Purines, Pyrimidines, and Imidazoles. Part XXVI.[1] Active Esters of Some 5-Aminoimidazole-4-carboxylic Acids and their Use in the Preparation of 5-Aminoimidazole-4-carboxyamides and their Nucleoside and Nucleotide Derivatives

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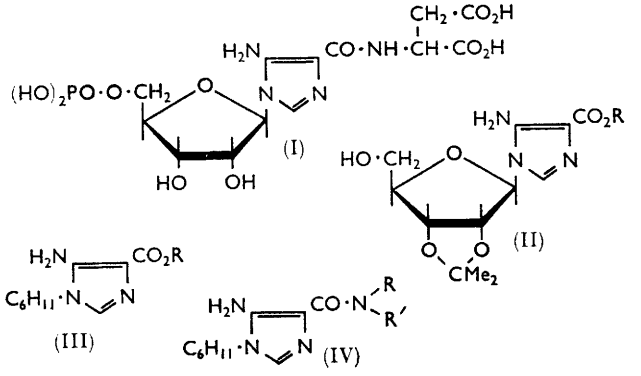
p-Nitrophenyl 5-amino-1-cyclohexylimidazole-4-carboxylate, prepared by reaction of the corresponding acid with *p*-nitrophenol and dicyclohexylcarbodi-imide, with a variety of amines and amino-acid esters gave amino-imidazole carboxyamides. The more reactive 2,4-dinitrophenyl esters of the cyclohexylimidazole and of 5-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxylic acid were prepared from 1-fluoro-2,4-dinitrobenzene and the appropriate sodium salts in dimethylformamide, and readily gave aminoimidazole carboxy-amides with amines or amino-acid esters. The ribosyl ester was also used for the synthesis of 5-amino-1-β-D-ribofuranosylimidazole-4-*N*-methylcarboxyamide 5′-phosphate and related *N*-diethyl and *N*-succino (SAICAR) carboxyamide derivatives by amidation followed by phosphorylation with pyrophosphoryl chloride. The related unsubstituted carboxyamide (AICAR) was also obtained by phosphorylation of the dinitrophenyl ester and reaction of the resulting ester phosphate with ammonia.

PART XIX [2] of this Series recorded the synthesis of the imidazole nucleotide peptide (I) (SAICAR), an important intermediate in the *de novo* biosynthesis of purine nucleotides, by acylating dimethyl L-aspartate with the aminoimidazole isopropylidene riboside carboxylic acid (II; R = H) and dicyclohexylcarbodi-imide in pyridine followed by phosphorylation with 2-cyanoethyl phos-

[1] Part XXV, M. Franks, C. P. Green, G. Shaw, and G. J. Litchfield, *J. Chem. Soc.* (*C*), 1966, 2270.
[2] G. Shaw and D. V. Wilson, *J. Chem. Soc.*, 1963, 1077.

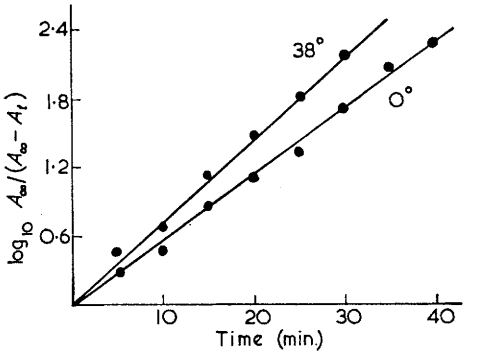
phate and dicyclohexylcarbodi-imide, and removal of protecting ester and isopropylidene groups by alkaline and acid hydrolysis. The intermediate carboxylic acid (II; R = H) is very unstable even in pyridine solution (for results of a preliminary investigation into the kinetics of decarboxylation of aminoimidazole carboxylic acids including the riboside, see ref.[3]), and this adds to the difficulties of the synthesis. In an attempt to overcome these difficulties we have sought alternative methods of synthesis which might be valuable especially for the preparation of SAICAR (I), its nucleoside derivatives, and analogues which we have required as possible metabolite antagonists and enzyme inhibitors in our general investigation of the enzyme systems associated with the whole sequence of purine nucleotide biosynthesis especially that leading to imidazole nucleotides.



