Purines, Pyrimidines, and Imidazoles Part 54.[1] Interconversion of Some Intermediates in the *de novo* Biosynthesis of Purine Nucleotides

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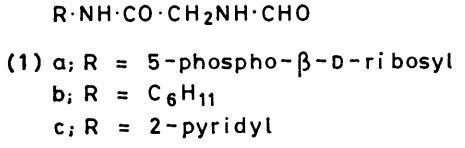
Ethyl and benzyl 5-amino-1-(2-pyridyl)imidazole-4-carboxylates, obtained from ethyl or benzyl α-amino-α-cyanoacetate, respectively, and ethyl formimidate hydrochloride followed by 2-aminopyridine, were converted into 5-amino-1-(2-pyridyl)imidazole-4-carboxylic acid which was decarboxylated *in situ* to 5-amino-1-(2-pyridyl)-imidazole. Reaction of 2-*N*-formylamino-*N*-(2-pyridyl)acetamide with ammonia and ammonium chloride gave 5-aminoimidazole and a similar reaction with the nucleotide 2-*N*-formylglycineamide ribotide similarly gave evidence for aminoimidazole formation. 4-Cyano-5-imidazolone, prepared by reaction of 2-cyano-*N*-formylacetamide with nitrous acid and reduction of the hydroxyimino derivative so produced, with ammonia and ammonium sulphite at 100 °C, gave 5-aminoimidazole-4-carboxamide. Implications of the reactions involved are discussed.

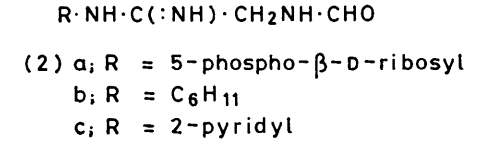
In earlier publications [2,3] we have considered the possibility that the sequence of enzyme controlled reactions leading to the *de novo* biosynthesis of purine nucleotides [4] may have evolved from progenitors of a more primitive nature. This pathway is of fundamental and philosophical importance since it is one of the two *de novo* routes to the monomer precursors of the nucleic acids. We have already demonstrated that most of the forward and reverse reactions in the pathway may be duplicated, almost all at the nucleotide level, by simple chemical means involving variation of pH, action of heat, or a simple coupling reaction with or without an anhydride reagent.[2,3,5]

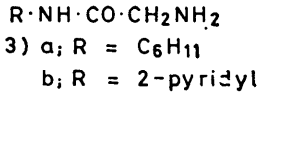
We now record the results of some experiments related to the conversion of the formylglycineamide ribotide (Ia)-(FGAR) into the analogous amidine ribotide (2a)(FGAM). The reverse reaction (FGAM ⟶ FGAR) is readily achieved [6] by mild hydrolysis with aqueous sodium hydrogencarbonate but the forward amination reaction is conceptually more difficult under the simple primitive conditions envisaged.

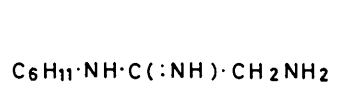
The enzymic synthesis of FGAM is accomplished [7] by the transfer of the amide nitrogen of L-glutamine to FGAR. Ammonium chloride may replace glutamine but recent studies [8] have indicated that ammonia rather than ammonium ions is the reactive intermediate since the pH optimum of the reaction is 8.0 and a sharp drop in activity occurs at slightly lower pH values.

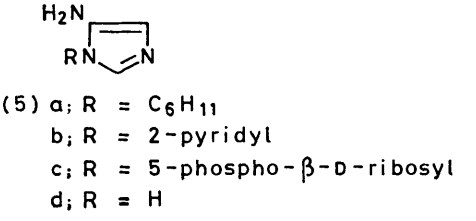
In preliminary experiments, because of the scarcity of the nucleotide FGAR (1a), experiments were carried out

