Syntheses of Some α-D-Ribofuranosylimidazoles Related to Purine Nucleotide Precursors

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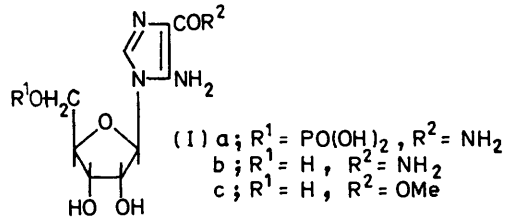
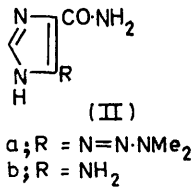
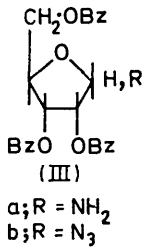
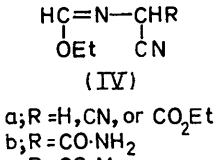
5-Amino-1-α-D-ribofuranosylimidazole-4-carboxamide [the α-anomer of the nucleoside corresponding to AICAR (5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide 5′-phosphate)] and a related 4-methoxycarbonyl compound have been synthesised from substituted ribofuranosyl azides possessing non-participating 2,3-cyclic carbonate protecting groups.

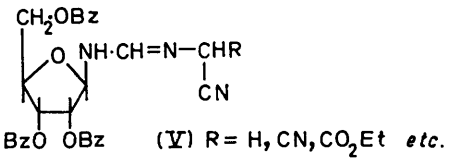
5-AMINO-1-β-D-RIBOFURANOSYLIMIDAZOLE-4-CARBOXAMIDE 5′-PHOSPHATE (AICAR) (Ia) is an important intermediate in the biosynthesis of inosinic acid and is also formed during conversion of ATP into histidine. Several syntheses of AICAR and related imidazole nucleosides and nucleotides have appeared [1,2] and a few analogues of the natural compound have been synthesised and tested for biological activity.[3,4] One of these (IIa) is







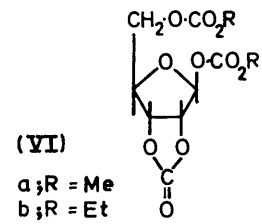


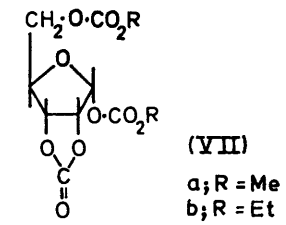


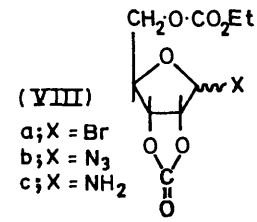
under clinical trial as an antitumour agent.[4] As all the known naturally occurring imidazole nucleosides and analogous synthetic glycosides have the β-configuration at C-1 of the sugar residue, it was of interest to obtain the α-anomers for enzymic studies and pharmacological testing.

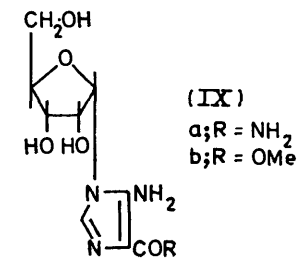
We have developed from the Shaw synthesis of β-D-ribofuranosylimidazoles [5] a satisfactory method for the synthesis of a range of α-anomers.[6] In the Shaw synthesis the imidazole ring is constructed by reaction between a glycosylamine (IIIa) and a complex imidate (IVa) to give, presumably, the intermediate (V), which cyclises spontaneously. Removal of protecting groups leads to the required compound. Although the reaction proceeds *via* a glycosylamine that mutarotates, no α-glycosides have ever been detected, probably because formation of a linear precursor of the α-compound is prevented by participation of the acyl group on C-2 of the sugar.

By using non-participating protecting groups, it has been possible to overcome this limitation. A suitable compound (VIa) was first prepared [7] by the action of methyl chloroformate on D-ribose. Two isomers were obtained by fractional crystallisation from ethyl acetate. The least soluble of these (described [7] as prisms, m.p. 180 °C, $[\alpha]_D^{20}$ −152°) was assigned the β-configuration (VIa) and the most soluble (described [7] as needles, m.p.









161 °C, $[\alpha]_D^{20}$ −76°) the α-structure (VIIa) on the evidence of i.r. spectra, optical rotation data, and the application of Hudsons Rules. In our hands, the yields of the pure isomers were low and, contrary to the original report, the less soluble isomer formed needles

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[1] G. Shaw, D. V. Wilson, and C. P. Green, *J. Chem. Soc.*, 1964, 2650; G. Shaw and D. V. Wilson, *ibid.*, 1963, 1077; L. B. Townsend, *Chem. Rev.*, 1967, **67**, 533.

[2] G. Shaw and D. V. Wilson, *J. Chem. Soc.*, 1962, 2937.

[3] I. E. Burrows, G. Shaw, and D. V. Wilson, *J. Chem. Soc.* (*C*), 1968, 40.