In particular, we have examined the use of active esters of aminoimidazole carboxylic acids as an alternative route to related carboxyamides. Earlier, we recorded the preparation of ethyl[4] and cyanomethyl[2] esters (III; R = Et, CH_2·CN), but neither of these compounds was very reactive towards amines, and even (III; R = CH_2·CN) reacted only sluggishly with cyclohexylamine at the boiling point of the amine, to give the corresponding cyclohexylamide (IV; R = H, R′ = C_6H_{11}), whereas with dimethyl L-aspartate at elevated temperatures no evidence for the formation of the carboxyamide was obtained.[2] We have also recorded the amidation of the methyl esters (II; R = Me) and its 5′-phosphate with ammonia, to give the related carboxyamides but only under forcing conditions.[5]

Accordingly, we turned our attention to the preparation of nitroaryl esters, and in initial experiments with the model acid (III; R = H) we obtained the *p*-nitrophenyl ester (III; R = *p*-$C_6H_4NO_2$) by reaction with *p*-nitrophenol and dicyclohexylcarbodi-imide in pyridine, and the 2,4-dinitrophenyl ester [III; R = 2,4-$C_6H_3(NO_2)_2$] from the sodium salt (III; R = Na) and 1-fluoro-2,4-dinitrobenzene in dimethylformamide. The structures of the esters were confirmed by microanalysis, and by their subsequent reactions with a variety of amines and amino-acid esters to give aminoimidazole carboxyamides. In particular, the *p*-nitrophenyl ester with ammonia, methylamine, ethylamine, diethylamine, diethyl L-aspartate, and ethyl aminoacetate gave products which were not generally isolated but characterised by paper and thin-layer chromatography as the corresponding carboxyamides [IV; R = R′ = H; R = H, R′ = Me, Et; R = R′ = Et; R = H, R′ = $CH(CO_2Et)$·CH_2·CO_2Et; R = H, R′ = CH_2·CO_2Et] by their ultraviolet absorption, visible absorption of the dyestuff produced in the Bratton–Marshall assay,[4] and the typical instability of the diazonium salt shown by the peptide derivatives. In the case of the reactions involving the amino-acid esters, the conditions (60—100° for 5 min. in various solvents) were insufficient for complete reaction, and unreacted *p*-nitrophenyl ester was observed during the chromatography. However, a similar series of reactions with the 2,4-dinitrophenyl ester gave the same carboxyamides as the *p*-nitrophenyl ester, but in this case complete conversion of ester into amide occurred with all the amines. The dinitrophenyl ester was therefore particularly valuable, and its mode of formation is advantageous since it avoids the use of the unstable free carboxylic acid.

Both the *p*-nitrophenyl and the 2,4-dinitrophenyl esters had almost identical and characteristic ultraviolet absorption spectra, and gave typical colours after

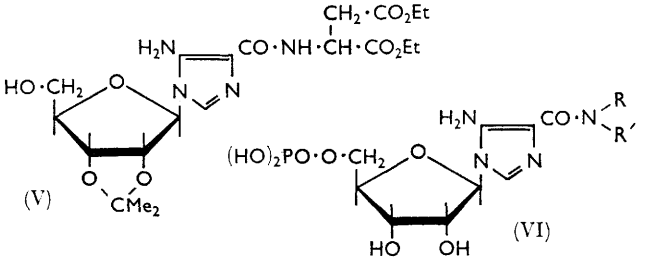


First-order plot of the diazotisation of 2,4-dinitrophenyl 5-amino-1-cyclohexylimidazole-4-carboxylate

diazotisation and coupling with naphthylethylene diamine (see Table 1). However, it is interesting that diazotisation of the 2,4-dinitrophenyl ester was slow and a maximum colour yield was obtained only after diazotisation for about 30—40 minutes at 0° or 20—30 minutes at 38°. In each set of experiments approximately the same colour yield was obtained, suggesting that the intermediate diazonium salt is stable at elevated temperatures (see Figure). The results contrast sharply with those given by the methyl or *p*-nitrophenyl esters, where diazotisation is substantially complete in 1 minute or so at 0°, and suggests that the *o*-nitro substituent is in some way interacting with the amino group of the imidazole ring.

