Purines, Pyrimidines, and Imidazoles. Part 50.[1] Inhibition of Adenylosuccinate AMP-Lyase No. 4.3.2.2. by Derivatives of *N*-(5-Amino-1-β-D-ribofuranosylimidazole-4-carbonyl)-L-aspartic Acid 5′-Phosphate (SAICAR) and Virazole 5′-Phosphate

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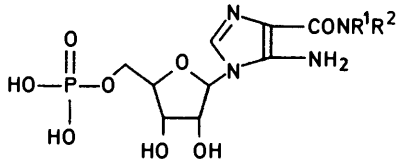
N-(5-Amino-1-β-D-ribofuranosyl-4-carbonyl)-L-aspartic acid 5′-phosphate (SAICAR) and some related imidazole peptides have been shown to form in low yield in aqueous solution from the appropriate imidazolecarboxylic acid, an amino-acid or ester and the soluble carbodi-imide 1-cyclohexyl-3-(4-ethylmorpholin-2-yl)carbodi-imide methotoluene-4-sulphonate. Benzyl aminocyanoacetate either with *N*-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-*N*′-dimethylformamidine, or with triethyl orthoformate followed by 2,3-*O*-isopropylidene-D-ribofuranosylamine, produced a readily separated mixture of benzyl 5-amino-1-(2,3-*O*-isopropylidene-α- and -β-D-ribofuranosyl)imidazole-4-carboxylates. Phosphorylation of the β-anomer and acid hydrolysis and hydrogenation of the product gave 5-amino-1-β-D-ribofuranosylimidazole-4-carboxylic acid 5′-phosphate, which was active as a substrate for the enzyme phosphoribosylaminoimidazole succinocarboxamide. The β-benzyl ester with acetic anhydride gave the 5′-*O*-acetate, with acetyl chloride the 5-*N*,5′-*O*-diacetate, and with dimethylformamide dimethyl acetal the 5-*N*-dimethylaminomethylene derivative. Hydrogenation of the β-anomer or its 5′-*O*-acetyl derivative and condensation of the resulting carboxylic acids with acetic anhydride gave 3-methyl-5-(2,3-*O*-isopropylidene-5-*O*-acetyl-β-D-ribofuranosyl)imidazo[4,5-*d*][1,3]oxazin-1-one. The 5′-*O*-acetyl ester was hydrogenated and the carboxylic acid condensed *in situ* with *N*-hydroxysuccinimide and dicyclohexylcarbodi-imide to produce *N*-succinimidyl 5-amino-1-(2,3-*O*-isopropylidene-5-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxylate which with dimethyl L-aspartate or diethyl *threo*-DL-β-methylaspartate produced the corresponding peptides, which were deacylated and phosphorylated to give, after removal of the isopropylidene groups, SAICAR and *threo*-DL-β-methyl-SAICAR respectively. The *threo*-derivative and virazole-5′-phosphate both inhibit adenylosuccinase and their effects on this enzyme and on SAICAR-kinosynthetase and AIR-carboxylase are compared.

THE enzyme adenylosuccinase (adenylosuccinate AMP-lyase no. 4.3.2.2.) is involved in two separate steps in the *de novo* biosynthetic pathway to purine nucleotides, the conversion of *N*-[5-amino-1-(β-D-ribofuranosyl)imidazole-



(1)

a ; R^1 = L—$CH(CO_2H)\cdot CH_2CO_2H$, R^2 = H
b ; R^1 = R^2 = H
c ; R^1 = L—*threo*—$CH(CO_2H)\cdot CHMeCO_2H$, R^2 = H
d ; R^1 = Me, R^2 = DL—$CH(CO_2H)\cdot CH_2CO_2H$

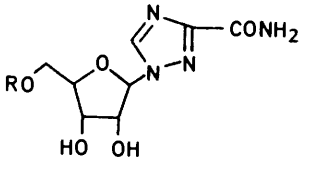
4-carbonyl]-L-aspartic acid 5′-phosphate (SAICAR) (1a) into 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide 5′-phosphate (AICAR) (1b) and also the conversion of succinoadenosine 5′-phosphate (succino-AMP) into adenosine 5′-phosphate (AMP).[2]

$$\text{SAICAR} \longrightarrow \text{AICAR} + \text{fumarate} \quad (1)$$

$$\text{succino-AMP} \longrightarrow \text{AMP} + \text{fumarate} \quad (2)$$

As part of a wider project we have been interested in the synthesis of compounds as potential inhibitors of the pathway enzymes and in an earlier part of this series we have recorded[3] that a simple derivative of SAICAR, namely *N*-[5-amino-1-(β-D-ribofuranosyl)imidazole-4-carbonyl)-L-*threo*-β-methylaspartic acid 5′-phosphate (1c) was a competitive inhibitor of adenylosuccinase isolated from yeast.

