81. 3-*Ribosyluric Acid. Part III.*[1] *Unambiguous Syntheses of 3-Ribosyluric Acid and Related Compounds.*

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The properties of an unambiguously synthesized 3-D-ribosyluric acid are shown to be essentially identical with those of the natural material. Various other 3- and 9-substituted uric acids, including 9-glucosyluric acid, have been synthesized.

In Part I [2] of this series it was shown that the " uric acid riboside " isolated from beef blood has a ribosyl group on the 3-position of the purine ring and not, as had been supposed previously, on the 9-position (cf. I). This conclusion was based on degradative evidence and on comparison of physical data obtained for the natural material and authentic samples of 3-, 7-, and 9-methyluric acid. However, since the proposed structure is unique for a naturally occurring ribosylpurine, synthetic confirmation was desirable. To this end, many potential routes to glycosyluric acids have been explored and an unambiguous synthesis of a 3-ribosyluric acid having properties almost identical (it is probably a mixture of α- and β-isomers) with those of the naturally occurring material has been achieved. In addition, an unambiguous synthesis of 9-D-glucosyluric acid provided material that was readily distinguished from the 3-isomer by its physical properties.

Condensation of a metal purine derivative with an acylglycosyl halide, the simplest method of preparing a glycosylpurine, usually gives the 9-isomer, although occasional formation of a 7-glycosylpurine has been reported.[3] This was the first synthetic procedure investigated in the present study since characterization of either isomer would lend support, albeit negative support, to the structure proposed for 3-D-ribosyluric acid. Attempts to apply this procedure to uric acid by using a variety of metal salts (potassium, lead, silver,

[1] Part II, Hatfield, Rinehart, and Forrest, *J.*, 1963, 899.
[2] Forrest, Hatfield, and Lagowski, *J.*, 1961, 963.
[3] Cf. Montgomery and Thomas, *Adv. Carbohydrate Chem.*, 1962, **17**, 301.

and mercury) and solvents (xylene and/or dimethyl sulphoxide) were unproductive, presumably owing to the limited solubilities of these salts. With 2,3,4,6-tetra-*O*-acetyl-D-glucosyl bromide and mercury urate in dimethyl sulphoxide, for example, the formation of traces of glucosyluric acids could be demonstrated paper chromatographically; on the basis of ultraviolet spectra, mixtures of the 9- and the 3-isomer appeared to have been produced.

2,3,5-Tri-*O*-acetyl-D-ribosyl bromide with chloromercuriuric acid in dimethyl sulphoxide gave a 3% yield of a compound which behaved like natural 3-ribosyluric acid in most of the tests previously used [1] (ultraviolet absorption spectra; colour reactions with *N*,2,6-trichloroquinone imine; rate of alkaline decomposition). However, the synthetic and the natural compound could be separated paper chromatographically. The poor yield of the synthetic compound limited its further investigation, but periodate oxidation indicated that the ribosyl portion had a pyranose ring rather than a furanose ring, as did the similarity of the pK_a values of the synthetic material to those of 3-D-glucosyluric acid (Table 2). It is probably 3-D-ribopyranosyluric acid (I; R = D-ribopyranosyl, R′ = H) and its formation may be a result of the long heating and high temperature used in the reaction. The general procedure, however, is obviously unsatisfactory for structural confirmation since formation of 3-isomers (still, of course, inferred at this point) was wholly unexpected.*

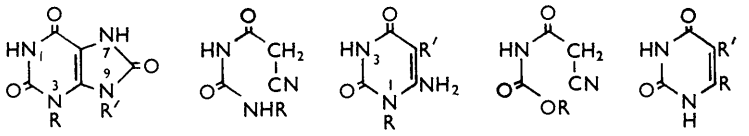
In other experiments designed to yield a 3- or 9-glycosyluric acid, insertion of the glycosyl residue into a pyrimidine rather than into a purine was attempted by similar techniques, *i.e.*, through a metal-pyrimidine intermediate. Thus 6-aminouracil, or some derivative of it, might be converted into a glycosylpyrimidine, which, in turn, might yield a 5,6-diamino-1-glycosylpyrimidine or a 5-amino-6-glycosylaminopyrimidine, and this could be treated with carbonyl chloride to give the uric acid derivative. For example, 6-amino-2,4-dibenzyloxy- and -2,4-dimethoxy-pyrimidine [4] were prepared from 6-amino-2,4-dichloropyrimidine [5] and the corresponding alcohol, and attempts were made to condense these intermediates with a glycosyl halide by using (*a*) silver carbonate in xylene (Koenigs–Knorr synthesis), and (*b*) lithium (prepared from phenyl-lithium) or magnesium salts (prepared from methylmagnesium iodide) of the pyrimidine and sugar halide. In additional experiments, the Hilbert–Johnson synthesis [6] with 2,3,4,6-tetra-*O*-acetyl-D-glucosyl bromide was attempted with the following pyrimidines and solvents: (*a*) silyl derivatives [7] of 6-aminouracil, 6-amino-2,4-dibenzyloxypyrimidine, and 6-amino-5-nitrosouracil in xylene; (*b*) 6-amino-2,4-dibenzyloxy-, 6-amino-2,4-dimethoxy-, and 6-chloro-2,4-dimethoxy-pyrimidine in xylene or acetonitrile. None of these methods led to a glycosylpyrimidine. Finally, 6-amino-2,4-dibenzyloxypyrimidine in alcohol containing a trace of acid did not react with glucose, as has been shown already for analogous compounds.[8]