[4] J. L. Skibba, G. Ramirez, D. D. Beal, and G. T. Bryan, *Cancer Res.*, 1969, **29**, 1944.

[5] G. Shaw, R. N. Warrener, D. N. Butler, and R. K. Ralph, *J. Chem. Soc.*, 1959, 1648.

[6] C. G. Beddows and D. V. Wilson, Abstracts of Chem. Soc. Autumn Meeting, 1968, p. G3.

[7] G. R. Barker, I. C. Gillam, P. A. Lord, T. Douglas, and J. W. Spoors, *J. Chem. Soc.*, 1960, 3885.

(m.p. 160—161 °C, $[\alpha]_D^{20}$ −75°). The more soluble isomer formed needles or prisms (m.p. 178—179 °C, $[\alpha]_D^{20}$ −152°), depending on the recrystallisation conditions.

Two isomers ($C_{12}H_{16}O_{10}$) having ethoxycarbonyl substituents were obtained from D-ribose and ethyl chloroformate in the presence of dilute sodium hydroxide. Both were ribofuranose derivatives (they could be converted into methyl ribofuranosides by treatment with hydrogen bromide then methanol and silver carbonate) and both had acyclic carbonate ($\nu_{max.}$ 1730 cm^{-1}) and cyclic carbonate ($\nu_{max.}$ 1805 cm^{-1}) substituents. The ethyl and methyl compounds differed in their stability to alkali. All the carbonate groups were removed and D-ribose was formed when the methyl carbonate derivative was exposed to N-ammonium hydroxide solution for 2 min at 100 °C, whereas similar treatment of the ethyl analogue removed only the cyclic carbonate group and it was necessary to use more vigorous conditions (0·5N-sodium hydroxide solution at 100° for 30 min) to hydrolyse the 1- and 5-ethoxycarbonyl groups of this compound.

The n.m.r. spectrum of the less soluble isomer (in dimethyl sulphoxide) shows a singlet at τ 3·85, indicative of a *trans*-relationship between the C-1 and C-2 protons, whereas the more soluble isomer shows a doublet at τ 4·2 (*J* 6·6 Hz), which may be interpreted as the consequence of a *cis*-relationship between the corresponding protons in this compound.[8] Thus the β-configuration (VIb) was assigned to the less soluble isomer (m.p. 160 °C, $[\alpha]_D^{20}$ −75°) and the α-structure (VIIb) to the more soluble isomer (m.p. 180 °C, $[\alpha]_D^{20}$ −152°).

N.m.r. evidence indicates that the correct configurations were assigned to the methoxy-compounds by Barker *et al.*[7] despite the incorrect optical rotations. Apparently both pairs of compounds represent exceptions to Hudsons Rules.

Treatment of the carbonate (VIb) with hydrogen bromide in dry dichloromethane at room temperature gave the glycosyl bromide (VIIIa) (mixture of anomers) as a syrup, which was converted into the glycosyl azide (VIIIb) with sodium azide in dimethylformamide at 100°. The product contained only 75% of the theoretical amount of nitrogen and did not crystallise. Attempts at purification by distillation *in vacuo* or by chromatography were not successful, but a sample was characterised by hydrolysing it to D-ribofuranosyl azide and identifying the product by direct comparison with an authentic sample prepared from tri-*O*-benzoyl-β-D-ribofuranosyl azide (IIIb; β-anomer).

The glycosylamine (VIIIc) produced by catalytic reduction of the azide (VIIIb) with hydrogen over palladium–charcoal was treated *in situ* with the imidate (IVb), and the reaction mixture was hydrolysed in sodium hydroxide to remove the protecting groups. T.l.c. showed that the products included 5-amino-imidazole-4-carboxamide (IIb), the nucleoside (Ib) corresponding to AICAR, and the required 5-amino-1-α-D-ribofuranosylimidazolecarboxamide (IXa). These three aminoimidazoles were separable from impurities and from each other by chromatography on thick paper. In later experiments larger amounts were obtained by a preliminary separation on AG 50W × 8 (H^+) resin followed by rechromatography on AG 1 × 8 (HCO_2^-) resin, with water as eluant.

The first fraction from the anion-exchange resin had u.v. and mass spectra identical with those of the β-compound (Ib); the result of a quantitative Bratton–Marshall test [9] was similar ($\lambda_{max.}$ 540 nm), aglycone (IIb) and D-ribose were formed by hydrolysis in acid, 1 mol. equiv. of periodate was consumed, and a compound spectroscopically similar to inosine was formed on formylation with formic acetic anhydride and ring closure of the product by heating in sodium hydrogen carbonate solution.

The new compound was readily distinguished from the β-anomer by t.l.c. and by high-voltage electrophoresis at pH 1·85, and it had a different optical rotation and m.p. Pure β-anomer could be isolated in crystalline form from the second peak from the column.

