Purines, Pyrimidines, and Imidazoles. Part 51.[1] New Syntheses of Some 5-Alkyl- and 5-Dialkyl-aminoimidazoles. 3-Alkylimidazolium Nucleosides and 3-Alkylpurines

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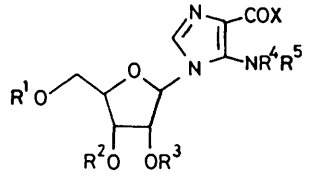
Ethyl 3-methyl-5-amino-1-(2,3-*O*-isopropylidene-α- and -β-D-ribofuranosyl)imidazolium-4-carboxylate iodides, the corresponding benzyl-β-D-ribofuranosyl ester, and benzyl 3-methyl-5-amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazolium-4-carboxylate iodide have been prepared by methylation of the corresponding nucleosides with methyl iodide. Their reactions with acids and bases have been examined. Ethyl 4(5)-methylamino- and dimethylamino-imidazole-5(4)-carboxylates have been prepared in a sequence of reactions from ethyl cyanoacetate and the former compound was converted into 3-methylhypoxanthine by heating with formamidine acetate and into 3-methylguanine by a sequence of reactions. The methylamino-derivative undergoes a Dimroth rearrangement when heated with aqueous ammonia, to produce 1-methyl-5-aminoimidazole-4-carboxamide.

As part of a wider research project we have been interested in the preparation of substances with structures similar to those of certain aminoimidazole nucleotides, namely the carboxylic acid (1a) and the related amide



(1) a; R^1 = HO—P(O)(OH)—, R^2 = R^3 = R^4 = R^5 = H, X = OH

b; R^1 = HO—P(O)(OH)—, R^2 = R^3 = R^4 = R^5 = H, X = NH_2

c; R^1 = HO—P(O)(OH)—, R^2 = R^3 = H, R^4 = H alkyl, R^5 = H, alkyl

d; R^1 = R^2 = R^3 = Ac, R^4 = R^5 = H, X = OCH_2Ph

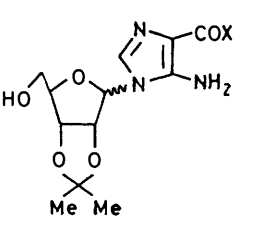
e; R^1 = R^2 = R^3 = R^4 = R^5 = H, X = OCH_2Ph

(1b), which are central intermediates in the purine nucleotide *de novo* biosynthetic pathway. Such compounds are potential inhibitors of the pathway enzymes and hence have possible chemotherapeutic applications in the control of cell growth. One such group of compounds would include 5-*N*-alkyl or 5-*NN*-dialkyl derivatives (1c) and in a series of preliminary experiments we have sought to produce nucleoside precursors of such compounds by direct alkylation of a pre-formed nucleoside.

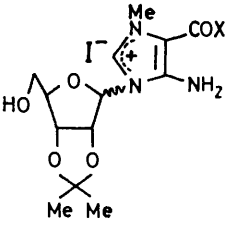
RESULTS AND DISCUSSION

Methylation of the aminoimidazole nucleoside ester (2a)[2] using methyl iodide at 98 °C over 2 h gave a crystalline product, $C_{15}H_{24}IN_3O_6$, which was readily isolated in good yield. The mass spectrum of the compound showed a peak at *m/e* 341 (*M* − 128) and a peak corresponding to a mono-methylated aglycone at *m/e* 169. The compound, however, was readily diazotised and gave a high colour yield in the Bratton–Marshall assay[3] indicating that the 5-amino-group had not been methylated. Accordingly the compound may be regarded as the 3-substituted quaternary salt (3a). Alkylation at position 3 rather than the alternative 1-position is favoured since this is the most basic nitrogen atom in the system.[4] The corresponding α-anomer (3b) was prepared in a similar manner from the α-nucleoside (2b) and its structure also confirmed by elemental analysis, mass spectrometry, and a positive reaction in the Bratton–Marshall assay.[3]

A comparison of the ^1H n.m.r. spectra of the anomeric pair of 3-methylated nucleosides [(3a) and (3b)] revealed that the H-1′ proton for the α-anomer (*i.e. cis* H-1′, H-2′) was downfield of the H-1 proton of the corresponding β-anomer (*i.e. trans* H-1′, H-2′) which is in agreement with the well known empirical rule.[5] The differences in the chemical shifts of the isopropylidene methyl groups of the α- and β-anomers are also in agreement with Imbach's rule.[6]



