570. *Purines, Pyrimidines, and Imidazoles. Part XVIII.*[1] *Synthesis of Some 5-Aminoimidazole-4-carboxylic Acids and 5-Amino-1-β-D-ribofuranosylimidazole-4-carboxylic Acid 5′-O-Phosphate.*

By G. Shaw and D. V. Wilson.

Some 1-substituted 5-aminoimidazole-4-carboxylic acids have been prepared by hydrolysis of corresponding esters, which were obtained by the reaction of ethyl *N*-(alkoxycarbonylcyanomethyl)formimidates with primary amines. The reactions have been adapted to include syntheses of 5-amino-1-β-D-ribofuranosylimidazole-4-carboxylic acid and its 5′-*O*-phosphate.

The aminoimidazolecarboxylic acid ribotide (I) is an important intermediate in the biosynthesis *de novo* of purine nucleotides in avian liver,[2] and has been prepared enzymically in an impure state by carboxylation of the aminoimidazole ribotide (II) with hydrogen carbonate in the presence of a carboxylase.[3]

The sole evidence for the β- and D-configuration of the compound and for the position of the phosphate group was the conversion of the substance into inosinic acid by a series of enzyme-controlled reactions which are assumed to have no effect on the glycosidic bond. The imidazole structure of the nucleotide was, however, confirmed by comparison of its ultraviolet absorption spectra at different pH values in water, the absorption spectrum of a coloured dye produced in the Bratton–Marshall assay [4] for arylamines, and the rate of decarboxylation in acid solution, with those of the corresponding aglycone (IV; R = R′ = H). The last compound has been detected in extracts of *Clostridium cylindrosporum* which had been incubated with xanthine at pH 8·4, and solutions of the compound have also been prepared by hydrogenation of 5-nitroimidazole-4-carboxylic acid with palladium–charcoal in a phosphate buffer at pH 8·5.[5]

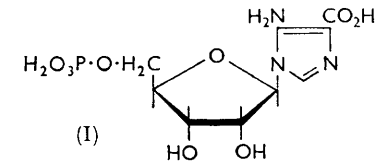
We have synthesised the ribotide (I) by an unambiguous route, an extension of that used for 1-substituted 5-aminoimidazole-4-carboxyamides, including the 1-ribofuranoside, in which the 1-substituent is derived from a primary amine.[6,7] A preliminary account has been recorded.[8]

When an ethereal solution of ethyl α-aminocyanoacetate (prepared by reduction of ethyl α-hydroxyiminocyanoacetate with aluminium amalgam in moist ether) was shaken for a few seconds with ethyl formimidate hydrochloride and water, the imidate (III; R = Et) was formed; it was isolated as an oil by evaporation but for most purposes the ethereal solution may be used directly. This imidate with cyclohexylamine in ether readily gave a good yield of the crystalline aminoimidazole ester (IV; R = C_6H_{11}, R′ = Et), the structure of which was confirmed by the absence of CN bands in the infrared spectrum, the ultraviolet absorption spectrum, the formation of a formyl derivative with formic acid and acetic anhydride, and positive reactions with the Bratton–Marshall and the Pauly reagent. The benzyl ester (IV; R = C_6H_{11}, R′ = CH_2Ph) was also prepared in a similar manner as a possible source, by hydrogenolysis, of the related acid, but subsequent work made this approach unnecessary.

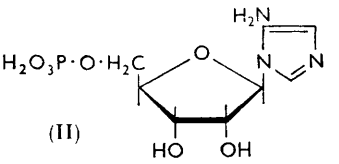
Alkaline hydrolysis of the ethyl ester (IV; R = C_6H_{11}, R′ = Et) gave an excellent yield of the acid (IV; R = C_6H_{11}, R′ = H) as sodium salt which with an acidic resin gave the free acid; this readily lost carbon dioxide, especially in acid solution, to give,

[1] Part XVII, Dewar and Shaw, *J.*, 1962, 583.
[2] Buchanan and Hartman, *Adv. Enzymol.*, 1959, **21**, 199; Hartman and Buchanan, *Ann. Rev. Biochem.*, 1959, **28**, 365; Baddiley and Buchanan, *Quart. Rev.*, 1957, **11**, 329.
[3] Lukens and Buchanan, *J. Biol. Chem.*, 1959, **234**, 1799; *J. Amer. Chem. Soc.*, 1957, **79**, 1511.
[4] Bratton and Marshall, *J. Biol. Chem.*, 1939, **128**, 537.
[5] Rabinowitz, *J. Biol. Chem.*, 1956, **218**, 175.
[6] Shaw, Warrener, Butler, and Ralph, *J.*, 1959, 1648.
[7] Shaw and Warrener, *Proc. Chem. Soc.*, 1958, 193.
[8] Shaw and Wilson, *Proc. Chem. Soc.*, 1961, 381.