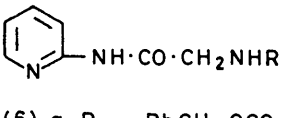


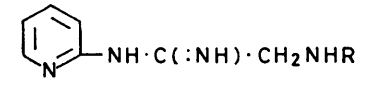


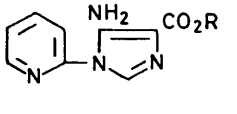


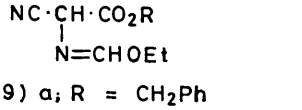












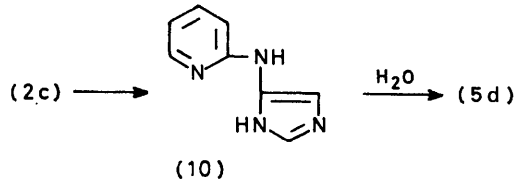
on the model FGAR analogue (1b) which we had earlier prepared [2] from *N*-formylglycine *p*-nitrophenyl ester and cyclohexylamine. The expected product of amination of (1b) would be the FGAM analogue (2b), which we had also synthesised earlier.[2] Specific detection of acyclic compounds of these types poses some practical problems since they do not have useful u.v. absorption and can only be detected by general reagents. The analogue (3a) however produces a purple colour with ninhydrin and the analogous amidine (4) is readily distinguished since it produces a bright yellow colour with this reagent.[2] In addition we have earlier shown [3] that the formylated amidine (2b) may be converted into the aminoimidazole (5a) under mild conditions, and such a compound might be expected to be the end product of any successful amination of the amide (1b). Accordingly, t.l.c. examination for diazotisable amines using the very sensitive Bratton–Marshall [9] assay has also been carried out on reaction mixtures.

The FGAR model (1b) was heated in aqueous buffered ammonia solutions at pH 8, in sealed tubes with ammonium salts at varying concentrations in the presence or absence of aqueous or alcoholic ammonia for times up to a month and temperatures ranging from 20 to 200 °C. In a series of fusion experiments the amide (1b) was heated with ammonium chloride, ammonium acetate, or ammonium dihydrogenphosphate in air or in a stream of ammonia. Several of these reactions were repeated with the addition of transition metals since the amidine (4) is known [10] to form a stable complex with copper which can additionally be distinguished from the amide analogue (3a) by alkaline hydrolysis which produces mainly glycineamide rather than glycine. However none of these experiments gave any evidence direct or indirect that the desired amination reaction was occurring.

The failure of these initial reactions suggested that the model used (1b) was not perhaps representative of the natural nucleotide (FGAR) (1a). In the latter compound the ribosyl group would tend to activate the carbonyl group to nucleophilic attack by an electron depletion process whereas the cyclohexyl group might reasonably be expected to act in the opposite sense. A better model analogue of the nucleotide FGAR should perhaps incorporate an electron withdrawing substituent in the *N*-substituent. It was decided therefore to use the 2-pyridyl analogue of FGAR, namely (1c).

2-Formylamino-*N*-(2-pyridyl)acetamide (1c) was readily prepared from 2-aminopyridine and *N*-formylglycine *p*-nitrophenyl ester. The formyl group could be removed with acid to produce (3b) which was also obtained from 2-aminopyridine with *N*-benzyloxycarbonylglycine and dicyclohexylcarbodi-imide to give the benzyloxycarbonyl derivative (6a) followed by deblocking with hydrogen bromide in acetic acid. Reaction of 2-aminopyridine with ethyl *N*-benzyloxycarbonylaminoacetimidate gave the acetamidine derivative (7a), catalytic hydrogenolysis of which then gave the amidine (7b). The amidine gave a bright yellow colour with the ninhydrin reagent analogous to that given by the cyclohexyl compound (3a) but it was less stable than the latter and after brief treatment with alkali, t.l.c. examination indicated the presence of glycine, glycineamide, and the amide (6b). In order to have samples of as many of the likely reaction products as possible the pyridine analogue (5b) of the natural *AIR* (5c) was prepared by acid catalysed decarboxylation of the amino-acid (8a). The latter compound was obtained by reaction of the formimidate [11,12] (9a) with 2-aminopyridine to produce the ester (8b) followed by catalytic hydrogenolysis to the amino-acid. The acid was also prepared by alkaline hydrolysis of the ethyl ester (8c), prepared from the formimidate [11] (9b) and 2-aminopyridine.

