Purines, Pyrimidines, and Imidazoles. Part XL.[1] A New Synthesis of a D-Ribofuranosylamine Derivative and its Use in the Synthesis of Pyrimidine and Imidazole Nucleosides

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2,3-*O*-Isopropylidene-D-ribofuranosylamine has been prepared in high yield as the stable crystalline toluene-*p*-sulphonate by reaction of D-ribopyranosylamine with acetone, 2,2-dimethoxypropane, and toluene-*p*-sulphonic acid. The anomeric configuration of the furanosylamine in various solvents has been investigated by n.m.r. spectroscopy and optical rotation measurements. In chloroform the β-form predominates whereas in dimethyl sulphoxide a high proportion of the α-form is present. A bis(isopropylideneribofuranosyl)amine of unknown anomeric configuration has also been prepared from the furanosylamine toluene-*p*-sulphonate; this has also been obtained as a by-product in some reactions.

Reaction of the furanosylamine with several, α-cyano, -acetyl, or -ethoxycarbonyl-β-ethoxy-*N*-ethoxycarbonylacrylamides occurs in both aqueous and non-aqueous solutions to give corresponding 5-substituted uracil α- and β-ribofuranosides. Assignments of anomeric configurations were confirmed by n.m.r. spectroscopy, by comparison with known compounds, and, in the case of 5-ethoxycarbonyluridine, by hydrolysis to the uridine-5-carboxylic acid and smooth decarboxylation of this to uridine by a new method which has also been used to decarboxylate uracil-5-carboxylic acid and its 1-methyl and 1-phenyl derivatives.

The furanosylamine with β-methoxy-α-methylacryloyl isothiocyanate also gave, *via* an intermediate acyclic thiourea riboside, 2-thiothymine 1-(isopropylidene-β-D-ribofuranoside).

Treatment of the furanosylamine either with ethyl *N*-[alkoxycarbonyl or carbamoyl(cyano)methyl]formimidates, or with ethylformimidate hydrochloride [to give a mixture of ethyl *N*-(2,3-*O*-isopropylidene-α- and β-ribofuranosyl)-formimidates], followed by ethyl α-amino(cyano)acetate, gave 5-amino-4-(alkoxycarbonyl or carbamoyl)imidazole isopropylidene-α- and β-ribofuranosides, from which the isopropylidene groups may be removed by aqueous acetic acid. Phosphorylation of the two anomeric esters gave corresponding 5-aminoimidazole-4-carboxylic acid α- and β-ribotides.

Structures of the aminoimidazole nucleosides and nucleotides were confirmed by comparisons with known compounds and by use of spectral (especially c.d.) techniques. Rates of reaction of several formimidates and acetimidates with cyclohexylamine have been examined.

In earlier parts of this series[2] we have described the synthesis of a variety of aminoimidazole (*i.e.* purine precursor) and pyrimidine nucleosides by reactions which in some ways parallel the *de novo* biosynthetic pathways of these materials, *viz.* condensation of a 2,3,5-tribenzoylribofuranosylamine (I) with various acyclic precursors of the heterocyclic ring systems. The

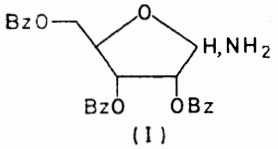
[1] Part XXXIX, D. H. Robinson and G. Shaw, *J.C.S. Perkin I*, 1972, 1715.

[2] (*a*) R. K. Ralph and G. Shaw, *J. Chem. Soc.*, 1956, 1877; (*b*) G. Shaw, R. N. Warrener, M. H. Maguire, and R. K. Ralph, 1958, 2294; (*c*) G. Shaw and R. N. Warrener, *ibid.*, 1959, 50; (*d*) G. Shaw, R. N. Warrener, D. N. Butler, and R. K. Ralph, *ibid.*, 1959, 1648; (*e*) G. Shaw and D. N. Butler, *ibid.*, 1959, 4040; (*f*) G. Shaw and D. V. Wilson, *ibid.*, 1962, 2937; (*g*) G. Shaw and D. V. Wilson, *ibid.*, 1963, 1077; (*h*) G. Shaw, D. V. Wilson, and C. P. Green, *ibid.*, 1964, 2650; (*i*) R. Carrington, G. Shaw, and D. V. Wilson, *ibid.*, 1965, 6864; (*j*) I. E. Burrows, G. Shaw, and D. V. Wilson, *J. Chem. Soc.* (*C*), 1968, 40; (*k*) R. Carrington and G. Shaw, *ibid.*, 1968, 1957; (*l*) M. Greenhalgh, G. Shaw, D. V. Wilson, and N. J. Cusack, *ibid.*, 1969, 2198; (*m*) J. M. Carpenter and G. Shaw, *ibid.*, 1970, 2016.

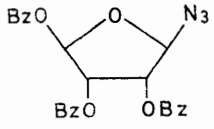
usefulness of these syntheses is dependent on the availability of the ribofuranosylamine. In all our earlier synthetic experiments we obtained the ribofuranosylamine tri-*O*-benzoate by reduction of the corresponding tribenzoylribofuranosyl azide [3] (II), which was prepared in turn by condensation of 2,3,5-tribenzoylribofuranosyl chloride with sodium azide. This route is tedious and suffers mainly from the requirement that the ribofuranosylamine has generally to be made *in situ* and used immediately, otherwise O→N migration of the 2-*O*-benzoyl group occurs. We now record a simple synthesis of a stable ribofuranosylamine derivative [4] which can be used for the direct preparation of many types of nucleosides.

Condensation of D-ribose with saturated methanolic ammonia at 0° readily gives the easily crystallised D-ribopyranosylamine (III) in >90% yield.[5] Evidence for the pyranose nature of this material came from its condensation with ethyl *N*-(α-cyano-β-ethoxyacryloyl)-carbamate (IVa) to afford the ribopyranosyluracil (V),[2a] which was shown to absorb 2 mol. equiv. of periodate thereby giving 1 mol. equiv. of formic acid. In contrast, the furanose derivative (VIa), subsequently prepared by a similar condensation of the benzoylated amine (I) with the carbamate (IVa) and hydrolysis of the derived nucleoside,[2b] absorbed 1 mol. equiv. of periodate with no acid production.

The ribopyranosylamine (III), when stirred with 2,2-dimethoxypropane, acetone, and toluene-*p*-sulphonic



(I)



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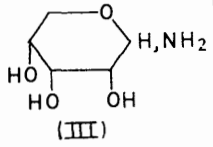
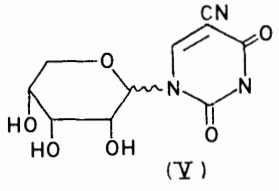


(III)

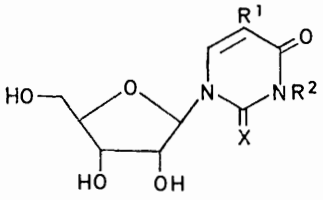
$R^1CH{:}CR^2CO{\cdot}NR^3{\cdot}CO_2Et$

	R^1	R^2	R^3
(IV) a;	OEt	CN	H
b;	OEt	COMe	H
c;	OEt	CN	p-ClC_6H_4
d;	OEt	CO_2Et	H
e;	NH_2	CO_2Et	H
f;	NHMe	CO_2Et	H
g;	NHPh	CO_2Et	H

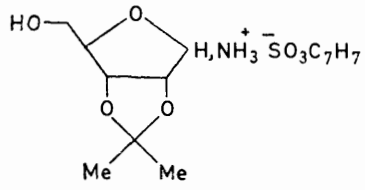


(V)

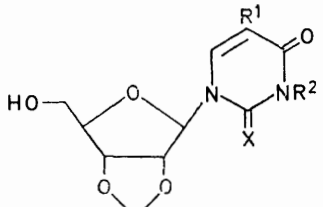
acid gave the crystalline isopropylideneribofuranosylamine as a toluene-*p*-sulphonate (VII). The structure of the compound followed from elemental analysis, mild acidic hydrolysis to give acetone, i.r. (characteristic doublet at 1380 cm^{-1}) and n.m.r. spectra, and chemical reactions. Table 1 shows the n.m.r. spectrum of



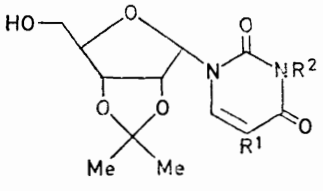
	R^1	R^2	X
(VI) a;	CN	H	O
b;	Me	H	S
c;	H	H	O
d;	COMe	H	O
e;	CN	p-ClC_6H_4	O



(VII)



	R^1	R^2	X
(VIII) a;	CN	H	O
b;	COMe	H	O
c;	CN	p-ClC_6H_4	O
d;	Me	H	S
e;	CO_2Et	H	O
f;	CO_2H	H	O
g;	H	H	O



	R^1	R^2
(IX) a;	CN	H
b;	CN	p-ClC_6H_4
c;	COMe	H

TABLE 1

N.m.r. data * for 2,3-*O*-isopropylidene-D-ribofuranosylamine toluene-*p*-sulphonate

	H-1 ($J_{1,2}$/Hz)		Me_2C	
Solvent	α	β	α	β
$(CD_3)_2SO$	5·13(3·5)	5·03(0)	1·32 1·53	1·30 1·44
$CDCl_3$		5·07(0)		1·16 1·38

* δ in p.p.m. from internal Me_4Si.

solutions in [^{2}H]chloroform and [$^2H_6$]dimethyl sulphoxide. In the former solvent the signal for the anomeric proton was a singlet and there were only two signals for the protons of the two isopropylidene methyl groups. This suggests that in this solvent the compound exists essentially as the pure β-anomer. The solution showed $[\alpha]_D$ −20·2° (*c* 1·5). In dimethyl sulphoxide, however, signals appeared for both α- and β-anomers and the ratio

[3] J. Baddilley, S. G. Buchanan, R. Hodges, and J. F. Prescott, *J. Chem. Soc.*, 1957, 4769.