presumably, the aminoimidazole (V; R = C_6H_{11}). This loss of carbon dioxide parallels the behaviour of the ribotide [3] (I) and is accompanied by loss of absorption, the final product showing only end-absorption. Dilute solutions of the sodium salt solution showed a similar, although slower, loss of absorption. The structure assigned to the acid was confirmed by the ultraviolet absorption spectrum and coupling, after diazotisation,



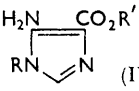
(I)



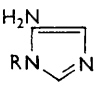
(II)

$EtO \cdot CH{:}N \cdot CH(CN) \cdot CO_2R$

(III)



(IV)



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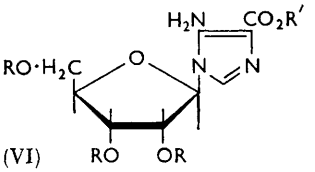
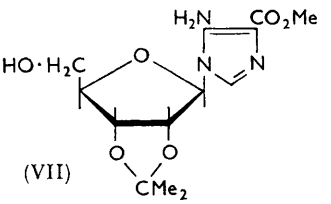


(VI)



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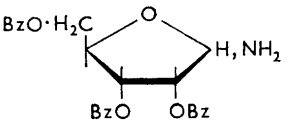

(VIII)

with β-naphthol or α-naphthylethylenediamine. No loss of the primary amino-group during the formation of the acid to give, say, an imidazolone was observed.

Similarly, reaction of the imidate (III; R = Et) with 2,3,5-tri-*O*-benzoylribofuranosylamine [9] (VIII) in ether at room temperature readily gave the crystalline nucleoside tribenzoate (VI; R = Bz, R′ = Et) which was diazotised in acetic acid solution and coupled with β-naphthol to give a red dye. A by-product was probably the aminoimidazole [IV; R = $\cdot CH(CN) \cdot CO_2Et$, R′ = Et] formed by partial hydrolysis of the imidate (III; R = Et) and subsequent condensation of the ethyl α-aminocyanoacetate so formed with the same imidate. An earlier paper [6] recorded a similar product from the analogous imidate $EtO \cdot CH{:}N \cdot CH(CN) \cdot CO \cdot NH_2$.

Attempts to prepare the pure aminoimidazole nucleoside (VI; R = H, R′ = Et) by debenzoylation of the tribenzoate (VI; R = Bz, R′ = Et) with sodium in ethanol were unsuccessful, and the product was contaminated with acidic impurities. Use of alcoholic ammonia was precluded in this reaction because of possible amide formation, but ethanolic triethylamine gave a solid, partially debenzoylated product, and analysis suggested that it was a monobenzoate, presumably the 5′-*O*-benzoate since it gave positive reactions with the periodate–Schiff reagent. Extensions of this reaction to the general nucleoside field are being examined. Hydrolysis of the tribenzoate (VI; R = Bz, R′ = Et) with dilute aqueous-alcoholic sodium hydroxide gave the acid (VI; R = R′ = H), isolated as sparingly soluble calcium and mercuric salts, which when prepared by addition of the metal chlorides to solutions of the sodium salt in water retained chloride ions.

Because of the difficulties encountered in the preparation of the pure nucleoside ester (VI; R = H, R′ = Et) the analogous crystalline methyl ester (VI; R = Bz, R′ = Me) was prepared from the imidate (III; R = Me) and the ribosylamine (VIII). This ester was then readily debenzoylated with sodium in methanol to give the crystalline nucleoside ester (VI; R = H, R′ = Me) which with acetone and toluene-*p*-sulphonic acid gave the crystalline isopropylidene derivative (VII) in excellent yield. This compound was phosphorylated by Tener's method,[10] namely, reaction with 2-cyanoethyl phosphate and

[9] Baddiley, Buchanan, Hodges, and Prescott, *J.*, 1957, 4769.
[10] Tener, *J. Amer. Chem. Soc.*, 1961, **83**, 159.

dicyclohexylcarbodi-imide in pyridine. The product was hydrolysed with aqueous acetic acid (to remove the isopropylidene group), and then with barium hydroxide solution, to give the barium salt of acid (I) which was purified by precipitation of an aqueous solution with ethanol.

