Purines, Pyrimidines, and Imidazoles. Part 56.[1] Some Aminoimidazole-carboxamidines and Derived Adenines

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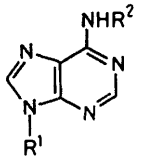
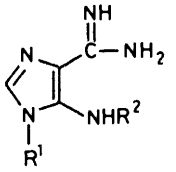
5-Amino-4-cyano-1-cyclohexylimidazole, prepared from aminomalononitrile, triethyl orthoformate, and cyclohexylamine with methanolic hydrogen chloride, produced methyl 5-amino-1-cyclohexylimidazole-4-carboximidate, which with ammonia gave 5-amino-1-cyclohexylimidazole-4-carboxamidine, formylation of which gave 9-cyclohexyl-N^6-formyladenine, which was also prepared by formylation of 9-cyclohexyladenine. 9-(2,3-Dihydroxypropyl)-N^6-formyladenine was produced in a similar series of reactions and the reaction of 5-aminoimidazole-4-carboxamidine with formic acetic anhydride has also been shown to produce not, as previously thought, the formylamidine, but N^6-formyladenine which could also be obtained by formylation of adenine, whereas adenosine was not *N*-formylated under the same conditions.

THE compound 9-β-D-arabinofuranosyladenine (1a) (Ara-A) is a valuable anti-tumour drug [2] but its activity *in vivo* is reduced by its rapid deamination with the enzyme adenosine deaminase. In recent years there have been several successful attempts to limit the effect of the enzyme by using the drug in the presence of an enzyme inhibitor such as Pentastatin (2′-deoxycoformycin) [3] or by using a precursor (prodrug) of the compound, such as the 2′-fluoro-2′-deoxy-derivative [4] which slowly produces Ara-A *in vivo* by slow hydrolysis. We have been interested in examining the preparation of aminoimidazole precursors of adenines which might similarly cyclise slowly to the purine in aqueous conditions. In particular we have sought to produce an *N*-formyl derivative of 5-aminoimidazole-4-carboxamidine of type (2). The synthesis of the *N*-formylamidine (2a) contaminated with a little adenine has been recorded [5] by formylation of the unsubstituted aminoimidazole-carboxamidine (2b) with formic acetic anhydride, and was reported to very readily cyclise to adenine when warmed with aqueous 0.5% sodium hydrogencarbonate for a short period. This result suggested that it might be possible to make the appropriate formylated arabinosyl amidine (2c) which would be expected to produce Ara-A slowly *in vivo*. In preliminary experiments we have examined the synthesis of some simple analogues of the required nucleoside.

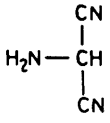
A few 1-substituted-5-aminoimidazole-4-carboxamidines have been prepared previously [6] and poorly characterised, in most cases by ring-opening *N*-alkoxyadenines, but we now report a simpler alternative synthesis.



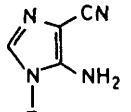
(1) a; R^1 = β-D-arabinofuranosyl, R^2 = H
b; R^1 = C_6H_{11}, R^2 = H
c; R^1 = C_6H_{11}, R^2 = CHO
d; R^1 = $CH_2CH(OH)CH_2OH$, R^2 = CHO
e; R^1 = $CH_2CH(OH)CH_2OH$, R^2 = H
f; R^1 = H, R^2 = CHO



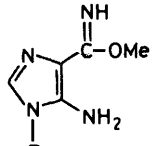
(2) a; R^1 = H, R^2 = CHO
b; R^1 = R^2 = H
c; R^1 = β-D-arabinofuranosyl, R^2 = CHO
d; R^1 = β-D-ribofuranosyl, R^2 = H
e; R^1 = C_6H_{11}, R^2 = H
f; R^1 = $CH_2CH(OH)CH_2OH$, R^2 = H



(3)



(4) a; R = C_6H_{11}
b; R = $CH_2CH(OH)CH_2OH$
c; R = $CH_2CH(OAc)CH_2OAc$

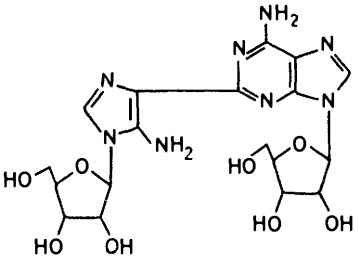


(5) a; R = C_6H_{11}
b; R = β-D-ribofuranosyl
c; R = $CH_2CH(OH)CH_2OH$

RESULTS AND DISCUSSION

Aminomalononitrile (3), prepared by a much simpler route than hitherto recorded,[7] and in better yield, from malononitrile, was treated with triethyl orthoformate during 10 min, followed by cyclohexylamine, to produce the 1-cyclohexyl-5-aminoimidazole-4-carbonitrile (4a) as a crystalline solid.