[4] Preliminary communication, N. J. Cusack and G. Shaw, *Chem. Comm.*, 1970, 1114.

[5] R. S. Tipson, *J. Org. Chem.*, 1961, **26**, 2462.

of β- to α-anomers, calculated from the integration both of the anomeric signals and of those corresponding to the two sets of methyl protons, was 1·7 : 1; the solution showed $[\alpha]_D$ −18·8° (*c* 1·2). In deuterium oxide the n.m.r. spectrum 15 min after dissolution indicated an anomeric ratio of 1·5 : 1 with the presence of smaller signals at δ 5·42, 5·35, 5·30 (possibly a doublet) 4·97, and 4·94, possibly resulting from hydrolysis to either ribose or (more likely) isopropylideneribose. The signals of equilibrated ribose [6] in deuterium oxide are at δ 5·42, 5·30, 4·99, and 4·91.

Specific assignments of signals can be made by consideration of coupling constants and chemical shifts.[7] In furanoid rings the coupling constants vary from 3 to 8 Hz for neighbouring *cis*-protons and from 0 to 8 Hz for neighbouring *trans*-protons; thus only when the coupling constants are less than 3 Hz can assignments be made. However a *cis* anomeric proton resonates at

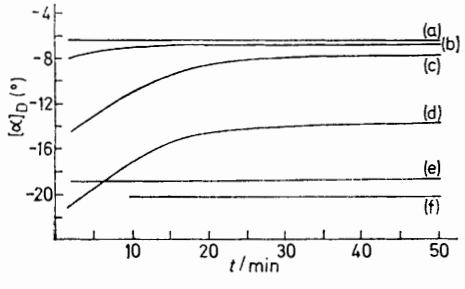


FIGURE 1 Specific rotation of 2,3-*O*-isopropylideneribofuranosylamine toluene-*p*-sulphonate: variation with time; (a) in aqueous 2M-Na_2CO_3; (b) in NaOMe–MeOH; (c) in NaOEt–EtOH; (d) in H_2O; (e) in Me_2SO; (f) in $CHCl_3$

lower field than a *trans* anomeric proton, so that assignments can be made if signals of both anomers are available. In our spectra of the isopropylideneribofuranosylamine toluene-*p*-sulphonate both methods can be applied. The anomeric region shows a clear doublet (*J* 3 Hz) at a lower field than a sharp singlet; these are therefore assigned to the *cis* (α) and *trans* (β) anomeric protons, respectively.

The specific rotations of alkaline solutions of the isopropylideneribofuranosylamine toluene-*p*-sulphonate in water, methanol, and ethanol all reached approximately the same value (−6·6, −6·8, and −7·7°, respectively) after 50 min, with the value for the ethanolic solution still rising to the equilibrium figure (Figure 1). Mutarotation is very fast in aqueous solution (almost instantaneous); in methanol it is just detectable over about 10 min and it is appreciably slower in ethanol. Under these conditions mutarotation is apparently related to solvent molecule size. These results indicate the occurrence of mutarotation of the free sugar amine in basic solution without hydrolysis, in contrast to the suggestion [8] that analogous pyranosylamines mutarotate very slowly under similar conditions; however these authors also comment on the slow hydrolysis compared to the much greater rate in weakly acidic solutions of pyranosylamines. By contrast the specific rotation of the furanosylamine toluene-*p*-sulphonate in water increases steadily to −14·5° during 15—20 min; this fact, together with the n.m.r. spectra in deuterium oxide confirms that some hydrolysis occurs under these conditions.

In preliminary experiments the reaction of the isopropylideneribofuranosylamine with the ethoxy-carbamate (IVa) in the presence of sodium methoxide in methanol gave the 5-cyanoisopropylideneuridine (VIIIa), which with aqueous acid gave the corresponding 5-cyanouridine (VIa). This compound was identical with an authentic sample prepared from the benzoylated amine (I) and the carbamate (IVa).[2b] In later experiments however, the presence was noted of a second u.v.-absorbing material similar to the β-derivative. This was readily separated and proved to be the α-form (IXa). The structures of the two isomers were confirmed by elemental analysis, mass spectra, n.m.r. spectroscopy (Table 2), and optical rotation data. The same mixture of anomers was also obtained, albeit in lower yield, when the preparation was carried out in aqueous sodium hydrogen carbonate solution. The isopropylidene-α-nucleoside could then be precipitated directly from the reaction mixture by adjustment to pH 7 whereas the β-form separated at pH 3. These are to our knowledge the first recorded syntheses of a nucleoside in aqueous solution from simple acyclic precursors.

Treatment of the ethoxy-carbamate (IVb) in methanolic sodium methoxide with the furanosylamine

TABLE 2

N.m.r. data * for some 3- and 5-substituted 1-α- and β-D-ribofuranosyluracils

	H-1′	Me_2C		H-6
5-Cyano-1-α-ribofuranosyluracil †	6·01			8·47
(IXa)	6·18	1·30	1·38	8·50
(VIa)	5·73			8·98
(VIIIa)	5·75	1·29	1·48	8·69
(IXc)	6·21	1·33		8·24
(VId)	5·76			8·68
(VIIIb)	5·84	1·30	1·49	8·56
(IXb)	6·16	1·28		8·12
(VIe)	5·57			9·09
(VIIIc)	5·80	1·29	1·48	8·78
(VIIId)	6·86	1·29	1·51	7·84
(VIIIe)	5·82	1·29	1·48	8·63

* δ Values; [$^2H_6$]dimethyl sulphoxide as solvent; Me_4Si as internal standard. † Obtained as a glass by hydrolysis of (IXa) with aqueous acetic acid.

gave the 5-acetylisopropylideneuridine (VIIIb); the mother liquors and the crude product appeared (t.l.c. and n.m.r.) to contain the anomeric α-form, which was not however isolated. The β-derivative was also precipitated in pure form directly from a reaction in aqueous

[6] R. V. Lemieux and J. D. Stevens, *Canad. J. Chem.*, 1966, **44**, 249.

[7] B. Capon and D. Thacker, *Proc. Chem. Soc.*, 1964, 399; S. J. Angyal and V. A. Pickles, *Austral. J. Chem.*, 1972, **25**, 1965; J. D. Stevens and H. G. Fletcher, *J. Org. Chem.*, 1968, **33**, 1799.

[8] H. S. Isbell and H. L. Frush, *J. Res. Nat. Bur. Stand.*, 1951, **46**, 132.

sodium carbonate solution. Hydrolysis of (VIIIb) with aqueous acid readily gave 5-acetyluridine (VId).

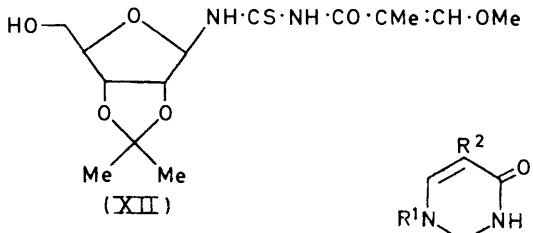
The carbamate (IVc), obtained from the acylcarbamate (Xa), on treatment with the furanosylamine (VII) gave a mixture of the α- and β-nucleosides (IXb) and (VIIIc), respectively, in a ratio of about 1 : 3. The products were readily separated by crystallisation and identified by elemental analysis, mass and n.m.r. spectra (Table 2), and comparison of rotations. Hydrolysis of the β-derivative in aqueous alcoholic acid gave the uridine derivative (VIe).

Reaction of the furanosylamine (VII) with β-methoxy-α-methylacryloyl isothiocyanate (XI) [9] gave the crystalline acylthiourea derivative (XII). The β-configuration is assumed from the ready cyclisation of the material to the thiothymine isopropylideneriboside (VIIId), which was readily hydrolysed to thiothymine riboside (VIb),

$R^1CH_2{\cdot}CO{\cdot}NR^2{\cdot}CO_2Et$

	R^1	R^2
(X) a;	CN	p-ClC_6H_4
b;	CO_2Et	H

MeO·CH:CMe·CO·NCS (XI)



(XII)

$R^1CO{\cdot}CH(CN){\cdot}N{:}CR^2{\cdot}OR^3$

	R^1	R^2	R^3
(XIV) a;	OEt	H	Et
b;	NH_2	H	Et
c;	NH_2	Me	Et
d;	NH_2	Me	C_6H_{11}
e;	NH_2	Me	bornyl

	R^1	R^2
(XIII) a;	H	CO_2Et
b;	Me	CO_2Et
c;	Ph	CO_2Et
d;	H	CO_2H
e;	Me	CO_2H
f;	Ph	CO_2H
g;	H	H
h;	Me	H
i;	Ph	H

identical with a sample prepared by an alternative route [2c] and whose β-structure was known from its conversion into thymidine *via* a cyclic thionucleoside intermediate.[2c]

We have also used the new ribofuranosylamine to prepare specific compounds whose configuration had been determined previously. In particular, the reaction of ethyl hydrogen malonate, with ethyl carbamate and acetic anhydride gave the acylcarbamate (Xb), which with triethyl orthoformate and acetic anhydride produced the ethoxymethylene derivative (IVd). This with ammonia, methylamine, and aniline in alcoholic solutions gave the corresponding acrylamide derivatives (IVe—g) as crystalline solids, which with ethanolic sodium ethoxide gave the 5-ethoxycarbonyluracils (XIIIa—c), respectively.

Hydrolysis of the uracil esters with aqueous sodium hydroxide, which was followed by titration studies, proved sluggish but ultimately gave the corresponding 5-carboxylic acids (XIIId—f). In contrast, treatment of the acyclic acrylamide esters (IVe—g) with sodium hydroxide caused hydrolysis and much more rapid cyclisation to the same uracil 5-carboxylic acids.