The structure of the ribotide was confirmed by analysis, and by a characteristic ultraviolet absorption spectrum in alkaline and acid solution very similar to that of the cyclohexyl derivative (IV; R = C_6H_{11}, R′ = H) and the corresponding nucleoside (VI; R = R′ = H), and in agreement with that recorded for the naturally occurring material [3] (see Table). The ribotide also decomposed in acid solution with loss of absorption, the final solution only showing the end-absorption characteristic of the aminoimidazole (II).[3,11] On the other hand, the absorption spectra for the aglycone [3,5] (IV; R = R′ = H) differed markedly from that of the ribotide (I), especially for acid solutions where the intensities in the 240 and 260 mμ regions are reversed; consequently the use of the aglycone as a model could be misleading. The synthetic ribotide (I) also gave positive tests in the Bratton–Marshall reaction and the absorption spectrum of the coloured derivative produced, while showing a peak at *ca.* 525 mμ similar to that recorded for the natural material,[3] has in addition a characteristic but less intense peak at 565—570 mμ, and the same behaviour is also found with the derivative (IV; R = C_6H_{11}, R′ = H).

Absorption spectra of some 5-aminoimidazole-4-carboxylic acids and esters.[a]

Compound	Solvent	$\lambda_{max.}$ (1) (mμ)	ε	$\lambda_{max.}$ (2) (mμ)	ε	Bratton–Marshall $\lambda_{max.}$ (mμ)
(IV; R = C_6H_{11}, R′ = Et)	EtOH	230—240 [b]	—	270	16,500	—
(VI; R = H, R′ = Me)	H_2O	—	—	269	12,900	525 [h]
,, ,,	0·25N-HCl	245—250 [b]	9800	269	11,700	—
(VII)	EtOH	244 [b]	5400	268	9600	—
(VI; R = Bz, R′ = Me)	EtOH	231	41,700	267—268	15,300	—
(VI; R = Bz, R′ = Et)	EtOH	230—231	43,000	267—268	15,800	—
(IV; R = C_6H_{11}, R′ = H)	H_2O	253	9100	—	—	520, 570 [h]
,, ,,	0·25N-HCl	244	6000	268	9500	
(VI; R = R′ = H)	H_2O	249	8300	—	—	—
,, ,,	pH 8·2 [c]	250	8600	—	—	—
,, ,,	0·25N-HCl	243	5600	267—268	7200	—
(IV; R = R′ = H) [d]	pH 9·0	260	9300	—	—	502 [i]
,, ,,	0·1M-KOH	260	7000	—	—	—
(I) [e]	H_2O	248	8100	—	—	525,
,,	pH 8·2 [c]	250	9700	—	—	565—570 [h]
,,	0·25N-HCl [g]	245	11,000	265	13,600	
(I) [f]	pH 8·2 [c]	249	~10,000	—	—	520
,,	0·25N-HCl	243	—	264	—	

[a] Measurements were made with Unicam S.P. 500 and Optica CF 4 recording spectrophotometers. [b] Inflection. [c] 0·01M-Tris chloride buffer. [d] Results by Rabinowitz [5] who also gives the 260 : 240 mμ absorption ratio at pH 5·0 as 0·88. [e] Synthetic material described in this paper. Naturally occurring material.[3] [g] The compound is unstable in acid solution and the spectrum was measured as soon as possible after preparation of the solution. After 2 hr. at room temperature ε_{265} was 6500 and after 20 hr. had dropped to 1200. [h] A solution of the test substance (*ca.* 1 mg.) in a phosphate buffer (15 ml.; prepared by addition of orthophosphoric acid to 4M-sodium dihydrogen phosphate to give a pH of 1·5) was treated with 0·1% sodium nitrite solution (10 ml.); after 5 min. 0·5% ammonium sulphamate solution (10 ml.) was added, followed 3 min. later by 0·1% *N*-α-naphthylethylenediamine dihydrochloride solution (10 ml.). The solution was made up to 100 ml. with water and the absorption measured after 30 min. All solutions were freshly prepared. [i] Recorded by Rabinowitz [5] and carried out in 5% perchloric acid solution.

The assignment of a β-configuration to the synthetic ribotide and its nucleoside precursors was confirmed by reaction of the methyl ester (VI; R = H, R′ = Me) with aqueous ammonia, to give the corresponding carboxyamide which was formylated, then cyclised with potassium hydrogen carbonate, to produce inosine, by modification of

[11] Levenberg and Buchanan, *J. Biol. Chem.*, 1957, **224**, 1005.