At this point the introduction of a sugar residue into an open-chain compound which could subsequently be converted into an appropriately substituted pyrimidine was investigated. Goodman [9] reported that ring closure of the previously prepared 1-cyanoacetyl-3-(2,3,4,6-tetra-*O*-acetyl-D-glucosyl)urea [10] (II; R = 2,3,4,6-tetra-*O*-acetyl-D-glucosyl) could be effected by using sodium ethoxide in ethanol. Starting with this

* Leonard and Laursen (*J. Amer. Chem. Soc.*, 1963, **85**, 2026) have reported a direct synthesis of 3-β-D-ribofuranosyladenine (3-isoadenosine), mixed with the 9-isomer, from adenine. The ribosyl portion was considered to be in the 3-position of the adenine nucleus on the basis of a comparison of ultraviolet spectral and pK_a values for this compound and adenine derivatives of known structure.

[4] Klötzer and Bretschneider, *Monatsh.*, 1956, **87**, 136.
[5] Smith and Christensen, *J. Org. Chem.*, 1955, **20**, 829.
[6] Cf. Fox and Wempen, *Adv. Carbohydrate Chem.*, 1959, **14**, 283.
[7] Birkofer, Richter, and Ritter, *Chem. Ber.*, 1960, **93**, 2804; Birkofer, Kühlthau, and Ritter, *ibid.*, p. 2810.
[8] Holland, Lythgoe, and Todd, *J.*, 1948, 965.
[9] Goodman, *Adv. Carbohydrate Chem.*, 1958, **13**, 215; personal communication.
[10] Johnson and Bergmann, *J. Amer. Chem. Soc.*, 1938, **60**, 1916.

reaction, a synthesis of 3-glucosyluric acid was realized in the following way. The product of ring closure, 6-amino-1-D-glucosyluracil (III; R = D-glucosyl, R′ = H), could not be nitrosated, but it coupled with *p*-chlorobenzenediazonium chloride to give the azo-compound (III; R = D-glucosyl, R′ = *p*-chlorophenylazo), which was hydrogenated in



(I) (II) (III) (IV) (V)

methanol with Adams catalyst to give the diamine (III; R = D-glucosyl, R′ = NH_2). This was treated with carbonyl chloride in strongly alkaline solution, yielding 3-D-glucosyluric acid (I; R = D-glucosyl, R′ = H) which was purified by paper chromatography and by ion-exchange. Periodate oxidation of this compound gave a product indistinguishable paper chromatographically from the periodate oxidation product of natural ribosyluric acid, providing confirmatory evidence for the location of the sugar residue on the pyrimidine rather than on the imidazole ring. The position of the sugar residue cannot be defined more precisely since this synthesis is based on the assumption that the structure of the starting material, 1-cyanoacetyl-3-(2,3,4,6-tetra-*O*-acetyl-D-glucosyl)urea, is correctly specified; its preparation from tetra-acetylglucosylurea and cyanoacetic acid, however, is not unambiguous since the possibility exists (although it is not very likely) that the resulting substituted urea is a 1,1- not a 1,3-derivative. If the former were the case, the end-product would be 1-D-glucosyluric acid. (The recently published [11] synthesis of 3-ribosyluric acid through the tetrasilyl derivative of uric acid also gives no definite information on the position of the ribosyl group, this being inferred entirely from physical data.) Further, even if a 1,3-disubstituted urea was obtained, this synthetic route does not lend itself to the synthesis of 3-D-ribosyluric acid because condensation of D-ribose with urea (unlike that of D-glucose) gives a low yield of a mixture of D-ribosylureas which can be separated and characterized only with difficulty.[12]

An alternative approach to a suitably substituted 1-ribosylpyrimidine in which the position of the sugar residue would be unambiguously known was therefore sought. Shaw and his co-workers,[13] in a series of papers devoted to the synthesis of 1-substituted uracils, described the following synthesis of uridine. β-Ethoxyacryloyl chloride was converted into the isocyanate (with silver cyanate). This reacted with ethanol to give ethyl *N*-ethoxyacryloylcarbamate which, on condensation with 2,3,5-tri-*O*-benzoyl-D-ribosylamine, ring closure of the intermediate, and debenzoylation, yielded uridine. In a modification of this synthesis designed to yield a 6-aminouracil derivative, the condensation of ethyl *N*-cyanoacetylcarbamate (IV; R = Et) [14] with 2,3,4,6-tetra-*O*-acetyl-D-glucosylamine was attempted under a large variety of conditions (*e.g.*, in dioxan or ethyl acetate, with varying amounts of triethylamine and, for various reaction times); none of these experiments was successful. In the earlier synthesis,[13] the intermediate was considered to be a substituted ethyl carbamate (ribose-NH·CH:CR·CO·NH·CO_2Et) which underwent ring-closure with elimination of ethanol. In the modification described here it was hoped that the amine would condense with the ethoxycarbonyl group of the carbamate (IV; R = Et) to give a substituted urea (*i.e.*, II; R = D-glucosyl) which could then undergo ring-closure to a 1-substituted uracil. Since the ethoxycarbonyl group did not condense with the amino-sugar the possibility of using the isocyanate (from cyanoacetyl chloride and silver cyanate) as an alternate pathway to the required substituted ureas was explored.

