.

TABLE 1. *Ultraviolet spectra of nitro-styrylglyoxalines in* 3 : 1 *ethanol–water.*

Glyoxaline derivative	$\lambda_{max.}$ (mμ) pH > 10	$\lambda_{max.}$ (mμ) pH < 1	ε pH > 10	ε pH < 1
4-Nitro-5-styryl (V)	280·5	269·5	24,760	21,240
	408	371	16,860	17,400
1-Methyl-5-nitro-4-styryl (XI; R = Me)	274	273·5	18,790	22,950
	384	384	10,710	16,160
1-Methyl-4-nitro-5-styryl (XII)	268	269	19,320	23,430
	367	366	12,320	12,840
5-Nitro-4-styryl-1-xylosyl (XI; R = xylosyl)	273	273	21,820	22,020
	384	384	15,900	17,910
5-Nitro-1-ribosyl-4-styryl (XI; R = ribosyl)	273·5	270	21,480	18,830
	385	372	14,980	15,190

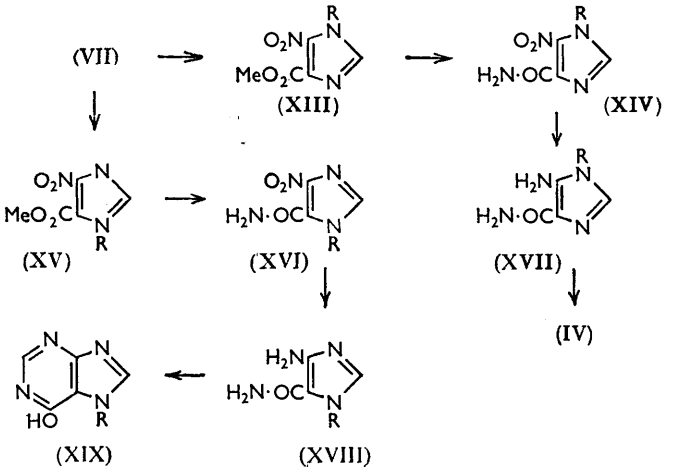
A similar reaction using tri-*O*-benzoylribofuranosyl chloride [3] was unsuccessful, but with the chloromercury salt a crystalline product was isolated after chromatography. Debenzoylation gave the ribosyl derivative (XI; R = β-D-ribofuranosyl), identified by its absorption spectra. It will be noticed (Table 1) that the spectrum in acid is the same as that of 4-nitro-5-styrylglyoxaline rather than that of the 1-methyl-5-nitro-compound. This is due to the great lability of the ribofuranosyl derivative towards acid. The spectrum in alkali is, however, sufficient for orientation. The synthetic glycosyl derivatives were assumed, by their mode of formation, to be β-compounds and to possess the same lactol ring structure as the parent glycosyl halides.

Attempts were made to oxidise the styryl compound (XI; R = tri-*O*-benzoylribofuranosyl) to the corresponding glyoxaline-4-carboxylic acid by neutral permanganate.[8,11] Great difficulties were encountered owing to solubility problems, incomplete oxidation, and the extreme acid-lability of 1-glycosyl-nitroglyoxalines.

Gulland and his co-workers [11] assumed that methylation of the silver salt of a glyoxaline was a reliable model for glycosylation. We have shown that in the nitro-styryl series this is not so. Although the earlier workers found that methylation of the silver salt of the nitro-ester (VII) gave the 1-methyl-4-nitro-compound (XV; R = Me) as the sole product, we considered that the nitro-ester (VII) might be a suitable starting point for our purpose. Condensation between the silver or chloromercury salt of the nitro-ester (VII) and tri-*O*-benzoylribofuranosyl chloride occurred in boiling xylene. Non-glycosyl compounds were removed by chromatography of the crude product on neutral alumina. A major fraction, presumably a mixture of esters (XIII and XV; R = ribofuranosyl), gave on prolonged treatment with methanolic ammonia a mixture which was examined by paper chromatography. Two components had the required properties, giving ribose and 5-nitroglyoxaline-4-carboxyamide (VIII) on mild acid-hydrolysis. Separation of the two isomers was achieved by chromatography on thick paper sheets, counter-current distribution having failed to give a clear-cut separation. One of the isomers was crystalline, and was converted by hydrogenation into a crystalline amino-compound differing from Greenberg and Spilman's ribosyl compound; [7] these synthetic compounds are shown below to have structures (XVI and XVIII respectively; R = ribofuranosyl).