When the FGAR model (1c) was heated with methanolic ammonia and an equivalent of ammonium chloride, t.l.c. examination of the reaction mixture showed the formation of a compound which gave a yellow colour with the ninhydrin reagent but this could not be positively identified as a glycineamidine derivative. However, the solution gave a strong orange colour ($\lambda_{max.}$ 495 nm) in the Bratton–Marshall assay, characteristic of 5-aminoimidazoles. The aminoimidazole (5b) produced a colour with $\lambda_{max.}$ 505 nm but had a different t.l.c. behaviour; 2-aminopyridine and 2,6-diaminopyridine gave no colour in the assay whereas 3-aminopyridine gave a red colour and had different t.l.c. characteristics. The material formed was however identical on t.l.c. examination with 5-aminoimidazole (5d) and the absorption spectrum of the dyestuff produced in the Bratton–Marshall assay was the same for both substances. 5-Aminoimidazole may be prepared by hydrogenation of 5-nitroimidazole [13] or by the acid catalysed decarboxylation of 5-aminoimidazole-4-carboxylic acid; it is very labile especially in an oxygen-containing atmosphere. 2-Acetylaminopyridine when similarly heated with ammonia and ammonium chloride at 100 °C for 30 min gave no trace of diazotisable amine. The formation of 5-aminoimidazole is readily explained by initial formation of the amidine (2c) followed by cyclisation to the pyridinylaminoimidazole (10) and hydrolysis of this compound (Scheme). 2-Pyridone was also detected (t.l.c.).



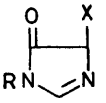
(10)

SCHEME

From the known extinction constants for the coloured dyestuff produced from 5-aminoimidazole (5d) and the Bratton–Marshall reagents, the yield of material obtained was calculated to be *ca.* 0.3%. Finally a similar reaction was carried out with the nucleotide (1a) when the solution was found to give a weak but positive Bratton–Marshall assay corresponding to the analogous

formation of 5-aminoimidazole (5d) or its nucleotide analogue (5c).

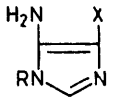
An alternative route to an aminoimidazole from the formylglycineamide derivative (1c) could involve prior formation of an imidazole (11a) and subsequent amination. To test this possibility the oxocarbonitrile (11b) was synthesised by conversion of 2-cyano-*N*-formylacetamide [14] (12) with nitrous acid to the hydroxyimino

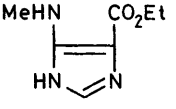


(11) a; R = 2-pyridyl, X = H
b; R = H, X = CN

$NC \cdot CH_2CO \cdot NH \cdot CHO$ (12)

$NC \cdot C(:NOH) \cdot CO \cdot NH \cdot CHO$ (13)





(14) a; R = H, X = $CONH_2$
b; R = H, X = CN
c; R = Me, X = $CONH_2$

(15)

derivative (13), hydrogenation of which using a platinic oxide catalyst or reduction with sodium dithionite gave the imidazolone (11b) directly, presumably, *via* intermediate amine formation. The structure of the imidazolone was confirmed by elemental analysis, mass and i.r. spectra, and u.v. absorption spectra similar to that of the 2-methyl derivative reported earlier.[15] When the imidazolone was heated with aqueous or methanolic ammonia no aminoimidazole could be detected. However under the general conditions of the Bucherer reaction, namely reaction with a solution of ammonium sulphite in aqueous ammonia at 100 °C overnight a u.v.-absorbing compound was produced which gave a strong Bratton–Marshall test (estimated yield 4%) for an aminoimidazole. The compound was identical on t.l.c. examination with 5-aminoimidazole-4-carboxamide (14a). The formation of the amide is not surprising since it has been shown [16] to form by hydrolysis of the aminoimidazolecarbonitrile (14b) with hot dilute alkali.