We wished to extend these studies to include the preparation of other substances similar to SAICAR, but also to investigate improved methods for the synthesis of peptide derivatives such as (1a) which have hitherto been



(2)

a ; R = $PO(OH)_2$
b ; R = H

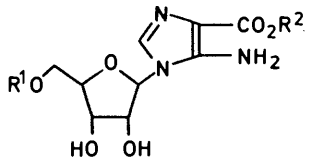
difficult to prepare. In addition we have been equally interested in comparing the effects of our compounds with that of virazole 5′-phosphate (2a), since this compound may be regarded as an imidazole nucleotide analogue, and the nucleoside derivative (2b) is a known anti-viral agent.[4]

RESULTS AND DISCUSSION

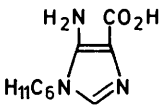
A major problem associated with the synthesis of SAICAR (1a) or analogous phosphorylated peptides is the partial cleavage of the acylaspartic acid which occurs during phosphorylation, resulting in the formation of the unstable carboxylic acid (3a); a mechanism for this reaction has been suggested.[3]

An alternative route to SAICAR-type compounds

would involve preparation of the aminoimidazole nucleotide (3a) and reaction of this with the appropriate amino-acid. Since nucleotides of type (3a) are essentially



(3)



(4)

a ; $R^1 = PO(OH)_2$, $R^2 = H$
b ; $R^1 = R^2 = H$
c ; $R^1 = PO(OH)_2$, $R^2 = CH_2Ph$

only water-soluble, we have examined condensation of such compounds with water-soluble carbodi-imides using conditions analogous to those reported for the acylation of glycine methyl ester.[5] In preliminary experiments the model aminoimidazolecarboxylic acid (4) was condensed with either L-aspartic acid, glycine, or glycine methyl ester in aqueous solution in the presence of 1-cyclohexyl-3-(4-ethylmorpholin-2-yl)carbodi-imide methotoluene-4-sulphonate. The resulting solutions were assayed by the Bratton–Marshall[6] method after acid hydrolysis under conditions[7] which are known to first decarboxylate, then ring-open, any unreacted acid (4). Small yields of products (*ca.* 6%) were obtained possessing the typical diazonium-salt instability associated with SAICAR-type compounds.[8,9] Optimum conditions of pH (6.5) and temperature (ambient) were also determined. Accordingly the experiments were repeated using dimethyl L-aspartate and the nucleoside (3b) when similar low yields (*ca.* 8%) of SAICAR-type material were obtained (Table 1). Using free L-aspartic acid, however, several products were obtained with characteristic (of SAICAR) diazotisation properties, and these

TABLE 1

Reaction [a] of 5-amino-1-cyclohexyl- (and β-D-ribofuranosyl)-imidazole-4-carboxylic acids with amino-acids and 1-cyclohexyl-3-(4-ethylmorpholin-2-yl)carbodi-imide methotoluene-4-sulphonate in aqueous solution

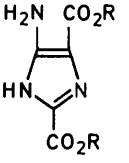
Amino-acid (Compound)	pH	Yield (%) after 2 h
Gly (4) [b]	5.5	1.8
Gly methyl ester (4) [b]	5.5	2.9
L-Asp (4) [b]	5.5	6.35
L-Asp (4)	3.5	0
L-Asp (4)	4.75	4.4
L-Asp (4)	5.5	6.7
L-Asp (4)	6.5	9.5
L-Asp (4)	7.0	5.8
L-Asp (4)	8.0	0
L-Asp (4) [c]	6.5	10
L-Asp (4) [d]	6.5	2.5
L-Asp (4) [e]	6.5	1
L-Asp (3b)	6.5	8

[a] Reactions were normally carried out with five molar equivalents of the amino-acid at 25 °C, and the products were assayed by the quantitative Bratton–Marshall method.[7] The products all showed the characteristic diazonium-salt instability associated with SAICAR.[8,9] [b] 1 equiv. of amino-acid used. [c] Additional 5 equiv. each of amino-acid and carbodi-imide added after 2 h. [d] At 0 °C. [e] At 50 °C.

are undoubtedly mixtures of aspartic acid–peptide derivatives. Finally the aminoimidazole nucleotide (3a)[10] was condensed with dimethyl L-aspartate in aqueous solution in the presence of the soluble carbodi-imide, but after base hydrolysis only a low (*ca.* 3.5%) yield of SAICAR could be detected, which after work-up was shown to be identical to an authentic sample of the nucleotide.

Apart from problems associated with phosphorylation the preparation of the desired carboxylic acid intermediate (3a) involves alkaline hydrolysis of an ethyl ester.[10] The isolation of material from the hydrolysate in sufficiently pure form for subsequent condensation with an amino-acid is tedious and generally leads to loss of material by decarboxylation of the unstable acid. Accordingly we have examined the use of benzyl esters as a source of the acids.