Repetition of the reaction sequence starting from 1,5-bis-*O*-ethoxycarbonyl-α-D-ribofuranose 2,3-cyclic carbonate (VIIb) gave no trace of either α- or β-nucleosides, the major product had chromatographic properties similar to those of a decomposition product of the imidate (IVb) but it was not identified. When a mixture of anomers of 1,5-bis-*O*-methoxycarbonyl-D-ribofuranose 2,3-carbonate was used as a starting material compounds (Ib), (IIb), and (IXa) were isolated in low yield.

An alternative route to the riboside (IXa) by way of the methyl ester (IXb) was also investigated because the ester is a promising intermediate for the synthesis of α-anomers of other naturally occurring imidazole precursors of purine nucleosides [*e.g.*, 5-amino-1-β-D-ribofuranosylimidazole 5′-phosphate (AIR), its 4-carboxylic acid derivative (carboxy-AIR), and *N*-(5-amino-1-β-D-ribofuranosylimidazole-4-carbonyl)-L-aspartic acid 5′-phosphate (SAICAR)].[1-3]

The reaction between ethyl *N*-[cyano(methoxycarbonyl)methyl]formimidate [2] (IVc) and the glycosylamine (VIIIc) gave a mixture of aminoimidazoles from which, after removal of protecting groups, the α- and β-anomers of methyl 5-amino-1-D-ribofuranosylimidazole-4-carboxylate (Ic) and (IXb) were isolated. They were characterised by spectroscopic and chromatographic comparisons and by conversion into the corresponding carboxamides (Ib) and (IXa) by treatment with ammonia.

EXPERIMENTAL

Evaporations were carried out with a Büchi rotary evaporator (water-pump vacuum; flask temperature below

[8] J. D. Stevens and H. G. Fletcher, *J. Org. Chem.*, 1968, **33**, 1799.

[9] A. C. Bratton and E. K. Marshall, *J. Biol. Chem.*, 1939, **128**, 537.

38°). Optical rotations were measured on a Perkin-Elmer 141 photoelectric polarimeter, n.m.r. spectra on a Varian A60 instrument, mass spectra on an A.E.I. MS902 spectrometer, and u.v. spectra on a Unicam SP 800 spectrophotometer. U.v. spectra ' in acid ' refer to solutions adjusted to pH 1·5—2·0 with hydrochloric acid, and those ' in alkali ' to solutions adjusted to pH 10·5 with dilute sodium hydroxide solution.

Ion-exchange separations were monitored by means of an LKB Uvicord I instrument operating at 253 nm; the resins were of an analytical grade supplied by Bio-Rad Laboratories, Richmond, California. DEAE and CM cellulose were Whatman microgranular grades.

Thin-layer chromatograms were run either on plates coated with CC41 cellulose with one of the following as developing solvent: (A) n-butanol–water (87 : 13), (B) 5% ammonium citrate (pH 4·4)–ethanol (18 : 83), (C) n-butanol–ethanol–water (4 : 1 : 5; upper layer), (D) n-butanol–ethanol–water (4 : 1 : 5; lower layer), (E) propan-2-ol–0·2N-ammonia solution (4 : 1), (F) ammonia solution (*d* 0·880)–water (4 : 1), (G) saturated ammonium hydrogen carbonate solution, (H) n-butanol–glacial acetic acid–water (4 : 2 : 5), (I) propan-2-ol–water–ammonia (*d* 0·880) (6 : 2 : 3), or on plates coated with Merck silica gel G with one of the following as solvent: (J) light petroleum (b.p. 40—60°)–benzene (1 : 9), (K) methanol–benzene (1 : 9).

Most electrophoretic separations were carried out at <10 °C on Whatman 3MM paper at a potential difference of 2—2·5 V cm^{-1} in pH 1·85 buffer [glacial acetic acid–95% formic acid–water (15 : 10 : 255 v/v)], pH 9·4 buffer [N-sodium hydrogen carbonate–N-sodium carbonate–water (56·8 : 14·4 : 929 v/v)], or pH 9·1 buffer (1% sodium tetraborate in water). Detection systems used for examining chromatograms and electrophoretograms included the Bratton–Marshall spray reagents, the ammonium molybdate reagent for phosphate esters,[10] treatment with Bromocresol Blue then borax solution, and ozonolysis followed by Tollens reagent.

Labile bromine was estimated by hydrolysing a sample of compound in N-sodium hydroxide solution at 100 °C for 30 min and titrating with silver nitrate the ionic bromine in the neutralised hydrolysate. Published procedures were used for micro-periodate titrations,[11] phosphate analysis,[12] and determinations of aminoimidazoles by the Bratton–Marshall test.[13]

1,5-*Bis*-O-*methoxycarbonyl*-α- *and* -β-D-*ribofuranose* 2,3-*Carbonate*.—D-Ribose (10 g) was treated with methyl chloroformate (33 ml) by the method of Barker *et al.*[7] The expected crystalline mixture of products (14 g; m.p. 87—110 °C) was obtained but, contrary to the published report, fractional crystallisation from ethyl acetate gave as the least soluble component 1,5-*bis*-*O*-methoxycarbonyl-β-D-ribofuranose 2,3-carbonate, which formed needles, m.p. 160—161°, $[\alpha]_D^{16}$ −75° (*c* 1·0 in $CHCl_3$) (Found: C, 41·2; H, 4·3. Calc. for $C_{10}H_{12}O_{10}$: C, 41·1; H, 4·1%); $\nu_{max.}$ 1750 (acyclic carbonate) and 1805 cm^{-1} (cyclic carbonate); τ (Me_2SO) 3·80 (s, H-1), 4·58 (s, H-2 and -3), 5·25 (q, H-4), 5·80 (m, CH_2), and 6·25 (d, CH_3).