The other component, present in equal amount in the original mixture, was not

crystalline, though chromatographically pure. Hydrogenation gave an amino-compound, isolated as its crystalline picrate. The free base was indistinguishable from a sample, kindly given to us by Professor G. R. Greenberg, in R_F values, ultraviolet absorption spectra, and the colour given by the Bratton–Marshall reagents.[16]



The orientation of the isomeric aminoglyoxaline carboxyamides was established in a number of ways. Greenberg and Spilman [7] showed that their compound (III) or (XVII; R = ribofuranosyl) could be converted into inosine (IV) by formylation of the amino-group, followed by ring-closure. Both synthetic aminoglyoxalinecarboxyamide isomers were treated in this way. One gave a nucleoside having R_F values and absorption spectra identical with those of inosine, in agreement with Greenberg and Spilman, whereas the other yielded a different nucleoside. The latter has the same spectrum as 7-methylhypoxanthine [17] (XIX; R = Me) and is undoubtedly the inosine isomer (XIX; R = ribofuranosyl) (see Table 2).

TABLE 2. *Ultraviolet spectra of 7- and 9-substituted hypoxanthines in water.*

	$\lambda_{max.}$ (mμ)		
	pH < 1	pH 7	pH > 10
Hypoxanthine [17]	248	—	262
Inosine [17]	247	—	254
Product from (XVII; R = ribosyl)	248	248	252
9-Methylhypoxanthine [17]	248	—	255
7-Methylhypoxanthine [17]	250	—	261
Product from (XVIII: R = ribosyl)	252	255	263

The methylated nitro-amides (XIV [11] and XVI; [18] R = Me), and the amino-amides (XVII and XVIII; [18] R = Me) were also prepared as reference compounds. Their ultraviolet spectra were compared with those of the corresponding ribosyl derivatives and the results (see Table 3) were in complete agreement with Greenberg and Spilman's structure.[7]

Two new syntheses of the amino-carboxyamide (III) from inosine (IV) have appeared [19] since the publication of our preliminary communication.[2]

Several points of interest arose during the synthesis of the methylated glyoxalines. When the silver salt of the nitro-ester (VII) was treated with methyl iodide methyl 1-methyl-4-nitroglyoxaline-5-carboxylate (XV; R = Me) was the sole product, in agreement with

[16] Bratton and Marshall, *J. Biol. Chem.*, 1939, **128**, 537.
[17] Gulland and Holiday, *J.*, 1936, 765.
[18] Sarasin and Wegmann, *Helv. Chim. Acta*, 1924, **7**, 713.
[19] Shaw, Abs. Int. Congr. Pure Appl. Chem., Paris, 1957; *J. Amer. Chem. Soc.*, 1958, **80**, 3899; Abs. 4th Internat. Congr. Biochem., Vienna, 1958, p. 6.