Reduction of the hydroxyimino-derivative NC·C(:NOH)·CO_2CH_2Ph (5)[10] with aluminium amalgam and water produced benzyl aminocyanoacetate NC·$CH(NH_2)$·CO_2R (6a; R = CH_2Ph, 6b, R = Et), isolated as a highly crystalline toluene *p*-sulphonate salt, in which form the material was readily stored. When a sample of the amino-ester (6a) was set aside for several days at 35 °C it produced the crystalline imidazole diester (7a), analogous to a compound (7b) formed in a similar manner



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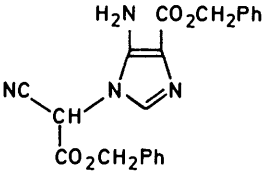
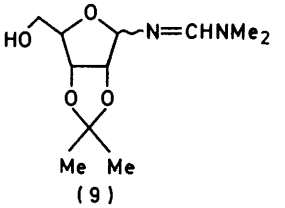


(8)

a ; R = CH_2Ph
b ; R = Et



(9)

a ; α—anomer
b ; β—anomer

from the diethyl ester.[11] Similarly the imidazole (8) was produced from the amino-ester (6a) and a half-equivalent of triethyl orthoformate. We have earlier recorded[12] the sole formation of what was considered to be the β-anomer (9b) by the reaction of isopropylidene ribofuranosylamine (10) with dimethylformamide dimethyl acetal, evidence for structure being based on its reactions. Fractional crystallisation of the reaction product has now been shown to produce both the crystalline α- (9a) and β-anomers (9b). It is interesting to note that different anomer mixtures may be obtained by variation of the reaction conditions. A short reaction

time favours the α-anomer and this presumably reflects the anomer concentrations in the glycosylamine. Longer reaction times favour firstly the β-anomer (9b) then after a long period the α-anomer (9a) as the final product.

The α- (9a) or β-formamidine (9b) with ethyl aminocyanoacetate (6b) also gave mixtures of (11a) and (11b) described earlier,[12] the ratio (β : α) of which varied from 1 : 1 (α-formamidine) to 3 : 1 (β-formamidine) (Table 2). Interestingly, the ribosides (11a) and (11b) were also produced, albeit in low yield, in aqueous solution from (12a) and (10) (Table 3).

TABLE 2

The reaction of *N*-[(dimethylamino)methylene] 2,3-*O*-isopropylidene-α- and -β-D-ribofuranosylamines with ethyl 2-amino-2-cyanoacetate [a]

Compound [b]	Time/min	α-D-riboside (11a) (%)	β-D-riboside (11b) (%)	β : α Ratio
(9a)	15	4.3	4.6	1.05 : 1
(9a)	90	6.5	6.5	1 : 1
(9b) [c]	15	6.1	11.6	1.9 : 1
(9b)	15	3.1	12.2	3.9 : 1
(9b) [d]	15	4.6	7.7	1.7 : 1
(9b)	45	5.5	13.4	2.5 : 1
(9b)	90	6.1	16.8	2.8 : 1

[a] Yields quoted refer to pure crystalline solids. [b] The reaction mixtures were dissolved in chloroform and the solutions washed with 2M-sodium hydroxide (*cf.* Experimental section). [c] Reaction mixture not washed with sodium hydroxide. [d] Solutions of the formamidine (9b) and ethyl 2-amino-2-cyanoacetate in acetonitrile were refluxed for 15 min before addition of acetic acid.

TABLE 3

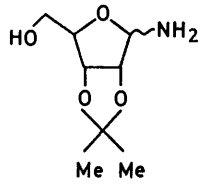
The preparation of 5-aminoimidazole-α- and -β-D-ribosides (11a and b) in aqueous solution

Reaction	Buffer solution	Total yield %	β : α Ratio [a]
(*i*) [b,c]	Sodium hydrogencarbonate	2.3	1.5 : 1
(*ii*) [b,c]	Sodium hydrogencarbonate	trace	
(*iii*) [b,d]	Sodium acetate	0.7	1 : 1
(*iv*) [b,d]	Sodium acetate	trace	
(*v*) [b,d,e]	Sodium acetate–acetic acid	1.0	1 : 1

[a] Ratio determined by t.l.c.–u.v. photometric method [b] Reactions: (*i*) (12) (0.185 g), (10) (0.36 g), and aqueous sodium hydrogencarbonate (10 ml, 2mM) were shaken and warmed to 70 °C, then set aside for 18h. (*ii*) (9b) (0.244 g), (6b) (0.198 g), and aqueous sodium hydrogencarbonate (10 ml, 2mM), were shaken and warmed to 70 °C, then set aside for 18 h. (*iii*) Repeat of (*i*) using sodium acetate (2mM). (*iv*) Repeat of (*ii*) using sodium acetate (2mM). (*v*) Repeat of (*iv*) using sodium acetate (2mM) in water (8 ml) followed by acetic acid (2 ml) after 10 min. [c] Assay by quantitative Bratton–Marshall technique. [d] Assay by a combined t.l.c.–u.v. photometric and quantitative Bratton–Marshall technique.[7] [e] Compound (8) (0.065 g) precipitated from the cooled reaction mixture.