[11] Birkofer, Ritter, and Kühlthau, *Angew. Chem.*, 1963, **75**, 209.
[12] Benn and Jones, *J.*, 1960, 3837.
[13] Shaw and Warrener, *J.*, 1958, 157; Shaw, Warrener, Maguire, and Ralph, *J.*, 1958, 2294.
[14] Conrod and Schulze, *Ber.*, 1909, **42**, 735.

Cyanoacetyl chloride[15] and silver cyanate gave the desired *N*-cyanoacetyl isocyanate (its formation was confirmed by converting it into ethyl *N*-cyanoacetylcarbamate, *i.e.*, by treating the reaction mixture with ethanol) but the yield was low and the compound was very unstable. Attempts to generate it *in situ* and cause it to react immediately with the appropriate amine were not satisfactory owing to the large amount of unchanged cyanoacetyl chloride always present. A second modification of this general scheme involved the preparation of phenyl *N*-cyanoacetylcarbamate (IV; R = Ph) which might be expected to condense with the amino-sugar more readily than its ethyl analogue. This compound (IV; R = Ph) was prepared in good yield from cyanoacetyl chloride[15] and phenyl carbamate,[16] although, unexpectedly, the reaction was much more sluggish than with urethane itself. An alternative cyanoethylation procedure, involving cyanoacetic acid and acetic anhydride, was much less satisfactory, the major product being phenyl *N*-acetylcarbamate with only a small amount of the desired product.

Phenyl *N*-cyanoacetylcarbamate (IV; R = Ph) is very reactive, condensing readily with amines such as aniline, 2-hydroxyethylamine, and 2,3,4,6-tetra-*O*-acetyl-D-glucosylamine in the presence of triethylamine to give the corresponding substituted ureas (II; R = Ph,[17] $CH_2 \cdot CH_2 \cdot OH$,[18] and 2,3,4,6-tetra-*O*-acetyl-D-glucosyl,[10] respectively). On treatment with sodium ethoxide in ethanol, 1-cyanoacetyl-3-2′-hydroxyethylurea (II; R = $CH_2 \cdot CH_2 \cdot OH$) cyclized to 6-amino-1-2′-hydroxyethyluracil (Na salt) and this was readily converted into the 5-nitroso-derivative (III; R = $CH_2 \cdot CH_2 \cdot OH$, R′ = NO). Reduction of the nitroso-compound with sodium dithionite gave a diamine which was treated, without isolation, with carbonyl chloride in dilute alkali, to give 3-2′-hydroxyethyluric acid (I; R = $CH_2 \cdot CH_2 \cdot OH$, R′ = H) in good yield. This compound had been synthesized previously[18] by a route involving condensation of 2-hydroxyethylurea with cyanoacetic acid followed by a series of steps essentially similar to those described above. Such a synthesis is, however, again open to the same criticism as our initial preparation of 3-D-glucosyluric acid (from D-glucosylurea). The synthesis described here proceeding from phenyl *N*-cyanoacetylcarbamate then represented an unambiguous synthesis of a 3-hydroxyalkyluric acid and provided a model for subsequent work with the sugar derivatives. The physical properties of 3-2′-hydroxyethyluric acid were very similar to those of the naturally occurring ribosyluric acid (see Tables 1 and 2).

1-Cyanoacetyl-3-(2,3,4,6-tetra-*O*-acetyl-D-glucosyl)urea prepared by the above method from 2,3,4,6-tetra-*O*-acetyl-D-glucosylamine and phenyl *N*-cyanoacetylcarbamate has a lower melting point than that reported by Johnson and Bergmann.[10] However, it undergoes ring-closure in presence of sodium ethoxide to give a glucosylpyrimidine. Paper chromatography showed this product to be a mixture of two components; the major component was chromatographically and spectrally (ultraviolet) identical with the material synthesized by Goodman's procedure.[9] This confirms, then, the correctness of the structure assigned to the substituted urea used in that procedure and justifies the assumption that the end-product of the synthesis proceeding through this urea is 3-D-glucosyluric acid.

Finally, phenyl *N*-cyanoacetylcarbamate was allowed to condense with 2,3,5-tri-*O*-benzoyl-D-ribosylamine[19] to give a urea derivative (II; R = 2,3,5-tri-*O*-benzoyl-D-ribosyl) which cyclized to the pyrimidine (III; R = D-ribosyl, R′ = H) on treatment with ethanolic sodium ethoxide. As expected, this product did not react with nitrous acid but could be converted, analogously to the glucosyl compound, through the *p*-chlorophenylazo-derivative and the diamine, into 3-D-ribosyluric acid (I; R = D-ribosyl, R′ = H). The product was purified by paper chromatography and by ion-exchange; it then

[15] Schroeter and Seidler, *J. prakt. Chem.*, 1922, **105**, 165; Schroeter and Finck, *Ber.*, 1938, **71**, 671.
[16] Kempf, *J. prakt. Chem.*, 1870, **1**, 402.
[17] Lifschitz, *Ber.*, 1922, **55**, 1619; D.R.-P. 175,415; cf. *Chem. Zentr.*, 1906, **77**, II, 1590.
[18] Nathan and Bogert, *J. Amer. Chem. Soc.*, 1941, **63**, 2567.
[19] Baddiley, Buchanan, Hodges, and Prescott, *J.*, 1957, 4769.

separated from water as a gelatinous mass which did not crystallize even when seeded with the natural material. This behaviour is reminiscent of that of the natural material when it is slightly impure and suggests that the synthetic material is probably a mixture of α- and β-isomers. In other respects it was substantially identical with the natural material (see Tables 1 and 2).