The nitrile with methanolic hydrogen chloride readily produced the carboximidate (5a) hydrochloride in good yield. Robins *et al.* have reported [8] that attempts to convert a similar carboximidate (5b) into the carboxamidine (2d) with ammonia led to the formation of the bis-

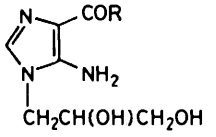


(6)

(imidazole) derivative (6), and these authors were unable to obtain the amidine.

We now find that treatment of the imidate (5a) hydrochloride with methanolic ammonia during 1 h gave an excellent yield of the carboxamidine (2e) hydrochloride, the structure of which was confirmed by elemental analysis, positive reaction in the Bratton–Marshall assay [9] for primary aromatic amines, and its ready conversion into 9-cyclohexyladenine (1b) when heated with ethyl orthoformate. However, when the amidine was formylated with formic acetic anhydride using conditions described for the formation of (2a), a compound was obtained which failed to give a positive Bratton–Marshall assay characteristic of aminoimidazoles, although it had a u.v. absorption spectrum very similar to that described for the postulated (2a). The compound was in fact 9-cyclohexyl-6-*N*-formyladenine (1c), and was produced in a separate synthesis by reaction of the aminonitrile (3) with triethyl orthoformate and ammonia, which gave 9-cyclohexyladenine (1b), and formylation of this with formic acetic anhydride. When the *N*-formyladenine (1c) was heated with aqueous sodium hydrogencarbonate it was smoothly converted into 9-cyclohexyladenine (1b).

In a similar series of experiments the (2,3-dihydroxypropyl)imidazolecarbonitrile (4b) was prepared from aminomalononitrile and triethyl orthoformate, followed by 2,3-dihydroxypropylamine. The carboxamide (7a), a potential product in some of the following reactions, was



(7) a; R = NH_2
b; R = OEt

prepared by reaction of ethyl aminocyanoacetate with triethyl orthoformate, followed by 2,3-dihydroxypropylamine, to give the ester (7b) which with aqueous ammonia at 100 °C gave the carboxamide (7a).

Acetylation of the nitrile (4b) with acetic anhydride–pyridine gave the diacetyl derivative (4c), which still produced a colour in the Bratton–Marshall assay procedure for primary aromatic amines indicating that acylation of the exocyclic amino-group had not occurred, and this is in agreement with results of similar acylation reactions of aminoimidazole derivatives. When a solution of the diacetate, in methanol saturated with hydrogen chloride, was set aside the carboximidate (5c) hydrochloride was rapidly precipitated with concomitant deacylation. The carboximidate with methanolic ammonia gave the carboxamidine (2f) which with formic acetic anhydride produced, after treatment with water, the formylated adenine (1d) which was readily converted into 9-(2,3-dihydroxypropyl)adenine (1e) with sodium hydrogencarbonate. This represents a new route to the latter compound, which has marked anti-viral properties.[10]

It seems clear from these experiments that the compound described [5] as the formylated carboxamidine (2a) is in fact N^6-formyladenine (1f). Confirmation of this was obtained by formylation of adenine with formic acetic anhydride which readily produced N^6-formyladenine (1f), which tended to contain a little adenine, and this product was identical with the material produced by formylation of the carboxamidine (2b).

The ease with which *N*-formyladenine is converted into adenine suggested that the corresponding N^6-formyl-9-arabinofuranosyladenine might be a useful prodrug of arabinofuranosyladenine. The preparation of 5′-*O*-formyladenosine has been recorded [11] by formylation of adenosine with formic acid and this compound is apparently less susceptible to deamination by adenosine deaminase than the non-formylated material. However it seemed reasonable that the N^6-formyl derivative might be more resistant to the deaminase.