Shaw's procedure.[12] A similar conversion of the carboxyamide into inosine has been mentioned by Greenberg and Spilman [13] and described in detail by Baddiley *et al.*[14]

The apparently exclusive formation of the β-nucleoside ester parallels earlier examples of the production of similar imidazolecarboxyamide [6] and pyrimidine nucleosides [15] in which a *trans*-relation exists between the heterocyclic group and the 2′-hydroxyl group of the sugar. A possible explanation of this effect has been outlined for the pyrimidine derivatives,[15] and the same arguments apply to the imidazole nucleosides where, in these syntheses, linear amidines, *e.g.*, sugar-NH·CH:N·CH(CN)·CO·NH_2 would be immediate precursors of the heterocyclic ring.

Experimental

Ethyl N-(*Cyano*-N-*ethoxycarbonylmethyl*)*formimidate*.—A solution of ethyl α-hydroxyimino-α-cyanoacetate [16] (10 g.) in ether (150 ml.) containing aluminium amalgam (from 2·5 g. of thin aluminium foil: domestic "Polyfoil" is excellent for this purpose and does not require preliminary washing to remove oxides or grease) was treated with water (5 ml.) portionwise during 20 min. so as to maintain steady refluxing; the reaction was complete in 45 min. The mixture was filtered and the solution (shown in a preliminary experiment to contain *ca.* 4·5 g. of ethyl α-amino-α-cyanoacetate) was shaken with ethyl formimidate hydrochloride (6·5 g.) and water (15 ml.) for a short time. Evaporation of the dried ether phase gave *ethyl* N-(*cyano*-N-*ethoxycarbonylmethyl*) *formimidate* (4·5 g.) as a pale yellow oil (Found: N, 15·0. $C_8H_{12}N_2O_3$ requires N, 15·2%).

Ethyl 5-*Amino*-1-*cyclohexylimidazole*-4-*carboxylate*.—To a dried ether solution of the foregoing formimidate (prepared from 10 g. of the hydroxyimino-derivative and used directly) was added cyclohexylamine (3·9 ml.). A bright red solution was obtained which rapidly precipitated a pink gum, and this soon crystallised. *Ethyl 5-amino-1-cyclohexylimidazole-4-carboxylate* (4·1 g.) recrystallised from aqueous ethanol as nacreous plates, m. p. 225—226° (Found: C, 60·9; H, 8·1; N, 17·75. $C_{12}H_{19}N_3O_2$ requires C, 60·8; H, 8·1; N, 17·7%). Extraction of the ethereal filtrate with dilute hydrochloric acid, and basification of the extract with sodium hydroxide solution precipitated a further quantity (1·5 g.) of the imidazole. The ester gave a deep red colour with diazotised sulphanilic acid; it was diazotised and then coupled with β-naphthol in alkaline solution to give a red dye.

Ethyl 1-*Cyclohexyl*-5-*formamidoimidazole*-4-*carboxylate*.—The foregoing imidazole (0·287 g.), formic acid (2·5 ml.), and acetic anhydride (2·5 ml.) were heated together on a water-bath for 20 min. The solution was evaporated *in vacuo* to a gum which crystallised when set aside with water for 3 days. The *formamide* (0·12 g.) separated from ethyl acetate as prisms, m. p. 155° (Found: C, 59·35; H, 7·6; N, 15·35. $C_{13}H_{19}N_3O_3$ requires C, 58·9; H, 7·25; N, 15·85%). A small amount of the amide (20 mg.) in aqueous ammonia (*d* 0·88) (0·2 ml.) was set aside for 2 days. The solution was evaporated to remove the excess of ammonia and acidified with acetic acid to pH 6, giving a crystalline precipitate of the aminoimidazole (12 mg.), m. p. and mixed m. p. 225°.

Benzyl 5-*Amino*-1-*cyclohexylimidazole*-4-*carboxylate*.—A mixture of ethyl cyanoacetate (113 g.), benzyl alcohol (108 g.), and a trace of sodium was heated at 170—200° (bath) and ethanol (44 ml.) was distilled off in 8 hr. Distillation of the residue gave benzyl cyanoacetate (108 g.), b. p. 133—134°/0·5 mm. (Found: C, 67·5; H, 5·1; N, 7·85. Calc. for $C_{10}H_9NO_2$: C, 68·55; H, 5·2; N, 8·0%). Jones and Ramage [17] give b. p. 130°/0·6 mm. and Bachmann and Cronyn [18] give b. p.141°/0·5 mm. A solution of the benzyl ester (80 g.) in acetic acid (133 ml.) and water (53 ml.) was treated with sodium nitrite (40 g.) in water (80 ml.), the temperature being kept at <10°. An oil was precipitated but redissolved to a clear yellow solution when all the nitrite had been added. The solution was kept at 0° for 5 hr., diluted with water (600 ml.), and acidified with 10N-hydrochloric acid (65 ml.), to give a crystalline precipitate.