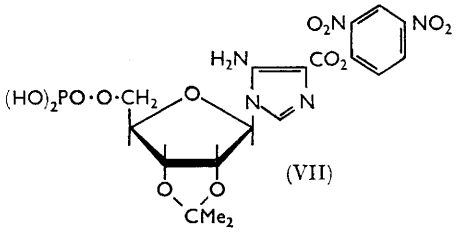
[3] G. J. Litchfield and G. Shaw, *Chem. Comm.*, 1965, 564.
[4] G. Shaw and D. V. Wilson, *J. Chem. Soc.*, 1962, 2937.
[5] G. Shaw, D. V. Wilson, and C. P. Green, *J. Chem. Soc.*, 1964, 2650.

In the same way, the 2,4-dinitrophenyl ester [II; R = 2,4-$C_6H_3(NO_2)_2$] of the isopropylidene riboside was prepared, and its structure was confirmed by elemental analysis and by its analogous ultraviolet absorption spectra and Bratton–Marshall colour reaction to the model, and also the characteristic delay in diazotisation. Condensation of the dinitrophenyl ester with diethyl L-aspartate, and phosphorylation of the product, presumably (V), with pyrophosphoryl chloride gave, after acid hydrolysis to remove the isopropylidene group and alkaline hydrolysis of the ester groups, the nucleotide peptide (I) (SAICAR), identical with an authentic sample. In a similar manner, reaction of the dinitrophenyl ester nucleoside with methylamine and diethylamine gave, after phosphorylation, the corresponding methyl (VI; R = H, R′ = Me) and diethyl (VI; R = R′ = Et) derivatives of 5-amino-1-β-D-ribofuranosylimidazole-4-carboxyamide 5′-phosphate (AICAR). In a related series of reactions condensation



(V)

(VI)



(VII)

of the isopropylidene acid (II; R = H) with pentachlorophenol and dicyclohexylcarbodi-imide gave, presumably, the pentachlorophenyl ester (II; R = C_6Cl_5) which, after phosphorylation with dibenzyl phosphorochloridate followed by condensation with diethyl L-aspartate, gave, after hydrolysis and hydrogenation, a small quantity of the peptide nucleotide (I) (SAICAR).

We have also prepared the phosphorylated dinitrophenyl ester (VII), albeit in low yield, by phosphorylation of the isopropylidene derivative [II; R = 2,4-$C_6H_3(NO_2)_2$] with pyrophosphoryl chloride. It was hoped that the compound would be useful as a route to peptide derivatives by reaction in aqueous solution, but preliminary experiments suggested that it was too readily hydrolysed under these conditions. However, a suspension of the nucleotide in ethanol with ammonia gave the carboxyamide (VI; R = R′ = H) (AICAR).

TABLE 1

Absorption spectra (λ in mμ) of some 5-aminoimidazole-4-carboxylic acid esters and carboxyamides *

						Bratton–Marshall			
Compound	pH	λ_{max} (1)	ε_{max} (1)	λ_{max} (2)	ε_{max} (2)	λ_{max} (1)	ε_{max}(1)	λ_{max} (2)	ε_{max} (2)
(III; R = *p*-$C_6H_4NO_2$)	1	250		285	3300	530	3850	570	7300
[III; R = 2,4-$C_6H_3(NO_2)_2$] ...	1	250		285	3500	530	4000	570	7500
[II; R = 2,4-$C_6H_3(NO_2)_2$] ...	1	250		285	3300	530		570	7350
(II; R = Me)	1	245—250	9800	269	11,700	525			
(VI; R = R′ = H) †	1	245		268		540			
(VI; R = H, R′ = Me)	1	244	8200	268	12,500	538	24,300		
(VI; R = R′ = Et)	1	244		267		540			

* The results given for the nitroaryl esters are observed and do not allow for hydrolysis which occurs under the conditions of the test. † The results refer to material mentioned in this Paper. Authentic AICAR had, at pH 1, λ_{max} (1) 245 mμ (ε 9600) λ_{max} (2) 268 mμ (ε 12,100), and Bratton–Marshall maximum at 540 mμ (ε 26,500).