The most soluble isomer was difficult to obtain completely free from the less soluble material, but repeated recrystallisation from ethyl acetate gave pure 1,5-bis-*O*-methoxycarbonyl-α-D-ribofuranose 2,3-carbonate as needles or prisms, m.p. 180°, $[\alpha]_D^{16}$ −152·2° (*c* 1·0 in $CHCl_3$) (Found: C, 41·2; H, 4·3. Calc. for $C_{10}H_{12}O_{10}$: C, 41·1; H, 4·1%); $\nu_{max.}$ 1750 (acyclic carbonate) and 1805 cm^{-1} (cyclic carbonate); τ (Me_2SO) 4·15 (d, *J* 6·6 Hz, H-1), 4·58—4·82 (m, H-2, -3, and -4), 5·80 (m, H-5 and CH_2), and 6·25 (d, CH_3).

Both compounds gave mass spectra with a strong line at *m/e* 217.

1,5-*Bis*-O-*ethoxycarbonyl*-α- *and* -β-D-*ribofuranose* 2,3-*Carbonate*.—A solution of D-ribose (20 g) in water (80 ml) was cooled to 1 °C and ethyl chloroformate (34 ml) was added. The cooled mixture was stirred vigorously while 2N-sodium hydroxide solution (*ca.* 33 ml) was added dropwise at such a rate that the temperature did not exceed 1°. After about 1 h the mixture showed a strong alkaline reaction to indicator which persisted on further stirring. The precipitate was filtered off, washed with cold water (5 ml) and ice-cold ethanol (5 ml), and recrystallised from ethanol (*ca.* 80 ml) to give the mixture of anomers (14·7 g). The mother liquors yielded a second crop (1·6 g) (total yield 76·5%).

A portion (4 g) of the mixture was twice recrystallised from ethyl acetate to give 1,5-*bis*-O-*ethoxycarbonyl*-β-D-*ribofuranose* 2,3-*carbonate* as prisms, m.p. 107—108°, $[\alpha]_D^{16}$ −71° (*c* 1·0 in $CHCl_3$) (Found: C, 45·3; H, 5·0. $C_{12}H_{16}O_{10}$ requires C, 45·0; H, 5·1%); $\nu_{max.}$ 1730 (acyclic carbonate) and 1805 cm^{-1} (cyclic carbonate); τ (Me_2SO) 3·85 (s, H-1), 4·60 (s, H-2 and -3), 5·25 (q, H-4), 5·85 (m, O·CH_2), 5·88—6·05 (m, C–CH_2), and 8·75 (t, C–CH_3).

The mother liquors from the recrystallisation were evaporated to dryness at 25° and the residue was recrystallised from absolute ethanol to give 1,5-*bis*-O-*ethoxycarbonyl*-α-D-*ribofuranose* 2,3-*carbonate* as prisms, m.p. 134—135°, $[\alpha]_D^{16}$ −154° (*c* 1·0 in $CHCl_3$) (Found: C, 45·5; H, 5·2; OEt, 27·0. $C_{12}H_{16}O_{10}$ requires C, 45·0; H, 5·1; OEt, 28·1%); τ ($CDCl_3$) 4·20 (d, *J* 6·6 Hz, H-1), 4·6—4·9 (m, H-2, -3, and -4), 5·68—6·10 (m, O·CH_2 and C–CH_2), and 8·75 (t, C–CH_3), *m/e* 231. The i.r. spectrum was indistinguishable from that of the β-anomer.

Treatment of D-*Ribose Carbonates with Alkali*.—(*a*) The mixture of the anomers of 1,5-bis-*O*-methoxycarbonyl-D-ribofuranose 2,3-carbonate (0·5 g) was heated for 5 min in N-ammonium hydroxide solution at 100 °C. The solution was evaporated to dryness. The solid was dissolved in anhydrous methanol; the solution was filtered and was shown to contain D-ribose by t.l.c. in systems (A), (J), and (K). The product reduced Fehlings solution, and did not show carbonate peaks in the i.r. spectrum.