[12] Shaw, *J. Biol. Chem.*, 1950, **185**, 439.
[13] Greenberg and Spilman, *J. Biol. Chem.*, 1956, **219**, 411.
[14] Baddiley, Buchanan, Hardy, and Stewart, *J.*, 1959, 2893.
[15] Shaw and Warrener, *J.*, 1958, 2294; Shaw, "Synthesis of Pyrimidine Nucleosides," "Current Trends in Heterocyclic Chemistry," Butterworths Scientific Publns., London, 1958.
[16] Conrad and Schulze, *Ber.*, 1909, **42**, 736.
[17] Jones, Ramage, and I.C.I., Ltd., B.P. 596,537/1948.
[18] Bachmann and Cronyn, "Chemistry of Penicillin," Princeton, 1949, p. 849.

Benzyl α-cyano-α-hydroxyiminoacetate (70 g.) separated from benzene–light petroleum as needles, m. p. 115° (Found: C, 59·0; H, 4·1; N, 13·6. Calc. for $C_{10}H_8N_2O_3$: C, 58·8; H, 3·95; N, 13·7%). Jones and Ramage [17] give m. p. 116°. The hydroxyimino-derivative (7·2 g.) in ether (100 ml.) was reduced with aluminium amalgam (from 1·25 g. of aluminium foil) and water (2·5 ml.), and the resulting ether solution was shaken with ethyl formimidate hydrochloride (3 g.) and water (10 ml.) for a short time. The dried ether phase with cyclohexylamine (1·5 ml.) gave a red solution and an oily precipitate. The precipitate with acetone left a solid which was removed. The filtrate, when treated with water, afforded a solid. The *imidazole ester* (0·5 g.) crystallised from aqueous ethanol as plates, m. p. 201—202° (Found: C, 67·95; H, 7·1; N, 14·1. $C_{17}H_{21}N_3O_2$ requires C, 68·2; H, 7·05; N, 14·05%).

5-Amino-1-cyclohexylimidazole-4-carboxylic Acid.—A solution of ethyl 5-amino-1-cyclohexylimidazole-4-carboxylate (0·54 g.) in N-sodium hydroxide (10 ml.) and ethanol (7 ml.) was boiled under reflux for 3 hr., then evaporated *in vacuo* to a crystalline residue. *Sodium 5-amino-1-cyclohexylimidazole-4-carboxylate dihydrate* (0·37 g.) crystallised from 95% ethanol as nacreous plates, m. p. 225° (decomp.) (Found: C, 45·0; H, 6·5; N, 15·55. $C_{10}H_{14}N_3NaO_2,2H_2O$ requires C, 44·95; H, 6·8; N, 15·7%). The sodium salt (0·1 g.) in water (1 ml.) was acidified with "ZeoKarb" 225 (H^+ form), and the filtered solution evaporated *in vacuo* at <35° to give the *acid hemihydrate*, m. p. 122—123° (effervescence) (Found: C, 55·15; H, 7·1; N, 19·1. $C_{10}H_{15}N_3O_2,\frac{1}{2}H_2O$ requires C, 55·05; H, 7·4; N, 19·25%). Solutions of the acid or the sodium salt gave a red colour with diazotised sulphanilic acid, and, after diazotisation, coupled with β-naphthol to form a red dye.