(2) a; X = OEt, β-anomer
b; X = OEt, α-anomer
c; X = OCH_2Ph, β-anomer

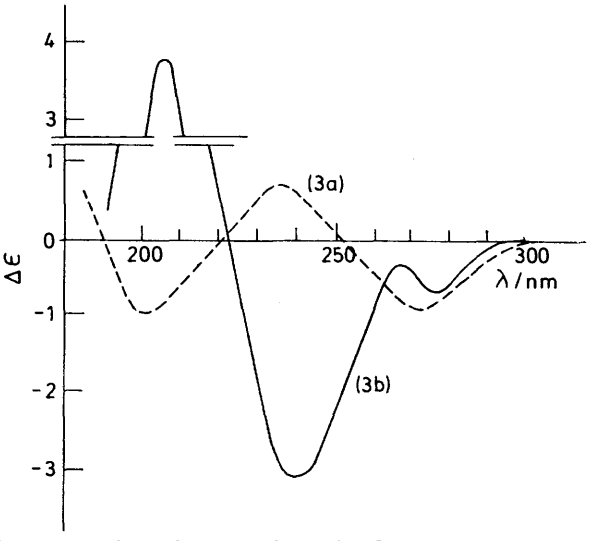


(3) a; X = OEt; β-anomer
b; X = OEt, α-anomer
c; X = OCH_2Ph; β-anomer

The ^1H n.m.r. spectra of the α- and β-quaternised nucleosides also showed considerable deshielding of the proton at C-2, shifting the peaks downfield to δ 9.03 and δ 9.04 respectively. This displacement constitutes a downfield shift of δ 1.83 and δ 1.34, respectively, relative to the peak observed under similar conditions for the

proton at C-2 of each non-quaternised α- and β-nucleoside [(2a) and (2b), respectively]. The peak for the proton at C-2 of each quaternised nucleoside [(3a) and (3b)] showed a sharp singlet in $(CD_3)_2SO$ but addition of D_2O caused rapid H–D exchange at C-2. The deshielding effect may be equated with an electron deficiency in the imidazole ring, caused by quaternisation at N-3.

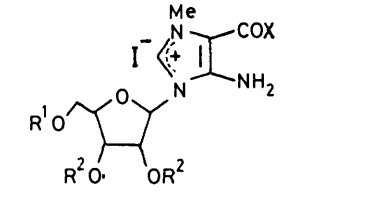
The c.d. curves of the two quaternised ribosides (Figure) were significantly different, confirming their



C.d. spectra of ethyl 3-methyl-5-amino-1-(2,3-*O*-isopropylidene-α- and -β-D-ribofuranosyl)imidazolium-4-carboxylate iodides (3a and b)

anomeric relationship. A comparison of the optical rotations of these compounds showed that they disobeyed Hudson's rules.[7]

The quaternary nucleoside derivatives of CAIR (4a) or AICAR (4b) are also of interest as potential enzyme



(4) a; $R^1 = HO-P(=O)(HO)-$, $R^2 = H$, X = OH

b; $R^1 = HO-P(=O)(HO)-$, $R^2 = H$, $X = NH_2$

c; $R^1 = R^2 = H$, $X = OCH_2Ph$

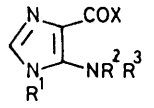
d; $R^1 = R^2 = Ac$, $X = OCH_2Ph$

inhibitors but attempts to hydrolyse the ester (3a) with sodium hydroxide to give the corresponding carboxylic acid resulted in rapid destruction of the imidazole, as evidenced by loss of u.v. absorption, and a negative Bratton–Marshall assay,[3] indicating loss of the diazotisable amino-group. In an attempt to overcome the problems associated with the lability of the ester to alkaline hydrolysis the benzyl ester (3c) was prepared as a crystalline solid by methylation of the isopropylidene ester (2c) [1] with methyl iodide. However, attempts to remove the isopropylidene group from the nucleoside with either aqueous acetic acid, trifluoroacetic acid, or ethylene glycol and toluene-*p*-sulphonic acid led to loss of the sugar, and only small amounts of a substance which might have been the required nucleoside (4c) could be detected. In order to modify the deblocking procedure the triacetyl derivative (1d) was prepared from (1e) and acetic anhydride in pyridine, and was obtained in crystalline form in high yield. Quaternisation of the acetate with methyl iodide proceeded readily with production of the methiodide (4d). However, attempts to deblock this compound with methanolic ammonia or sodium methoxide in methanol led to loss of imidazole absorption. These various reactions serve to emphasise the marked lability of the sugar–aglycone bond to acid treatment, and the quaternised imidazole to treatment with bases. The latter reaction may have value for the preparation of acyclic nucleosides since such compounds are probably produced during the alkaline fission of the quaternised imidazole ring system.

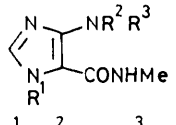
The failure to produce 5-*N*-methylaminoimidazole nucleosides by direct methylation prompted us to turn our attention to the synthesis of 5-*N*-alkyl- or dialkylaminoimidazole-4-carboxylic acid derivatives unsubstituted at N-1, since such compounds offer a potential route to the required corresponding 1-glycosylimidazoles by direct glycosylation procedures. In a recent publication [8] we have shown that it is possible to glycosylate a similar unsubstituted imidazole namely (5a) with 2,3,5-tri-*O*-benzylarabinofuranosyl chloride to produce the 1-substituted nucleoside (6) as a major product.