(*b*) 1,5-Bis-*O*-ethoxycarbonyl-β-D-ribofuranose 2,3-carbonate (1 g) was suspended in N-ammonium hydroxide solution and the mixture was heated until all the ester had dissolved (4—5 min), then evaporated to dryness. The product was redissolved in anhydrous methanol (*ca.* 10 ml) and the residual ammonium hydrogen carbonate was removed by centrifuging (2000 rev. min^{-1} for 5 min). The The solution was added to a column of cellulose (25 × 2·5 cm diam.) and eluted with methanol; 50 ml fractions were collected. Fraction (i) had [α] −0·228°, fraction (ii) −1·198°, and fraction (iii) −0·208°. Fraction (ii) gave syrupy 1,5-bis-*O*-ethoxycarbonyl-β-D-ribofuranose (Found: C, 46·3; H, 6·2. $C_{11}H_{18}O_9$ requires C, 46·5; H, 6·35%); $\nu_{max.}$ 1750 cm^{-1} (acyclic carbonate) (no 1805 cm^{-1} peak).

[10] S. Burrows, F. St. R. Grylls, and J. S. Harrison, *Nature*, 1952, **170**, 800.
[11] J. Dixon and C. Lipkin, *Analyt. Chem.*, 1954, **26**, 1092.
[12] D. N. Fogg and N. T. Wilkinson, *Analyst*, 1958, **83**, 401.
[13] J. C. Rabinowitz, *Methods Enzymology*, 1963, **6**, 712.

The product did not reduce Fehlings solution, and was chromatographically pure [t.l.c. in systems (J) and (K)].

(*c*) 1,5-Bis-*O*-ethoxycarbonyl-β-D-ribofuranose 2,3-carbonate (1 g) was heated in 0·5N-sodium hydroxide solution at 100 °C for 30 min. After neutralisation with Zeokarb 225 (H^+) resin, the solution was evaporated to dryness; a solution of the residue in anhydrous methanol was filtered and evaporated to dryness. The solid had identical chromatographic behaviour to D-ribose, did not show 1750 and 1805 cm^{-1} peaks in the i.r. spectrum, and reduced Fehlings solution.

2,3-O-*Carbonyl*-5-O-*ethoxycarbonyl*-αβ-D-*ribofuranosyl Bromide*.—1,5-Bis-*O*-ethoxycarbonyl-β-D-ribofuranose 2,3-carbonate (5 g) was suspended in anhydrous dichloromethane (80 ml) and dry hydrogen bromide was passed through the solution for 3 h. Evaporation of the solution yielded the *glycosyl bromide* as an amorphous solid (4·8 g, 98%), $[\alpha]_D^{16}$ +27·3° (*c* 3·5 in $CHCl_3$) (Found: Br, 26·2. $C_9H_{11}BrO_7$ requires Br, 25·7%).

This glycosyl bromide (1 g) was dissolved in dry methanol (10 ml) in which was suspended silver carbonate (1 g). The solution was filtered and the filtrate was evaporated to a syrup, which was heated in N-sodium hydroxide (10 ml) for 10 min at 100° to remove protecting groups. The cooled solution was neutralised with Zeokarb 225 (H^+) resin, filtered, and evaporated to give methyl αβ-D-ribofuranoside as a gum, identified by direct comparison with an authentic sample [t.l.c. in the systems (E), (G), (H), and (I) (detection by the Bromocresol Purple–borax method)].

2,3-O-*Carbonyl*-5-O-*ethoxycarbonyl*-αβ-D-*ribofuranosyl Azide*.—A solution of freshly prepared 2,3-*O*-carbonyl-5-*O*-ethoxycarbonyl-αβ-D-ribofuranosyl bromide (4·8 g) in anhydrous dimethylformamide (60 ml) was treated with sodium azide (2·4 g) and the mixture was heated at 100 °C for 1·5 h. The mixture was cooled and filtered and the filtrate evaporated to a syrup. The last traces of solvent were removed at 0·2 mmHg and 20 °C; hot ethyl acetate (5 ml) was added, the mixture was again filtered, and the filtrate was evaporated to yield the impure *glycosyl azide* as a syrup (3·5 g, 75%) (Found: C, 40·6; H, 4·2; N, 12·4. $C_9H_{11}N_3O_7$ requires C, 39·6; H, 4·0; N, 15·4%). Attempts at purification by distillation under reduced pressure or by chromatography failed. The impure product had $\nu_{max.}$ 1805 and 1750 (acyclic and cyclic carbonates) and 2105 cm^{-1} (azide); the rest of the spectrum was almost identical with that of 1,5-bis-*O*-ethoxycarbonyl-β-D-ribofuranose 2,3-carbonate. The n.m.r. spectrum had signals at τ 3·92 and 4·24 suggesting the presence of a mixture of anomers.

The glycosyl azide (0·5 g) was heated with 0·5N-sodium hydroxide (10 ml) for 30 min at 100 °C. The cooled solution was neutralised with Zeokarb 225 (H^+) resin, filtered, and evaporated. The i.r. spectrum of the residue lacked peaks attributable to cyclic or acyclic carbonate substituents, but had a strong azide peak at 1805 cm^{-1}.

The product(s) contained a major compound which had the same chromatographic behaviour as the hydrolysis product of authentic β-D-ribofuranosyl azide in systems (A), (B), (D), and (E).