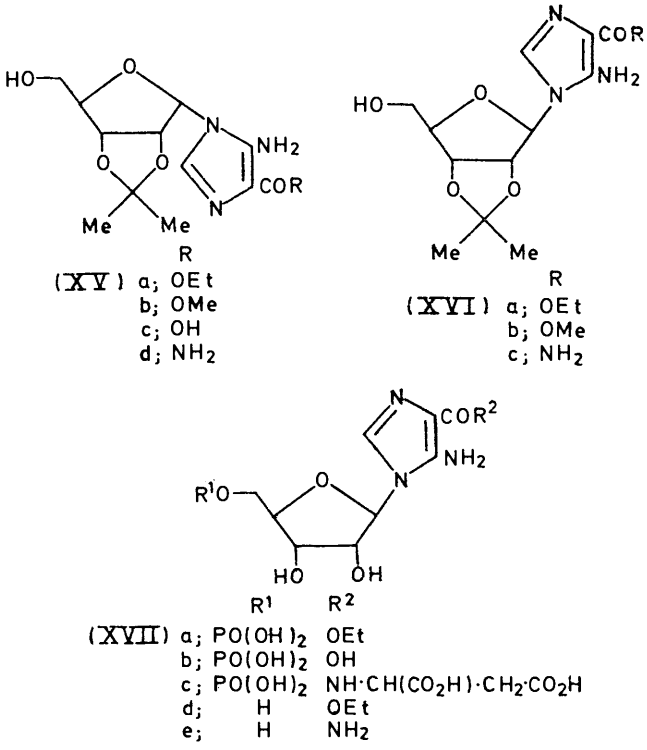
Similarly the ethoxyacrylamide (IVd) and the isopropylideneribofuranosylamine (VII) gave, presumably *via* an intermediate acyclic riboside, 5-ethoxycarbonylisopropylideneuridine (VIIIe), which was hydrolysed to the acid (VIIIf). If the initial reaction mixture was treated with aqueous sodium hydroxide the 5-carboxylic acid (VIIIf) was produced directly. The structures assigned both to the ester and the carboxylic acid were confirmed by decarboxylation of the acid to afford isopropylideneuridine (VIIIg), which when hydrolysed with aqueous acetic acid gave uridine (VIc). In these particular experiments no α-forms were isolated but their formation is not excluded.

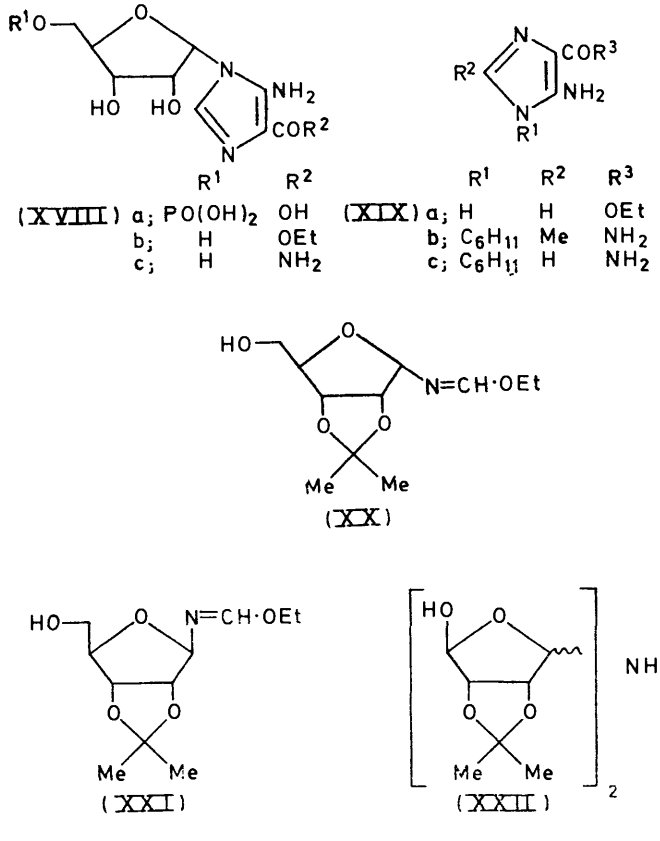
The decarboxylation of this group of compounds has been investigated in detail. Uracil-5-carboxylic acid may be decarboxylated by heating the solid to 260—280°. However such conditions were considered too severe to apply to the nucleoside derivatives and alternative methods were sought. The simple uracils (XIIId—f) were readily decarboxylated when heated with quinoline and copper powder at 210—220°, with *NN*-dimethylaniline at 160—180°, or (best) with *N*-ethylmorpholine at 130°. This last method has proved readily applicable to nucleoside derivatives, and offers a valuable route to uracil nucleosides *via* the 5-carboxylic acids.

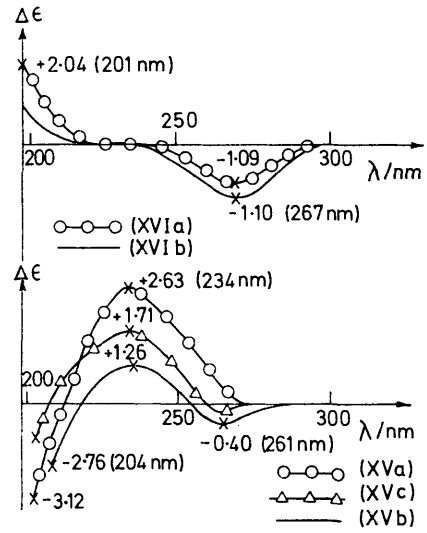
The furanosylamine has equally proved of great value in the synthesis of aminoimidazole nucleosides related to intermediates in purine nucleotide biosynthesis. Thus reaction of the furanosylamine (VII) with the imidate (XIVa) (formed by refluxing ethyl cyanoacetate and triethyl orthoformate in acetonitrile) either in ethanol with sodium ethoxide or in acetonitrile with triethylamine gave roughly equal amounts of the aminoimidazole α- and β-ribosides (XVa) and (XVIa), respectively, which were readily obtained as crystalline solids. The structures of the anomers followed from elemental analysis, characteristic u.v. spectra, Bratton–Marshall assay,[10] mass spectra, i.r. spectra (doublet at 1380 cm^{-1}, CMe_2) and c.d. measurements (Figures 2 and 3 and Table 3). The c.d. curve of the proposed β-form was almost identical with that of the previously prepared methyl ester, and different from that of the α-form; Hudson's rules [11] are obeyed. After hydrolysis, (XVIa) was converted into inosine by amination followed by formylation with formic acetic anhydride and cyclisation of the *N*-formyl derivative with potassium hydrogen carbonate solution.[2f] Also phosphorylation of the β-form with pyrophosphoryl chloride and mild acidic

[9] G. Shaw and R. N. Warrener, *J. Chem. Soc.*, 1958, 153.
[10] C. Bratton and E. K. Marshall, *J. Biol. Chem.*, 1939, **128**, 537.
[11] C. S. Hudson, *J. Amer. Chem. Soc.*, 1909, **31**, 66.

hydrolysis to remove the isopropylidene group gave the monophosphate ester (XVIIa), which after alkaline hydrolysis to form the carboxylic acid gave C-AIR (XVIIb),[2f] active as a substrate in the enzymic conversion into SAICAR (XVIIc). The α-form was similarly phosphorylated and hydrolysed but the resultant α-C-AIR (XVIIIa) was inactive in the enzyme system. Both nucleotides readily and characteristically







FIGURES 2 and 3 C.d. spectra of some anomeric imidazole ribonucleosides (crosses mark maxima and minima)

underwent rapid decarboxylation in aqueous acid solution. In a further set of reactions the corresponding α- and β-methyl esters (XVb) and (XVIb) were prepared; one of these was identical (mixed m.p., i.r., t.l.c.) with the material produced in an earlier synthesis [2f] using the ribofuranosyl azide (II) as a source of ribofuranosylamine.[12]

The isopropylidene groups in the ethyl esters (XVa) and (XVIa) were removed by heating with aqueous 10% acetic acid to give the nucleosides (XVIId) and (XVIIIb). The β-form was completely hydrolysed (t.l.c.) after about 1·5 h, whereas the α-form required 3 h. The difference in reactivity is presumably due to the closer proximity of the imidazole and isopropylidene ring systems in the α-form. Acidic hydrolysis of the β-form led to only small amounts of aglycone (XIXa), whereas the α-form gave larger quantities of this material.

Direct alkaline hydrolysis of the α-ethyl ester (XVa) with sodium hydroxide gave a crystalline sodium salt of the acid (XVc), the structure of which followed from elemental analysis, u.v. spectral comparisons, positive Bratton–Marshall test, i.r. spectra (doublet at 1380 cm^{-1}, CMe_2), a c.d. curve similar to that of the corresponding methyl ester (Figure 3) and loss of carbon dioxide on heating with acid. Decarboxylation was accompanied by changes of u.v. and Bratton–Marshall assay absorptions to those characteristic of the decarboxylated material.