Syntheses of the required 5-alkylamino- or 5-dialkylamino-imidazole-4-carboxylic acid derivatives unsubstituted at N-1 do not appear to have been recorded. However, some 1-substituted-4(5)-alkylamino- and 4(5)-dialkylamino-imidazole derivatives have been prepared. Thus caffeine has been converted to caffeidine (7a) by alkaline hydrolysis and caffeidine has been reported to be further methylated with methyl iodide to produce the dimethylaminoimidazole (7b) which was also obtained by methylation of the aminoimidazole (7c).[9] Methylation of N^6N^6-diethyl-9-methyladenine with methyl iodide produced 5-diethylamino-3,9-dimethylpurinium iodide (8), which was readily cleaved by sodium hydroxide to give 1-methyl-5-methylaminoimidazole-4-carboxamide (5b).[10] The latter compound has also been prepared from 1-methyl-5-aminoimidazole-4-carboxamide (5c) with formic acetic anhydride and subsequent reduction of the 1-methyl-5-formamidoimidazole-4-carboxamide (5d) formed, with lithium aluminium hydride; the yield was <2%. Ring-opening of 3,9-dialkyl-N^6N^6-diethyladenines was also found to produce

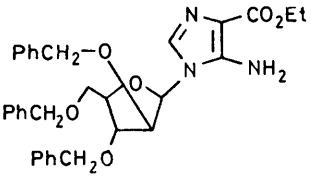
1-substituted-5-*N*-methylformamidoimidazole-4-carboxamides.[11]



(5) a; $R^1 = R^2 = R^3 = H$, X = OEt
b; $R^1 = R^2 = Me$, $R^3 = H$, $X = NH_2$
c; $R^1 = R^2 = R^3 = H$, $X = NH_2$
d; $R^1 = Me$, $R^2 = CHO$, $R^3 = H$, $X = NH_2$
e; $R^1 = H$, $R^2 = R^3 = Me$, X = OEt
f; $R^1 = H$, $R^2 = Me$, $R^3 = CH_2Ph$, X = OEt
g; $R^1 = R^2 = H$, $R^3 = Me$, X = OEt
h; $R^1 = Me$, $R^2 = R^3 = H$, $X = NH_2$



(7) a; $R^1 = R^2 = Me$; $R^3 = H$
b; $R^1 = R^2 = R^3 = Me$
c; $R^1 = Me$; $R^2 = R^3 = H$



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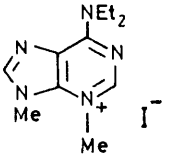
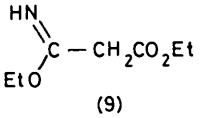


(8)

In order to produce unambiguous syntheses of the desired 5-alkylamino-imidazoles we decided to investigate their preparation from acyclic precursors. The imidate (9) [12] with ethanolic dimethylamine gave the corresponding dimethylamidine (10a), which with an aqueous



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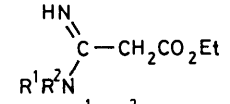
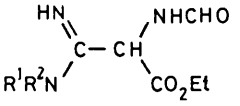
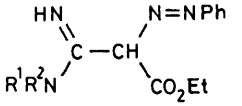


(10) a; $R^1 = R^2 = Me$
b; $R^1 = Me$, $R^2 = H$
c; $R^1 = Me$, $R^2 = CH_2Ph$



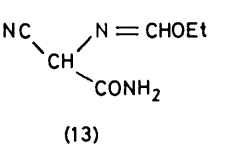


(11) a; $R^1 = R = Me$
b; $R^1 = Me$, $R^2 = CH_2Ph$

solution of benzenediazonium chloride at pH 4 afforded the azo-compound (11a) in 77% yield. Reductive formylation of (11a) with zinc–formic acid led to the formation of ethyl 2-formamido-*N*-dimethylacetamidine-2-carboxylate (12a), which when heated at 130 °C for 0.5 h cyclised to give the required ethyl 4(5)-dimethylaminoimidazole-5(4)-carboxylate (5e) in 58% yield. Similar attempts to prepare ethyl 4(5)-methylaminoimidazole-5 (4)-carboxylate from the imidate (9) and ethanolic methylamine *via* an intermediate ethyl *N*-methylacetamidine-2-carboxylate gave mixtures of several products after further treatment with benzenediazonium chloride.

Presumably (9) with a primary amine can produce *N*-monosubstituted amidines capable of reacting with a further equivalent of primary amine leading ultimately to mixtures of products. This second substitution reaction cannot occur with a secondary amine. Accordingly ethyl *N*-benzyl-*N*-methylacetamidine-2-carboxylate (10c) was prepared from (9) and *N*-benzyl-*N*-methylamine. The azo-compound (11b) was formed in 77% yield from the amidine (10c) and benzenediazonium chloride and was reduced by zinc–formic acid to give ethyl 2-formamido-*N*-benzyl-*N*-methylacetamidine-2-carboxylate (12b), which when heated at 130 °C for 1 h gave ethyl 4(5)-*N*-benzyl-*N*-methylaminoimidazole-5(4)-carboxylate (5f). The product gave an intense red colour reaction with the Pauly reagent.[13] The benzyl group was readily removed by catalytic hydrogenation over palladium–carbon to give the required ethyl 4(5)-methylaminoimidazole-5(4)-carboxylate (5g).