In preliminary experiments we examined the formylation of adenosine with formic acetic anhydride. A mixture of formyl derivatives was obtained, presumably *O*-formyl derivatives since the absorption spectrum of the reaction mixture was identical to that of adenosine. The results surprisingly indicate that whereas free adenine is readily *N*-formylated, adenosine is not readily *N*-formylated under the same conditions since even after heating there was no suggestion of *N*-formylation.

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 SP800 spectrophotometer, and mass spectra with an A.E.I. MS-902 spectrometer. Silica gel 60 F 0.25 mm pre-coated aluminium 254 plates (Merck) were used for t.l.c. with (A) $CHCl_3$–MeOH (9 : 1); (B) $CHCl_3$–MeOH (1 : 1); (C) butanol–acetic acid–water (12 : 3 : 5) as development solvent systems. Imidazoles were detected on t.l.c. plates by u.v. absorbance or the Bratton–Marshall test.

Aminomalononitrile (3).—To a cold solution of malononitrile (22 g) in water (500 ml) containing sodium nitrite (20 g) and sodium acetate (136 g) maintained at 0 °C in an ice–salt freezing mixture, a cold solution of concentrated sulphuric acid (15 g) in water (50 ml) was slowly added with stirring. The solution was set aside overnight at 4 °C, then treated slowly with concentrated sulphuric acid (49 g) with stirring and strong cooling. The solution was extracted with ether (4 × 100 ml) and the extract added dropwise to amalgamated aluminium foil (14 g, 0.52 ml) in dry THF (250 ml). The mixture was stirred using an ice-bath to maintain the temperature just below reflux. After completion of the addition the mixture was stirred for an additional 15 min, then water (25 ml) was slowly added and the mixture was refluxed for 1 h, filtered through a Celite pad, and the residues washed with ether (2 × 100 ml) and THF (2 × 100 ml). The filtrate was concentrated to 200 ml and to it was added toluene-*p*-sulphonic acid (40 g, 0.232 mol) suspended in diethyl ether (300 ml). Sufficient ether was added to make a total volume of 1 l. The solution was cooled at 4 °C for 2 h to give a crystalline precipitate, which was collected and washed well with dry ether (2 × 100 ml)

to give the amine toluene-*p*-sulphonate (33 g, 52%), m.p. 173 °C (lit., m.p. 175—176 °C).

5-Amino-1-cyclohexylimidazole-4-carbonitrile (4a).—Dry ammonia was passed through a suspension of aminomalononitrile toluene-*p*-sulphonate (9 g) in dry acetonitrile (400 ml) with stirring and cooling for 20 min. Precipitated ammonium toluene-*p*-sulphonate was removed and the filtrate evaporated to *ca.* 200 ml, then triethyl orthoformate (5.3 g) was added and the solution refluxed for 10 min. The cooled solution with cyclohexylamine (3.5 g) was set aside at room temperature overnight. T.l.c. examination (system A) showed a single spot (R_F 0.70). The solution was evaporated to a solid residue, and the imidazole (2.8 g, 37%) recrystallised from aqueous ethanol as needles, m.p. 188—189 °C (lit.,[12] m.p. 188—190 °C) (Found: C, 63.2; H, 7.5; N, 23.5%; M^+, 190. Calc. for $C_{10}H_{13}N_4$: C, 63.15; H, 7.4; N, 23.45%; *M*, 190).

Methyl 5-Amino-1-cyclohexylimidazole-4-carboximidate Hydrochloride (5a).—A solution of 5-amino-1-cyclohexylimidazole-4-carbonitrile (1 g) in methanol (20 ml) was saturated with dry hydrogen chloride at 0 °C. A voluminous precipitate was observed after *ca.* 15 min. This was collected and washed with dry acetone. The *carboximidate hydrochloride* (0.9 g, 66%) crystallised from water–acetone as needles, m.p. 237—239 °C (Found: C, 47.4; H, 7.0; N, 20.4; Cl, 19.3. $C_{11}H_{19}ClN_4O{\cdot}0.5H_2O$ requires C, 47.7; H, 7.03; N, 20.25; Cl, 19.25%); $\lambda_{max.}$ (H_2O) 292 nm.