EXPERIMENTAL

Unless otherwise stated, evaporations were carried out in a Buchi rotary evaporator, under a water-pump vacuum, with a flask temperature of 40° or less.

Paper chromatograms were run on unwashed Whatman No. 1 paper in the following systems: (A) n-butanol–acetic acid–water (12 : 3 : 5); (B) n-butanol saturated with water; (C) n-propanol–0·2N-ammonia (3 : 2); (D) isobutyric acid–ammonia (*d* 0·88)–water (66 : 1 : 33). Spots were detected under an ultraviolet lamp, by the modified Bratton–Marshall spray reagents,[4] and by the ammonium molybdate reagent.[6] Thin-layer chromatograms were run on cellulose (Whatman CC 41) coated glass plates (20 × 20 cm.) in the solvent systems A and B, and spots detected by an ultraviolet lamp or realised by a modified Bratton–Marshall test. In this, after the solvent front had travelled 12 cm., the dried plates were immersed in nitrogen dioxide fumes (from a commercial cylinder), then sprayed with 0·5% ethanolic ammonium sulphamate followed by a spray with a 0·1% ethanolic solution of *N*-1-naphthylethylenediamine dihydrochloride; diazotisable amines appeared as purple spots.

Ion-exchange separations were performed in an apparatus, all Teflon or glass, equipped with a Buchler micropump and an LKB Uvicord 4701A ultraviolet absorptiometer with a flow cell of 3 mm. light path for continuous recording of column eluates at 253·7 mμ or a Vanguard model 1056 double-beam automatic ultraviolet analyser with a continuous recording at a variety of wavelengths between 200 and 400 mμ. All resins used for ion-exchange chromatography were an analytical grade of Dowex from Bio-Rad Laboratories, Richmond, California. Spectra were measured on Perkin-Elmer 137UV or Unicam SP 500 spectrophotometers.

[6] S. Burrows, F. S. M. Grylls, and J. S. Harrison, *Nature*, 1952, **170**, 800.

p-*Nitrophenyl* 5-*Amino*-1-*cyclohexylimidazole*-4-*carboxylate*.—A solution of sodium 5-amino-1-cyclohexylimidazole-4-carboxylate dihydrate [4] (0·5 g.) in 50% aqueous pyridine (10 ml.) was passed down a column (8 × ½ in.) of the pyridine form of ZeoKarb 225 resin, and the column eluted with aqueous pyridine and fractions containing diazotisable amine (30—80 ml.) collected. The solution was evaporated to half volume several times with dry pyridine, the final volume being *ca.* 10 ml. To this dried solution was added dicyclohexylcarbodi-imide (4 g.) followed by *p*-nitrophenol (0·34 g.), and the mixture set aside at room temperature for 3 days. Water (2 ml.) was then added, and after 1 hr. at room temperature the mixture was evaporated and the residue further evaporated with water to remove pyridine. The residue was triturated with N-hydrochloric acid, the mixture filtered, and *p*-nitrophenol removed with ether (6 × 25 ml.). The aqueous acid solution at 0° was adjusted to pH 9 with sodium hydrogen carbonate, to give a fine yellow precipitate which was collected by centrifugation, washed, and dried. The p-*nitrophenyl ester* (0·11 g.) was purified by precipitation of a solution in chloroform with light petroleum (b. p. 40—60°). It was finally obtained as a yellow powder, m. p. 266—270° (decomp.) (Found: C, 58·25; H, 5·5; N, 17·0. $C_{16}H_{18}N_4O_4$ requires C, 58·3; H, 5·45; N, 17·0%).