Ethyl 5-Amino-1-β-D-2′,3′,5′-tri-O-benzoylribofuranosylimidazole-4-carboxylate.—(*a*) 2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl azide (3 g.) in ethyl acetate (200 ml.) was hydrogenated over platinic oxide (0·3 g.) for 2 hr. and the mixture treated with an ethereal solution of ethyl *N*-cyano-*N*-(ethoxycarbonylmethyl)formimidate (1·5 g.; prepared as described above), and then evaporated *in vacuo* to a pink gum. This was dissolved in methylene chloride and washed with dilute sodium hydroxide solution, then dried (Na_2SO_4) and evaporated to dryness *in vacuo*. The residue was dissolved in benzene (*ca.* 20 ml.), and the solution rendered barely turbid with light petroleum (b. p. 40—60°). A crystalline precipitate soon separated and was collected after a few hours. The *imidazole* (0·9 g.) recrystallised from ethanol as prisms, m. p. 206—207° (decomp.) (Found: C, 63·95; H, 4·85; N, 7·05. $C_{32}H_{29}N_3O_9$ requires C, 64·1; H, 4·9; N, 7·0%). The compound gave a cherry-red colour with diazotised sulphanilic acid and could be diazotised in acetic acid solution and then coupled with alkaline β-naphthol to give a blood-red dye. (*b*) The last reaction was repeated, but the first formed gum was dissolved in benzene (*ca.* 20 ml.) and seeded, to give after a short time a crystalline precipitate of the imidazole (1 g.), m. p. and mixed m. p. 206—207° (decomp.). The mother-liquors were set aside overnight; a solid had separated; *ethyl 5-amino-1-(α-cyano-α-ethoxycarbonylmethyl)imidazole-4-carboxylate* (0·1 g.) crystallised from ethanol or acetone as needles, m. p. 175° (decomp.) (Found: C, 49·65; H, 5·4; N, 21·0. $C_{11}H_{14}N_4O_4$ requires C, 49·6; H, 5·3; N, 21·05%). The compound gave an orange-red colour with Pauly's reagent, and a red colour on diazotisation and coupling with β-naphthol; it was also soluble in dilute sodium hydroxide solution.

Ethyl 5-Amino-1-β-D-5′-O-benzoylribofuranosylimidazole-4-carboxylate.—The foregoing tribenzoate (0·36 g.) in absolute ethanol (50 ml.) and triethylamine (0·5 ml.) was boiled under reflux for 5 hr. The solution was evaporated *in vacuo* and the residue, which had a strong odour of ethyl benzoate, was rendered turbid with ether and set aside overnight; a solid *ester* (0·14 g.) separated but did not readily recrystallise (Found: C, 50·7; H, 6·55; N, 10·1. $C_{18}H_{21}N_3O_7,2H_2O$ requires C, 50·6; H, 5·9; N, 9·85%).

5-Amino-1-β-D-ribofuranosylimidazole-4-carboxylic Acid.—A solution of the foreging tribenzoate (0·2 g.) in ethanol (3 ml.) and 0·5N-sodium hydroxide (3 ml.) was boiled under reflux for 4 hr., then evaporated *in vacuo*, and the residue was dissolved in water and neutralised with 0·5N-hydrochloric acid (3 ml.). Precipitated benzoic acid was extracted with ether, and the aqueous phase adjusted to pH 7·5 with sodium hydroxide solution, then evaporated to *ca.* 2 ml. *in vacuo*. This solution (1 ml.) with aqueous calcium chloride gave a precipitate of the *calcium salt* of the imidazole (0·08 g.) which was washed with a little water, ethanol, and ether (Found: C, 26·2; H, 4·55; N, 10·85. $C_9H_{12}N_3O_6CaCl,4H_2O$ requires C, 26·5; H, 4·95; N, 10·35%). The remainder of the solution with mercuric chloride precipitated a *mercuric salt* (Found: C, 19·35; H, 2·75; N, 8·05. $C_9H_{12}N_3O_6HgCl,3H_2O$ requires C, 19·65; H, 3·3; N, 7·65%).

Methyl 5-*Amino*-1-(2,3,5-*tri*-O-*benzoyl*-β-D-*ribofuranosyl*)*imidazole*-4-*carboxylate*.—Water (1·7 ml.) and a solution of methyl α-cyano-α-hydroxyiminoacetate [16] (2·94 g.) in ether (20 ml.) were added alternately to aluminium amalgam (from 0·9 g. of aluminium) and ether (20 ml.) at a sufficient rate to maintain steady refluxing; the reaction was complete after 30—40 min. The mixture was filtered and the solid was collected and washed with ether (4 × 25 ml.). The combined filtrate and washings (shown in a preliminary experiment to contain about 1·5 g. of methyl α-amino-α-cyanoacetate) were shaken for 2 min. with ethyl formimidate hydrochloride (2·2 g.) and water (5 ml.). The ether phase was collected and the aqueous phase washed with ether (10 ml.). The combined ether extracts were added to a freshly prepared solution of 2,3,5-tri-*O*-benzoyl-D-ribofuranosylamine (from 3 g. of 2,3,5-tri-*O*-benzoylribofuranosyl azide) in ethyl acetate (200 ml.). The solution was set aside overnight, then evaporated *in vacuo* to a pink gum which was dissolved in dichloromethane (20 ml.) and washed with 0·5N-sodium hydroxide (30 ml.). The aqueous layer was extracted further with dichloromethane (15 ml.) and discarded. The combined and dried (Na_2SO_4) extracts were evaporated *in vacuo* to a gum which rapidly crystallised when treated with warm methanol (5 ml.) and then cooled to 0°. The *tribenzoate* (0·71 g.) recrystallised from methanol as rods, m. p. 223—225° (decomp.) (Found: C, 63·0; H, 4·45; N, 7·2. $C_{31}H_{27}N_3O_9$ requires C, 63·6; H, 4·65; N, 7·2%).