5-*Amino*-1-α-D-*ribofuranosylimidazole*-4-*carboxamide from* 2,3-O-*Carbonyl*-5-O-*ethoxycarbonyl*-αβ-D-*ribofuranosyl Azide*.—2,3-*O*-Carbonyl-5-*O*-ethoxycarbonyl-αβ-D-ribofuranosyl azide (4 g), anhydrous ethyl acetate (40 ml), and pre-reduced 5% palladium–charcoal (1·2 g) were shaken under hydrogen at room temperature until i.r. spectroscopy showed the absence of azide ($\nu_{max.}$ *ca.* 2105 cm^{-1}). The reduction generally took about 30 min. Ethyl *N*-[carbamoyl(cyano)methyl]formimidate [5] (1·1 g) was then added and the mixture was shaken for a further 2 h, subsequently left at room temperature overnight, heated under reflux for 30 min, cooled, filtered, and evaporated to a syrup (4·3 g). A portion of this (1·3 g) dissolved in 0·5N-sodium hydroxide (5 ml) was boiled under reflux for 30 min, cooled, neutralised with Zeokarb 225 resin (H^+), and then placed on a column (2·5 × 28 cm) of AG 50W × 8 (200—400 mesh) resin (H^+). The column was washed with water (690 ml) and elution was started with N-ammonium hydroxide. Evaporation at 30° of the ammoniacal eluate (450 ml) gave a syrup, which was dissolved in water (10 ml) and placed on a column (3·5 × 25 cm) of AG 1 × 8 (200—400 mesh) resin (HCO_2^-). The products were eluted with water at 60 ml h^{-1} (10 ml fractions).

Fractions 15—34 were combined and freeze-dried to give 5-*amino*-1-α-D-*ribofuranosylimidazole*-4-*carboxamide* $[\alpha]_D^{16}$ +6·2° (*c* 0·1 in H_2O) [Found: C, 41·6; H, 5·45; N, 21·5%; *m/e* 258·0957 (±10 p.p.m.). $C_9H_{14}N_4O_5$ requires C, 41·85; H, 5·4; N, 21·7%; *M*, 258·0964]; $\lambda_{max.}$ (in acid) 246 and 267—268 nm (ε 11,000), $\lambda_{max.}$ (H_2O) 267—268 nm (12,000), $\lambda_{max.}$ (in alkali) 267—268 nm (12,400); the amide gave a purple solution ($\lambda_{max.}$ 540 nm) in the Bratton–Marshall test and consumed 0·98 mol. equiv. of periodate. On electrophoresis, a single spot was observed that migrated −0·4 cm kV^{-1} h^{-1} in borate buffer (pH 9·1), +1·0 cm kV^{-1} h^{-1} in pH 9·4 buffer, and +4·9 cm kV^{-1} h^{-1} in pH 1·85 buffer. The α-nucleoside gave 5-aminoimidazole-4-carboxamide on hydrolysis in 2N-hydrochloric acid at 100 °C for 10 min; with formic acetic anhydride it was cyclised to α-inosine (see later); and with aqueous picric acid, a crystalline picrate (m.p. 166°) was formed.

Fractions 35—50 were combined and evaporated at 20° to a gum which was shown by t.l.c. in systems (A), (B), (C), and (D) to contain mainly 5-*amino*-1-β-D-*ribofuranosylimidazole*-4-*carboxamide* with a trace of 5-aminoimidazole-4-carboxamide. Rechromatography on a column (1 × 98 cm) of the formate resin removed the aglycone, and the β-ribofuranoside was isolated by freeze-drying appropriate fractions. It gave needles (24 mg) from water, and had $\lambda_{max.}$ (in acid) 267—268 nm (ε 11,000) (absorbance ratios A_{260}/A_{250} 1·13, A_{260}/A_{280} 1·38), $\lambda_{max.}$ (neutral solution) 267—268 nm (ε 12,200) (A_{260}/A_{250} 1·34, A_{260}/A_{280} 1·36), $\lambda_{max.}$ (in alkali) 267—268 nm (ε 12,600) (A_{260}/A_{250} 1·35, A_{260}/A_{280} 1·34). The product had chromatographic properties identical with those of authentic material and it migrated at the same rate on electrophoresis at pH 9·4, 9·1, and 1·85.

Fractions 51—85 yielded 5-aminoimidazole-4-carboxamide, identified by comparison with an authentic sample in the systems (A), (C), (E), and (F), and by electrophoresis at three pH values.

9-α-D-*Ribofuranosylhypoxanthine* (' α-*Inosine* ').—5-Amino-1-α-D-ribofuranosylimidazole-4-carboxamide (5·8 mg), acetic anhydride (0·5 ml), and 98% formic acid (0·5 ml) were heated at 100 °C for 2 h. The cooled mixture was evaporated to a syrup which was then heated at 100 °C for 30 min with 0·3N-potassium hydrogen carbonate (1 ml). Potassium ions were removed by adding small amounts of Zeokarb 225 resin (H^+) until the solution reached pH 7. The resin was filtered off and washed with 2N-ammonium hydroxide (1 ml). The filtrate and washings were combined and evaporated to give α-inosine as a syrup which

formed a single u.v.-absorbing spot on chromatography in systems (A), (B), (C), and (E), and had $\lambda_{max.}$ (in acid) 249 nm (A_{250}/A_{260} 1·62, A_{260}/A_{280} 4·09), $\lambda_{max.}$ (neutral solution) 253—254 and 225 nm (A_{250}/A_{260} 1·06, A_{260}/A_{280} 5·39), $\lambda_{max.}$ (in alkali) 253—254 and 225 nm (A_{250}/A_{260} 1·05, A_{260}/A_{280} 5·55).