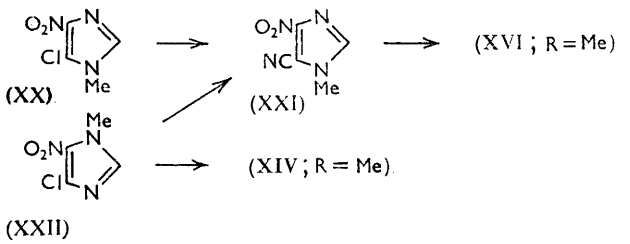
Allsebrook *et al.*[11] Treatment of this with methanolic ammonia at 0° gave the carboxyamide (XVI; R = Me) described by Sarasin and Wegmann.[18] On the other hand, by chromatography of the methylation product of the silver salt of the nitro-amide (VIII) a

TABLE 3. *Ultraviolet spectra of glyoxaline-4-carboxyamides in water.*

Glyoxaline-4-carboxyamide	$\lambda_{max.}$ (mμ) pH < 1	pH 7	pH > 10
1-Methyl-4-nitro- (XVI; R = Me)	300	299	300·5
4-Nitro-1-ribosyl- (XVI; R = ribosyl)	295	294	295·5
1-Methyl-5-nitro- (XIV; R = Me)	303·5	303·5	304·5
5-Nitro-1-ribosyl- (XIV; R = ribosyl)	304	301	301
4-Amino-1-methyl- (XVIII; R = Me)	266, 239 *	271	271
4-Amino-1-ribosyl- (XVIII; R = ribosyl)	267, 241	273	274
5-Amino-1-methyl- (XVII; R = Me)	267, 240	266·5	267
5-Amino-1-ribosyl- (XVII; R = ribosyl)	267, 247	266	267

* Shoulder.

small amount of the 5-carboxyamide (XVI; R = Me) was isolated, together with the 4-carboxyamide. Allsebrook *et al.*[11] reported the 4-carboxyamide as the sole product. We attempted to prepare the latter by an extension of Sarasin and Wegmann's method [18] for the preparation of the 5-carboxyamide (XVI; R = Me). These workers described



the reaction of 5-chloro-1-methyl-4-nitroglyoxaline (XX) with potassium cyanide in ethanol to give the cyanide (XXI) which was subsequently hydrolysed to the carboxyamide (XVI; R = Me).

When the isomeric 4-chloro-1-methyl-5-nitroglyoxaline (XXII), rigorously purified by chromatography, was treated similarly no reaction was observed. When the reactants were heated for 20 hours in dimethylformamide at 120—130° a substantial yield of the cyanide (XXI) was produced, but none of the required compound. The formation of a rearranged product is noteworthy and we are unaware of a precedent.[20] The possibility was considered that the apparent methyl migration was a result of methylation by dimethylformamide with subsequent decomposition of the quaternary compound, but the rearranged product could be isolated (in small yield) when no solvent was used. In the absence of solvent, a very small amount of 1-methyl-5-nitroglyoxaline-4-carboxyamide (XIV; R = Me) was isolated by chromatography.

Reference has been made above to the acid-lability of ribosylnitroglyoxalines. In addition, it was found during experiments on the isolation of the ribosyl derivatives of 5-nitroglyoxaline-4-carboxyamide that 5-nitro-1-ribosylglyoxaline-4-carboxyamide (XIV; R = ribosyl) was converted into the aglycone [(VIII) or (XIV; R = H)] by the action of sodium methoxide in methanol. The sugar product had the chromatographic properties of a methyl riboside. The reaction, which recalls the behaviour of an aryl glycoside [21] rather than a ribosylglyoxaline, has not been investigated further.

[20] Cf. Hofmann, " Imidazole and its Derivatives," Part I, Interscience Publ. Inc., New York, 1953.
[21] Ballou, *Adv. Carbohydrate Chem.*, 1954, **9**, 59.

Experimental

Infrared spectra were determined for potassium bromide discs.