5-Amino-1-cyclohexylimidazole-4-carboxamidine Hydrochloride (2e).—Methyl 5-amino-1-cyclohexylimidazole-4-carboximidate hydrochloride (0.5 g) was dissolved in saturated methanolic ammonia (20 ml). The reaction mixture was heated at 100 °C for 1 h. The cooled solution was evaporated to give a solid residue, which was dissolved in methanol (30 ml) and treated with charcoal. The clarified filtrate was evaporated to a small volume, and cooling gave crystals of the *amidine* hydrochloride (0.3 g, 64%) which was recrystallised from methanol as needles, m.p. 302—305 °C (Found: C, 49.2; H, 7.6; N, 28.6. $C_{10}H_{17}N_5{\cdot}$HCl requires C, 49.3; H, 7.45; N, 28.75%); $\lambda_{max.}$ (H_2O) 283 nm.

N⁶-Formyl-9-cyclohexyladenine (1c).—(a) 5-Amino-1-cyclohexylimidazole-4-carboxamidine hydrochloride (0.05 g) and sodium formate (0.019 g) dissolved in formic acid–acetic anhydride (1 : 1) (5 ml) was set aside at room temperature. After 2 h the solution was evaporated to dryness and the residue triturated with water (5 ml) to a solid. The *formyladenine* (0.020 g) crystallised from methanol as needles, m.p. 228 °C (Found: C, 58.7; H, 6.2; N, 28.4. $C_{12}H_{15}N_5O$ requires C, 58.75; H, 6.15; N, 28.55%); $\lambda_{max.}$ (H_2O) 272 nm.

(*b*) A solution of 5-amino-1-cyclohexylimidazole-4-carbonitrile (0.1 g) in triethyl orthoformate (15 ml) was refluxed for 7 h. Excess of triethyl orthoformate was removed by evaporation *in vacuo* and the residue treated with saturated methanolic ammonia (15 ml) then set aside overnight. The clarified solution was evaporated to a solid. *9-Cyclohexyladenine* (0.1 g) crystallised from methanol as needles, m.p. 197—200 °C (Found: C, 60.7; H, 7.1; N, 32.1%; M,$^+$ 217. $C_{11}H_{15}N_5$ requires C, 60.85; H, 6.95; N, 32.25%; *M*, 217).

9-Cyclohexyladenine (0.020 g) was treated with formic acid–acetic anhydride (1 : 1) (2 ml) and set aside at room temperature overnight, then evaporated to a gum which gave a solid when triturated with water (2 ml). The solid was filtered off, washed with ice-cold water and dried. The formyladenine (0.018 g) crystallised from ethanol as needles, m.p. 226—228 °C, $\lambda_{max.}$ (H_2O) 272 nm, identical (t.l.c., i.r.) with material prepared under (*a*).

Ethyl 5-Amino-1-(2,3-dihydroxypropyl)imidazole-4-carboxylate (7b).—A solution of ethyl 2-amino-2-cyanoacetate (2.8 g) and triethyl orthoformate (3.56 g) in acetonitrile (75 ml) was refluxed for 45 min. To the warm solution was added 1-aminopropane-2,3-diol (1.95 g) in ethanol (3 ml). The pale orange solution was left at room temperature overnight to give a solid precipitate. T.l.c. examination (system B) showed a u.v. and Bratton–Marshall active spot (R_F 0.71). The *aminoimidazole* (2.3 g, 47%) was recrystallised from ethyl acetate as needles, m.p. 113 °C (Found: C, 46.9; H, 6.7; N, 18.3%; M^+, 229. $C_9H_{15}N_3O_4$ requires C, 47.15; H, 6.60; N, 18.35%; *M*, 229).