The imidazoles (5e, f, and g) were found to be unstable when boiled in dilute mineral acid or dilute sodium hydroxide solution, and in each case a total loss of imidazole occurred. Reaction of (5g) with conc. aqueous ammonia in a sealed tube at 120 °C for one week produced 1-methyl-5-aminoimidazole-4-carboxamide (5h) and not the expected 4(5)-methylaminoimidazole-5(4)-carboxamide. The imidazole (5g) apparently undergoes a Dimroth rearrangement [14] in which base-catalysed ring-opening occurs to give ethyl 2-formamido-*N*-methylacetamidine-2-carboxylate which may then cyclise, and after conversion of the ester group to an amide, produces (5h) which is stable. The structure assigned to (5h) was confirmed by comparison with an authentic sample prepared by the method of Shaw [15] from the formimidate (13) and methylamine. The



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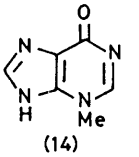

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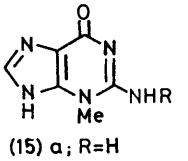
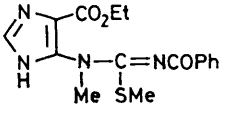


(15) a; R=H
b; R=PhCO



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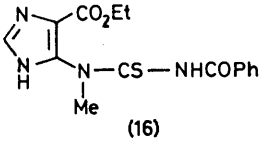

(16)

reaction of (5e) with conc. aqueous ammonia at 120 °C in a sealed tube also led to total loss of imidazole, which may also be explained by ring-opening to give ethyl 2-formamido-*NN*-dimethylacetamidine-2-carboxylate (12a) and further hydrolysis of this to acyclic derivatives in the alkaline conditions.

Reaction of (5g) with excess formamidine acetate afforded 3-methylhypoxanthine (14),[16] identical with an authentic specimen. Similar attempts to prepare 3-methylguanine (15a), by reaction of (5g) with cyanamide or guanidine hydrochloride in various solvents, were unsuccessful. However, a modified method of Yamasaki [17] was employed for the synthesis of 3-methylguanine.[18] Treatment of (5g) with benzoyl isothiocyanate afforded ethyl 4(5)-(3-benzoyl-1-methylthioureido)imidazole-5(4)-carboxylate (16) which with methyl iodide in the presence of sodium hydroxide gave ethyl 4(5)-(3-benzoyl-1,*S*-dimethyl-1-isothioureido)imidazole-5(4)-carboxylate (17) in 52% yield. The *S*-methyl derivative (17) was converted to the *N*-benzoylguanine (15b) by treatment with 2% ethanolic ammonia at 4 °C for one week. Debenzoylation was effected by treatment with hot dilute hydrochloric acid to give, after neutralisation, 3-methylguanine (15a) identical with an authentic sample.[18]

It is interesting to note that the imidazole (17) cyclises under mild basic conditions to give the purine, in contrast to the corresponding non-*N*-methylated imidazole which first produces an acyclic guanidine derivative.

EXPERIMENTAL

Evaporations were carried out with a Büchi rotary evaporator, under water pump vacuum with a flask temperature ⩽40 °C unless otherwise stated. U.v. absorption spectra were measured with a Unicam SP 800 spectrophotometer, i.r. spectra with a Perkin-Elmer 157 spectrophotometer, n.m.r. spectra with a JEOL JNM-MH-100 spectrometer using $SiMe_4$ 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. Circular dichroism spectra were provided by Professor W. Klyne and Dr. P. Scopes of Westfield College, University of London, whom we thank. Silica gel 0.05—0.20 mm, 325—70 mesh from Machery Nagel and Co. was used for column chromatography, and silica gel $60F_{254}$ 0.25 mm precoated glass plates from Merck were used for t.l.c. with (A) $CHCl_3$–MeOH (9 : 1); (B) toluene–ethyl acetate (4 : 1); (C) n-butanol–acetic acid–water (12 : 3 : 5); and (D) n-propanol–ammonia–water (6 : 6 : 1) as development solvent systems. Imidazoles were detected on t.l.c. plates by the Pauly Spray,[13] u.v. absorbance, or the Bratton–Marshall test.[3]