1-Methyl-5-nitro-4-styrylglyoxaline and 1-Methyl-4-nitro-5-styrylglyoxaline.—4-Nitro-5-styrylglyoxaline (3 g.) in 50% aqueous ethanol (200 c.c.) containing sodium hydroxide (0·62 g., 1·1 mol.) was treated with mercuric chloride (4·2 g., 1·1 mol.) in the same solvent (100 c.c.) at the b. p. The yellow precipitate became orange on cooling. The salt was filtered off, washed with water, ethanol, and ether, and dried at 55° *in vacuo* (yield 6·2 g., 98%). The salt was suspended in dry, sulphur-free xylene (250 c.c.) from which 100 c.c. were removed by distillation at atmospheric pressure. Methyl iodide (6 g., 3 mol.) was added, and the mixture boiled under reflux for 2·5 hr., cooled, and evaporated to dryness. The residue was extracted (Soxhlet) with hot ethyl acetate, and the evaporated extract chromatographed from benzene on Grade 0 alumina. Two orange bands were eluted, with benzene–ether and chloroform–methanol respectively. The first gave 1-methyl-5-nitro-4-styrylglyoxaline (0·41 g.), m. p. 213—214° (from ethyl acetate) (Allsebrook *et al.*[11] give m. p. 214—215°). The second gave 1-methyl-4-nitro-5-styrylglyoxaline (0·54 g.), m. p. 143—144° (from ethyl acetate), (Found: C, 63·1; H, 4·9. Calc. for $C_{12}H_{11}O_2N_3$: C, 62·9; H, 4·8%) (Allsebrook *et al.*[11] give m. p. 150—151°).

5-Nitro-4-styryl-1-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)glyoxaline.—4-Nitro-5-styrylglyoxaline (3·3 g.) was dissolved in aqueous ethanol (1 : 2; 2·5 l.). Silver nitrate (2·5 g.) in the same solvent (50 c.c.) was added with vigorous stirring while both solutions were boiling. 6N-Ammonia was added to pH 7 and the orange precipitate of silver salt was digested at 50° for 7 hr. in the dark. After cooling, the precipitate (4·3 g., 87%) was filtered off, washed with water, ethanol, and ether, and dried *in vacuo*. Light was excluded from this product.

The silver salt (3·6 g.) was suspended in dry sulphur-free xylene (500 c.c.), and solvent (100 c.c.) was removed by distillation. The suspension was cooled, 2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl bromide (3·6 g.) in xylene (100 c.c.) added, and the mixture was boiled under reflux for 0·5 hr., with stirring. Silver salts were removed by filtration, and xylene was evaporated from the filtrate under reduced pressure. The gummy residue crystallised from methanol to give the *xylosyl compound* as yellow needles (2·7 g., 51%), m. p. 149—150° (Found: C, 55·6; H, 5·4; N, 8·4. $C_{22}H_{23}O_9N_3$ requires C, 55·8; H, 4·9; N, 8·9%).

5-Nitro-4-styryl-1-β-D-xylopyranosylglyoxaline.—The above triacetate (0·32 g.) was deacetylated with methanolic ammonia (100 c.c., saturated at 0°) during 18 hr. at 0°. The solid residue obtained after evaporation was recrystallised from methanol, giving the *xylosyl derivative* as yellow needles (0·19 g., 80%), m. p. 185—187° (Found: C, 55·5; H, 5·3; N, 11·6. $C_{16}H_{17}O_6N_3$ requires C, 55·3; H, 4·9; N, 12·1%).

5-Nitro-4-styryl-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)glyoxaline.—The above mercury salt of 4-nitro-4-styrylglyoxaline (3·9 g.) with "Hyflo Supercel" silica (5 g.), was suspended in sulphur-free xylene, 100 c.c. of which were removed by distillation. To the boiling solution was added 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl chloride [from the 1-*O*-acetyl derivative (5 g.)] in xylene (100 c.c.). The mixture was boiled for 0·5 hr., then filtered while hot, and the volume of the filtrate reduced to about 50 c.c. by evaporation under reduced pressure. Addition of light petroleum (b. p. 60—80°) caused precipitation of a sticky solid, removed by filtration. The solid was treated with chloroform (150 c.c.), filtered from some 4-nitro-5-styrylglyoxaline (0·46 g.; m. p. 260—280°), and shaken with 30% potassium iodide solution, and the chloroform layer was dried ($MgSO_4$). Evaporation of the filtered solution left a gum (5 g.) which was chromatographed in benzene (100 c.c.) on neutral alumina (600 g.). A yellow band was eluted with benzene–chloroform (3 : 1), giving, from ethanol, the *ribosyl derivative* as yellow needles (2·3 g., 41%), m. p. 115—118° (Found: C, 67·8; H, 4·9; N, 6·1. $C_{37}H_{29}O_9N_3$ requires C, 67·4; H, 4·4; N, 6·4%).