TABLE 1.

Chromatographic behaviour of uric acid derivatives * in various solvents.

Acid	Propan-1-ol–1% aq. NH_3 (2 : 1)	Bu^nOH–5N-AcOH (2 : 1)	3% NH_4Cl	4% Na Citrate
Synthetic 3-D-ribosyluric	0·49, R, Y	0·20, NC, Y	0·60, NC, Y	0·53, NC, Y
Natural 3-D-ribosyluric	0·49, R, Y	0·20, NC, Y	0·60, NC, Y	0·52, R, Y
9-(Tetra-*O*-acetyl-D-glucosyl)-uric	0·76, Y, Y	0·76, NC, Y	0·89, NC, Y	0·82, Y, Y
9-D-Glucosyluric	0·28, Y, Y	0·09, NC, Y	0·74, NC, Y	0·67, Y, Y
3-D-Glucosyluric	0·27, Y, Y	0·10, NC, Y	0·70, NC, Y	0·65, Y, Y
3-2′-Hydroxyethyluric	0·41, Y, Y	0·21, NC, Y	0·63, NC, Y	0·52, Y, Y

* R_F values (1st entry) and colour reactions with *N*,2,6-trichloroquinone imine before (2nd entry) and after (3rd entry) spraying with alkaline buffer. NC, no colour; R, red; Y, yellow.

TABLE 2.

Ultraviolet absorption spectra and pK_a values.

Uric acid derivative	pH of solvent	$\lambda_{max.}$ (mμ)	$10^{-3}\varepsilon$	$\lambda_{min.}$ (mμ)	$10^{-3}\varepsilon$	pK_a in H_2O
Synthetic 3-D-ribosyl-	1·0	288, 233	11·4, 6·4	257, 221	2·9, 5·6	
	8·6	298, 219, 241sh	16·5, 7·9, 2·9	260	2·1	—
	14·0	297	14·5	252	1·03	
Natural 3-D-ribosyl- [2]	1·0	288, 233	12·4, 6·8	256, 220	2·9, 5·7	6·0
	7·0	298, 243sh	17·7, 2·9	257	2·3	10·9
	14·0	297	16·5	254	1·6	
3-D-Glucosyl-	2·03	289, 233	12·1, 6·2	257, 219	2·5, 4·7	5·76
	7·8	297, 240sh	18·7, 2·3	258	2·1	10·90
	14·0	298	16·3	256	1·3	
3-2′-Hydroxyethyl-	2·03	287, 233	12·3, 7·8	258, 218	3·1, 4·7	—
	8·6	297, 217, 241sh	18·6, 13·8, 3·5	260	2·5	
	14·0	292	17·0	256	2·0	
3-D-Ribopyranosyl-	1·0	289, 230sh	—	256	—	
	7·0	297	—	262	—	5·60
	14 0	298	—	259	—	10·95
9-(Tetra-*O*-acetyl-D-glucosyl)-	2·03	286, 234	10·7, 9·1	256, 214	3·16, 4·8	—
	8·6	294, 240	11·0, 10·5	264	2·7	
9-D-Glucosyl-	2·03	287, 234	11·5, 8·3	257, 215	2·95, 4·27	4·38
	7·8	293, 239	11·2, 10·5	264, 218	2·3, 4·27	10·65
	14·0	303, 249	9·5, 11·0	274, 222	3·5, 0·81	

In the course of this study, 9-glucosyluric acid was synthesized by the following route. 6-Chloro-5-phenylazouracil (V; R = Cl, R′ = PhN_2) [20] condensed with 2,3,4,6-tetra-*O*-acetyl-D-glucosylamine to give the 6-(glucosylamino)uracil (V; R = 2,3,4,6-tetra-*O*-acetyl-D-glucosylamino, R′ = PhN_2), and catalytic reduction of the azouracil yielded the corresponding diamine. Treatment of a bicarbonate solution of the diamine with carbonyl chloride gave the acetyl derivative of 9-D-glucosyluric acid (I; R = H, R′ = 2,3,4,6-tetra-*O*-acetyl-D-glucosyl) which was readily converted into 9-D-glucosyluric acid (I; R = H, R′ = D-glucosyl) by hydrolysis with sodium ethoxide. The pure glucosyluric acid was obtained by ion-exchange chromatography; it has the properties expected of a 9-alkyluric acid and could be readily distinguished from either the natural or the synthetic ribosyluric acid, as well as from 3-D-glucosyluric acid (Tables 1 and 2).

EXPERIMENTAL

Melting points were observed on a hot-stage microscope and were corrected.

Where possible, compounds were checked for purity by paper chromatography on Whatman's No. 1 paper with the solvents described in Table 1. Their presence was detected on the

[20] Pfleiderer and Nübel, *Chem. Ber.*, 1960, **93**, 1406.

chromatogram in ultraviolet light (maximum emission, 254 mμ) and/or, for uric acid derivatives, the colour reagent *N*,2,6-trichloroquinone imine–alkaline buffer described previously.[1]

pK_a values were determined spectrophotometrically by Mattoo's method.[21]

Ultraviolet absorption spectra were recorded at pH values at which one specific ionic species (from pK_a values) is present.