The aminoimidazole ester nucleosides could also be obtained by a variation of the foregoing synthesis, *viz.*

[12] Preliminary communication, D. H. Robinson and G. Shaw, *Experientia*, 1972, **28**, 763.

by first condensing the isopropylideneribofuranosylamine (VII) with ethyl formimidate hydrochloride to give a mixture of the α- and β-ribosyl imidates (XX) and (XXI). Evidence for these structure came from the i.r. spectrum of the mixture [doublet at 1380 cm^{-1} (CMe_2); two C:N bands at 1695 and 1665 cm^{-1}], mass and n.m.r. spectra, and the reaction of the mixture with ethyl α-amino(cyano)acetate to give an anomeric mixture of aminoimidazole ribosides similar to those the aglycone (XIXa) were also detected (t.l.c.) during the syntheses of the aminoimidazole ribosides from the ribosylamine toluene-*p*-sulphonate. Formation of the aglycone presumably results from reaction of evolved ammonia with the imidate (XIVa). This is supported by the fact that imidazole formation is rather slow. Experiments, using cyclohexylamine as a model, have shown that reaction with the imidate (XIVb) is complete in about 5 h (Figure 4) and this would allow time for

TABLE 3

Spectral data for some 5-aminoimidazole ribofuranosides

Nucleoside	$\lambda_{max.}$/nm(ε)	λ_{max} of Bratton–Marshall [d] dyestuff (nm)	$[\alpha]_D^{20}$ (*c*) [a]	N.m.r. data (δ) H-1′	$J_{1',2'}$/Hz	Solvent
(XVIa)	267 (11,060) [b]	508	−97° (0·3)	5·99	4 (±0·05)	$(CD_3)_2SO$
(XVa)	267 (12,900) [b]	512	−70° (0·3)	5·88	4(±0·5)	
(XVIc)	267 (11,700) [b]	536	−72° (1·1)	5·84	4 (±0·5)	$(CD_3)_2SO$–D_2O (4 : 1)
(XVd)	267 (12,500) [b]	526	−56° (1·3)	5·96	3 (±0·5)	
(XVIb)	275 (9660) [b]	520	−100° (0·3)			
(XVb)	268 (11,420) [b]	507	−63° (0·3)			
(XVIId)	269 (11,190) [b]	517	−60° (1·3)			
(XVIIIb)	271 (11,040) [b]	527	+14° (1·5)			
(XVc)	248 (11,150) [b]	519	−33° (1·2)			
(XVc)	266 (10,700) [c]					
	244 (8400) [c]					

[a] Me_2SO. [b] MeOH. [c] In 0·1N-HCl by extrapolation of the absorption–time curve to zero time. [d] A sample of the imidazole in a phosphate buffer (15 ml) at pH 1·5 was treated with 0·1% sodium nitrite solution (10 ml). After 5 min 0·5% ammonium sulphamate solution (10 ml) was added, followed 3 min later by 0·1% *N*-α-naphthylethylenediamine dihydrochloride solution (10 ml). The solution was made up to 100 ml with water and the absorption measured after 15 min.

obtained in the foregoing syntheses but in higher overall yield.

In a similar manner, condensation of the ribofuranosylamine (VII) with the amide imidate (XIVb), either in ethanol with sodium ethoxide or in acetonitrile with triethylamine, gave a mixture of the aminoimidazolecarboxamide ribosides (XVd) and (XVIc) in roughly equal amounts; they were readily separated on silica gel columns. The structures assigned followed from comparison of c.d. measurements with those of the corresponding ethyl esters, optical rotations (Hudson's rules obeyed), u.v. and i.r. spectra, Bratton–Marshall assay, and elemental analysis of either the bases or the derived crystalline picrates. The two carboxamides were identical (t.l.c.) with the products produced by heating the corresponding ethyl esters with strong aqueous ammonia. In addition when the picrates were heated for 30 min at 100° in water the solution produced crystalline picrates of the free nucleosides (XVIIIc) and (XVIIe), identical (mixed m.p., i.r. spectra, t.l.c.) with materials prepared previously from an azidoribose carbonate.[13]

During some of these syntheses small amounts of the bis(isopropylideneribofuranosyl)amine (XXII) were isolated. This could be prepared directly from the ribofuranosylamine (VII) and methanolic sodium methoxide. Its structure was confirmed by elemental analysis, i.r. spectra (doublet at 1380 cm^{-1}, CMe_2), mass spectra, and acidic hydrolysis to give ribose as the only sugar (t.l.c.). The anomeric configuration of the product is as yet unknown.

In addition to the diribosylamine, small amounts of simultaneous formation of the diribosylamine and ammonia, and consequently the aglycone.

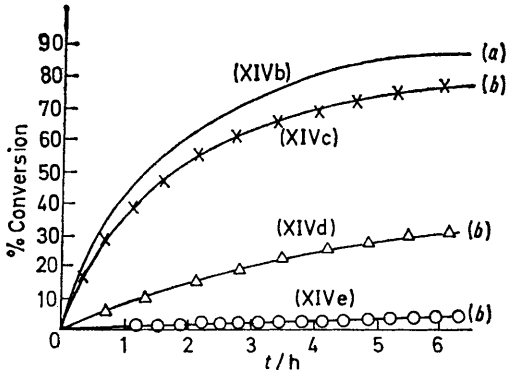


FIGURE 4 Reaction of cyclohexylammine with some imidates. (*a*) Samples were taken over 6 h from a solution of cyclohexylamine (1·0 g) and the formimidate (XIVb) (1·6 g) in acetonitrile (25 ml) at 20° and made up to 100 ml in methanol; the optical density was measured at 265 nm against a methanol blank. The amount of aminoimidazole formed was estimated by use of an extinction coefficient value of 13,500, derived from the pure aminoimidazole (XIXc). (*b*) Solutions of cyclohexylamine (1·0 g) and 1 mol. equiv. of each the acetimidates (XIVc—e) were made up to 25 ml in acetonitrile at 60°. Samples were removed and assayed as in (*a*) by use of an extinction coefficient value of 12,300, derived from the pure imidazole (XIXb)

Furthermore, a study of a series of reactions of cyclohexylamine with the acetimidates (XIVc—e) to give (XIXb) had shown that the rate of reaction is greatly

[13] C. G. Beddows and D. V. Wilson, *J.C.S. Perkin I*, 1972, 1773.

affected by the nature of R^2, in the order ethyl > cyclohexyl > bornyl (Figure 4).

EXPERIMENTAL

Evaporations were carried out with a Büchi rotary evaporator, under water-pump vacuum with a flask temperature below 40° unless otherwise stated. U.v. spectra were measured with a Unicam SP 800 spectrophotometer, i.r. spectra with a Perkin-Elmer 157 spectrophotometer, n.m.r. spectra with a J.E.O.L. JNM-MH-100 spectrometer (tetramethylsilane or 3-trimethylsilylpropane-1-sulphonic acid as internal standard), mass spectra with an A.E.I. MS 902 spectrometer, and optical rotations with a Perkin-Elmer 141 polarimeter. C.d. spectra were provided by Professor W. Klyne and Dr. P. M. Scopes (Westfield College, University of London), whom we thank. Silica gel (0·05—0·20 mm, 325—370 mesh; Machere Nagel and Co.) was used for column chromatography, and Silica gel GF_{254} (0·25 mm precoated glass plates; Merck) was used for t.l.c. ($CHCl_3$–MeOH, 9 : 1 as developing solvent). Ion-exchange separations were performed in an all-Teflon or -glass apparatus equipped with a Buchler micropump and an LKB Uvicord 4701A u.v. absorptiometer, with a flow cell of 3 mm light path for continuous recording of column eluates at 253·7 nm.

D-*Ribosylamine* (III).—D-Ribose (10 × 20 g) was added to a stirred, cooled solution of ammonia in methanol (200 ml). During the addition ammonia was bubbled through the mixture, which was then transferred to a heavy-gauge polythene bag and stored at 4°. After 2 weeks the product (180 g, 90%), m.p. 128—129°, was broken up, collected, washed with methanol, and dried *in vacuo*. Levene *et al.*[14] give m.p. 138°, but Tipson,[5] who gives m.p. 128—129° suggests that Levene's material is the diribopyranosylamine produced during purification. Our experiments support this.

2,3-O-*Isopropylidene*-D-*ribofuranosylamine Toluene*-p-*sulphonate* (VII).—Powdered D-ribopyranosylamine (40·8 g, 0·275 mol) was added to a vigorously stirred solution of dry toluene-*p*-sulphonic acid monohydrate (105 g, 0·55 mol) in 2,2-dimethoxypropane (286 ml, 2·2 mol) and dry acetone (1000 ml). The solution was stirred for 15 h, then evaporated to about half its volume, and dry ether (about 500 ml) was added to render the solution almost turbid. Crystallisation began within 20 min and the mixture was kept overnight at 0°. The product (VII) was then collected, washed with dry ether, and dried *in vacuo* to give *needles* (78 g, 80%), m.p. 128—129° (decomp.) (Found: C, 50·1; H, 6·35; N, 3·8. $C_{15}H_{23}NO_7S$ requires C, 49·9; H, 6·45; N, 3·9%). It crystallised from ethanol as a *monoethanolate* [δ 1·06 (t, *J* 7·0 Hz) and 3·43 (q, *J* 7·0 Hz)], which readily lost ethanol to give needles, m.p. 131° (decomp.).

A solution of the product in dilute hydrochloric acid was boiled under a short column. The distillate was treated with 2,4-dinitrophenylhydrazine in ethanol–phosphoric acid to give acetone dinitrophenylhydrazone, identical (mixed m.p.) with authentic material.

5-*Cyano*-1-(2,3-O-*isopropylidene*-α- *and* β-D-*ribofuranosyl*)-*uracils*.—(*a*) A solution of 2,3-*O*-isopropylideneribofuranosylamine toluene-*p*-sulphonate (VII) (10·8 g, 30 mmol) in methanol (100 ml) was treated with methanolic 2M-sodium methoxide (15 ml). Ethyl *N*-(α-cyano-β-ethoxyacryloyl)-carbamate (IVa) [15] (6·35 g, 30 mmol) was added, and the solution was left for 1 h, then treated with more 2M-sodium methoxide (15 ml) and set aside overnight. The solution was evaporated and a solution of the residue in water (50 ml) was acidified with acetic acid at 0°. The precipitate was collected and washed with water; t.l.c. showed this to be a mixture. Fractional crystallisation from a large volume of ethanol gave the *uracil* β-*nucleoside* (VIIIa) as white needles, m.p. 226°, R_F 0·60, δ 5·75 (*J* 2 Hz, H-1′) (Found: C, 50·5; H, 4·9; N, 13·6%; M^+, 309. $C_{13}H_{15}N_3O_6$ requires C, 50·5; H, 4·9; N, 13·6%; *M*, 309), *m/e* 294 $(M - Me)^+$, 251 $(M - Me_2CO)^+$, 234 $(M - Me_2CO_2)$,$^+$ 173 $(M$ − cyanouracil$)^+$, and 138 (cyanouracil + 2)$^+$, all characteristic of isopropylideneribofuranosyluracils.[16] The mother liquors were evaporated to a small volume and cooled to yield a sample enriched in material corresponding to the lower of two t.l.c. spots. Recrystallisation from a large volume of 95% ethanol gave as a first fraction, pure *uracil* α-*nucleoside* (IXa) as white needles, m.p. 267—268°, R_F 0·53, δ 6·18 (*J* 4 Hz, H-1′) (Found: C, 50·75; H, 4·95; N, 13·5%; M^+, 309).