Reaction of the p-*Nitrophenyl Ester with Ammonia and Amines*.—Solutions of small amounts of the foregoing *p*-nitrophenyl ester in different solvents (including ethanol, ethyl acetate, methyl cyanide, and dimethylformamide) were heated on a water-bath for 5 min. with ammonia, methylamine, ethylamine, diethylamine, diethyl L-aspartate, and ethyl aminoacetate. Evidence of reaction was followed by observing changes in the coloured dyestuff produced in the Bratton–Marshall assay, and reaction products were observed by thin-layer chromatography. In all cases spots corresponding to the corresponding carboxyamides were observed (Table 2), accompanied,

TABLE 2

Thin-layer and paper chromatographic data on some 5-aminoimidazole-4-carboxyamides and *p*-nitrophenyl esters

Compound	Solvent *	R_F †
(III; R = *p*-$C_6H_4NO_2$)	A,B	0·93, 0·87
[II; R = 2,4-$C_6H_3(NO_2)_2$]	A,B	0·87, 0·81
(IV; R = R′ = H)	A,B	0·78, 0·65
(IV; R = H, R′ = Me)	A,B	0·82, 0·68
(IV; R = H, R′ = Et)	A,B	0·8, 0·68
(IV; R = R′ = Et)	A,B	0·82, 0·68
[IV; R = H, R′ = $CH(CO_2Et)\cdot CH_2\cdot CO_2Et$]	A,B	0·76, 0·63
(IV; R = H, R′ = $CH_2\cdot CO_2Et$)	A,B	0·78, 0·65
(VI; R = H, R′ = Me)	A,C,D	0·12, 0·08, 0·51
(VI; R = R′ = Et)	A,C,D	0·12, 0·08, 0·51
(VI; R = R′ = H)	A,C,D	0·12, 0·08, 0·5

* See introduction to Experimental section. † The first eight results refer to thin-layer chromatogram results after a 12 cm. run, and the last three to paper chromatograms.

especially when the amino-acid esters were used, by spots of unreacted *p*-nitrophenyl ester. The aminoimidazole carboxyamides produced had a Bratton–Marshall colour maximum at 540 mμ, except for the peptide derivatives, which had maxima at 550—560 mμ, and the latter compounds both showed the characteristic instability of the diazonium salt observed with SAICAR (I).[2]

2,4-*Dinitrophenyl* 5-*Amino*-1-*cyclohexylimidazole*-4-*carboxylate*.—Sodium 5-amino-1-cyclohexylimidazole-4-carboxylate dihydrate (0·3 g.) in the minimum quantity of dimethylformamide with 1-fluoro-2,4-dinitrobenzene in dimethylformamide (1 ml.) gave a deep red solution which was set aside at room temperature for 30 min. then added to water (50 ml.), to give a heavy yellow precipitate. This was collected by centrifugation, washed with water, dried, and purified by precipitation from chloroform solution with light petroleum (b. p. 40—60°). The 2,4-*dinitrophenyl ester* (0·32 g.) was obtained as a yellow microcrystalline powder, m. p. (decomp.) from 280° (Found: C, 51·3; H, 4·6; N, 18·7. $C_{16}H_{17}N_5O_6$ requires C, 51·3; H, 4·55; N, 18·7%).

Diazotisation of the 2,4-*Dinitrophenyl Ester*.—A stock solution of the foregoing ester, freshly prepared by dissolving 4·68 mg. in N-hydrochloric acid (1 ml.) and diluting the solution to 25 ml., was stored at 0°. Aliquots (1 ml.) of the solution were allowed to diazotise for various lengths of time by addition of 1% sodium nitrite solution (0·1 ml.). At the end of the period, ammonium sulphamate (0·4 ml. of 0·5% solution) was added, and 30 sec. later 0·1% *N*-1-naphthylethylenediamine dihydrochloride (0·2 ml.). Each coloured solution was accurately diluted to 5 ml., and its optical density determined at 570 mμ. The measurements were carried out in a silica cell (1 cm. light path) against a blank composed of the reagents. The experiments were repeated at various temperatures (Figure).