Condensation of benzyl aminocyanoacetate (6a) with the β-formamidine (9b) in acetonitrile containing a little acetic acid similarly gave a mixture of the α- and β-anomeric imidazole nucleosides (11c) and (11d), respectively, which were separated in crystalline form by chromatography on silica gel. The structures assigned to the compounds were confirmed by elemental analysis, mass, u.v., and n.m.r. spectra, the formation of a coloured dye in the Bratton–Marshall assay, and subsequent reactions. In addition the ^{1}H n.m.r. spectra of the pair of anomers was in accord with the empirical rule for ribosides which states [13] that H-1′ resonates at lower field in the α-anomer than the β-anomer. Also, comparison of chemical shifts of the CMe_2 groups in each anomer indicated that $\Delta\delta$ values were in accord with Imbach's rule.[14] In addition, optical rotations agreed with Hudson's rules.

The same mixture of anomeric isopropylidene nucleosides, (11c) and (11d), was also obtained in better yield



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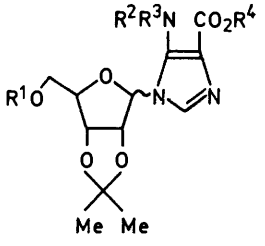
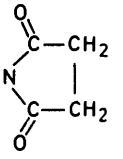


(11)

a ; $R^1 = R^2 = R^3 = H$, $R^4 = Et$ — α-anomer
b ; $R^1 = R^2 = R^3 = H$, $R^4 = Et$ — β-anomer
c ; $R^1 = R^2 = R^3 = H$, $R^4 = CH_2Ph$ — α-anomer
d ; $R^1 = R^2 = R^3 = H$, $R^4 = CH_2Ph$ — β-anomer
e ; $R^1 = R^2 = R^3 = R^4 = H$ — β-anomer
f ; $R^1 = Ac$, $R^2 = R^3 = H$, $R^4 = CH_2Ph$ — β-anomer
g ; $R^1 = R^2 = Ac$, $R^3 = H$, $R^4 = CH_2Ph$ — β-anomer
h ; $R^1 = Ac$, $R^2 = R^3 = R^4 = H$ — β-anomer
i ; $R^1 = R^2 = Ac$, $R^3 = R^4 = H$ — β-anomer
j ; $R^1 = R^2 = R^4 = Ac$, $R^3 = H$ — β-anomer



k ; $R^1 = Ac$, $R^2 = R^3 = H$, R^4 = — β-anomer

$NC \cdot CH(N{:}CHOEt) \cdot CO_2R$

(12)

a ; R = Et
b ; $R = CH_2Ph$

by condensation of the isopropylidene ribofuranosylamine (10) with the formimidate (12b) prepared *in situ* from (6a) and triethyl orthoformate and, given seed crystals, the β-anomer was readily separated from the mixture without chromatography. The structure assigned to the nucleoside (11d) was further confirmed by phosphorylation with pyrophosphoryl chloride, and

removal of the isopropylidene group by acid treatment to produce the nucleotide ester (3c), hydrogenation of which with either platinum(IV) oxide or palladium catalysts then produced the β-anomeric imidazolecarboxylic acid nucleotide (3a), which was active as a substrate for the enzyme phosphoribosylaminoimidazole succinocarboxamide and identical with an authentic sample of the nucleotide prepared by a different route.[10]

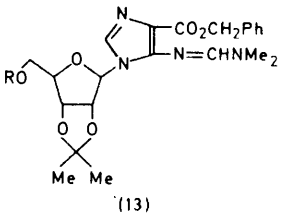
The ready removal of the benzyl group by hydrogenolysis confirmed the usefulness of the imidazole benzyl esters as a source of the carboxylic acids and prompted us to extend their use.

Hydrogenolysis of the isopropylidene ester (11d) with palladium–charcoal produced the carboxylic acid (11e) which was condensed with dimethyl *N*-methyl-DL-aspartate in the presence of dicyclohexylcarbodi-imide, and the resulting peptide phosphorylated with pyrophosphoryl chloride to produce, after deblocking with acid and then base, two very similar nucleotides, presumably diastereoisomeric forms of DL-*N*-methyl-SAICAR (1d) the structures of which were confirmed by hydrolysis to produce *N*-methylaspartic acid, phosphate, and glycine, by their u.v. absorption spectra and by the absorption spectra of the dye produced by each nucleotide in the Bratton–Marshall assay. Neither of the *N*-methyl nucleotides however had any inhibitory activity against a sample of SAICAR-kinosynthetase or a sample of adenylosuccinase, both prepared from avian liver.