3-D-*Ribopyranosyluric Acid* (I; R = D-ribosyl, R′ = H).—Chloromercuriuric acid (0·53 g.; prepared [22] from equimolar amounts of uric acid, sodium hydroxide, and mercuric chloride) was adsorbed on Celite,[23] dried *in vacuo*, and added to redistilled dimethyl sulphoxide (25 ml.). The suspension was refluxed for 2 hr., then cooled to 60°, and a solution of 2,3,5-tri-*O*-acetyl-D-ribofuranosyl chloride [24] (from 0·31 g. of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose [25]) in anhydrous xylene (20 ml.) was added.[3] After 5 hr. at 115°, the mixture was filtered and concentrated to 10 ml., and the brown syrupy residue was chromatographed on filter paper (Whatman's No. 17; 3 sheets; ascending) with propan-1-ol–1% aqueous ammonia (3 : 2). The product was detected by its absorption of ultraviolet light (254 mμ) or by its colour reaction on a representative strip with *N*,2,6-trichloroquinone imine and alkaline buffer, eluted from the paper with water, and rechromatographed [Pr^{n}OH–1% aq. NH_3 (3 : 2); ascending]. The uric acid derivative was obtained from the final chromatogram as an amorphous solid (13·7 mg., 3·4%, estimated spectroscopically) which was spectrally similar (Table 2) to natural 3-D-ribosyluric acid but was readily distinguished from it paper chromatographically. Deacetylation was apparently effected by the solvent used for chromatography. Only one band developed a yellow colour with the *N*,2,6-trichloroquinone imine reagent, the acetylated derivative would have a higher R_F value than the deacetylated compound in this solvent and could have been readily detected.

If we assume a molecular weight of 300, the ribosyl derivative consumed 1·9 mol. of periodate. The pK values (Table 2) are very close to those of 3-D-glucosyluric acid in which the sugar residue is in the pyranose form. Further, the rate of decomposition of this compound in 0·1N-sodium hydroxide very closely parallels that observed for natural 3-D-ribosyl- and 3-methyl-uric acid. On the other hand, this compound was resistant to hydrolysis by 4N-sulphuric acid or by *L. pentosus* in conditions which effected cleavage of the sugar residue from natural 3-D-ribosyluric acid.[2] R_F values are as follows (natural ribosyluric acid given in parentheses): Pr^{n}OH–1% aq. NH_3 (3 : 2), 0·62 (0·51); NMe_2·CHO–Bu^{n}OH–H_2O (1 : 1 : 1), 0·68 (0·63); 3% NH_4Cl, 0·60 (0·60).

6-*Amino*-2,4-*dibenzyloxypyrimidine*.—6-Amino-2,4-dichloropyrimidine [5] (1·6 g.) was added to a solution of sodium (0·5 g.) in benzyl alcohol (40 ml.), and the mixture was heated at 140—160° (internal temperature) for 4½ hr. After removal of the excess of alcohol *in vacuo* below 120°, the residue was dissolved in a minimum of methanol, the solution decolourized with charcoal, and warm water added until the solution became turbid. The product, which slowly separated on cooling, recrystallized from ethanol–water to give the *pyrimidine* (2·45 g., 79%), m. p. 108° (Found: C, 70·6; H, 5·3; N, 14·4. $C_{18}H_{17}N_3O_2$ requires C, 70·3; H, 5·6; N, 13·8%).

6-*Amino*-1-D-*glucosyluracil* (III; R = D-glucosyl, R′ = H).—To a solution of 1-cyanoacetyl-3-(2,3,4,6-tetra-*O*-acetyl-D-glucosyl)urea (10 g.) [prepared by acetylation [26] of glucosylurea [27] and treatment of the product with cyanoacetic acid and acetic anhydride [10]] in hot absolute ethanol (1 l.) was added a solution from sodium (1 g.) in ethanol (50 ml.), whereupon a white precipitate began to separate. The solid which separated after cooling was collected, washed with absolute ethanol, and recrystallized from ethanol–water. This sodium salt (6·72 g., 76%) was used without further purification.

Ring closure of 1-cyanoacetyl-3-(2,3,4,6-tetra-*O*-acetyl-D-glucosyl)urea, prepared by the alternative procedure [from phenyl *N*-cyanoacetylcarbamate; see below], gave a mixture of two components; the major one was identical with the material described here.

6-*Amino*-5-p-*chlorophenylazo*-1-D-*glucosyluracil* (III; R = D-glucosyl, R′ = *p*-chlorophenylazo).—The above glycosyluracil (1 g.) in water (10 ml.) was treated with sodium hydrogen carbonate (0·6 g.) in a small amount of water. A solution (6 ml.) of the diazonium salt from

[21] Mattoo, *Trans. Faraday Soc.*, 1958, **54**, 19.
[22] Davoll and Lowy, *J. Amer. Chem. Soc.*, 1951, **73**, 1650.
[23] McEvoy, Weiss, and Baker, *J. Amer. Chem. Soc.*, 1960, **82**, 205.
[24] Davoll, Lythgoe, and Todd, *J.*, 1948, 967.
[25] Zinner, *Chem. Ber.*, 1950, **83**, 153.
[26] Helferich and Kosche, *Ber.*, 1926, **59**, 69.
[27] Hynd, *Biochem. J.*, 1926, **20**, 205.

p-chloroaniline [0·5 g. in 6N-hydrochloric acid (2·4 ml.), treated with sodium nitrite (0·3 g.)] was added slowly at 0° with stirring and exclusion of light. After 1 hr., the red precipitate was collected, washed, and dried; a further quantity was obtained from the mother-liquors after refrigeration overnight. The azo-compound (0·8 g., 58%) was used without further purification.