(*b*) To a solution of 2,3-*O*-isopropylideneribofuranosylamine toluene-*p*-sulphonate (VII) (0·36 g, 1·0 mmol) in saturated aqueous sodium hydrogen carbonate (10 ml) was added ethyl *N*-(α-cyano-β-ethoxyacryloyl)carbamate (IVa) (0·21 g, 1·0 mmol) and the mixture was warmed gently to give a green solution, which was left at room temperature overnight. The solution was cooled in ice and the pH was adjusted to 7 with 2M-hydrochloric acid to yield a white precipitate of 5-cyano-(2,3-*O*-isopropylidene-α-D-ribofuranosyl)uracil (IXa) (0·052 g, 15·5%), which crystallised from 95% ethanol as needles, m.p. and mixed m.p. 269° [with the material prepared in (*a*)]. The mother liquor was further acidified to pH 3 to yield the β-isomer (0·025 g, 7·8%), which crystallised from 95% ethanol as needles, m.p. and mixed m.p. 216°.

5-*Cyano*-1-β-D-*ribofuranosyluracil* (VIa).—A suspension of the isopropylidene β-nucleoside (VIIIa) (4·9 g, 16 mmol) in aqueous 30% acetic acid (50 ml) was heated on a boiling water-bath for 3 h, then evaporated to dryness. The residue was repeatedly evaporated with water to remove acetic acid. When a solution of the remaining foam in ethanol (20 ml) was boiled for a few minutes, 5-cyano-β-D-ribofuranosyluracil (3·4 g, 80%) separated as needles, m.p. and mixed m.p. 185° (decomp.), $[\alpha]_D^{25}$ −4·7°. In 24 h at 25° the compound had consumed 1·1 mol. equiv. of 0·10N-sodium periodate, without the formation of formic acid.

5-*Acetyl*-2′,3′-O-*isopropylideneuridine* (VIIIb).—(*a*) A solution of 2,3-*O*-isopropylidene-D-ribofuranosylamine toluene-*p*-sulphonate (VII) (10·8 g, 30 mmol) in methanol (50 ml) was treated with methanolic 2M-sodium methoxide (15 ml), followed by ethyl *N*-(α-acetyl-β-ethoxyacryloyl)-carbamate (IVb) (6·8 g, 30 mmol), and the solution was left for 1 h. More 2M-sodium methoxide (15 ml) was added and the solution was boiled under reflux for 2 h and then evaporated to dryness. A solution of the residue in water (50 ml) was acidified at 0° with acetic acid, and the precipitated gel was collected, washed with ice-cold water, and dried overnight *in vacuo* to give a crystalline powder shown (t.l.c.) to be a mixture, presumably of α- and β-forms (4·1 g, 42%), m.p. 178°. Crystallisation from methanol gave the

[14] P. A. Levene and F. B. La Forge, *J. Biol. Chem.*, 1915, **20**, 433.

[15] G. Shaw, *J. Chem. Soc.*, 1955, 1834.

[16] P. Brown, G. R. Pettit, and R. K. Robins, *Org. Mass Spectrometry*, 1969, **2**, 521; J. J. Dolhun and J. L. Wiebers, *ibid.*, 1970, **3**, 669.

β-*nucleoside derivative* (VIIIb) as needles, m.p. 189—190° (Found: C, 51·7; H, 5·4; N, 8·7%; M^+, 326. $C_{14}H_{18}N_2O_7$ requires C, 51·6; H, 5·5; N, 8·6%; *M*, 326). The compound absorbed 1 mol. equiv. periodate and did not liberate formic acid. The *phenylhydrazone* (65%) separated from aqueous methanol as needles, m.p. 176° (Found: C, 57·6; H, 5·85; N, 13·4%; M^+, 416. $C_{20}H_{24}N_4O_6$ requires C, 57·7; H, 5·8; N, 13·45%; *M*, 416).

(*b*) 2,3-*O*-Isopropylideneribofuranosylamine toluene-*p*-sulphonate (VII) (3·61 g, 10 mmol) and the carbamate (IVb) (2·29 g, 10 mmol) were shaken in aqueous 2M-sodium carbonate (50 ml) and left at room temperature overnight. The solution was then cooled and acidified with acetic acid to yield 5-acetyl-2′,3′-*O*-isopropylideneuridine (0·65 g, 20%), which crystallised from methanol as white plates, identical [m.p. and mixed m.p. 190—195°; n.m.r. spectra (Table 2)] with the product from (*a*).

5-*Acetyluridine* (VId).—A suspension of the foregoing isopropylideneuridine (4·3 g) and Dowex 50 (H^+) ion-exchange resin (5 g) in water (60 ml) was heated at 100° with stirring for 3 h, filtered, and evaporated to a foam, which crystallised when treated with boiling ethanol for a few minutes. 5-*Acetyluridine* (3·2 g, 86%) was obtained as prisms, m.p. 165—167° (Found: C, 46·2; H, 4·8; N, 9·9%; M^+, 286. $C_{11}H_{14}N_2O_7$ requires C, 46·2; H, 4·90; N, 9·8%; *M*, 286).

Ethyl N-p-*Chlorobenzylcarbamate*.—To an ice-cooled stirred suspension of *p*-chlorobenzylamine (24 g, 0·17 mol) in 0·1M-sodium hydroxide (200 ml), ethyl chloroformate (16·4 ml, 0·17 mol) was added dropwise. The white solid formed was stirred for 1 h at 0°, kept at room temperature overnight, then collected, washed with 2M-hydrochloric acid and water, and recrystallised from light petroleum (b.p. 60—80°), to give the carbamate as white needles (25·8 g, 71%), m.p. 59° (lit.,[17] 62°).

Ethyl N-p-*Chlorobenzyl*-N-*cyanoacetylcarbamate* (Xa).—Ethyl *N*-*p*-chlorobenzylcarbamate (25·8 g, 0·12 mol), dry cyanoacetic acid, (10·3 g, 0·12 mol) and phosphoryl chloride (11·1 ml, 0·12 mol) were heated together at 70° for 1·5 h and left at room temperature overnight. The yellow solid produced was triturated under ice-cold water three times and recrystallised from 95% ethanol to give the carbamate (Xa) as white needles (17·1 g, 53%), m.p. 124—125°.

Ethyl N-(p-*Chlorobenzyl*)-N-(α-*cyano*-β-*ethoxyacryloyl*)-*carbamate* (IVc).—A mixture of the carbamate (Xa) (2·56 g, 10 mmol), triethyl orthoformate (3·0 ml, 20 mmol), and acetic anhydride (5 ml, 50 mmol) was boiled under reflux for 1 h. The solution was evaporated to an oil, which was distilled *in vacuo* to give the carbamate (IVc) as a viscous oil (2·1 g, 67%), b.p. 210° at 0·5 mmHg (Found: M^+, 336. Calc. for $C_{16}H_{17}ClN_2O_4$: *M*, 336).

3-p-*Chlorobenzyl*-5-*cyanouracil*.—A small amount of the carbamate (IVc) was dissolved in saturated methanolic ammonia. The solution was left overnight, then evaporated to dryness and the residue crystallised from ethanol on addition of water. The cyanouracil was obtained as off-white plates, m.p. 183° (Found: M^+, 261. Calc. for $C_{12}H_8ClN_3O_2$: *M*, 261), $\lambda_{max.}$ (MeOH–HCl) 273 nm (ε 7510), $\lambda_{max.}$ (MeOH–NaOH) 296 nm (ε 10,210).

3-p-*Chlorobenzyl*-5-*cyano*-1-*cyclohexyluracil*.—The carbamate (IVc) (0·426 g, 1·3 mmol) and cyclohexylamine (0·5 ml, 4 mmol) were dissolved in absolute ethanol (3 ml) by gentle warming and left at room temperature overnight. The solution was evaporated to an oil, which slowly crystallised; the crystals were swirled with ether (5 ml), collected, and washed twice with ether to give the cyclohexyluracil as white needles (0·22 g, 50%), m.p. 160° (Found: M^+, 343. Calc. for $C_{18}H_{18}ClN_3O_2$: *M*, 343), *m/e* 261 (*M* − cyclohexyl + 1) and 125 (*p*-chlorobenzyl).

3-p-*Chlorobenzyl*-5-*cyano*-1-(2,3-O-*isopropylidene*-α- *and* β-D-*ribofuranosyl*)*uracils*.—Methanolic 0·98M-sodium methoxide (16 ml) was added to a solution of the isopropylidenefuranosylamine salt (VII) (5·7 g, 15·8 mmol) and the carbamate (IVc) (5·3 g, 15·8 mmol) in methanol (20 ml). The solution was boiled for 10 min and cooled to give a precipitate of the β-*nucleoside derivative* (VIIIc), which was collected and washed with methanol. It formed needles (3·5 g, 51%), m.p. 222° (Found: C, 55·9; H, 4·75; N, 9·45. $C_{20}H_{20}ClN_3O_6$ requires C, 55·35; H, 4·65; N, 9·7%), δ 5·80 (*J* 3 Hz, H-1′), $[\alpha]_D$ −25·0° (*c* 1·4 in Me_2SO). The filtrate was evaporated to a foam, which was swirled with methanolic 4% dichloromethane (15 ml), and the precipitated sodium toluene-*p*-sulphonate was collected and washed twice with methanolic dichloromethane (5 ml). The filtrate was evaporated to a foam which crystallised on addition of ethanol to give the α-*nucleoside derivative* (IXb) which separated from 95% ethanol as white needles (1·1 g, 16%), m.p. 185° (Found: C, 55·35; H, 4·75; N, 9·5%), δ 6·16 (*J* 5 Hz, H-1′), $[\alpha]_D$ −62·7° (*c* 1·0 in Me_2SO).