This latter route to 5-aminoimidazole from the formyl amide (1c) may be less likely mechanistically than that involving intermediate amidine formation since it would require prior formation of the 5-amino-1-pyridylimidazole (5b) which was not detected in the reaction mixture by the very sensitive Bratton–Marshall test. The pyridyl derivative (5b) would in any case have to hydrolyse directly to (5d) which seems unlikely or undergo a Dimroth rearrangement to (10) which is more likely to undergo hydrolysis. We have recently shown [1] that the Dimroth rearrangement can occur in such systems but appears to favour the ring-substituted derivative. Thus amination of ethyl 5-methylaminoimidazole-4-carboxylate (15) led exclusively to the 1-methyl-carboxamide (14c). Although there is no evidence to suggest that the reverse reaction occurs it cannot be completely excluded, not least because 5-alkylaminoimidazoles are much less readily detected than the corresponding 5-amino-derivatives.

EXPERIMENTAL

Evaporations were carried out with a Büchi rotary evaporator under water pump vacuum with a flask temperature ⩽40 °C. I.r. spectra were recorded with a Perkin-Elmer 157 spectrophotometer, mass spectra with an A.E.I. M S902 spectrometer, and u.v. absorption spectra with a Unicam SP 800 spectrophotometer. Cellulose (Whatman CC41) coated (0.25 mm) glass plates were used for t.l.c. with (A) Bu^nOH–AcOH–water (12 : 3 : 5) and (B) Bu^tOH–EtCOMe–water–0.88 ammonia (4 : 3 : 2 : 1) as development solvent systems. Compounds were detected by (a) a Hanovia Chromatolite lamp at 254 nm, (b) by a modified Bratton–Marshall [9] test in which dried chromatograms were immersed in nitrogen dioxide for 1 min then sprayed with 0.5% aqueous ammonium sulphamate followed by 0.1% aqueous α-naphthylethylene diamine (diazotisable amines appeared immediately as orange, red, or purple spots), and (c) by a spray with 0.1% solution of ninhydrin in acetone and development overnight in the dark.

2-*Formylamino*-N-(2-*pyridyl*)*acetamide* (1c).—A solution of *N*-formylglycine *p*-nitrophenyl ester (1.3 g) and 2-aminopyridine (0.55 g) in ethyl acetate (30 ml) was boiled under reflux for 8 h. The cooled solution gave a crystalline precipitate which was collected and washed with ethyl acetate. The *acetamide* (0.7 g) recrystallised from methanol as prisms, m.p. 183—185 °C (Found: C, 54.0; H, 5.3; N, 24.35%; M^+, 179. $C_8H_9N_3O_2$ requires C, 53.65; H, 5.05; N, 23.45%; *M*, 179). Examination by t.l.c. showed the absence of ninhydrin-active material. The compound had R_F 0.70 (system A) and 0.83 (system B); $\lambda_{max.}$ (MeOH) 275, 235, and 205 nm.

2-*Benzyloxycarbonylamino*-N-(2-*pyridyl*)*acetamide* (6a).—A solution of *N*-benzyloxycarbonylglycine (4.18 g), 2-aminopyridine (1.88 g), and dicyclohexylcarbodi-imide (4.12 g) in tetrahydrofuran (100 ml) was left for 2 h at room temperature, treated with a few drops of acetic acid, filtered from precipitated dicyclohexylurea, the precipitate washed with tetrahydrofuran and the combined filtrates evaporated to a crystalline residue. The *benzyloxycarbonyl derivative* (1.3 g) recrystallised from ethanol as needles, m.p. 148—150 °C (Found: C, 63.45; H, 5.4; N, 15.1. $C_{15}H_{15}N_3O_3$ requires C, 63.15; H, 5.30; N, 14.75%).

2-*Amino*-N-(2-*pyridyl*)*acetamide* (6b).— (a) A solution of 2-formylamino-*N*-(2-pyridyl)acetamide (0.1 g) in N-hydrochloric acid (10 ml) was heated at 100 °C for 15 min, then evaporated to dryness, and the residue evaporated twice with water to a solid. 2-*Amino*-N-(2-*pyridyl*)*acetamide dihydrochloride* (31 mg) separated from ethanol as needles, m.p. 230 °C (decomp.) (Found: C, 37.7; H, 5.0; N, 18.55. $C_7H_{11}Cl_2N_3O$ requires C, 37.5; H, 4.95; N, 18.75%). The compound gave a strong purple colour with ninhydrin, and had R_F 0.6 and 0.4 (system A) and 0.94 (system B). The compound ran as two spots in system A because of dissociation of the pyridinium chloride moiety and exchange of

chloride for acetate present in the solvent. If the water in system A was replaced by N-hydrochloric acid, then the compound ran as one spot (R_F 0.4). A trace of glycineamide, R_F 0.3 (system A) and 0.4 (system B), was also present.