Attempted Preparation of 5-*Amino*-1-α-D-*ribofuranosylimidazole*-4-*carboxamide from* 1,5-*Bis*-O-*ethoxycarbonyl*-α-D-*ribofuranose* 2,3-*Carbonate*.—The 1,5-bis-*O*-ethoxycarbonyl-α-D-ribofuranose 2,3-carbonate (5 g) was carried through the sequence of reactions already described for the β-anomer and the product, after treatment with ethyl N-[carbamoyl(cyano)methyl]formimidate and hydrolysis with 0·5N-sodium hydroxide, was chromatographed on AG 1 × 8 (200—400 mesh) resin (HCO_3^-) as before.

No trace of α- or β-nucleoside was detected. The major product had R_F values 0·14 in system (A), 0·31 in system (C), 0·32 in system (E), and 0·40 in system (F); on electrophoresis it migrated 0·8 cm kV^{-1} h^{-1} at pH 9·1 (in presence of borate), and 3·9—4·4 cm kV^{-1} h^{-1} at pH 1·85. These values are similar to those given by a decomposition product formed when the formimidate reacts with ammonia and water.

5-*Amino*-1-α-D-*ribofuranosylimidazole*-4-*carboxamide from* 1,5-*Bis*-O-*methoxycarbonyl*-αβ-D-*ribofuranose* 2,3-*Carbonate*.—The methoxycarbonyl compound was converted into the corresponding glycosyl azide by a procedure similar to that already described for the ethoxycarbonyl compound. Analysis showed the intermediate glycosyl bromide to contain only 80% of the required bromine and the azide prepared from it was only 50% pure.

The syrupy azide (4 g) and a suspension of 5% palladium–charcoal (1·2 g) in ethyl acetate (80 ml) were shaken under hydrogen until no azide was detected by i.r. spectroscopy (3 h). Ethyl *N*-[carbamoyl(cyano)methyl]formimidate (1 g) was added to the suspension immediately, and the mixture was shaken for 10 min and left overnight at room temperature. The catalyst was filtered off and washed with ethyl acetate (10 ml). The filtrate and washings were combined and evaporated to a syrup (4·2 g), a portion (2 g) of which and N-ammonium hydroxide (10 ml) were heated together at 100° for 2 min. The mixture was evaporated to dryness and the residue in water (3 ml) was placed on a column (2·5 × 15 cm) of AG 50W × 8 (200—400 mesh) resin (H^+). Some impurities were removed by washing with water (650 ml) and the products were eluted with N-ammonium hydroxide (400 ml). The ammoniacal eluate was evaporated to a gum which was redissolved in water (5 ml) and re-chromatographed on a column (3·5 × 20 cm) of AG 1 × 8 (200—400 mesh) resin (HCO_3^-); water (35 ml h^{-1}) was used as eluant (7·5 ml fractions). Appropriate fractions containing u.v.-absorbing material were combined and evaporated to dryness at 30°.

Fractions 14—31 gave a gum which solidified on freeze-drying from aqueous solution. The compound (8·4 mg) had $\lambda_{max.}$ (in acid) 246 and 267 nm (ε 11,000), $\lambda_{max.}$ (water) 267—268 nm (ε 12,000), $\lambda_{max.}$ (in alkali) 267—268 nm (ε 12,400); $\lambda_{max.}$ 542 nm in the Bratton–Marshall test. In 21 h it consumed 0·97 mol. equiv. of periodate. T.l.c. in systems (A), (C), (E), and (F) showed it to be identical with the 5-amino-1-α-D-ribofuranosylimidazole-4-carboxamide already prepared.

Fractions 32—45 gave the corresponding β-nucleoside, identified by direct t.l.c. comparison with authentic material in systems (A), (B), and (C).

Fractions 44—77 contained only 5-aminoimidazole-4-carboxamide.

Methyl 5-*Amino*-1-α-D-*ribofuranosylimidazole*-4-*carboxylate*.—Ethyl *N*-[cyano(methoxycarbonyl)methyl]formimidate [2] (0·7 g) was added to a solution of 5-*O*-ethoxycarbonyl-αβ-D-ribofuranosylamine 2,3-carbonate [prepared as already described from 2,3-*O*-carbonyl-5-*O*-ethoxycarbonylribofuranosyl azide (1 g)] in ethyl acetate (15 ml). The mixture was shaken for 2 h and kept at room temperature overnight. Filtration and evaporation left a syrup (1 g), which was heated with 0·5N-sodium hydroxide (20 ml) for 15 min, cooled, and neutralised immediately with Zeokarb 225 resin (H^+). The entire mixture was added to a column (1 × 12 cm) of the same resin. The column was washed with water (100 ml) to remove impurities, and then with N-ammonium hydroxide (500 ml). The ammoniacal eluate was evaporated to dryness at 28°; the residue in water (10 ml) was placed on a column (1 × 60 cm) of AG 1 × 8 (200—400 mesh) resin (HCO_3^-). Products were eluted with water (35 ml h^{-1}; 8·8 ml fractions).