3-p-*Chlorobenzyl*-5-*cyano*-1-β-D-*ribofuranosyluracil* (VIe).—A solution of the isopropylidene-β-nucleoside (VIIIc) (0·46 g, 1·1 mmol) in ethanol (100 ml), water (25 ml), and acetic acid (15 ml) was heated at 100° for 8 h, then evaporated to dryness. The residue was shaken with water (75 ml) and the solution filtered and evaporated. The residue crystallised from aqueous ethanol to give the β-nucleoside (0·23 g, 55%) as needles, m.p. 110—112° (Found: M^+, 393. Calc. for $C_{17}H_{16}ClN_3O_6$: *M*, 393).

N-(2′,3′-O-*Isopropylidene*-β-D-*ribofuranosyl*)-N′-(β-*methoxy*-α-*methylacryloyl*)*thiourea* (XII).—Methanolic 0·98M-sodium methoxide (6·5 ml, 6·4 mmol) was added to 2,3-*O*-isopropylidene-D-ribofuranosylamine toluene-*p*-sulphonate (VII) (2·3 g, 6·4 mmol) in ethanol (25 ml), to give a white precipitate of sodium toluene-*p*-sulphonate. To the suspension was added β-methoxy-α-methylacryloyl isothiocyanate [9] (1·0 g, 6·4 mmol), and the mixture was shaken at room temperature for 30 min then evaporated to dryness. The residue was swirled with methanolic 4% dichloromethane (25 ml); the mixture was filtered and evaporated to a gum which crystallised in 95% ethanol after addition of water and was recrystallised from acetone–water to give the *thiourea derivative* (XII) (0·7 g, 32%) as white needles, m.p. 125° (Found: C, 48·25; H, 6·7; N, 8·1; S, 9·5%; M^+, 346. $C_{14}H_{22}N_2O_6S$ requires C, 48·55; H, 6·4; N, 8·1; S, 9·25%; *M*, 346).

1-(2,3-O-*Isopropylidene*-β-D-*ribofuranosyl*)-2-*thiothymine* (VIIId).—A solution of the thiourea derivative (XII) (0·45 g, 1·3 mmol) in M-sodium hydroxide (2·5 ml) was heated at 100° for 30 min, cooled, and acidified with 2M-hydrochloric acid. The precipitate was collected, washed with water, and dried *in vacuo* to give the thiothymine riboside (0·215 g, 52·6%), m.p. 187° (Found: M^+, 314. Calc. for $C_{13}H_{18}N_2O_5S$: *M*, 314).

2-*Thiothymine* 1-β-D-*ribofuranoside* (VIb).—The isopropylideneriboside (VIIId) (0·085 g, 0·17 mmol) in aqueous 30% acetic acid (1 ml) was heated at 100° for 1 h. The solution was evaporated to a white solid, a portion of which was crystallised from ethyl acetate to give 2-thiothymine 1-β-D-ribofuranoside (0·012 g) as spherical clusters, m.p.

[17] T. Curtius, *J. prakt. Chem.*, 1914, **89**, 531.

210—214°, identical (i.r. and mixed m.p.) with an authentic sample.[2c]

Ethyl N-*Ethoxycarbonylacetylcarbamate* (Xb).—Ethyl hydrogen malonate (13·2 g, 0·1 mol) and acetic anhydride (15·0 g, 0·15 mol) were warmed together at 75° for 15 min. Ethyl carbamate (8·9 g, 0·1 mol) was added and the solution was maintained at 75° for 3 h. Evaporation to half volume and cooling gave the *acylcarbamate* as a crystalline solid, which crystallised from ethyl acetate or petroleum (b.p. 80—100°) as prisms, m.p. 59° (16·2 g, 80%) (Found: C, 47·3; H, 6·45; N, 6·9. $C_8H_{13}NO_5$ requires C, 47·4; H, 6·4; N, 6·9%).

Ethyl N-(α-*Ethoxycarbonyl*-β-*ethoxyacryloyl*)*carbamate* (IVd).—Triethyl orthoformate (350 g, 2·3 mol) and acetic anhydride (450 ml, 4·5 mol) were warmed together at 75° for 10 min; the acylcarbamate (Xb) (469 g, 2·3 mol) was added. The solution was boiled under reflux for 1 h and cooled to give the *carbamate* (IVd) (230 g, 39%) as needles, m.p. 159° (from ethyl acetate) (Found: C, 51·1; H, 6·6; N, 5·4. $C_{11}H_{17}NO_6$ requires C, 51·0; H, 6·55; N, 5·4%).

5-*Ethoxycarbonyluracil* (XIIIa).—Ammonia was passed into a solution of the carbamate (IVd) (3 g, 11·5 mmol) in methanol at 5—10° for 30 min to give a white precipitate of ethyl *N*-(β-amino-α-ethoxycarbonylacryloyl)carbamate (IVe) (2·8 g), m.p. 164° (resolidified) (Found: M^+, 230. Calc. for $C_9H_{14}N_2O_5$: *M*, 230). To a solution of this product (0·92 g, 4 mmol) in ethanol (20 ml) was added ethanolic sodium ethoxide (3 ml, 4·35 mmol); the solution was warmed on a steam-bath for 5 min, and cooled to give a white gel which dissolved after adjustment of the pH to 6·8 with N-hydrochloric acid (6 ml). 5-Ethoxycarbonyluracil separated as an amorphous solid which crystallised from ethanol–ethyl acetate as plates, m.p. 287° (Found: M^+, 184. Calc. for $C_9H_{11}N_2O_5$: *M*, 184).

5-*Carboxyuracil* (XIIId).—(*a*) The carbamate (IVe) (1·45 g, 5·6 mmol) was dissolved in aqueous sodium hydroxide (20 ml, 70 mmol) and kept at room temperature for 30 min. The sodium salt of 5-carboxyuracil separated as an amorphous white solid (1·0 g, 95%), which was dissolved in hot water (30 ml); the pH was adjusted to 6·0 with 2M-hydrochloric acid to give a white precipitate of 5-carboxyuracil, m.p. 223° (from water) (Found: M^+, 156. Calc. for $C_5H_4N_2O_4$: *M*, 156).

(*b*) A small amount of 5-ethoxycarbonyluracil in aqueous M-sodium hydroxide was kept at room temperature for 1 h. The pH was adjusted to 6·0 with 2M-hydrochloric acid, to give 5-carboxyuracil, identical with the sample from (*a*).

Uracil (XIIIg).—A small amount of 5-carboxyuracil was heated in *N*-ethylmorpholine to 120—130° over 20 min; gas was evolved and the solid dissolved. Uracil was precipitated from the cooled solution with ether and purified by dissolution in M-sodium hydroxide solution and neutralisation with 2M-sulphuric acid; the product was identical (i.r., t.l.c., mixed m.p.) with an authentic sample.

5-*Ethoxycarbonyl*-1-*methyluracil* (XIIIb).—Methylamine (2·5 ml of a 25% ethanolic solution, 20 mmol) was added to a solution of the carbamate (IVd) (5·2 g, 20 mmol) in ethyl acetate (3 ml); the solution was kept for 12 h at room temperature and evaporated to dryness to give *ethyl* N-(α-*ethoxycarbonyl*-β-*methylaminoacryloyl*)*carbamate* (IVf) as a solid, m.p. 120°, which crystallised as plates from ethyl acetate–petroleum (b.p. 60—80°) (Found: C, 49·2; H, 6·6; N, 11·5. $C_{10}H_{16}N_2O_5$ requires C, 49·1; H, 6·6; N, 11·5%).

To a solution of the carbamate (IVf) (1 g, 4 mmol) in ethanol (10 ml) was added a 2% solution of sodium ethoxide in ethanol (8 ml, 7 mmol); the solution was warmed on a steam-bath for 15 min, neutralised with 2M-hydrochloric acid, evaporated to half volume, and cooled, to yield 5-ethoxycarbonyl-1-methyluracil as a crystalline solid, m.p. 237° (from ethyl acetate) (Found: M^+, 198. Calc. for $C_8H_{10}N_2O_4$: *M*, 198).

5-*Carboxy*-1-*methyluracil* (XIIIe).—(a) *From* 5-*ethoxycarbonyl*-1-*methyluracil*. The ester (XIIIb) (1·98 g, 10 mmol) was dissolved in aqueous sodium hydroxide (20 ml containing 1·6 g, 70 mmol), and left at room temperature. (The reaction was followed by titration of samples against 0·1M-sulphuric acid and found to be complete in 1 h.) The solution was neutralised with Dowex 50 W × 8 resin (H^+) to give a white precipitate of 5-*carboxy*-1-*methyluracil* which crystallised from water as needles, m.p. 269—270° (Found: 41·5; H, 3·4; N, 16·9. $C_6H_6N_2O_4$ requires C, 42·3; H, 3·6; N, 16·5%).

(b) *From the carbamate* (IVf). The carbamate (1 g, 4·6 mmol) was dissolved in aqueous sodium hydroxide (10 ml containing 0·45 g, 19·5 mmol) at room temperature. After 10 min the pH was adjusted to 6·0 with Dowex W × 8 resin (H^+) and the solution was evaporated to half volume to give a crystalline precipitate of 5-carboxy-1-methyluracil, m.p. 270° identical (i.r., t.l.c.) with the foregoing material.

1-*Methyluracil* (XIIIh).—5-Carboxy-1-methyluracil, when boiled in *N*-ethylmorpholine as described for the unsubstituted compound, decarboxylated to give 1-methyluracil, identical (i.r., m.p. and mixed m.p.) with an authentic sample.

1-*Phenyluracil* (XIIIi).—A solution of the carbamate (IVd) (1·3 g, 5 mmol) and aniline (0·5 g, 5 mmol) in benzene (15 ml) was heated on a water-bath for 15 min then cooled to give crystals of ethyl *N*-(β-anilino-α-ethoxycarbonylacryloyl)carbamate (IVg) (1·4 g, 92%), m.p. 113°. To a solution of this (1·4 g, 4·5 mmol) in ethanol (10 ml) was added sodium ethoxide (7 mmol) in ethanol (8 ml); the mixture was warmed on a steam-bath for 15 min and the pH was adjusted to 6·8 with 2M-hydrochloric acid to give a precipitate of 5-ethoxycarbonyl-1-phenyluracil, which crystallised from alcohol as needles, m.p. 294° (decomp.) (Found: M^+, 260. Calc. for $C_{13}H_{12}N_2O_3$: *M*, 260).