(b) The foregoing formylamide (0.1 g) in a solution of dry hydrogen chloride (0.04 g) in dry methanol (1.2 ml) was shaken gently at room temperature when it dissolved and crystals separated after 1 h. The solution was set aside at room temperature overnight, the crystals collected and washed with a small volume of ice-cold methanol. The dihydrochloride (50 mg, 40%) was identical with material prepared under (a).

(c) 2-Benzyloxycarbonylamino-*N*-(2-pyridyl)acetamide (0.28 g) was treated with a 45% w/v solution of hydrogen bromide in acetic acid (5 ml), and the mixture shaken gently at room temperature for 1 h, with protection from moisture. There was an evolution of gas, and the crystalline form of the solid changed. Dry ether (20 ml) was added, the solid precipitate collected, washed several times with ether and dried *in vacuo* over sodium hydroxide. The *amine dihydrobromide* (300 mg) crystallised from methanol as needles, m.p. 288 °C (decomp.) (Found: C, 27.05; H, 3.65; N, 13.65. $C_7H_{11}Br_2N_3O$ requires C, 26.85; H, 3.55; N, 13.4%).

2-Benzyloxycarbonylaminoacetonitrile.—An improved yield was obtained using the following modification of the original procedure.[17] To a solution of aminoacetonitrile hydrogensulphate (77.0 g) in 2M-sodium hydroxide (500 ml) was added sodium hydrogencarbonate (63 g) followed by benzyloxycarbonyl chloride (78 ml) dropwise over 2 h at ≤15 °C with vigorous stirring. After stirring for a further 1 h at room temperature, the mixture was poured into ice–water and the heavy semi-solid product washed several times with water by decantation. A solution of the material in hot propan-2-ol (400 ml) was treated with decolouring charcoal, filtered through Hyflo Supercel, and cooled to 0 °C. An equal volume of light petroleum (b.p. 40—60 °C) was added and crystallisation initiated by the addition of a small piece of solid carbon dioxide. The mixture was diluted with more light petroleum (2 000 ml), and after 30 min the product was collected and washed with light petroleum. 2-Benzyloxcarbonylaminoacetonitrile (47 g, 50%) recrystallised from propan-2-ol–light petroleum ether as needles, m.p. 59—60 °C (lit.,[17] 60 °C).

Ethyl 2-Benzyloxycarbonylaminoacetimidate Hydrochloride.—A solution of the foregoing nitrile (3.0 g) in dry ethanol (16 ml) was cooled to 0 °C, treated with a saturated solution of dry hydrogen chloride in dry ether (7.5 ml) and kept at 0 °C for 2 h when a precipitate was obtained. Dry ether (150 ml) was added, and the precipitate collected, washed with dry ether and dried *in vacuo* over sodium hydroxide. The imidate hydrochloride (4.0 g, 93%) was obtained as a fluffy solid, m.p. 113—114° (decomp.) (lit.,[17] 115 °C).

2-Benzyloxycarbonylamino-N-(2-pyridyl)acetamidine (7a).—A suspension of the foregoing imidate hydrochloride (1.37 g) in dry acetonitrile (25 ml) was treated with 2-aminopyridine (0.47 g) acetonitrile (5 ml). The suspension dissolved rapidly to give a cloudy solution which was quickly clarified by filtration through Hyflo Supercel, then left at room temperature for 3 days. A crystalline deposit was harvested and washed with acetonitrile. The *benzyloxycarbonylamidine hydrochloride* (1.88 g, 67%) recrystallised from acetonitrile as prisms, m.p. 176—178 °C (decomp.) (Found: C, 56.35; H, 5.5; N, 17.8. $C_{15}H_{17}ClN_4O_2$ requires C, 56.15; H, 5.35; N, 17.45%); $\lambda_{max.}$ (MeOH) 278, 238, and 208 nm.