3-D-*Glucosyluric Acid* (I; R = D-glucosyl, R′ = H).—The theoretical amount of hydrogen was taken up when a mixture of the azo-compound (III; R = D-glucosyl, R′ = *p*-chlorophenylazo) (2 g.), Adams catalyst (200 mg.), and absolute methanol (50 ml.) was reduced at 35°. The catalyst was removed and the filtrate evaporated *in vacuo* under nitrogen. The residue was thoroughly washed with chloroform–carbon tetrachloride (1 : 1), and the yellow-brown solid was collected and dissolved in warm water (15 ml.). Without delay carbonyl chloride was slowly bubbled through the solution while the pH was maintained between 2 and 9 by addition of N-sodium hydroxide; at 10-min. intervals the solution was warmed to 80°. After 30 min., the suspension was adjusted to pH 6 with glacial acetic acid, the precipitate was discarded, and the yellow-red filtrate was purified by paper chromatography (2 sheets of Whatman's No. 17; ascending) with the solvent Pr^nOH–1% aq. NH_3 (2 : 1). The product was detected on the developed chromatogram by its strong absorption (R_F 0·25—0·3) of ultraviolet light (254 mμ) or by its colour reaction on spraying a representative strip with *N*,2,6-trichloroquinone imine followed by alkaline buffer. The appropriate band was cut out and eluted with 1% aqueous ammonia; evaporation of the eluate *in vacuo* gave the ammonium salt (280 mg., 17%). This salt was dissolved in a little water and the solution (pH 8—9) passed through a short column of Dowex I (acetate form) ion-exchange resin. After washing the column with dilute ammonium acetate, followed by water until the eluate was non-fluorescent, the product was eluted with 0·05N-acetic acid. Evaporation of the solvent and recrystallization of the white residue twice from acetone–water gave the *glucosyluric acid*, m. p. 232—235° (decomp.) (Found, for material dried at 80° over P_2O_5: C, 39·3; H, 4·7; N, 16·2. $C_{11}H_{14}N_4O_8,\frac{1}{2}H_2O$ requires C, 39·0; H, 4·5; N, 16·5%). The pK_a values of this compound (Table 2) are somewhat lower (0·4 unit) than those of 3-methyluric acid but are similar to those for natural 3-ribosyluric acid.

Periodate oxidation (directly on the origin of the paper chromatogram) gave a product whose R_F values were compared with those of the product obtained similarly from natural ribosyluric acid (glucosyl derivative first): Pr^nOH–1% aq. NH_3 (3 : 2), 0·28, 0·25; NMe_2·CHO–Bu^nOH–H_2O (1 : 1 : 1), 0·31, 0·32 [spots detected in ultraviolet light (254 mμ); they gave no colour with the *N*,2,6-trichloroquinone imine reagent].

Phenyl N-*Cyanoacetylcarbamate* (IV; R = Ph).—Freshly distilled cyanoacetyl chloride [15] (15·7 g.) in dry benzene (20 ml.) was treated with carefully dried (over P_2O_5) phenyl carbamate [16] (18·5 g.), and warmed to 70—75° with exclusion of moisture. Evolution of hydrochloric acid began in about 15 min., and in 45 min. dissolution was complete. After 80 min., the solution was cooled and treated with absolute ethanol (30 ml.), and the solvents were removed *in vacuo*. The residue was freed from hydrochloric acid by repeated dissolution in ethanol and evaporation *in vacuo*. It was finally taken up in a minimum of ethanol, and hot water was added. The oily precipitate slowly solidified, and the *phenyl carbamate* (20·8 g., 76%) was collected and recrystallized from water or ethyl acetate–light petroleum, then having m. p. 103—105° (Found: C, 58·8; H, 4·1; N, 13·4. $C_{10}H_8N_2O_3$ requires C, 58·8; H, 4·0; N, 13·7%).

1-*Cyanoacetyl*-3-*phenylurea* (II; R = Ph).—The above phenyl carbamate (100 mg.) was warmed with aniline (0·05 ml.) in a mixture (2—3 ml.) of ethyl acetate and triethylamine (11 : 3) for 2—3 min. at 65°. The resulting white precipitate, recrystallized from methanol, had m. p. 208—210° (lit.,[17] 198°, 216°) (Found: N, 20·7. Calc. for $C_{10}H_9N_3O_2$: N, 20·7%).

6-*Amino*-1-2′-*hydroxyethyluracil* (III; R = CH_2·CH_2·OH, R′ = H).[18]—In a similar preparation ethanolamine (0·15 ml.) was condensed with the above phenyl carbamate (500 mg.) to give 1-cyanoacetyl-3-2′-hydroxyethylurea (II; R = CH_2·CH_2·OH) (250 mg., 60%) which was recrystallized from absolute ethanol. To a suspension of this urea derivative (510 mg.) in hot absolute ethanol (5 ml.) was added a solution from sodium (150 mg.) in ethanol (23 ml.); a white precipitate slowly separated from the resultant clear solution. After 30 minutes' heating at 100°, the mixture was cooled and the precipitate was collected, washed with ethanol, and dissolved in water. The aqueous solution was acidified with acetic acid; the resulting crystalline material (410 mg., 80%), when washed with acetone and recrystallized (charcoal) from water, had m. p. 253—254° (decomp.) (Found: C, 42·0; H, 5·9; N, 25·5. Calc. for $C_6H_9N_3O_3$: C, 42·1; H, 5·3; N, 24·6%).