5-Nitro-1-(β-D-ribofuranosyl)-4-styrylglyoxaline.—The above tribenzoate (0·3 g.) was dissolved in methanol (100 c.c.) saturated with ammonia at 0° and kept at 0° for 16 hr. The gummy residue left after evaporation of solvent was crystallised from methanol to give the *ribosyl compound* (0·16 g., 70%) as yellow needles, m. p. 255—256° (Found: C, 55·2; H, 5·4; N, 11·8. $C_{16}H_{17}O_6N_3$ requires C, 55·3; H, 4·9; N, 12·1%).

Silver Salt of Methyl 5-Nitroglyoxaline-4-carboxylate, and Condensation with Tri-O-benzoyl-ribofuranosyl Chloride.—The methyl ester [8] (1·3 g.; prepared from the acid [8,22]) was dissolved in 50% aqueous ethanol (100 c.c.) and mixed, with vigorous stirring, with a solution of silver

[22] Kuler and Gireva, *Zhur. priklad. Khim.*, 1957, **30**, 811.

nitrate (1·3 g.) in the same solvent (50 c.c.) at the b. p. The gelatinous suspension was brought to pH 7 with dilute ammonia and kept at 60° for 2 hr. " Hyflo Supercel " silica (3·6 g.) was added with shaking, and the mixture allowed to cool. Solids were filtered off, washed with water, ethanol, and ether, and then dried (P_2O_5) *in vacuo* at 50° (yield, 1·2 g., 62%). The salt was suspended in dry sulphur-free xylene, and 100 c.c. were removed by distillation at atmospheric pressure. Tri-*O*-benzoylribofuranosyl chloride [from 1-*O*-acetyl compound (4·0 g.)] in dry xylene (70 c.c.) was added and the mixture was boiled under reflux for 1 hr. with stirring. The solid was filtered off while hot, cooled, and filtered again to remove a slight opalescence. The filtrate was evaporated under reduced pressure and the gummy residue was dissolved in chloroform and then shaken with aqueous sodium hydrogen carbonate, followed by water. The chloroform solution was dried (Na_2SO_4) and evaporated to a syrup (4·35 g.) which was chromatographed in benzene on neutral alumina (250 g.). A yellow band was eluted with chloroform and gave a syrup (3·8 g.) on evaporation. The syrup was treated with methanolic ammonia (50 c.c.) at 0° for 90 hr., and evaporated to a syrup (2·7 g.), which was dissolved in a mixture of water (30 c.c.) and ethanol (10 c.c.). This solution was extracted twice with ether (5 c.c.), and the aqueous layer subjected to descending paper chromatography on sheets of Whatman No. 3 paper in solvent B. The compounds corresponding to bands at R_F 0·5 and 0·6, detected by their absorption of ultraviolet light, were extracted with water, and the solids from each isolated by freeze-drying.

The first band gave a residue (0·219 g.) which was dissolved in hot methanol, which was then filtered, and further impurities were removed by precipitation from the cold solution by addition of acetone. Solvents were removed *in vacuo* to give syrupy 5-*nitro*-1(β-D-*ribofuranosyl*)*glyoxaline*-4-*carboxyamide* chromatographically pure (see Tables 3 and 5).