2,4-*Dinitrophenyl* 5-*Amino*-1-(2,3-O-*isopropylidene*-β-D-*ribofuranosyl*)*imidazole*-4-*carboxylate*.—Methyl 5-amino-1-(2,3-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxylate [4,5] (0·415 g.) was boiled under reflux with N-sodium hydroxide (4 ml.) and ethanol (12 ml.) for 1 hr. The solution was evaporated to dryness, and the residue in water (10 ml.) adjusted to pH 7·4 with N-hydrochloric acid. The solution was evaporated to low volume and further evaporated several times with ethanol, then finally evaporated to dryness, and the residue dissolved in a small volume of dimethylformamide. To this was added a solution of 1-fluoro-2,4-dinitrobenzene (0·3 g.) in dimethylformamide (2 ml.), and the mixture set aside at room temperature for 1 hr. The dark red-brown solution was added to water (50 ml.), to give a heavy yellow precipitate. This was collected by centrifugation, washed with water, and dried. The 2,4-*dinitrophenyl ester* (0·21 g.) was purified by precipitation from a chloroform solution with light petroleum (b. p. 40—60°), and was obtained as a yellow powder, m. p. (decomp.) from 280° (Found: C, 46·45; H, 4·1; N, 15·1. $C_{18}H_{19}N_5O_{10}$ requires C, 46·5; H, 4·1; N, 15·1%). The spectral properties (Table 1) and behaviour when diazotised were very similar to those of the analogous cyclohexyl derivative.

N-(5-*Amino*-1-β-D-*ribofuranosylimidazole*-4-*carbonyl*)-L-*aspartic Acid* 5′-*Phosphate* (*SAICAR*).—(*a*) A solution of the foregoing 2,4-dinitrophenyl ester (0·325 g.) in ethyl acetate (10 ml.) with diethyl L-aspartate (0·21 g.) was boiled under reflux for 1 hr. The cooled solution was extracted with saturated aqueous sodium hydrogen carbonate (3 × 10 ml.). The organic phase was washed with water, evaporated, and water removed by evaporation with benzene and finally toluene. The residue was dissolved in a very small amount of dichloromethane, cooled to −40°, and sealed from the atmosphere with a serum cap. Through the cap was injected pyrophosphoryl chloride (0·187 g.), and the mixture allowed to warm slowly to room temperature and set aside for 1 hr. Water (50 ml.) was added, and the solution adjusted to pH 7 with lithium hydroxide

then to pH 3 with acetic acid and heated on a water-bath for 1 hr. The solution was evaporated to dryness and the residue heated on a water-bath with 0·5N-lithium hydroxide (40 ml.) for 1 hr. The cooled solution was adjusted to pH 7·2 with hydrobromic acid, treated with barium bromide (1 g.), evaporated to *ca.* 30 ml., treated with ethanol (150 ml.), cooled to 4°, and set aside overnight. The resulting precipitate was collected by centrifugation, washed with ethanol, and dried. The solid (0·96 g.) in water (180 ml.) was filtered, and the filtrate pumped on to a column (12 cm. × ½ cm.) of Dowex 1 × 8 resin in the Br^- form. The column was washed with water and eluted with 0·008N-hydrobromic acid. Ultraviolet absorbing material was collected in 10-ml. fractions between 0·88 and 1·01 litres of eluate (fraction X) and between 1·4 and 1·58 litres (fraction Y). The fractions were each neutralised to pH 7·3 with barium hydroxide solution and reduced in volume to *ca.* 40 ml., treated with ethanol (200 ml.), cooled to 0°, and set aside overnight. The precipitated solids were collected, washed with ethanol and ether, and dried, to give from fraction X 0·056 g. and from fraction Y 0·038 g. Paper chromatography of the two materials showed that fraction Y was SAICAR and fraction X the corresponding nucleoside. The structures were further confirmed by the Bratton–Marshall assay, in which each compound had $\lambda_{max.}$ 555—560 mμ and showed the characteristic instability of the diazonium salt if coupling was delayed for a few min.