5-Amino-1-(2,3-dihydroxypropyl)imidazole-4-carboxamide (7a).—A solution of ethyl 5-amino-1-(2,3-dihydroxypropyl)-imidazole-4-carboxylate (1 g) in aqueous ammonia solution (*d* 0.88, 15 ml) was heated at 100 °C for 2 days. T.l.c. examination (system B) showed absence of starting material and presence of a u.v. and Bratton–Marshall active spot (R_F 0.5). Evaporation of the solution afforded a gum which was dissolved in hot methanol (30 ml) and the solution treated with decolourising charcoal. The clarified filtrate was evaporated to a small volume, which gave crystals when cooled. The *aminoimidazole* (0.6 g, 70%) was recrystallised from ethanol as needles, m.p. 150 °C (Found: C, 39.0; H, 6.2; N, 26.6%; M^+, 200. $C_7H_{12}N_4O_3{\cdot}0.75H_2O$ requires C, 39.35; H, 6.35; N, 26.2%; *M*, 200).

5-Amino-4-cyano-1-(2,3-dihydroxypropyl)imidazole (4b).—Dry ammonia was passed through a suspension of aminomalononitrile toluene-*p*-sulphonate (5 g) in dry acetonitrile (250 ml) with stirring and cooling for 20 min. Precipitated ammonium toluene-*p*-sulphonate was removed and the filtrate evaporated to *ca.* 150 ml, then triethyl orthoformate (2.9 g) was added and the solution refluxed for 10 min. To the cooled solution was added 1-aminopropane-2,3-diol (1.6 g) in ethanol (2 ml), and the reaction mixture was set aside at room temperature overnight. T.l.c. examination (system A) showed a Bratton–Marshall active spot (R_F 0.15). The solution was evaporated to dryness to a foam (Found: M^+, 182. $C_7H_{10}N_4O_2$ requires *M*, 182). The crude material (2 g) was subsequently used in the following reaction.

5-Amino-4-cyano-1-(2,3-di-O-acetylpropyl)imidazole (4c).—To a cold stirred solution of the foregoing nitrile (2 g) in dry pyridine (10 ml), an excess of acetic anhydride (10 ml) was added dropwise. After *ca.* 3 h, the starting material had disappeared and t.l.c. examination (system A) showed the presence of a new Bratton–Marshall active spot (R_F 0.51). The solution was evaporated (bath temperature <30 °C) to dryness and re-evaporated with water (2 × 10 ml). The resultant gum was dissolved in chloroform (75 ml) and the solution washed with cold sodium hydrogencarbonate solution (30 ml). The dried (Na_2SO_4) chloroform extract was evaporated to a solid residue. The *aminoimidazole diacetate* (1.5 g, 52%) crystallised from ethyl acetate as short needles, m.p. 140 °C (Found: C, 49.4; H, 5.1; N, 20.9%; M^+, 266. $C_{11}H_{14}N_4O_4$ requires C, 49.6; H, 5.3; N, 21.05%; *M*, 266).

Methyl 5-Amino-1-(2,3-dihydroxypropyl)imidazole-4-carboximidate (5c).—A solution of 5-amino-4-cyano-1-(2,3-di-*O*-acetylpropyl)imidazole (1 g) in methanol (20 ml) was saturated with dry hydrogen chloride at 0 °C and allowed to stand at the same temperature. After *ca.* 1 h, a white solid separated, which was collected and washed with dry

ether. The *imidazole hydrochloride* (0.6 g, 64%) crystallised from water–acetone, m.p. 134—136 °C (Found: C, 35.1; H, 5.9; N, 20.7; Cl, 21.4. $C_8H_{14}N_4O_3 \cdot 1.7HCl$ requires C, 34.8; H, 5.7; N, 20.3; Cl, 21.85%); $\lambda_{max.}$ 293 nm (H_2O).

5-Amino-1-(2,3-dihydroxypropyl)imidazole-4-carboxamidine (2f).—Methyl 5-amino-1-(2,3-dihydroxypropyl)imidazole-4-carboximidate hydrochloride (0.5 g) was dissolved in saturated methanolic ammonia (20 ml), and the reaction mixture was heated at 100 °C for 1 h. The pink solution was evaporated to a solid residue which was dissolved in methanol (30 ml) and treated with decolourising charcoal. The clarified filtrate was evaporated to a small volume which gave crystals when cooled. The *amidine hydrochloride* (0.35 g, 74%) recrystallised from methanol as needles, m.p. 193—194 °C (Found: C, 35.8; H, 6.1; N, 30.0; Cl, 14.9. $C_7H_{13}N_5O_2 \cdot HCl$ requires C, 35.65; H, 6.0; N, 29.7; Cl, 15.05%); $\lambda_{max.}$ 281 nm (pH 1); $\lambda_{max.}$ 283 nm (pH 9); $\lambda_{max.}$ 266 nm (pH 13).