The second solid (0·244 g.) was treated similarly to remove flocculent impurities and crystallised from methanol as needles. 4-*Nitro*-1-(β-D-*ribofuranosyl*)*glyoxaline*-5-*carboxyamide* (0·131 g.) had m. p. 175—177°, $[\alpha]_D$ −0·6° (*c* 1·02 in H_2O) (Found: C, 37·2; H, 4·8; N, 18·9. $C_9H_{12}O_7N_4$ requires C, 37·5; H, 4·2; N, 19·4%). The ribosyl derivative consumed 1·1 mol. of sodium periodate to give a dialdehyde, $[\alpha]_D$ +183·9° (*c* 1·01 in H_2O), which slowly separated from solution (see Tables 3 and 5).

5-*Amino*-1-(β-D-*ribofuranosyl*)*glyoxaline*-4-*carboxyamide*.—The above 5-nitro-compound (0·186 g.) in water (10 c.c.) was shaken with pre-reduced Adams platinum oxide (0·114 g.) in hydrogen at atmospheric pressure for 1 hr.: uptake was then complete. The catalyst was removed by filtration and the filtrate evaporated to a syrup, which was examined by paper chromatography. The major product, which absorbed ultraviolet light and behaved as a diazotisable amine, had the same R_F values as the natural compound. The syrup (0·02 g.) in water (3 c.c.) was treated at 60° with aqueous picric acid (9 c.c., saturated at 0°). After cooling, the picrate was filtered and washed with a little water. It had m. p. 213—214° (Greenberg and Spilman give no m. p.) (Found, in sample dried *in vacuo* at 70°: C, 36·8; H, 3·6; N, 19·8. Calc. for $C_{15}H_{17}O_{12}N_7$: C, 37·0; H, 3·5; N, 20·1%).

The picrate was converted into the free base by using Dowex-1 (formate) and Dowex-50 (NH_4^+) resins by Greenberg and Spilman's method. The resulting aqueous solution was freeze-dried to a white powder, $[\alpha]_D$ −50·3° (*c* 1·0 in H_2O) (Greenberg and Spilman [7] give no $[\alpha]_D$). From ethanol–water (4 : 1) crystals (0·002 g.) were obtained. R_F values are shown in Table 5 and light absorption data in Table 3.

4-*Amino*-1-(β-D-*ribofuranosyl*)*glyoxaline*-5-*carboxyamide*.—The above 4-nitro-compound (0·121 g.) in water (10 c.c.) was hydrogenated at atmospheric pressure over Adams platinum catalyst [from oxide (0·1 g.)]. After 3 hr. the catalyst was filtered off and the filtrate evaporated to small volume and finally freeze-dried. The residue crystallised from methanol as *needles* (0·05 g.), m. p. 187—189° (Found: C, 41·3; H, 5·7. $C_9H_{14}O_5N_4$ requires C, 41·9; H, 5·4%). See Tables 5 and 3 for R_F values and ultraviolet absorption data respectively.

Inosine.—The above 5-amino-compound (0·01 g.) was dissolved in 98% formic acid (1 c.c.) and treated with a mixture of acetic anhydride (0·8 c.c.) and 98% formic acid (2·3 c.c.). The mixture was warmed at 30° for 2·5 hr., then cooled to 4° for 16 hr. The solution was freeze-dried and the residue heated at 90° for 4 hr. in 0·05M-potassium hydrogen carbonate (6 c.c.). The resulting solution was examined by paper chromatography, which showed the presence of starting material and hypoxanthine, as well as inosine, identified by R_F value and ultraviolet absorption spectra (see Tables 2 and 6).

7-β-D-*Ribofuranosylhypoxanthine*.—The above 4-amino-compound (0·013 g.) was treated

in the same way as the 5-amino-compound. The products were hypoxanthine and a hypoxanthine riboside differing from inosine and having ultraviolet absorption spectra indistinguishable from those of 7-methylhypoxanthine (see Tables 2 and 6).