Methyl 5-*Amino*-1-β-D-*ribofuranosylimidazole*-4-*carboxylate*.—A suspension of the foregoing tribenzoate (1·43 g.) in methanol (150 ml.) was treated with sodium (0·06 g.) in methanol (5 ml.) and set aside at room temperature overnight. The excess of sodium was removed with " ZeoKarb 225 " resin (H^+ form; methanol-washed); the resin was filtered off and extracted with 2N-ammonia (4 × 20 ml.). The methanol filtrate and ammonia washings were combined and evaporated *in vacuo* to a gum which was dissolved in water (20 ml.) and washed with ether (15 ml.). The aqueous phase was evaporated *in vacuo* to dryness and twice evaporated with absolute ethanol (10 ml.). The final residue with warm ethyl acetate gave a crystalline powder. The *imidazole riboside hemihydrate* (0·6 g.) formed prisms, m. p. 152°, which did not readily recrystallise (Found: C, 42·8; H, 5·4; N, 14·4. $C_{10}H_{15}N_3O_6,\frac{1}{2}H_2O$ requires C, 42·6; H, 5·7; N, 14·9%).

Methyl 5-*Amino*-1-2′,3′-O-*isopropylidene*-β-D-*ribofuranosylimidazole*-4-*carboxylate*.—The foregoing nucleoside (0·273 g.), toluene-*p*-sulphonic acid (1·9 g.; dried over NaOH and P_2O_5), and dry acetone (30 ml.) were shaken together at room temperature during 2 hr.; this gave a clear solution which was added dropwise with stirring to cold N-sodium hydrogen carbonate (30 ml.). The resulting suspension was evaporated *in vacuo* (bath-temperature $<35°$) to a solid which was twice evaporated with benzene (20 ml.) to remove water. The dry residue was extracted with boiling chloroform (5 × 20 ml.; 10 min. for each extraction) under reflux. Evaporation of the combined chloroform extracts gave the *isopropylideneriboside* (0·282 g.) which crystallised from ethyl acetate as prisms, m. p. 161—162° (Found: C, 49·8; H, 6·2; N, 13·2. $C_{13}H_{19}N_3O_6$ requires C, 49·85; H, 6·1; N, 13·4%).

5-*Amino*-1-β-D-*ribofuranosylimidazole*-4-*carboxylic Acid* 5′-O-*Phosphate*.—(*a*) A solution of 2-cyanoethyl phosphate [10] in pyridine (2 ml. containing 2 mmoles) was added to the foreging isopropylideneriboside (0·16 g.) in pyridine (5 ml.), and the mixture evaporated to a gum which was evaporated with anhydrous pyridine (4 × 5 ml.) and finally dissolved in pyridine (5 ml.). The solution was treated with dicyclohexylcarbodi-imide (1·2 g.) and set aside at room temperature for 2 days. Water (3 ml.) was then added and after 1 hr. the solution was evaporated *in vacuo* to dryness and again evaporated with water (10 ml.). The residue was heated at 100° for 1·5 hr. with 10% acetic acid (20 ml.), evaporated to dryness *in vacuo*, and again evaporated after addition of water (10 ml.). The residue was extracted with cold water (4 × 20 ml.), insoluble material was filtered off, and the combined aqueous extracts were evaporated. The residue was heated at 100° for 3·25 hr. with 0·5N-sodium hydroxide (20 ml.), to give a pale yellow solution and some tar. The mixture was filtered and the filtrate passed through a column of " ZeoKarb 225 " resin (H^+ form), the first eluted acid fraction being collected (water as eluant). The eluate (75 ml.) was immediately adjusted to pH 7·5 with aqueous barium hydroxide; suspended solids were centrifuged off and washed with water and again removed. The combined aqueous solutions were evaporated to 12 ml., then treated with ethanol (30 ml.), to give a gelatinous precipitate which after 2 hr. at 0° was collected by centrifugation and washed with ethanol and ether. The crude barium imidazole phosphate (0·144 g.) was purified by extraction with water (2 × 6 ml.), and the solution clarified and treated with ethanol (2 × 18 ml.). The first fraction (42 mg.) was washed with ethanol and