Ethyl 3-Methyl-5-amino-1-(2,3-O-isopropylidene-β-D-ribofuranosyl)imidazolium-4-carboxylate Iodide (3a).—A solution of ethyl 5-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-imidazole-4-carboxylate [2] (1.1 g, 3.3 mmol) and methyl iodide (0.48 g, 3.3 mmol) in acetonitrile (20 ml) was heated in a sealed tube at 98 °C for 2 h. T.l.c. examination (system A) showed one fairly intense Bratton–Marshall active spot at R_F 0.36 with some streaking and absence of the starting material at R_F 0.54. The cooled dark brown solution was evaporated to a gum which, when dissolved in absolute ethanol (2 ml), soon yielded a crystalline solid. The *imidazole-nucleoside quaternary ammonium salt* was recrystallised from absolute ethanol as prisms (0.83 g, 53%), m.p. 150 °C (Found: C, 38.55; H, 5.1; N, 8.75; I, 26.9. $C_{15}H_{24}IN_3O_6$ requires C, 38.4; H, 5.2; N, 8.95; I, 27.05%); $[\alpha]_D^{20}$ −47° (*c* 1.0% in DMSO); $\lambda_{max.}$ (methanol) 270 nm (ε 10 720); the mass spectrum showed a peak at *m/e* 341 (*i.e.* $M^+ - 128$) and an aglycone peak at *m/e* 169; δ[$(CD_3)_2SO$] 6.00 (H-1′, d, $J_{1',2'}$ 4 Hz), 1.36 and 1.57 (CMe_2), 9.04 (H-2, s), and 3.88 (s, Me).

A small amount of the solid (20 mg) in 2M-sodium hydroxide solution (10 ml) was heated at 100 °C for 1 h. The resulting solution showed a faint trace of Bratton–Marshall activity. T.l.c. examination showed a sulphuric acid spray reagent spot at R_F 0.21 and a faint Bratton–Marshall active spot at R_F 0.32.

Ethyl 3-Methyl-5-amino-1-(2,3-O-isopropylidene-α-D-ribofuranosyl)imidazolium-4-carboxylate Iodide (3b).—A solution of ethyl 5-amino-1-(2,3-*O*-isopropylidene-α-D-ribofuranosyl)-imidazole-4-carboxylate [2] (1.1 g, 3.3 mmol) and methyl iodide (0.48 g, 3.3 mmol) in acetonitrile (20 ml) gave as in the foregoing experiment the *quaternary salt*, which crystallised from absolute ethanol as prisms (950 mg, 60%), m.p. 171 °C (Found: C, 38.5; H, 5.2; N, 8.6%; I, 25.6. $C_{15}H_{24}IN_3O_6 \cdot 0.25C_2H_5OH$ requires C, 38.7; H, 5.35; N, 8.75; I, 26.4%); $[\alpha]_D^{20}$ −62° (*c* 1.0% in DMSO); $\lambda_{max.}$ (methanol) 271 nm (ε 10 480); *m/e* 341 ($M^+ - 128$) and 169 (aglycone peak); δ[$(CH_3)_2SO$] 6.25 (H-1′, d, $J_{1',2'}$ 4 Hz), 1.21 and 1.28 (CMe_2), 9.03 (H-2, s), and 3.97 (Me, s).

Benzyl 3-Methyl-5-amino-1-(2,3-O-isopropylidene-β-D-ribofuranosyl)imidazolium-4-carboxylate Iodide (3c).—A solution of benzyl 5-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxylate [1] (1 g) and distilled methyl iodide (0.55 g) in dry acetonitrile (20 ml) gave as in the foregoing experiment the quaternary salt (0.64 g) which crystallised from ethanol as short prisms, m.p. 169 °C (Found: C, 43.25; H, 4.85; N, 7.5; I, 21.1. $C_{20}H_{26}IN_3O_6 \cdot H_2O$ requires C, 43.75; H, 4.75; N, 7.65; I, 23.1%); *m/e* 404 ($M^+ - 128$) 217 (aglycone peak).

Benzyl 5-Amino-1-β-D-ribofuranosylimidazole-4-carboxylate (1e).—Dry toluene-4-sulphonic acid monohydrate (2.5 g) was added to a suspension of benzyl 5-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxylate (5 g) in freshly distilled ethylene glycol (10 ml) and dry methanol (10 ml) and stirred to dissolution. The yellow solution was left at ambient temperature for *ca.* 36 h. The loss of starting material at R_F 0.61 (system A) was monitored by t.l.c. After careful neutralisation with ammonia solution (density 0.88 g cm^{-3}), the solvent was removed and the reaction mixture triturated three times with a mixture of dry acetone (10 ml) and dry ether (50 ml). To the thick residual syrup was added water (10 ml) and the mixture set aside at 4 °C. A crystalline precipitate was collected and washed with ice-cold water (2 × 10 ml). The *benzyl ester riboside* (3.7 g) was recrystallised from water as needles, m.p. 164 °C (Found: C, 54.65; H, 5.7; N, 11.8%. $C_{16}H_{19}N_3O_6$ requires C, 55.0; H, 5.5; N, 12.05%).