6-*Amino*-1-2′-*hydroxyethyl*-5-*nitrosouracil* (III; R = CH_2·CH_2·OH, R′ = NO).[18]—To a

solution of the preceding aminouracil (285 mg.) in hot water (20 ml.) was added sodium nitrite (140 mg.), followed by sufficient 5N-acetic acid to render the mixture weakly acid. After 5 min. at 100° the mixture was cooled and the precipitated blue-violet nitroso-compound (290 mg., 87%) was collected; recrystallized from water, it decomposed at >250° (Found: N, 27·7. Calc. for $C_6H_8N_4O_4$: N, 28·0%).

3-2′-*Hydroxyethyluric Acid* (I; R = CH_2·CH_2·OH, R′ = H).—The nitroso-compound (130 mg.) was suspended in a small amount of hot water and reduced with sodium dithionite. Carbonyl chloride was then bubbled into the colourless solution, sodium hydrogen carbonate being added to maintain the pH of the solution at or near 7. At the conclusion of the reaction (15 min.) the solution was adjusted to pH 2 with 2N-hydrochloric acid. The compound which separated on cooling was collected, washed with water and acetone, and recrystallized from water (charcoal) containing a trace of hydrochloric acid to give 3-2′-hydroxyethyluric acid, m. p. 343—344° (decomp.) (lit.,[18] 315—325°) (Found: C, 39·5; H, 4·0; N, 25·3. Calc. for $C_7H_8N_4O_4$: C, 39·6; H, 3·8; N, 26·4%).

1-*Cyanoacetyl*-3-(2,3,4,6-*tetra*-O-*acetyl*-D-*glucosyl*)*urea* (II; R = 2,3,4,6-tetra-*O*-acetyl-D-glucosyl).—Phenyl *N*-cyanoacetylcarbamate (IV; R = Ph) (0·7 g.), 2,3,4,6-tetra-*O*-acetyl-D-glucosylamine (1·5 g.),[28] and a mixture (5 ml.) of ethyl acetate and triethylamine (11 : 3) were allowed to react at 70—80° for 15 min. A small precipitate formed during this time. The mixture was cooled and light petroleum (b. p. 40—60°) was added, whereupon a syrup separated. After decantation of the solvents, the syrup was crystallized from ethyl acetate–light petroleum, giving the urea derivative as white crystals (1·2 g., 76%), m. p. 112—115° (lit.,[10] 135°) (Found: C, 45·4; H, 5·3; N, 8·6. Calc. for $C_{18}H_{23}N_3O_{11},H_2O$: C, 45·5; H, 5·3; N, 8·8%).

In the presence of sodium ethoxide in ethanol, this product cyclized, as described above; two pyrimidines were obtained, the major component being identical with 6-amino-1-D-glucosyluracil prepared by Goodman's procedure.

1-*Cyanoacetyl*-3-(2,3,5-*tri*-O-*benzoyl*-D-*ribosyl*)*urea* (II; R = 2,3,5-tri-*O*-benzoyl-D-ribosyl).—2,3,5-Tri-*O*-benzoyl-D-ribosyl azide[19] (4·9 g.) was reduced in ethyl acetate. The solution of the amine in ethyl acetate (total vol., 45 ml.) was treated with phenyl *N*-cyanoacetylcarbamate (2·2 g.), followed by triethylamine (1 ml.) in ethyl acetate (10 ml.), and the resulting clear solution was heated at 60—70° for 1 hr. Addition of light petroleum (b. p. 40—60°) to the cooled solution caused precipitation of a syrup. After decantation of the solvents, the syrup was dissolved in chloroform, and the solution was decolourized with charcoal and evaporated to dryness. This procedure was repeated several times, but without the charcoal treatment. Finally, light petroleum was added to a chloroform solution of the residue to give a white solid (5 g., 88% based on the azide) which was used without further purification.

6-*Amino*-1-D-*ribosyluracil* (III; R = D-ribosyl, R′ = H).—An ethanol solution of sodium ethoxide was added dropwise to the above crude urea derivative (3·3 g.) in warm ethanol (30 ml.) until turbidity persisted, and the mixture was kept at 80° for 30 min. with careful exclusion of moisture. More sodium ethoxide solution was added during this time to keep the mixture alkaline. The precipitate which separated on cooling was collected by centrifugation and washed with cold alcohol and ether (yield, 1·3 g., 80%). Only partial hydrolysis of this compound was effected by 2N-hydrochloric acid at 100° for 1 hr., but with 6N-hydrochloric acid under the same conditions the formation of 6-aminouracil and barbituric acid was demonstrated by paper chromatography. The compound had an ultraviolet absorption maximum at 248 mμ.

6-*Amino*-5-p-*chlorophenylazo*-1-D-*ribosyluracil* (III; R = D-ribosyl, R′ = *p*-chlorophenylazo).—The sodium salt of the above uracil (1 g.) in sodium hydrogen carbonate solution (0·6 g. in 25 ml.) was treated with diazotized *p*-chloroaniline [0·5 g., treated with concentrated hydrochloric acid (1·2 ml.) and sodium nitrite (0·3 g.); final volume, 8 ml.; excess of nitrite destroyed with urea]. The mixture, protected from light, was stirred for several hours at 0°, and the resulting precipitate was collected and washed with a little water. Some additional material was obtained by refrigerating the original mixture overnight. The combined material was recrystallized from acetone–water, to give the red *p*-chlorophenylazouracil (960 mg.), probably as the ammonium salt; it had m. p. 247—248° (decomp.) (sintering at 240°) (Found: N, 19·8. $C_{15}H_{15}ClN_5O_6,NH_4$ requires N, 20·2%).