ether and dried. The *barium salt* of the imidazole nucleotide was finally obtained as a white solid (Found: C, 17·4; H, 3·55; P, 4·45; Ba, 36·2. $C_9H_{11}Ba_{1\frac{1}{2}}N_3O_9P,5H_2O$ requires C, 17·1; H, 3·35; P, 4·9; Ba, 32·55%). (*b*) The reactions described under (*a*) were repeated with the isopropylidenenucleoside (0·282 g.) but after hydrolysis with acetic acid the residue was heated at 100° with 0·5N-barium hydroxide (40 ml.) for 3 hr. The cooled solution was adjusted to pH 8·2 with N-sulphuric acid and the solid centrifuged off. The supernatant fluid was treated with ethanol (180 ml.) and was kept at 0° overnight, giving a precipitate which was collected in the centrifuge and washed with ethanol and ether; the first solid was extracted with cold water (5 × 40 ml.) and the combined extracts were evaporated to 25 ml. and treated with ethanol (100 ml.) to give a precipitate which was collected and washed with ethanol and ether. The two solid fractions, which both had $\lambda_{max.}$ 245 mμ at pH 8·2, were combined and purified by precipitation of an aqueous extract with ethanol. The solvated *barium salt* was obtained as a white powder (31 mg.) (Found: C, 19·05; H, 4·0; N, 6·05; Ba, 30·65. $C_9H_{11}Ba_{1\frac{1}{2}}N_3O_9P,5H_2O,C_2H_6O$ requires C, 19·45; H, 4·0; N, 6·2; Ba, 30·4%). The absorption spectra and the spectra of the coloured substance produced with the Bratton–Marshall reagents were the same for the compounds produced as in (*a*) and (*b*). Each compound also gave the same orange-red colour when diazotised and coupled with an alkaline solution of β-naphthol.

Conversion of Methyl 5-Amino-1-β-D-ribofuranosylimidazole-4-carboxylate into Inosine.—A solution of the nucleoside methyl ester (12 mg.) in aqueous ammonia (3 ml.; *d* 0·88) was heated in a sealed tube at 78—80° for 15 hr., then evaporated *in vacuo* to a clear gum which was freed from the last traces of ammonia by evaporation with water (1 ml.). A portion of the gum in a drop of water with saturated aqueous picric acid gave a crystalline picrate whereas the starting material does not give a picrate under these conditions. The remainder of the gum was heated in 98% formic acid (0·2 ml.) and acetic anhydride (0·1 ml.) at 35° during 2 hr., then evaporated *in vacuo* at room temperature. The residue was warmed with 0·05M-potassium hydrogen carbonate (6 ml.) on a steam-bath for 2 hr. A portion of the final solution was adjusted to pH 4 with acetic acid, then concentrated *in vacuo* and used for paper chromatography on Whatman No. 1 paper (ascending) in a solvent system composed of butanol–acetic acid–water (4 : 1 : 5) (upper layer). Compounds were detected as dark spots under ultraviolet light and as reddish-purple spots with the modified Bratton–Marshall spray of Baddiley *et al.*[14] The solution was found to contain inosine as the sole ultraviolet-absorbing spot, which was also negative with the Bratton–Marshall reagent, some unchanged methyl ester, and some of the corresponding amide. The spot corresponding to inosine, an authentic sample of which was chromatographed at the same time, was cut out and eluted with water, and its absorption spectrum measured. It had $\lambda_{max.}$ 250 and $\lambda_{min.}$ 224 mμ at pH 4 with optical-density ratios at 250 : 260 mμ 1·62 and at 280 : 260 mμ 0·29; $\lambda_{max.}$ 253—254 and $\lambda_{min.}$ 228 mμ at pH 12 with optical-density ratios at 250 : 260 mμ 1·055 and at 280 : 260 mμ 0·22. The figures are in excellent agreement with those published for inosine.[19]

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[19] Chargaff and Davidson, "The Nucleic Acids," Academic Press Inc., New York, 1955.