We have earlier [15] used active esters derived from aminoimidazolecarboxylic acids as a route to peptide derivatives, and more recently *N*-succinimidyl esters have been used in a similar manner.[16] Reaction of the benzyl isopropylidene derivative (11d) with acetic anhydride readily gave the 5′-*O*-acetyl derivative (11f) and with acetyl chloride the 5′-*O*,5-*N*-diacetyl derivative (11g). The amino-group in either the nucleoside (11d) or the mono-*O*-acetate (11f) could also be protected by reaction with dimethylformamide dimethyl acetal when the corresponding dimethylaminomethylene derivatives (13a and b) respectively were obtained. The structures assigned were confirmed by elemental analysis, mass spectra, u.v. absorption spectra and negative reactions (for NH_2) in the Bratton–Marshall assay.

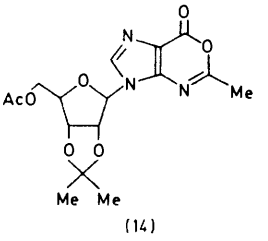
Reaction of the acids (11e or h) prepared in solution by hydrogenation of the corresponding benzyl esters with 1 and 2 equiv. of acetic anhydride, respectively, in pyridine solution produced the oxazin-7-one (14) in a manner analogous to a previously recorded [17] preparation of the acetylated derivative (15). The mechanism suggested earlier [17] for the formation of (15), namely a direct cyclodehydration of the acetylaminocarboxylic acid (16), seems unlikely since our attempts to produce an oxazinone by heating a solution of the acid (11i) in pyridine were unsuccessful. Excess of acetic anhydride is essential for the reaction to proceed successfully and we suggest that the reaction involves the formation of a diacetyl derivative (11j) and loss of acetic acid from this (Scheme).

Condensation of the 5′-*O*-acetyl derivative (11h) with *N*-hydroxysuccinimide in the presence of DCC produced the crystalline succinimidyl ester (11k). This reacted smoothly with dimethyl L-aspartate to give the peptide (17a) which with sodium methoxide in methanol produced the isopropylidene peptide (17b). Similarly the



(13)

a ; R = H
b ; R = Ac



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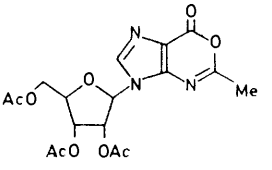

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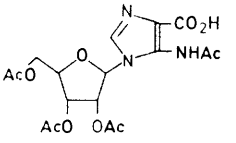
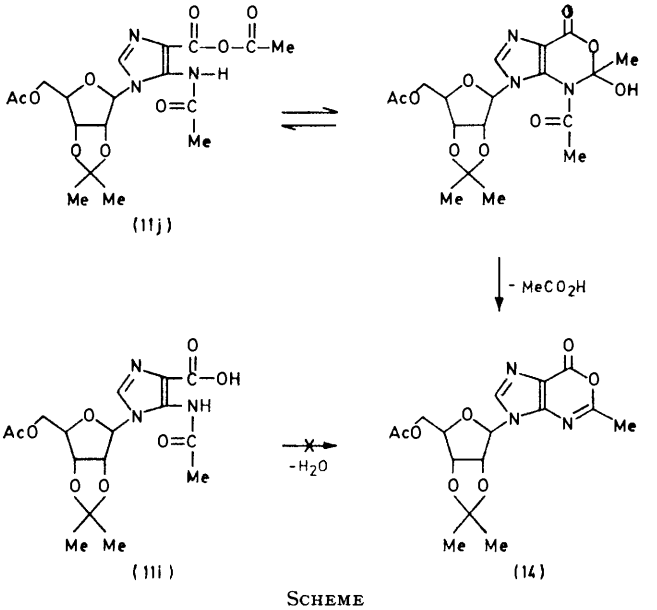


(16)

succinimidyl ester (11k) with diethyl *threo*-DL-β-methyl aspartate gave the 5′-acetyl derivative (17c) which was converted to the deacylated derivative (17d). In contrast neither dimethyl *N*-methyl nor *N*-benzyl

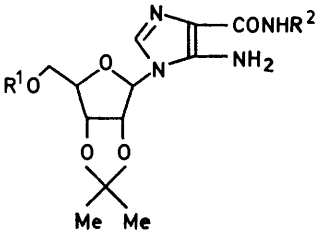


SCHEME

aspartate could be persuaded to react with the succinimidyl ester.