Benzyl 5-Amino-1-(2,3,5-O-triacetyl-β-D-ribofuranosyl)-imidazole-4-carboxylate (4d).—To a stirred solution of benzyl 5-amino-1-β-D-ribofuranosylimidazole-4-carboxylate (2 g) in dry pyridine (15 ml) maintained at 0 °C was added acetic anhydride (8.76 g) in dry pyridine (10 ml) over 45 min. The temperature was raised to 10 ± 5 °C and maintained for 6 h, after which all the starting material had reacted. The solution was evaporated to dryness. Evaporation was repeated with toluene (2 × 10 ml) and water (3 × 15 ml) to give a white solid, m.p. 130—132 °C; the *nucleoside* (2.5 g) recrystallised from ethanol as needles, m.p. 130—132 °C (Found: C, 55.3; H, 5.3; N, 8.85. $C_{22}H_{25}N_3O_9$ requires C, 55.6; H, 5.3; N, 8.85%).

Benzyl 3-*Methyl*-5-*amino*-1-(2,3,5-O-*triacetyl*-β-*ribofuranosyl*)*imidazolium*-4-*carboxylate Iodide* (3d).—A solution of benzyl-5-amino-1-(2,3,5-*O*-triacetyl-β-D-ribofuranosyl)imidazole-4-carboxylate (1 g) and distilled methyl iodide (0.45 g) in dry acetonitrile (20 ml) was heated in a sealed tube at 100 °C for 3 h. T.l.c. examination showed an intense Bratton–Marshall streaking spot at R_F 0.20 and two faint spots at R_F 0.74, which corresponded to the starting material, and at R_F 0.35. The cooled solution was evaporated to a gum which was dissolved in chloroform (2 ml) and applied to a silica gel column (2 × 40 cm). The *triacetyl quaternary ammonium salt* was eluted by 8% methanol in chloroform and evaporated to give a solid foam (516 mg, 43%), homogeneous on t.l.c. (Found: C, 43.85; H, 4.45; N, 6.6; I, 20.65%; *m/e*, 490. $C_{23}H_{28}IN_3O_9 \cdot 0.5H_2O$ requires C, 44.1; H, 4.65; N, 6.7; I, 20.25%; *M* − 128, 490).

2-*Ethoxycarbonyl*-NN-*dimethylacetamidine* (10a).—Ethyl 2-ethoxycarbonylacetimidate hydrochloride [12] (40 g, 0.2M) was dissolved in a 33% solution of dimethylamine in ethanol (100 ml). The warm solution was set aside at room temperature for 1 h, then dry ether (1.0 l) was added to precipitate dimethylamine hydrochloride, which was removed. The solvent was evaporated off and the residual oil dissolved in ether (500 ml) and the solution added slowly with cooling and stirring to a solution of hydrogen chloride (8 g) in ether (50 ml), and a solid soon precipitated. When the addition was complete the mixture was set aside at 4 °C for 1 h, and the *dimethylamidine hydrochloride* (35 g), m.p. 140 °C, was collected, washed with ether, and dried *in vacuo*; ν(C=O) 1 740 cm^{-1}, ν(C=N) 1 678 cm^{-1} (Found: C, 42.8; H, 7.85; N, 14.3; Cl, 18.5%; *m/e*, 158. $C_7H_{14}N_2O_2 \cdot HCl$ requires C, 43.2; H, 7.75; N, 14.4; Cl, 18.2%; *M* − HCl, 158).

2-*Ethoxycarbonyl*-NN-*dimethyl*-2-*phenylazoacetamidine* (11a).—A solution of aniline (40 ml) in 6N-hydrochloric acid (240 ml) was diazotised below 5 °C by the gradual addition of a solution of sodium nitrite (31.8 g) in water (200 ml); 5 min after the addition was complete the excess of nitrous acid was decomposed by the addition of ammonium sulphamate (40 g) in water (150 ml). After a further 5 min the solution was poured into a solution of 2-ethoxycarbonyl-*NN*-dimethylacetamidine hydrochloride (60 g) in water (200 ml) and the pH of the solution adjusted to 4 by the addition of a saturated solution of sodium acetate. A brown oil soon precipitated and the mixture was set aside overnight, then extracted with chloroform (3 × 150 ml) and the combined extracts dried (Na_2SO_4) and evaporated to a brown gum; this was dissolved in a little benzene, the solution diluted with ether to incipient turbidity, and then set aside at 4 °C overnight. The *phenylazo-amidine hydrochloride* (63.4 g) formed yellow needles, m.p. 183 °C, after washing with benzene–ether (1 : 1) (Found: C, 52.0; H, 6.4; N, 18.6%; *m/e*, 262. $C_{13}H_{18}N_4O_2 \cdot HCl$ requires C, 52.25; H, 6.4; N, 18.75%; *M* − HCl, 262).