2-Amino-N-(2-pyridyl)acetamidine (7b).—A solution of the foregoing benzyloxycarbonyl derivative (320 mg) in methanol (10 ml) containing hydrogen chloride (37 mg) and a suspension of 10% palladium-charcoal (100 mg), was stirred under hydrogen at 0 °C. After 10 min the filtered solution was evaporated to dryness and the residue collected and washed with ether. The *amidine dihydrochloride* (170 mg) was obtained as a crystalline powder, m.p. 218 °C (decomp.) (Found: C, 37.65; H, 5.2; N, 25.2. $C_7H_{12}Cl_2N_4$ requires C, 37.65; H, 5.4; N, 25.1%).

Ethyl 5-Amino-1-(2-pyridyl)imidazole-4-carboxylate (8c).—A solution of ethyl 2-hydroxyimino-2-cyanoacetate[18] (10.0 g) in dry ether (150 ml) covering aluminium amalgam (from aluminium foil, 2.5 g) was treated with water (5.0 ml) in portions so as to maintain a steady reflux. After 60 min the mixture was filtered, the residue washed with ether, and the combined filtrates dried (Na_2SO_4) and evaporated. The residue in acetonitrile (50 ml) was shaken with ethyl formimidate hydrochloride (4.4 g) for 20 min, the filtered solution treated with 2-aminopyridine (2.2 g), and left overnight at room temperature. The red solution was evaporated and the residue dissolved in dilute hydrochloric acid. The solution was clarified by filtration through Hyflo Supercel, cooled, and made strongly alkaline with 4N-sodium hydroxide, then saturated with sodium chloride. The solution was extracted with ether (5 × 100 ml), the combined extracts dried (K_2CO_3), and evaporated until crystals appeared. After standing overnight, the crystals were collected and washed with hot light petroleum. The *imidazole ester* (0.84 g) crystallised from nitromethane as prisms, m.p. 119—121 °C (Found: C, 56.8; H, 5.25; N, 24.25. $C_{11}H_{12}N_4O_2$ requires C, 56.9; H, 5.2; N, 24.15%). The material gave a strong purple colour with the Bratton–Marshall reagent, $\lambda_{max.}$ 530 nm (ε 21 700).

Benzyl 5-Amino-1-(2-pyridyl)imidazole-4-carboxylate (8b).—A solution of benzyl 2-hydroxyimino-2-cyanoacetate[12] (14.3 g) in ether (200 ml) covering aluminium amalgam (from aluminium foil, 2.5 g) was treated portionwise with water (5 ml) to maintain reflux over 45 min. The mixture was filtered, the residue washed with ether, and the combined extracts dried (Na_2SO_4), and evaporated. The residue in acetonitrile (50 ml) was shaken with ethyl formimidate hydrochloride (4.4 g) for 20 min, the mixture filtered, and the filtrate treated with 2-aminopyridine (2.2 g) and left at room temperature overnight. The solution was evaporated and the residue dissolved in dilute hydrochloric acid, and the solution made strongly alkaline with 4N-sodium hydroxide and extracted with chloroform (5 × 50 ml). The combined extracts were dried (K_2CO_3) then concentrated to *ca.* 20 ml and treated with dry ether (100 ml) which gave a solid precipitate. The *imidazole benzyl ester* (1.4 g) crystallised from ethanol as plates, m.p. 142 °C (Found: C, 65.2; H, 4.9; N, 19.1. $C_{16}H_{14}N_4O_2$ requires C, 65.3; H, 4.80; N, 19.05%).