(*b*) Methyl 5-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxylate (0·586 g.) was boiled under reflux with N-sodium hydroxide (12 ml.) and ethanol (12 ml.) for 1 hr. Pyridine (5 ml.) was added, and the mixture placed on a column of ZeoKarb 225 (pyridine form) resin which was eluted with 50% aqueous pyridine. Fractions (20 ml.) containing diazotisable amine were combined and evaporated to 5 ml., then six times concentrated to half volume after adding pyridine (6 × 5 ml.). The final solution in pyridine (20 ml.) was set aside for 3 days with dicyclohexylcarbodi-imide (4 g.) and pentachlorophenol (0·62 g.) at room temperature. Water (5 ml.) was then added, and after 2 hr. the solvent was evaporated and the residue extracted with ether until free from arylamines (Bratton–Marshall assay). The extract was evaporated, and the residue dissolved in pyridine (15 ml.), cooled to 0°, mixed with a solution of dibenzyl phosphorochloridate (0·765 g.) in pyridine (5 ml.), and set aside overnight at room temperature. The solution was evaporated to dryness, and the residue dried by evaporation with ethanol and benzene, then dissolved in methyl cyanide (10 ml.) and diethyl L-aspartate (0·3 g.), and the solution boiled under reflux for 1 hr. The mixture was evaporated, and the residue in methanol (10 ml.) and 10% aqueous acetic acid (30 ml.) heated on a water-bath for 1 hr. The solution was evaporated, and the residue heated on a water-bath for 1 hr. with 0·5N-lithium hydroxide (40 ml.). The cooled solution was adjusted to pH 7·2 with 2N-hydrobromic acid and reduced with hydrogen over palladised charcoal (0·4 g.) for 2 hr. The filtered solution was treated with barium bromide (1 g.) and evaporated to 20 ml., filtered, and the filtrate treated with ethanol (150 ml.). The mixture was set aside overnight and the precipitate collected by centrifugation. Paper chromatography of the product showed the presence of SAICAR, R_F 0·16 (system A) and the related nucleoside R_F 0·41 (system A) which were run at the same time. The structures were further confirmed by ultraviolet absorption spectra of eluted spots and of the characteristic unstable diazonium salt formed by each compound. The yield of nucleotide estimated by spectrophotometry was 0·068 g.

5-Amino-1-β-D-ribofuranosylimidazole-4-N-methylcarboxyamide 5′-Phosphate.—The foregoing 2,4-dinitrophenyl ester (0·5 g.) was set aside with 30% aqueous methylamine (5 ml.) for 2 hr. at room temperature. The mixture was evaporated to dryness, and the residue in ethyl acetate washed with sodium hydrogen carbonate solution. The organic phase was evaporated, and the residue dried by evaporation with benzene (25 ml.) then toluene (25 ml.). The resulting stiff gum was made mobile with a few drops of dichloromethane, cooled to −40°, and mixed with pyrophosphoryl chloride (0·32 g.), then slowly allowed to warm to room temperature, and finally set aside for a further 30 min. The mixture was treated with water (50 ml.), and the pH adjusted to 7·2 with lithium hydroxide and then to pH 3 with 10% aqueous acetic acid. The solution was heated on a water-bath for 1 hr., cooled, and the pH adjusted to 7·3 with barium hydroxide solution. Barium bromide (1 g.) was added, and the solution evaporated to about 20 ml., treated with ethanol (100 ml.), and set aside at 4° for 2 hr. The resulting precipitate was collected by centrifugation, washed with ethanol and ether, and dried. The product (0·98 g.) was dissolved in water (180 ml.), the solution filtered, and the filtrate pumped on to a column of Dowex 1 × 8 Br^- form resin (12 × ½ cm.), and the column washed with water (450 ml.) and then eluted with 0·008N-hydrobromic acid. Ultraviolet absorbing material appeared between 0·24 and 0·38 litres (fraction X) and between 0·68 and 0·82 litres (fraction Y). Fraction X gave a negative reaction with the Bratton–Marshall test reagents, whereas fraction Y was positive. Fraction Y was adjusted to pH 7·6 with barium hydroxide solution, and the volume reduced to *ca.* 30 ml. Barium bromide (1 g.) was added, and the clarified solution treated with ethanol (150 ml.) and set aside overnight at 4°. The precipitate which had separated was collected by centrifugation, washed with ethanol and ether, and dried. The *solvated imidazole nucleotide barium salt* was obtained as a white powder which retained a little barium carbonate (Found: C, 19·7; H, 3·3; Ba, 32·2; N, 8·75, P, 4·85. $C_{10}H_{15}BaN_4O_8P,3H_2O,\frac{1}{2}BaCO_3$ requires C, 19·7; H, 3·3; Ba, 32·15; N, 8·75; P, 4·85%).