Ethyl 4(5)-*Dimethylaminoimidazole*-5(4)-*carboxylate* (5e).—The foregoing acetamidine hydrochloride (55 g), was added in portions during 1 h to a well stirred suspension of zinc dust (91 g), in 98% formic acid (500 ml). The azo-compound was rapidly decolourised and the reaction mixture became warm. After the addition was complete the mixture was boiled briefly, filtered while hot, and the residue washed with formic acid. The combined filtrates were evaporated to a gum which was freed of formic acid by repeated co-evaporation with water. The residue was dissolved in water (150 ml) and the solution exposed to a stream of hydrogen sulphide. Zinc sulphide was removed by filtration through a Celite pad and the filtrate evaporated to a gum which was dried by repeated co-evaporation with propanol. The residue was dissolved in a little methanol and treated with methanolic hydrogen chloride until acid. The immediate addition of a large volume of anhydrous ether precipitated a gum from which the supernatant solution was decanted. The gum was heated at 130 °C for 0.5 h, and the resultant hard glass cooled, dissolved in a little warm ethanol, and the solution treated with a large volume of ether to precipitate the *imidazole hydrochloride* as a white solid (23.7 g), m.p. 150 °C, which was collected by filtration, washed with ether, and dried *in vacuo*. A suspension of the hydrochloride (2.2 g) in chloroform (50 ml) was treated with sodium hydrogencarbonate (1.0 in 25 ml water). The chloroform layer was collected and the aqueous layer extracted with chloroform (3 × 25 ml). The combined chloroform extracts were dried (Na_2SO_4), filtered, and evaporated to a gum which was set aside at 0.1 mmHg over phosphorous pentaoxide for 48 h to give the imidazole as a waxy solid, which gave an intense red colour reaction with the Pauly reagent. A methanolic solution of the imidazole with methanolic picric acid gave a crystalline precipitate of the *picrate*, m.p. 165 °C (Found: C, 40.4; H, 3.8; N, 19.85%; *m/e*, 183 and 229. $C_{14}H_{16}N_6O_9$ requires C, 40.8; H, 3.9; N, 20.4%; *M*, 412).

2-*Ethoxycarbonyl*-N-*benzyl*-N-*methylacetamidine* (10c).—The foregoing acetimidate hydrochloride (20 g) was added to *N*-benzyl-*N*-methylamine (24 g) and the mixture stirred to produce a paste to which was added ethanol (150 ml). The mixture was stirred to give a clear solution which was set aside at room temperature for 1 h then solvent was removed by evaporation. A large excess of ether was added to the gum and the precipitate removed. The ice-cooled ether solution was treated with an excess of ethereal hydrogen chloride to give an immediate precipitate. Excess of solvent was removed by decantation and replaced by fresh ether (200 ml). The mixture was set aside at 4 °C for 1 h to give the *imidazole hydrochloride* (19.5 g) as a solid precipitate, m.p. 158 °C; ν(C=O) 1 750 cm^{-1}; ν(C=N) 1 675 cm^{-1} (Found: C, 57.55; H, 6.9; N, 10.2%; *m/e*, 234. $C_{13}H_{18}N_2O_2 \cdot HCl$ requires C, 57.65; H, 7.05; N, 10.35%; *M* − HCl, 270.5).

2-*Ethoxycarbonyl*-2-*phenylazo*-N-*benzyl*-N-*methylacetamidine* (11b).—A solution of aniline (12.9 g, 0.14 mol), in 6N hydrochloric acid (80 ml), was diazotised below 5 °C by the gradual addition of sodium nitrite (10.3 g, 0.15 mol) in water (50 ml); 5 min after the addition was complete a solution of ammonium sulphamate (13 g, 0.08 mol) in water (50 ml) was added. After a further 5 min the solution was poured into a solution of the foregoing benzymethylacetamidine hydrochloride (27.1 g) in water (200 ml) and the pH of the solution adjusted to 4 by the gradual addition of a saturated solution of sodium acetate. An orange solid precipitated; after 5 h this was collected by filtration, washed with ether, dissolved in ethanol and the solution treated with ether to incipient turbidity, and then set aside at 4 °C overnight. The *phenylazo-hydrochloride* (28.7 g) was obtained as yellow needles, m.p. 208 °C (Found: C, 60.55; H, 6.1; N, 14.8%; *m/e* 338. $C_{19}H_{22}N_4O_2 \cdot HCl$ requires C, 60.85; H, 6.2; N, 14.95%; *M* − HCl, 338).

Ethyl 4(5)-N-*Benzyl*-N-*methylaminoimidazole*-5(4)-*carboxylate* (5f).—The foregoing 2-phenylazo-acetamidine hydrochloride (6.0 g) was added portionwise during 1 h to a well

stirred suspension of zinc dust (7.91 g) in formic acid (43 ml). The imidazole (1.22 g) was produced as for compound (5e). It formed needles, m.p. 80 °C (Found: C, 65.0; H, 6.75; N, 16.0%; M^+, 259. $C_{14}H_{17}N_3O_2$ requires C, 64.85; H, 6.6; N, 16.2%; M, 259). The product gave an intense red colour with the Pauly reagent.

Ethyl 4(5)-Methylaminoimidazole-5(4)-carboxylate (5g).—A solution of *N*-benzyl-*N*-methylaminoimidazole-5(4)-carboxylate (0.6 g) in ethyl acetate (20 ml) and acetic acid (10 drops) was shaken with Pd–C (0.1 g, 10%) and hydrogen for 4 h, when t.l.c. indicated that all the starting material had disappeared. The mixture was filtered and the solvent evaporated to a white solid. The *imidazole* (0.35 g) recrystallised from ethyl acetate as needles, m.p. 126 °C (Found: C, 49.95; H, 6.5; N, 24.8%; M^+, 169. $C_7H_{11}N_3O_2$ requires C, 49.7; H, 6.55; N, 24.85%; M, 169). The product gave an intense red colour with the Pauly reagent.