5-Amino-1-(2-pyridyl)imidazole-4-carboxylic Acid (8a).—(a) A suspension of the foregoing imidazole benzyl ester (300 mg) and 10% palladium–charcoal (50 mg) in methanol (30 ml) was stirred at 0 °C under hydrogen at atmospheric pressure for 4 h, when the uptake of hydrogen had ceased. The solution obtained gave an amorphous precipitate. This was dissolved in a solution of sodium (23 mg) in methanol (10 ml), the mixture filtered and evaporated to a gel which

partially crystallised on standing. The gel was dissolved in a little methanol and the remaining crystals (88 mg) quickly collected by centrifugation, washed with acetone and dry ether, and dried *in vacuo*. The supernatant with dry ether precipitated further material (86 mg), which was collected by centrifugation, washed with acetone and ether and dried *in vacuo*. The *sodium imidazolecarboxylate* (72%) had m.p. 255—256 °C (Found: C, 47.6; H, 3.25; N, 24.65. $C_9H_7N_4NaO_2$ requires C, 47.8; H, 3.1; N, 24.8%). The product gave a strong purple colour with the Bratton–Marshall reagent, $\lambda_{max.}$ 509 (ε 27 000) and 570 nm (inflection). The compound had $\lambda_{max.}$ (0.1N-$KHCO_3$) 262 (ε 11 100) and 225 (14 100) nm. The material was homogeneous on t.l.c. and had R_F 0.63 (system A) and 0.51 (system B).

(b) A solution of the foregoing imidazole ethyl ester (232 mg) in ethanol (1.0 ml) and 1N-sodium hydroxide (1.0 ml) was boiled under reflux for 3 h, when t.l.c. examination showed hydrolysis of the ester to be complete. The solution was evaporated to dryness, and a solution of the residue in water (0.3 ml) maintained at 100 °C whilst ethanol was added to precipitate a gel which soon solidified. The sodium carboxylate (225 mg) was collected, washed with ethanol, acetone, and dry ether, and dried *in vacuo* over phosphorus pentaoxide. It had m.p. 247—249° (decomp.) and was identical with material prepared under (a) above.

5-Amino-1-(2-pyridyl)imidazole (5b).—A solution of the foregoing sodium carboxylate (100 mg) in 0.25M-acetate buffer of pH 5.1 (40 ml) was maintained at 50 °C for 60 min. The solution was cooled to 0 °C and saturated aqueous picric acid (30 ml) added to precipitate a flocculent solid, which was filtered off and washed with a little ice–water. The imidazole *picrate* (35 mg) crystallised from water as yellow needles, m.p. 156—158 °C (decomp.) (Found: C, 43.5; H, 2.95; N, 24.8. $C_{14}H_{11}N_7O_7$ requires C, 43.2; H, 2.85; N, 25.2%). The picrate gave a red colour with the Bratton–Marshall reagent, $\lambda_{max.}$ 505 nm, and had R_F 0.65 (system A) and 0.89 (system B).

Reaction of 2-Formylamino-N-(2-pyridyl)acetamide with Ammonia.—2-Formylamino-*N*-(2-pyridyl)acetamide (17.9 mg), ammonium chloride (10.6 mg), 10% palladium–charcoal (10 mg), and saturated methanolic ammonia solution (0.5 ml) were heated in a sealed tube at 100 °C for 30 min. The contents were lyophilised, and a solution of the residue in water adjusted to pH 5. Assay of a portion of the solution by the Bratton–Marshall procedure gave an orange dyestuff, $\lambda_{max.}$ 495 nm [5-amino-1-(2-pyridyl)imidazole had $\lambda_{max.}$ 505 nm]. On t.l.c. examination the Bratton–Marshall positive material had R_F 0.45 (system A) and 0.66 (system B). Authentic 5-amino-1-(2-pyridyl)imidazole had R_F 0.65 (system A) and 0.89 (system B). The experiment was repeated with 2-acetylaminopyridine (12.8 mg) but no Bratton–Marshall positive material was formed. 3-Aminopyridine had R_F 0.53 (system A) and 0.88 (system B) and gave a red colour with the Bratton–Marshall reagent, $\lambda_{max.}$ 505 nm. The compound which most closely fitted the unknown Bratton–Marshall active material was 4(5)-aminoimidazole which had R_F 0.48 (system A) and 0.67 (system B), and gave an orange dyestuff, $\lambda_{max.}$ 495 nm, in the Bratton–Marshall assay (0.3% yield assuming ε 17 000). A similar reaction was carried out using the barium salt of formylglycineamide ribotide (5 mg). Assay of the resultant solution showed the presence of a Bratton–Marshall positive compound ($\lambda_{max.}$ 495 nm).