(*a*) The carbamate (IVg) (1·3 g, 4·2 mmol) was dissolved, by warming, in aqueous sodium hydroxide (15 ml, 11 mmol), and the pH of the solution was adjusted to 6·5 with 2M-hydrochloric acid to give a white precipitate of 5-carboxy-1-phenyluracil (1·0 g, 100%), which crystallised from water as needles, m.p. 284° (Found: M^+, 232. Calc. for $C_{11}H_8N_2O_4$: *M*, 232).

(*b*) A small amount of 5-ethoxycarbonyl-1-phenyluracil was dissolved in aqueous sodium hydroxide (10 ml, 7·5 mmol). The solution was kept at room temperature for 1 h then neutralised with 2M-hydrochloric acid to give 5-carboxy-1-phenyluracil, identical (i.r., t.l.c.) with the foregoing sample. A suspension of the acid (1·0 g, 4·3 mmol) in *N*-ethylmorpholine (5 ml) was heated at 120° for 15 min, during which time rapid evolution of gas was observed. 1-Phenyluracil was filtered off and recrystallised from methyl acetate to give plates, m.p. 321° (0·7 g, 86%) (Found: C, 64·2; H, 4·1; N, 14·8. Calc. for $C_{10}H_{18}N_2O_2$: C, 63·9; H, 4·2; N, 14·9%).

5-*Ethoxycarbonyl*-1-(2,3-O-*isopropylidene*-β-D-*ribofuranosyl*)*uracil* (VIIIe).—To a solution of 2,3-*O*-isopropylideneribofuranosylamine toluene-*p*-sulphonate (10·8 g, 30 mmol) in methanol (30 ml) was added a solution of sodium ethoxide (30 mmol) in ethanol (34·5 ml) followed by the carbamate

(IVd) (9·0 g, 35 mmol). The solution was kept at room temperature for 2 h then warmed on a steam-bath for 10 min. More ethanolic sodium ethoxide (34·5 ml, 30 mmol) was added and the solution was left at room temperature overnight, heated on a water-bath, for 0·5 h, and evaporated to a foam.

The foam was dissolved in water (40 ml), the pH was adjusted to 5·8 with 2M-sulphuric acid, and the solution was filtered, concentrated, and seeded with a crystal. The *product* (VIIIe) (3·4 g, 32%) crystallised over several days and was recrystallised from water to give short needles, m.p. 196° (Found: C, 48·1; H, 5·75; N, 7·6. $C_{15}H_{20}N_2O_8$ requires C, 50·5; H, 5·6; N, 7·9%).

5-Carboxy-1-(2,3-O-isopropylidene-β-D-ribofuranosyl)-uracil (VIIIf).—(*a*) To a solution of 2,3-*O*-isopropylideneribofuranosylamine toluene-*p*-sulphonate (2·4 g, 6·7 mmol) in methanol (10 ml) were added ethanolic sodium ethoxide (6·7 ml, 6·7 mmol) and the carbamate (IVd) (1·7 g, 6·6 mmol). The solution was kept at room temperature for 1 h and evaporated to dryness. The residue was dissolved in water (15 ml) and sodium hydroxide (0·8 g, 35 mmol) was added; the solution was kept at room temperature overnight then neutralised with Dowex 50W-X8 resin (H^+) and evaporated to dryness. The residue crystallised from nitromethane–petroleum to give the *acid* (VIIIf), m.p. 186—192° (decomp.) (Found: C, 47·9; H, 4·5; N, 8·75. $C_{13}H_{16}N_2O_8$ requires C, 47·5; H, 4·8; N, 8·55%).

(*b*) A solution of the ester (VIIIe) (1·6 g, 4·5 mmol) and sodium hydroxide (0·72 g, 31 mmol) in water (18 ml) was heated on a water-bath for 2 h, then left at room temperature overnight. The pH was adjusted to 6·0 with Dowex 50W-X8 resin (H^+) and the solution was evaporated to a foam which crystallised from nitromethane–petroleum to give the acid (VIIIf) (1·1 g, 75%), identical with the sample from (*a*).

Uridine.—A suspension of the acid (VIIIf) (2 g, 6·1 mmol) in *N*-ethylmorpholine (15 ml) was heated to 130—135° for about 1 h (until evolution of gas ceased). The suspension was filtered and the solid (1·7 g) was washed with ether and boiled under reflux in aqueous 30% acetic acid (20 ml) for 3 h. The resulting solution was evaporated to dryness, acetic acid was removed by evaporation with water (3 × 15 ml), and the water was removed by evaporating with toluene (3 × 15 ml). The resulting dry foam was shaken with absolute ethanol; the suspension was filtered and ether was added to the filtrate to precipitate uridine (0·9 g, 60%), identical with an authentic sample (i.r., u.v., m.p., mass spectra, and t.l.c.) (Found: C, 44·3; H, 4·85; N, 11·6. Calc. for $C_9H_{12}N_2O_6$: C, 44·6; H, 4·90; N, 11·5%).

Ethyl 5-Amino-1-(2,3-O-isopropylidene-α- and β-D-ribofuranosyl)imidazole-4-Carboxylates.—A solution of Ethyl α-amino-α-cyanoacetate [1] (8 g, 60 mmol) and triethyl orthoformate (11 ml, 66 mmol) in acetonitrile (160 ml) was boiled under reflux for 45 min, cooled, and added to a mixture of 2,3-*O*-isopropylideneribofuranosylamine toluene-*p*-sulphonate (18 g, 50 mmol) and ethanolic sodium ethoxide (150 ml, 0·05 mol). The mixture was set aside at room temperature overnight. The red solution was then filtered from sodium toluene-*p*-sulphonate and evaporated to a foam. This was dissolved in chloroform (150 ml) and the solution was washed with 2N-sodium hydroxide (2 × 30 ml) and saturated aqueous sodium chloride (30 ml). The organic phase was separated, dried (Na_2SO_4), and evaporated to a pale orange gum (10 g).

(*a*) A solution of the foregoing gum in ethyl acetate (10 ml) was stored overnight at 0°. Crystals separated of the *imidazole α-nucleoside* (XVa), and were recrystallised from ethanol to give prisms (3·5 g, 22%), m.p. 188—190° (Found: C, 51·35; H, 6·35; N, 12·65%; M^+, 327. $C_{14}H_{21}N_3O_6$ requires C, 51·35; H, 6·45; N, 12·85%; *M*, 327).

The mother liquors, after 2 weeks at 0° produced crystals of the *β-nucleoside* (XVIa), which were recrystallised from ethanol to give prisms (2·5 g, 15%), m.p. 180—182° (Found: C, 51·4; H, 6·4; N, 12·7%; M^+, 327).

(*b*) The foregoing gum was dissolved in chloroform (5 ml) and applied, in two batches, to a silica gel column (3 × 30 cm). The β-imidazole ester was eluted by 2% ethanol in chloroform and the α-imidazole ester by 5% ethanol in chloroform. Crystallisation from ethanol gave the imidazole β-nucleoside (3·4 g, 21%) and the imidazole α-nucleoside (4·6 g, 28%), as prisms in each case.

The reaction was repeated with acetonitrile as solvent and triethylamine as base. The α- and β-nucleosides were formed in approximately the same amounts.

(*c*) A suspension of 2,3-*O*-isopropylidene-D-ribofuranosylamine toluene-*p*-sulphonate (3·6 g, 10 mmol) and ethyl formimidate hydrochloride (1·2 g, 11 mmol) was shaken with a solution of triethylamine (1·4 ml, 10 mmol) in acetonitrile (50 ml) for 20 min. The mixture was filtered and evaporated to a gum. This was dissolved in chloroform (20 ml); the solution was washed with water (2 × 20 ml), dried (Na_2SO_4), and evaporated to give a mixture of ethyl *N*-(2,3-*O*-isopropylidene-α- and β-D-ribofuranosyl)-formimidates (XX) and (XXI) as an oil (2·1 g) (Found: M^+, 244. Calc. for $C_{11}H_{19}NO_5$: *M*, 245), $\nu_{max.}$ 1695m and 1665s cm^{-1} (C=N), δ 7·68 (N=CH) and a complex pattern of methyl protons indicative of an αβ-mixture.

A portion of the foregoing gum (1·5 g, 6·1 mmol) and ethyl α-amino-α-cyanoacetate (0·77 g, 6·0 mmol) were boiled under reflux in acetonitrile (20 ml) for 15 min. The solution was evaporated to a gum and chromatographed as before to give the α- (0·7 g, 37%) and β- (0·74 g, 37%) nucleosides as crystalline solids, identical with those produced from the preformed imidates.

Methyl 5-Amino-1-(2,3-O-isopropylidene-β- and α-D-ribofuranosyl)imidazole-4-carboxylates.—The methyl esters were obtained in a similar manner to the ethyl esters, and the anomers were readily separated on a silica column. The β-nucleoside was eluted by 2% methanol in chloroform and the α-nucleoside by 5% methanol in chloforom. The β-nucleoside (XVIb) crystallised from ethyl acetate as pale cream prisms (15%), m.p. 161—162° (Found: M^+, 313. Calc. for $C_{13}H_{19}N_3O_4$: *M*, 313). The *α-nucleoside* (XVb) crystallised from ethanol as elongated prisms (20%), m.p. 188—189° (Found: C, 49·75; H, 6·05; N, 13·35%; M^+, 313. $C_{13}H_{19}N_3O_4$ requires C, 49·85; H, 6·1; N, 13·45%; *M*, 313).