3-Methylhypoxanthine (14).—Ethyl 4(5)-methylaminoimidazole-5(4)-carboxylate (1.69 g) was boiled under reflux in a solution of butanol (40 ml) and ethanol (10 ml). Formamidine acetate portions (1.0 g) were added at 15-min intervals and the reaction followed by t.l.c. A total of 6 g of formamidine acetate was required to remove starting material. The solution was cooled and evaporated to a gum, which was freed from traces of butanol by repeated evaporation with water. The residue was triturated with water (15 ml) and the solid collected by filtration. *3-Methylhypoxanthine monohydrate* (0.9 g) was recrystallised from water as needles, m.p. 320 °C (decomp.) (Found: C, 42.6; H, 4.8; N, 32.6%; M^+, 150. Calc. for $C_6H_6N_4O,H_2O$: C, 42.85; H, 4.8; N, 33.3%; $M - H_2O$, 150).

Ethyl 4(5)-(3-Benzoyl-1-methylthioureido)imidazole-5(4)-carboxylate (16).—To a solution of ethyl 4(5)-methylaminoimidazole-5(4)-carboxylate (1.8 g) in THF (50 ml) and triethylamine (2.2 g) was added benzoyl isothiocyanate (3.6 g). The mixture was heated on a steam-bath for 2 h, then cooled in ice to afford a yellow crystalline solid which was collected. The *imidazole* (0.188 g) was recrystallised from THF a yellow needles, m.p. 224 °C (decomp.) (Found: C, 54.7; H, 4.3; N, 16.7; S, 9.9%; *m/e*, 314. $C_{15}H_{16}N_4O_3S$ requires C, 54.2; H, 4.85; N, 16.85; S, 9.65%; M, 332).

Ethyl 4(5)-(3-Benzoyl-1,S-dimethylisothioureido)imidazole-5(4)-carboxylate (17).—To a solution of ethyl 4(5)-(3-benzoyl-1-methylthioureido)imidazole-5(4)-carboxylate (1.66 g) in methanol (10 ml) was added 0.1N aqueous sodium hydroxide (100 ml) and methyl iodide (1.0 ml). The solution was stirred at room temperature for 2 h, adjusted to pH 7 with acetic acid, and extracted with chloroform (3 × 25 ml). The combined chloroform extracts were dried (Na_2SO_4), filtered, and evaporated to a colourless solid which was freed of acetic acid by repeated evaporations with propanol and toluene. The *imidazole* (0.9 g) was obtained as a white solid, m.p. 104 °C (Found: C, 55.6; H, 5.3; N, 15.8; S, 9.4%; M^+, 346. $C_{16}H_{18}N_4O_3S$ requires C, 55.5; H, 5.25; N, 16.15; S, 9.25%; M, 346).

3-Methyl-N-benzoylguanine (15b).—A solution of ethyl 4(5)-(3-benzoyl-1,*S*-dimethylisothioureido)imidazole-5(4)-carboxylate (0.5 g) in 2% ethanolic ammonia (20 ml) was set aside at 4 °C for one week. The odour of methyl mercaptan became apparent and a solid slowly precipitated. It was collected, washed with diethyl ether, and dried *in vacuo*. The *guanine* (0.262 g) formed white needles, m.p. 326 °C (decomp.) which retained water (Found: C, 57.2; H, 4.1; N, 25.9; M^+, 268. $C_{13}H_{11}N_5O_2 \cdot 0.25H_2O$ requires C, 57.1; H, 4.15; N, 25.6%; M, 269).

3-Methylguanine (15a).—A solution of *N*-benzoyl-3-methylguanine (0.10 g) in 1N hydrochloric acid (17 ml) was heated under reflux for 2 h to slowly give a solution. The mixture was cooled, adjusted to pH 7 by the addition of 6N sodium hydroxide, and set aside at 4 °C overnight to give a solid precipitate identical (t.l.c. and i.r.) with an authentic [18] sample of 3-methylguanine.

1-Methyl-5-aminoimidazole-4-carboxamide (5h).—A solution of ethyl 4(5)-methylaminoimidazole-5(4)-carboxylate (0.169 g) in ammonia (density 0.88 g cm^{-3}) was heated to 120 °C in a sealed tube for one week, after which time evaporation of the solvent afforded a gum, which was applied to a column of silica gel and eluted with chloroform–methanol (9 : 1). Evaporation of the major Pauly-active fraction afforded a solid. The imidazole (0.84 g) crystallised from ethanol as needles, m.p. 254 °C, identical (i.r.) to an authentic sample of 1-methyl-5-aminoimidazole-4-carboxamide prepared by the method of Shaw.[15]

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