5-Nitroglyoxaline-4-carboxyamide.—Methyl 5-nitroglyoxaline-4-carboxylate (0·05 g.) in methanolic ammonia (50 c.c., saturated at 0°) was kept at room temperature for 4 days. Paper chromatograms showed complete disappearance of the ester. After removal of solvent, crystallisation from water gave the amide (0·038 g., 80%), m. p. 290°. Windaus and Langenbeck [8] give m. p. 291°. Similarly, methyl 1-methyl-4-nitroglyoxaline 5-carboxylate gave the amide,[18] m. p. 258—260°, in high yield.

Methylation of the Silver Salt of 5-Nitroglyoxaline-4-carboxyamide.—The silver salt was treated with methyl iodide according to Allsebrook *et al.*[11] The crude methylation product (0·8 g.) was treated with chloroform–methanol (9 : 1; 500 c.c.) and filtered. The insoluble residue was washed with dilute ammonia to dissolve any unmethylated material and recrystallised from water to give 1-methyl-5-nitroglyoxaline-4-carboxyamide (0·17 g.), m. p. 228—230°. Allsebrook *et al.*[11] give m. p. 234°. The above chloroform–methanol solution was chromatographed on Grade 0 alumina. Elution with chloroform–methanol gave first 1-methyl-4-nitroglyoxaline-5-carboxyamide with m. p. 258° after crystallisation from water. It was identified by comparison of its infrared spectrum with that of an authentic sample. Later fractions contained a mixture of 4- and 5-nitro-amide; the final fraction had m. p. 231—232° and was the pure 5-nitro-amide.

5-Amino-1-methylglyoxaline-4-carboxyamide.—1-Methyl-5-nitroglyoxaline-4-carboxyamide (0·25 g.) in water (100 c.c.) was hydrogenated over Adams catalyst (0·1 g.) until hydrogen uptake was complete. The catalyst was filtered off and the filtrate evaporated to dryness. The residue was extracted with hot ethanol from which the *amine* (0·04 g., 20%) crystallised on cooling. The pink product had m. p. 255—257°, and alcoholic solutions became red on warming[7] (Found: C, 42·4; H, 5·5; N, 40·1. $C_5H_8ON_4$ requires C, 42·8; H, 5·8; N, 40·0%).

Reactions with 4-Chloro-1-methyl-5-nitroglyoxaline and Potassium Cyanide.—In the following experiments the chloro-compound, m. p. 76—77°, had been purified by chromatography on alumina.

(*a*) When the reaction was carried out in boiling ethanol according to Sarasin and Wegmann's method [18] for the isomeric 5-chloro-compound only starting material could be isolated.

(*b*) The 4-chloro-compound (0·5 g.) was dissolved in dimethylformamide (40 g.). Potassium cyanide (0·3 g.) and potassium iodide (0·05 g.), both finely ground, were added and the mixture was heated for 20 hr. at 120—130° with continuous stirring. The dimethylformamide was distilled off under reduced pressure and the residue was shaken with chloroform (400 c.c.). The mixture was filtered, the filtrate was evaporated to dryness, and the residue crystallised from acetone–light petroleum as a pale-green solid (0·25 g.), m. p. 110—115°. After recrystallisation from methanol it had m. p. 139—140°, undepressed in admixture with authentic 5-cyano-1-methyl-4-nitroglyoxaline.[18] The infrared spectra were identical.

(*c*) The 4-chloro-compound (1·0 g.) was mixed with finely ground potassium cyanide (0·8 g.) and potassium iodide (0·1 g.) in a small Pyrex tube. The mixture was kept at 110—120° for 24 hr. with vigorous stirring and the resulting brown mass was extracted with hot chloroform (500 c.c.). Removal of solvent left a brown residue (0·88 g.) which crystallised from aqueous methanol to give starting material (0·43 g.), m. p. 75—76°. The methanolic mother-liquors were evaporated to dryness, and the residue chromatographed in benzene on Grade 0 alumina (15 g.). Elution with benzene–ether removed a further quantity of starting material, and subsequent elution with chloroform gave a yellow solid (0·035 g.) which, after recrystallisation from benzene–light petroleum and from ethanol, had m. p. 136—137°, identified as 5-cyano-1-methyl-4-nitroglyoxaline by mixed m. p. and infrared spectrum. Elution with chloroform–methanol (1 : 1) gave a yellow solid (0·03 g.), which was recrystallised from acetone–light petroleum and from water to give 1-methyl-5-nitroglyoxaline-4-carboxyamide, m. p. 231—232°, identified by infrared spectrum.