Fractions 32—42 contained an unidentified compound which gave a purple colour in the Bratton–Marshall test.

Fractions 43—64 were combined and evaporated at 25° to a syrup which was dissolved in water (5 ml) and chromatographed on AG 1 × 8 (200—400 mesh) resin (HCO_3^-). The column (1 × 60 cm) was washed with water and the main fraction was freeze-dried to give methyl 5-amino-1-β-D-ribofuranosylimidazole-4-carboxylate (14·3 mg) as a fluffy solid (Found: C, 43·6; H, 5·3; N, 15·5. Calc. for $C_{10}H_{15}N_3O_6$: C, 43·9; H, 5·49; N, 15·4%); $\lambda_{max.}$ (in acid) 245 and 267 nm (ε 11,300); $\lambda_{max.}$ (in water) 267—268 nm (ε 11,600); $\lambda_{max.}$ (in alkali) 268 nm (ε 11,850), in agreement with published data. It gave the same R_F values as an authentic sample in systems (A), (B), (D), (E), and (H). A sample (2·3 mg) was converted into 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide by heating with ammonium hydroxide (*d* 0·880; 1 ml) in a sealed tube at 80° for 16 h. The product had the same R_F values as authentic material in system (A), (C), and (E).

Fractions 65—75 were combined and evaporated to a syrup which was dissolved in water (3 ml) and chromatographed on a column (1 × 60 cm) of the formate resin. The main component eluted with water was methyl 5-aminoimidazole-4-carboxylate, identified by comparison with an authentic sample.

Fractions 76—106 were combined and evaporated at 25° to a syrup which was dissolved in water (5 ml) and chromatographed on a column (1 × 60 cm) of the formate resin. The main u.v.-absorbing component eluted with water (emerging between 255 and 325 ml) was freeze-dried to give *methyl* 5-*amino*-1-α-D-*ribofuranosylimidazole*-4-*carboxylate* (30 mg) as a fluffy solid (Found: C, 43·6; H, 5·6; N, 15·2. $C_{10}H_{15}N_3O_6$ requires C, 43·9; H, 5·5; N, 15·4%). The compound gave a single u.v.-absorbing, Bratton–Marshall positive spot, R_F 0·45 in system (A), 0·74 in system (B), 0·63 in system (C), 0·66 in system (E), and 0·69 in system (H). It had $\lambda_{max.}$ (acid) 245 and 266 nm (ε 11,300); $\lambda_{max.}$ (neutral) 267—268 nm (ε 11,600); $\lambda_{max.}$ (alkali) 267—268 nm, and gave a red colour ($\lambda_{max.}$ 567 nm) in the Bratton–Marshall test. A sample was hydrolysed by heating with 2N-hydrochloric acid at 100° for 30 min. T.l.c. in systems (A), (B), and (E) showed methyl 5-aminoimidazole-4-carboxylate as the only u.v.-absorbing product.

A further sample (4·3 mg) was heated with ammonia (*d* 0·880; 1 ml) in a sealed tube at 80° for 16 h. The

mixture was cooled and evaporated to a gum, $\lambda_{max.}$ (in acid) 245 and 266 nm; $\lambda_{max.}$ (in water) 266 nm; $\lambda_{max.}$ (in alkali) 266 nm; $\lambda_{max.}$ 540 nm in the Bratton–Marshall test. It gave the same R_F values on t.l.c. in systems (A), (B), and (E) as the aforementioned 5-amino-1-α-D-ribofuranosylimidazole-4-carboxamide. The picrate formed from this carboxamide had m.p. 165° (*cf.* m.p. 166° for the picrate of the carboxamide prepared by the direct method).

αβ-D-*Ribofuranosyl Azide*.—2,3-*O*-Carbonyl-5-*O*-ethoxycarbonyl-αβ-D-ribofuranosyl azide (0·5 g) and 0·5N-sodium hydroxide (10 ml) were heated at 100° for 30 min. The solution was cooled and neutralised with Zeokarb 225 resin (H^+). Filtration and evaporation left a syrup, which showed no i.r. peaks at 1805 and 1750 cm^{-1} (cyclic and acyclic carbonates) but a strong peak at 2105 cm^{-1} (azide). The major component had R_F 0·04—0·14 in system (K) and appeared to be identical with β-D-ribofuranosyl azide prepared by a published procedure.[14]

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[14] J. Baddiley, J. G. Buchanan, R. Hodges, and J. F. Prescott, *J. Chem. Soc.*, 1957, 4769.