Studies on the mechanism of action of the broad-spectrum antiviral agent virazole (2b), an imidazole nucleoside analogue have shown [4] that it is a potent inhibitor of both RNA and DNA synthesis in various cell cultures infected with RNA or DNA viruses. Moreover, virazole 5′-phosphate, shown to be produced *in vivo* and possibly the active form of the drug, has been

shown to be a competitive inhibitor of the enzyme IMP-dehydrogenase isolated from *E. Coli* or from Ehrlich ascites tumour cells.[4] The results suggested that the antiviral activity of virazole might be due to inhibition of GMP biosynthesis in an infected cell, at the step involving the conversion of IMP into xanthosine 5′-phosphate. Since inhibition of any of the enzymes involved in purine nucleotide *de novo* biosynthesis would presumably lead to inhibition of nucleic acid synthesis we have been interested in comparing the effects of virazole 5′-phosphate with those of *threo*-β-methyl SAICAR (1c) on appropriate pathway enzymes. The *threo*-derivative (1c) has earlier been shown [3] to be a competitive inhibitor of adenylosuccinase from yeast and this has been confirmed with enzyme isolated from avian liver. In addition similar examination of virazole 5′-



(17)

a ; R^1 = Ac, R^2 = L—CH(CO_2Me)·CH_2CO_2Me
b ; R^1 = H , R^2 = L—CH(CO_2Me)·CH_2CO_2Me
c ; R^1 = Ac, R^2 = *threo*—DL—CH(CO_2Et)·$CHMeCO_2Et$
d ; R^1 = H , R^2 = *threo*—DL—CH(CO_2Et)·$CHMeCO_2Et$

phosphate reveals that this nucleotide also inhibits the enzyme (Figure 1). On the other hand, neither the *threo*-derivative nor virazole 5′-phosphate inhibited SAICAR-kinosynthetase, although virazole 5′-phosphate

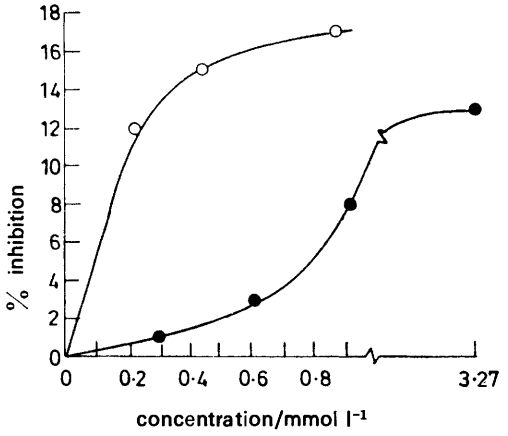


FIGURE 1 Inhibition of adenylosuccinase by virazole-5′-phosphate (○) and β-methyl-SAICAR (●) [*N*-(5-amino-1-β-D-ribofuranosylimidazole-4-carbonyl)-DL-*threo*-β-methylaspartic acid 5′-phosphate]

inhibited AIR-carboxylase to some extent whereas the *threo*-derivative was inactive against this enzyme (Figure 2).

These preliminary results suggest that the source of antiviral activity associated with virazole may be more complex than hitherto imagined, and that activity at several positions in the purine nucleotide biosynthetic pathway may be an important prerequisite for useful activity.

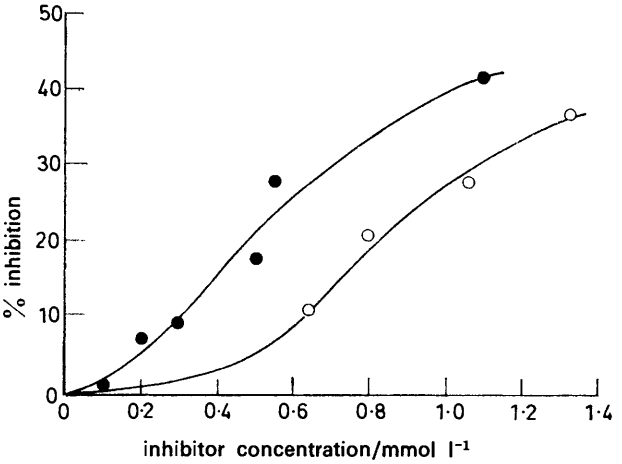


FIGURE 2 Inhibition of AIR-carboxylase by virazole-5′-phosphate (○) and β-xylo-CAIR [18] (5-amino-1-β-D-xylofuranosylimidazole-4-carboxylic acid 5′-phosphate) (●)

EXPERIMENTAL

Evaporations were carried out with a Büchi rotary evaporator, under water-pump vacuum with a flask temperature below 40 °C unless otherwise stated. U.v. spectra were measured with a Unicam SP 800 spectrophotometer, i.r. spectra with a Perkin-Elmer 157 spectrophotometer, ^1H n.m.r. spectra with a JEOL JNM-MH-100 spectrophotometer (δ in p.p.m., $SiMe_4$ as internal standard), mass spectra with an A.E.I. MS-903 spectrometer, and optical rotations with a Perkin-Elmer 141 polarimeter. Silica gel (0.05—0.20 mm, 325—370 mesh; Machery Nagel and Co.) was used for column chromatography. Thin-layer chromatograms were run either on silica gel $60F_{254}$ (0.25-mm thick) pre-coated glass plates (Merck) in the systems; (A) chloroform–methanol (9 : 1); (B) butan-1-ol–acetic acid–water (12 : 3 : 5); or on Cellulose D.C. (0.1-mm thick) pre-coated glass plates (Merck) in the systems; (C) solvent (B); or (D) propan-2-ol–ammonia–water (6 : 3 : 1).