Paper Chromatography.—Unwashed Whatman No. 4 paper was used for analytical chromatograms and unwashed Whatman No. 3 for preparative work. Irrigation was normally by the ascending technique unless otherwise stated. Solvents were allowed to dry by evaporation at room temperature. The following solvent systems were used: (A) n-butyl alcohol (4)–acetic acid (1)–water (5) (upper layer); (B) ethyl acetate (5)–n-propyl alcohol (3)–water (2); (C) n-butyl alcohol (40)–ethyl alcohol (10)–water (49)–ammonia (*d* 0·88) (1) (upper layer); (D)

n-butyl alcohol (4)–water (1)–diethylene glycol (1); (E) 5% aqueous sodium dihydrogen phosphate covered by a thin layer of isopentyl alcohol.[23]

Most compounds were located by examination of the chromatogram under ultraviolet light. Glyoxalines lacking a nitro-group or a substituent on a ring-nitrogen atom reacted with the Pauly spray.[24] Free sugars were detected by aniline phthalate [25] and glycosides by the periodate–Schiff reagent spray.[26] The following modification of the Bratton–Marshall spray was used for the detection of the aminoglyoxalines: Papers were hung in a chamber filled with nitrous fumes (from sodium nitrite and acetic acid) for 5 min., removed, and sprayed with ammonium sulphamate (0·5%) in 70% aqueous ethanol. After 3 min. they were sprayed with ethanolic *N*-1-naphthylethylenediamine dihydrochloride (0·1%). The colours obtained from aromatic primary amines ranged from red to purple. The colour appeared immediately after treatment with the coupling reagent.

TABLE 4. R_F *Values of amino- and nitro-glyoxalines in solvent A.*

		Colour with	
	R_F	Pauly reagent	B.–M. reagent
5-Nitroglyoxaline-4-carboxylic acid	0·35 *	—	—
Methyl 5-nitroglyoxaline-4-carboxylate	0·87	—	—
5-Nitroglyoxaline-4-carboxyamide	0·63	—	—
5-Aminoglyoxaline-4-carboxyamide	0·46	Blue	Purple
1-Methyl-4-nitroglyoxaline-5-carboxyamide	0·66	—	—
1-Methyl-5-nitroglyoxaline-4-carboxyamide	0·63	—	—
4-Amino-1-methylglyoxaline-5-carboxyamide	0·46	—	Purple
5-Amino-1-methylglyoxaline-4-carboxyamide	0·50	—	Purple

* Often as a double spot.

TABLE 5. R_F *Values of ribosylglyoxalines.*

	R_F in				Colour with	
	A	B	C	D	IO_4^-/Schiff reagent	B.–M. reagent
4-Nitro-1-ribosylglyoxaline-5-carboxyamide	0·45	0·60	0·44	—	Blue	—
5-Nitro-1-ribosylglyoxaline-4-carboxyamide	0·37	0·50	0·35	—	Blue	—
4-Amino-1-ribosylglyoxaline-5-carboxyamide	0·34	—	—	—	Blue	Purple
5-Amino-1-ribosylglyoxaline-4-carboxyamide	0·40	—	—	0·37	Purple	Purple

TABLE 6. R_F *Values of hypoxanthines.*

	R_F in			R_F in			R_F in	
	A	E		A	E		A	E
Hypoxanthine	0·46	0·57	Inosine	0·36	0·72	7-Ribosylhypoxanthine	0·36	0·68

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