[28] Helferich and Mitrowsky, *Ber.*, 1952, **85**, 1.

3-D-*Ribosyluric Acid* (I; R = D-ribosyl, R′ = H).—The azopyrimidine (1 g.) was reduced in the presence of Adams catalyst (150 mg.) during 24 hr. The resulting yellow solution was treated with carbonyl chloride as described above for the preparation of 3-glucosyluric acid, and the product was similarly isolated by using a column of Dowex I (acetate form) ion-exchange resin. Attempts to recrystallize the product from water led to the formation of a gelatinous precipitate which did not crystallize even when seeded with the crystalline natural material. 3-D-Ribosyluric acid (29 mg.) was precipitated as a white solid by addition of acetone to the aqueous solution. Comparisons of its physical properties and chromatographic behaviour with those of the natural material are given in Tables 1 and 2. From the ultraviolet absorption spectrum of the natural material, the synthetic product is calculated to be about 94% pure. Because of this fact, the small amount of material available, and the technique used to isolate it, the compound was not analysed.

5-*Phenylazo*-6-(2,3,4,6-*tetra*-O-*acetyl*-D-*glucosylamino*)*uracil* (V; R = 2,3,4,6-tetra-*O*-acetyl-D-glucosylamino, R′ = phenylazo).—2,3,4,6-Tetra-*O*-acetyl-D-glucosylamine [28] (8 g.) and 6-chloro-5-phenylazo-uracil [20] (4 g.) was added to dry dioxan (45 ml.), and the whole was kept at 80° for 1 hr., precautions being taken to exclude light. The temperature was then raised to 100—105° for a further 1 hr. The dark red solution was cooled, poured over ice, cautiously acidified to pH 2 with 2N-hydrochloric acid, and the mixture extracted twice with chloroform. The chloroform extract was dried and evaporated *in vacuo*; the residue was dissolved in a minimum of chloroform and the solution treated with light petroleum (b. p. 40—60°) to incipient crystallization. After 2 days at 4°, the yellow precipitate (7·3 g., 80%) was collected, washed with carbon tetrachloride, and recrystallized from chloroform–light petroleum, to give the pure *phenylazouracil*, m. p. 200—201° (Found: C, 51·7; H, 4·7; N, 12·8. $C_{24}H_{27}N_5O_{11}$ requires C, 51·3; H, 4·8; N, 12·5%).

9-(2,3,4,6-*Tetra*-O-*acetyl*-D-*glucosyl*)*uric Acid* (I; R = H, R′ = 2,3,4,6-tetra-*O*-acetyl-D-glucosyl).—The azo-compound (500 mg.) was reduced in methanol (20 ml.) in the presence of Adams catalyst (50 mg.), and the reduction mixture evaporated to dryness *in vacuo* under helium. The residue was washed with carbon tetrachloride, collected, and dissolved in a small amount of acetone. To this was added a solution of sodium hydrogen carbonate (5 g.) in water (30 ml.), and carbonyl chloride was bubbled into the mixture for 30 min.; after the reaction had proceeded for 5 min. the mixture was warmed briefly to 80°. The warm solution was then filtered and adjusted to pH 3 by addition of 2N-hydrochloric acid, whereupon a yellow precipitate separated. The suspension was extracted with a large quantity of ethyl acetate and the organic phase was dried and the solvent removed *in vacuo*. Trituration of the residue with ether gave a crystalline compound (240 mg., 44%) which was collected and purified in the following way. The crude product was extracted with a small amount of ethyl acetate, and the residue was taken up in warm chloroform, treated with Celite, and filtered. Addition of dry benzene to the filtrate gave a small flocculent precipitate which was removed, and the clear solution was then treated with light petroleum. The resulting cream-coloured precipitate was recrystallized repeatedly from chloroform–light petroleum (b. p. 40—60°) (charcoal) and finally from ethyl acetate or absolute ethanol, to give the *tetra-acetylglucosyluric acid*, m. p. 194—196° (sintering at 190°) (Found: C, 45·0; H, 5·0; N, 11·2. $C_{19}H_{22}N_4O_{12},\frac{1}{2}H_2O$ requires C, 45·0; H, 4·8; N, 11·0%).

9-D-*Glucosyluric Acid* (I; R = H, R′ = D-glucosyl).—The acetyl compound (300 mg.) was hydrolysed with sodium ethoxide in ethanol (40 ml.) for 15 min. at 100°. The white precipitate which separated on cooling was collected by centrifugation, washed with ethanol and acetone, and dissolved in water (20 ml.). This solution was placed on a Dowex I column (5 × 2·5 cm.) in the acetate form. The column was washed with dilute ammonium acetate solution (pH 8) followed by water, and the product was eluted with 0·1N-acetic acid (*ca.* 4 l.). Evaporation of the eluate *in vacuo* gave a residue which was recrystallized from water–acetone, to yield the pure *glucosyluric acid*, m. p. >250° (Found: C, 37·6; H, 4·5; N, 16·2. $C_{11}H_{14}N_4O_8,H_2O$ requires C, 37·9; H, 4·6; N, 16·1%).

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