Ion-exchange separations were performed in all-Teflon or glass apparatus equipped with a Buchler Micropump and a Vanguard Automatic U.v. Analyser (model 1056) for continuous recording of column eluates at 267 nm.

Peptide Synthesis in Aqueous Solutions.—(*a*) To sodium 5-amino-1-cyclohexylimidazole-4-carboxylate [19] (46.2 mg) in water (15 ml) was added 1-cyclohexyl-3-(4-ethylmorpholin-2-yl)carbodi-imide methotoluene-4-sulphonate (0.78 g) and 2N-hydrochloric acid to pH 5.5. A solution of the sodium salt (0.2 mmol) in water was added slowly with stirring, and maintenance of pH 5.5 by the addition of 2N-hydrochloric acid. The reaction was stirred for 2 h at room temperature, and then further imide (0.78 g) added and the pH adjusted to 5.5.

Samples (0.5 ml) were withdrawn at 1-h intervals, added to disodium hydrogen orthophosphate–phosphoric acid buffer (4M, pH 1.5) and heated at 100 °C for 1.5 h. The cooled sample was assayed by the quantitative Bratton–

Marshall assay. The resulting solution showed some of the diazonium-salt instability typical of SAICAR and its analogues.[8,9]

The experiment was repeated with glycine, glycine methyl ester, and L-aspartic acid (Table 1).

The reaction was repeated using 5 equiv. of disodium L-aspartate. Further addition of disodium L-aspartate had no appreciable effect on the yield. The reaction was also shown to be temperature-sensitive; when performed at 0 or 50 °C the reaction yield was lowered.

(*b*) The reaction was repeated as in (*a*) at pH 6.5 using sodium 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxylate (56.2 mg). The diazotisation behaviour of the reaction product (8% by assay) was similar in nature to that of the model reaction product.

(*c*) To 5-amino-2-(β-D-ribofuranosyl)imidazole-4-carboxylic acid 5′-phosphate (67.8 mg) in water (20 ml) was added the carbodi-imide (0.78 g) and the pH adjusted to 6.5. Dimethyl L-asparate (1 mmol) was added in water (2 ml), the reaction stirred for 1 h, and further imide (0.78 g) then added. The solution was stirred for 23 h, acidified to pH 1.5 using 6N-hydrochloric acid, and heated at 100 °C for 20 min. The solution was adjusted to pH 12 with 4M-sodium hydroxide and then heated at 100 °C for 1 h. The cooled solution was adjusted to pH 7.5 with 6N-hydrochloric acid, diluted to 2 l with water, and applied to a column of Bio-Rad AG1X2 resin (OH^- form, 0.9 × 25 cm). The column was washed with water (500 ml) and eluted with 0.008N-hydrochloric acid to give four products, one of which was similar to SAICAR (1a) on t.l.c. [systems (C) and (D)]. Vigorous acid hydrolysis of the sample gave glycine and L-aspartic acid as the only ninhydrin-active products in a manner analogous to authentic SAICAR.

Benzyl 2-Amino-2-cyanoacetate Toluene-4-sulphonate Salt.—To freshly prepared aluminium amalgam (15.9 g, 18% molar excess) in dry ether (500 ml) was added benzyl 2-cyano-2-hydroxyiminoacetate[10] (102 g) in dry ether (500 ml). The mixture was placed in an ice-water bath and water (32 ml) added at a rate so as not to exceed a gentle reflux (*ca.* 30 min). The mixture was stirred for a further 30 min after addition of water was completed. The mixture was then filtered through Celite and the residue extracted with ether (4 × 100 ml). The combined ether solutions were evaporated to *ca.* 700 ml. The solution was treated with dry toluene-4-sulphonic acid monohydrate (120 g) in ether (600 ml) and methanol (100 ml), and then set aside at 4 °C for 18 h. The solid precipitate which formed was collected, washed with dry ether (2 × 200 ml), and dried *in vacuo* over phosphorus pentaoxide. The *amine toluene-4-sulphonate* (60.6 g) was recrystallised from ethanol as needles, m.p. 168—170 °C (Found: C, 56.55; H, 5.1; N, 7.65; S, 9.05. $C_{17}H_{18}N_2O_5S$ requires C, 56.35; H, 5.00; N, 7.71; S, 8.85%); $\nu_{max.}$ 1 785 (C=O), 2 290w (C≡N), and 1 610s cm^{-1} (C=N).