N⁶-Formyl-9-(2,3-dihydroxypropyl)adenine (1d).—5-Amino-1-(2,3-dihydroxypropyl)imidazole-4-carboxamidine hydrochloride (0.058 g) and sodium formate (0.016 g) were treated with formic acid–acetic anhydride (1 : 1) (5 ml). After 2 h at room temperature, the solution was evaporated to dryness and the residue triturated with water (5 ml) to give a solid which was collected. The *formyladenine* (0.028 g, 51%) crystallised from methanol as needles, m.p. 178 °C (Found: C, 45.2; H, 4.7; N, 29.2. $C_9H_{11}N_5O_3$ requires C, 45.55; H, 4.65; N, 29.55%); $\lambda_{max.}$ (H_2O) 272 nm.

N⁶-Formyladenine (1f).—Adenine (0.1 g) was heated on a steam-bath with formic acetic anhydride (2 ml) for 3 h and the solution evaporated to a syrup which soon crystallised. *N*-Formyladenine (0.07 g) crystallised from aqueous methanol as needles, m.p. 250 °C (Found: C, 43.5; H, 4.4; N, 42.15. $C_6H_5N_5O$ requires C, 43.65; H, 4.25; N, 42.4%). The product was identical (t.l.c., i.r.) with the product prepared by a published method, but identified as 5-formamidoimidazole-4-carboxamidine. The formyladenine had $\lambda_{max.}$ 272 nm.

Similar attempts to produce N^6-formyladenosine by the formylation of adenosine with formic acetic anhydride gave material with $\lambda_{max.}$ 261 nm, with no evidence for maximum absorption at the higher wavelength of 272 nm typical of *N*-formyladenines.

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REFERENCES

[1] Part 55, G. Mackenzie and G. Shaw, *J. Chem. Res.*, 1980, (*S*), 254; (*M*), 3201.

[2] P. M. Schwartz, C. Shipman, jun., and J. C. Drach, *Antimicrob. Agents Chemother.*, 1976, **10**, 64; F. M. Schabel, jun., M. W. Trader, and W. R. Laster, jun., *Proc. Amer. Assoc. Cancer Res.*, 1976, **17**, abstract 181; G. A. Le Page, L. S. Worth, and A. P. Kimbal, *Cancer Res.*, 1976, **36**, 1481; C. E. Cass and T. H. A-Yeung, *Cancer Res.*, 1976, 1486.

[3] R. P. Agarwal, T. Spector, and R. E. Parks, jun., *Biochem. Pharmacol.*, 1977, **26**, 359.

[4] J. A. Montgomery, S. D. Clayton, and A. T. Shortnacy, *J. Org. Chem.*, 1979, **16**, 157.

[5] E. Shaw, *J. Biol. Chem.*, 1950, **185**, 439.

[6] J. A. Montgomery and J. H. Thomas, *J. Medicin. Chem.*, 1972, **15**, 182; T. Fujii, T. Itaya, T. Saito, and M. Kawanishi, *Chem. Pharm. Bull.* (*Japan*), 1978, **26**, 1929.

[7] J. P. Ferris and L. E. Orgel, *J. Amer. Chem. Soc.*, 1966, **88**, 3829.

[8] P. C. Srivastava, G. A. Ivanovics, R. J. Rousseau, and R. K. Robins, *J. Org. Chem.*, 1975, **40**, 2920.

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

[10] E. De Clercq, J. Descamps, P. De Somer, and A. Holy, *Science*, 1978, **200**, 563.

[11] A. J. Repta, B. J. Rawson, R. D. Shaffer, K. B. Sloan, N. Bodor, and T. Higuchi, *J. Pharm. Sci.*, 1975, **64**, 392.

[12] M. Greenhalgh, G. Shaw, D. V. Wilson, and N. J. Cusack, *J. Chem. Soc.* (*C*), 1969, 2198.