Ethyl 5-Amino-1-β-D-ribofuranosylimidazole-4-carboxylate (XVIId).—A solution of the isopropylidene-β-nucleoside (XVIa) (1 g) in aqueous 10% acetic acid (20 ml) was heated at 100° for 1·5 h. The orange solution was cooled and evaporated to a gum, which was evaporated with water (3 × 20 ml) and ethanol (2 × 20 ml). The residual gum was dissolved in ethanol and placed on a silica gel column (1·5 × 30 cm). The imidazole β-riboside was eluted by 15% methanol in chloroform and obtained as a white, brittle foam (0·74 g, 84%) (Found: M^+, 287. Calc. for $C_{11}H_{17}N_3O_6$: *M*, 287). A portion of the β-nucleoside was

converted into inosine by successive amination, formylation, and cyclisation with potassium hydrogen carbonate as described elsewhere.[2f]

Ethyl 5-Amino-1-(α-D-ribofuranosyl)imidazole-4-carboxylate (XVIIIb).—The isopropylidene-α-nucleoside (XVa) (11 g) was heated in aqueous 10% acetic acid (20 ml) at 100° for 3·5 h. Evaporation, then further evaporation of the residue with water and ethanol, left material which was chromatographed on a silica gel column (1·5 × 30 cm); the product was eluted with 25% methanol in chloroform. Evaporation gave the product as a white foam (6·2 g, 70%) (Found: M^+, 287. Calc. for $C_{11}H_{17}N_3O_6$: M, 287).

Sodium 5-Amino-1-(2,3-O-isopropylidene-α-D-ribofuranosyl)imidazole-4-carboxylate.—The isopropylidene-α-nucleoside ester (XVa) (1 g) was heated at 100° in sodium hydroxide solution (0·5N; 20 ml) for 3 h. The pale yellow solution was filtered and evaporated and the residue was dissolved in aqueous ethanol (2 ml); after 5 days at 0° the solid was collected, washed with cold ethanol, and recrystallised from aqueous ethanol to give the *sodium salt* (600 mg, 65%) as rods, m.p. 91° which retained water and alcohol. The salt was stored at −18° (Found: C, 40·75; H, 5·9; N, 10·35. $C_{12}H_{16}N_3NaO_6$,2·5H_2O,EtOH requires C, 40·8; H, 6·65; N, 10·2%), *m/e* 255 ($M^+ - CO_2$).

(*a*) A solution of the sodium salt (5·69 mg) in 0·1N-hydrochloric acid (100 ml) was kept at 25°. The u.v. absorbance at 266 nm decreased by 73% during 6 h.

(*b*) The salt (1 mg) in 0·1N-hydrochloric acid (1 ml) was warmed on a water-bath for 5 min. A slight effervescence was observed. A Bratton–Marshall test on the resulting solution produced a salmon pink colour, $\lambda_{max.}$ 489 nm, characteristic of simple 5-aminoimidazoles. A similar sample heated for 30 min gave a negative Bratton–Marshall test, indicating cleavage of the aminoimidazole ring.

5-Amino-1-(2,3-O-isopropylidene-α- and β-D-ribofuranosyl)imidazole-4-carboxamides.—A solution of ethyl *N*-(carbamoylcyanomethyl)formimidate [1] (3·1 g, 20 mmol) and 2,3-*O*-isopropylideneribofuranosylamine toluene-*p*-sulphonate (7·2 g, 20 mmol) in ethanolic sodium ethoxide (60 ml, 20 mmol) was set aside at room temperature overnight. The precipitated sodium toluene-*p*-sulphonate was filtered off, and the filtrate was evaporated to a red gum (6·4 g).

The gum (5 g) was dissolved in chloroform (4 ml) and ethanol (1 ml) and applied in two batches to a silica gel column (2 × 30 cm) equilibrated with ethanolic chloroform (1%). The β-ribofuranosylimidazolecarboxamide was eluted by 10% ethanol in chloroform and the α-anomer by 15% ethanol in chloroform. The fractions were separately evaporated to give the β-nucleoside (XVIc) as a white, brittle foam (1·50 g, 28%), $[\alpha]_D^{20}$ −72° (*c* 1·1 in Me_2SO) (Found: M^+, 298. Calc. for $C_{12}H_{18}N_4O_5$: M, 298). The *α-nucleoside* (XVd) also gave a white foam, which when warmed in a mixture of ethyl acetate (5 ml) and ethanol (0·5 ml) readily crystallised as rods (1·8 g, 35%), m.p. 182—183° (Found: C, 48·2; H, 6·15; N, 18·55%; M^+, 298. $C_{12}H_{18}N_4O_5$ requires C, 48·3; H, 6·1; N, 18·8%; M, 298).

A similar reaction in acetonitrile and triethylamine also gave the α- and β-nucleosides.

An excess of saturated methanolic picric acid was added to a solution of the β-nucleoside (XVIc) (500 mg) in methanol (10 ml). The *picrate* formed yellow needles (0·73 g, 83%), m.p. 122—124° (from methanol) (Found: C, 40·85; H, 4·0; N, 18·75. $C_{18}H_{21}N_7O_2$ requires C, 41·0; H, 4·0; N, 18·6%). The *α-nucleoside picrate*, similarly prepared (85%), had m.p. 234—235° (decomp. from 195°) (from methanol) (Found: C, 40·9; H, 4·15; N, 18·7%).

A solution of the isopropylidene-β-nucleoside (XVIc) picrate (100 mg) in water (10 ml) was heated on a water-bath for 20 min. The cooled solution gave the β-nucleoside (XVIIe) picrate (62 mg, 64%), m.p. and mixed m.p. with an authentic specimen 164° (decomp.). The isopropylidene-α-nucleoside (XVd) picrate, similarly heated for 1 h, gave the α-nucleoside (XVIIIc) picrate (60 mg, 61%), m.p. and mixed m.p. with an authentic specimen 168—170° (from water).

Ethyl 5-Amino-1-β- and α-D-ribofuranosylimidazole-4-carboxylate 5′-O-Phosphates.—A solution of ethyl 5-amino-1-β-D-ribofuranosylimidazole-4-carboxylate (1 g) in dry acetonitrile (25 ml) was cooled to −40°. Pyrophosphoryl chloride [18] (2·5 ml) at 0° was slowly added with stirring, and the mixture was allowed to warm to room temperature and left for 3 h. The red solution was cooled to −20° and added dropwise to an ice cold solution of barium acetate (12 g) in water (30 ml); the total volume was adjusted to 200 ml with water, and the pH was quickly adjusted to 6·5 with saturated barium hydroxide solution (350 ml) The solution was boiled for 5 min and filtered through a pad of Supercel. The residue was re-extracted with boiling water (2 × 20 ml) and the filtrates were combined and evaporated to *ca.* 100 ml. The solution was adjusted to pH 3 with acetic acid (20 ml), heated at 100° for 1·5 h, and evaporated to dryness; the residue was re-evaporated with water (3 × 20 ml) to remove traces of acetic acid. The solid was dissolved in water (500 ml) and brought to pH 7·5 with aqueous ammonia (10 drops; *d* 0·88); the solution was filtered, diluted to 2 l, and applied to a column of BIORAD AG1 × 2 (Br^-) (1·5 × 25 cm). The column was washed overnight with water and elution was commenced with 0·02N-hydrobromic acid at 60 ml h^{-1}. The major u.v.-absorbing product was eluted between 2300 and 2600 ml. The pH of the eluate was adjusted to 8 with saturated barium hydroxide solution and the solution was evaporated to *ca.* 50 ml. The precipitate was removed at the centrifuge and washed with water (2 × 10 ml). The supernatant liquors were combined and treated with M-barium bromide (2·5 ml) and ethanol (200 ml). After 2 h at 0° the precipitate was collected at the centrifuge, washed with ethanol (2 × 20 ml) and ether (20 ml), and dried to give the 5′-*O*-phosphate as a white barium salt (1·0 g, 65%). The product gave an intense Bratton–Marshall colour, and was identical (u.v., i.r., t.l.c.) with material prepared by an alternative route.[2f]

The analogous α-nucleoside phosphate was prepared similarly with the exception that the product was heated for 3 h with dilute acetic acid to remove the isopropylidene group. The phosphate was isolated as a cream-coloured barium salt (0·82 g, 53%); it had the same general properties as the β-nucleotide.

The β-nucleotide, when treated with 0·5N-sodium hydroxide, gave the corresponding 4-carboxylic acid (C-AIR) which was active as a substrate in the enzymic conversion of C-AIR into SAICAR. The α-nucleotide under these conditions gave a similar material (α-C-AIR), which was inactive in the enzyme assay.

Both nucleotide carboxylic acids, when warmed briefly with dilute aqueous acid, gave solutions of the decarboxyl-

[18] P. C. Crofts, I. M. Downie, and R. B. Heslop, *J. Chem. Soc.*, 1960, 3673.

ated materials. Bratton–Marshall assay gave a dyestuff with $\lambda_{max.}$ 500 nm.

Bis-(2,3-O-*isopropylidene*-D-*ribofuranosyl*)*amine*.— 2,3-*O*-Isopropylidene-D-ribofuranosylamine toluene-*p*-sulphonate (3·6 g, 10 mmol) in methanol (50 ml) containing N-sodium methoxide (10 ml) was left at room temperature overnight and then warmed at 40° for 3 h. Ammonia was evolved in the early stages. The solution was evaporated to a gum which was dissolved in chloroform (30 ml). The solution was washed with water (30 ml), dried (Na_2SO_4), and evaporated to a gum. This in ethyl acetate (2 ml) after 14 days had deposited the *diribosylamine* as prisms (450 mg, 25%), m.p. 153—154°, $[\alpha]_D^{20}$ −114° (*c* 0·4 in Me_2SO) (Found: C, 53·15; H, 7·65; N, 4·05%; M^+, 361. $C_{16}H_{27}NO_8$ requires C, 53·2; H, 7·5; N, 3·9%; *M*, 361).

We thank the S.R.C. (B. J. H. and D. H. R.), and Smith Kline & French (N. J. C. and P. W. R.), for research studentships.

[3/445 *Received*, 27*th February*, 1973]