5-Amino-1-β-D-ribofuranosylimidazole-4-N-diethylcarboxyamide 5′-Phosphate.—The foregoing 2,4-dinitrophenyl ester (0·56 g.) was set aside for 2 hr. with diethylamine (1 g.). The mixture was evaporated to dryness, and the residue in ethyl acetate washed with sodium hydrogen carbonate solution. Evaporation of the organic phase left a gum which was phosphorylated with pyrophosphoryl chloride (0·34 g.) as in the preceding experiment, to give a crude barium salt (1·03 g.). Ion-exchange chromatography of this as in the last experiment gave three fractions between 0·13 and 0·146 litres (fraction X), 0·18 and 0·213 litres (fraction Y), and 0·72 and 0·98 litres (fraction Z). The first fraction gave a negative test in the Bratton–Marshall assay whereas the other two were positive. Paper chromatography of fraction Y and Z indicated that Z was the diethylcarboxyamide 5′-phosphate and Y the corresponding nucleoside, and this was confirmed by their ultraviolet absorption spectra and spectra of the dyestuffs formed in the Bratton–Marshall test (Table 1). The nucleotide fraction was worked up as in the preceding experiment, to give a barium salt (33 mg.).

Phosphorylation of 2,4-Dinitrophenyl 5-Amino-1-(2,3-isopropylidene-β-D-ribofuranosylimidazole)-4-carboxylate.—The dinitrophenyl ester (0·187 g.) and pyrophosphoryl chloride (0·263 g.) were triturated together at −40°, and the mixture allowed to warm to room temperature over 30 min. The dark solution was diluted with water (50 ml.), and adjusted to pH 7·3 with barium hydroxide solution. The clarified solution was reduced to *ca.* 20 ml., diluted with ethanol (100 ml.), and set aside at 4° for 1 hr.; a fine precipitate had separated. The product was washed with ethanol and ether, and dried, to give a pale yellow solid (0·028 g.) which gave a single absorbing and phosphate positive spot on paper chromatograms, R_F 0·23 (system A) and 0·08 (system B). It also had the same ultraviolet and Bratton–Marshall dye absorption maxima as the corresponding nucleoside. A suspension of the finely ground ester phosphate (0·02 g.) in ethanol (5 ml.) was saturated with dry ammonia, then set aside overnight. The solvent was removed, and the residue washed with ethanol, dissolved in water, and chromatographed on a column (3 × ½ cm.) of Dowex 1 × 8 Br^- form resin. Elution with 0·008N-hydrobromic acid gave two fractions which appeared between 0·94 and 0·98 litres (fraction X) and 1·02 and 1·06 litres (fraction Y). Fraction Y was neutralised to pH 7·2 with barium hydroxide solution, evaporated to a small volume, clarified, and diluted with ethanol (3—4 volumes), to give 6·8 mg. of a barium salt which had the same ultraviolet absorption spectra, Bratton–Marshall assay behaviour, and paper chromatographic properties as an authentic sample of 5-amino-1-β-D-ribofuranosylimidazole-4-carboxyamide 5′-phosphate (AICAR) (Tables 1 and 2). Fraction X was not distinguished on paper chromatograms from an authentic specimen of the nucleoside corresponding to AICAR.

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