2-Cyano-2-hydroxyimino-N-formylacetamide (13).—A solution of 2-cyano-*N*-formylacetamide (22.4 g) and sodium nitrite (14.0 g) in water (250 ml) was stirred at 5—10 °C during the dropwise addition of 2.5N-hydrochloric acid (40 ml). The pH was monitored with a glass electrode at ⩾4. The yellow precipitate was collected. The *hydroxyimino-amide* (16 g, 57%) crystallised from water as needles, m.p. 187—188 °C (decomp.) (Found: C, 34.1; H, 2.3; N, 29.65. C $H_3N_3O_3$ requires C, 34.05; H, 2.15; N, 29.7%); $\lambda_{max.}$ (MeOH) 298 and 230 nm. A solution of the product in dilute sodium hydroxide solution gave an intense violet colour with aqueous iron(II) sulphate.

4(5)-Oxoimidazole-5(4)-carbonitrile (11b).—(a) A solution of the foregoing hydroxyimino-compound (1.41 g) in ethanol (50 ml) covering pre-reduced Adams platinum oxide (0.5 g) was magnetically stirred under hydrogen at room temperature and pressure overnight. An uptake of hydrogen of 430 ml was observed (theory 448 ml). The precipitated product was dissolved by heating, and the filtered solution evaporated to a light yellow solid, which recrystallised from methanol (120 ml), after treatment with decolourising charcoal, as light yellow needles, m.p. 250 °C (decomp). The *imidazolone* (0.65 g, 60%) crystallised from water as needles, m.p. 360 °C (decomp.) (Found: C, 44.2; H, 2.85; N, 38.65%; M^+, 109. $C_4H_3N_3O$ requires C, 44.05; H, 2.75; N, 38.55%; *M*, 109); $\nu_{max.}$ 2 230 cm^{-1} (C≡N stretch); $\lambda_{max.}$ 258, 229 (0.1N-HCl), and 252 nm (0.1N-NaOH).

(b) A stirred suspension of 2-cyano-*N*-formylacetamide in water (25 ml) containing sodium nitrite (14 g) was maintained at 5—10 °C and carefully acidified by dropwise addition of 2.5M-HCl (*ca.* 40 ml) to pH 4. The reaction mixture was allowed to warm to room temperature (*ca.* 17 °C) to give a clear orange-yellow solution. To the stirred solution was added sodium dithionite (76.8 g) in portions. The mixture effervesced strongly, decolourised and the temperature rose to 70 °C. After 1 h the temperature had fallen to 30 °C. The mixture was set aside at 4 °C overnight to give a crystalline precipitate. The oxoimidazole (16.55 g, 76%) was collected. The product was identical with material prepared under (a).

4(5)-Aminoimidazole-5(4)-carboxamide (14a).—Sulphur dioxide (10 g) was absorbed in cooled concentrated ammonia solution (40 ml). A portion of the resulting mixture (0.4 ml) equivalent to sulphur dioxide (1.6mM) and ammonia (6mM), was mixed with the foregoing oxoimidazole (109 mg, 1.0mM) and heated in a sealed tube at 100 °C. The mixture was cooled and lyophilised to a green powder. Examination by t.l.c. showed the presence of starting material accompanied by a u.v. absorbing spot, R_F 0.56 (system A) and 0.60 (system B) which gave a purple colour with the Bratton–Marshall spray reagent, identical with an authentic sample of 4(5)-aminoimidazole-5(4)-carboxamide run at the same time, and dissimilar to an authentic sample of 4(5)-aminoimidazole-5(4)-carbonitrile, which had R_F 0.71 (system A) and 0.72 (system B), and gave a purple-blue colour with Bratton–Marshall spray reagent. Assay of the lyophilised reaction mixture with the Bratton–Marshall reagents produced a purple dyestuff, $\lambda_{max.}$ 508 and 550 nm (inflection) and a yield of 4% of the amide was indicated.

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