To the toluene-4-sulphonate (7.2 g) suspended in chloroform (50 ml) was added 2N-sodium hydroxide solution (10 ml) and the mixture shaken. The organic layer was separated and the aqueous layer re-extracted with chloroform (3 × 30 ml). The dried (Na_2SO_4) solution was evaporated to an oil which was dissolved in dry ether (100 ml), the solution filtered through Celite, and then evaporated to give the free amine as an oil (2.8 g); δ 4.47 (H-2) and 1.96 (NH_2).

Dibenzyl 5-Aminoimidazole-2,4-dicarboxylate (7a).—Benzyl 2-amino-2-cyanoacetate (5 g) was set aside for 5 d at 35 °C, when it became semi-crystalline. Trituration of the product with acetonitrile yielded a solid, which was washed with acetonitrile and dry ether. The combined filtrates and washings were evaporated to *ca.* 10 ml and the addition of dry ether (5 ml) gave a further solid precipitate. The *imidazoledicarboxylate hemihydrate* crystallised from DMSO–water as needles, m.p. 198—200 °C (Found: C, 63.2; H, 4.85; N, 11.85%; M^+, 351. $C_{19}H_{17}N_3O_4 \cdot \frac{1}{2}H_2O$ requires C, 63.35; H, 5.05; N, 11.75%; *M*, 351).

Benzyl 5-Amino-1-(1-cyano-1-benzyloxycarbonylmethyl)-imidazole-4-carboxylate (8).—A solution of benzyl 2-amino-2-cyanoacetate (5 g), triethyl orthoformate (1.85 g), benzenesulphonic acid (2 mg), and acetonitrile (20 ml) was refluxed for 1 h, and the solid which crystallised on cooling was collected. The *imidazole* (2.1 g) was recrystallised from acetonitrile as needles, m.p. 150—152 °C (Found: C, 64.75; H, 4.60; N, 14.45. $C_{21}H_{18}N_4O_4$ requires C, 64.6; H, 4.65; N, 14.35%); δ 7.38 (2 H-5 + H-2), 5.1 and 5.32 (2 CH_2), and 2.06 (NH_2, disappears on addition of D_2O).

N-[*(Dimethylamino)methylene*]-2,3-O-*isopropylidene*-α- *and* -β-D-*ribofuranosylamines* (9a) *and* (9b).—(*a*) To a solution of 2,3-*O*-isopropylidene-D-ribofuranosylammonium toluene-4-sulphonate (13.61 g)[19] in dry methanol (40 ml) was added dimethylformamide dimethyl acetal (2.15 g) and triethylamine (1.4 ml). The mixture was refluxed for 1.25 h, cooled, and evaporated to a gum. This was dissolved in chloroform (20 ml), washed with 2N-sodium hydroxide solution (4.5 ml), dried and evaporated to a waxy solid which was recrystallised from ethyl acetate–ether (1 : 1). The α-D-*ribofuranosyl formamidine* crystallised first, as fine hair-like crystals (0.35 g), m.p. 139—140 °C (Found: C, 54.15; H, 8.15; N, 11.35%; M^+, 244. $C_{11}H_{20}N_2O_4$ requires C, 54.10; H, 8.25; N, 11.45%; *M*, 244). The ^1H n.m.r. spectrum in $(CD_3)_2SO$ had signals at δ 5.34 (H-1′) ($J_{1'.2'}$ 4 Hz), 1.36, 1.56 (CMe_2) (Δδ 0.2), 7.64 (H-2), and 2.96 (NMe_2); $[\alpha]_D^{25}$ −30.6° (*c* 1.0 in CH_2Cl_2). The β-D-ribofuranosyl formamidine crystallised from the mother liquor as prisms (0.88 g), m.p. 106—107 °C (Found: C, 54.30; H, 8.15; N, 11.55%; M^+, 244. Calc. for $C_{11}H_{20}N_2O_4$; C, 54.1; H, 8.25; N, 11.45%; *M*, 244). The ^1H n.m.r. spectrum in $(CD_3)_2SO$ had signals at δ 5.03 (H-1′) ($J_{1'.2'}$ 3 Hz), 1.28, 1.48 (CMe_2) (Δδ 0.2), 7.38 (H-2), and 2.86 (NMe_2); $[\alpha]_D^{25}$ −76.9° (*c* 1.0 in CH_2Cl_2).

(*b*) Synthesis (*a*) was repeated replacing the methanol with an equal volume of chloroform; yields obtained were: the α-D-ribofuranosylformamidine 0.45 g (16%), and the β-D-ribofuranosylformamidine 0.85 g (35%).

(*c*) Synthesis (*a*) was repeated on a 0.025-mol scale and the crude reaction mixture set aside for 16 h after heating, before recovery of the products. Yields were: the α-D-ribofuranosylformamidine 2.95 g (48%) and the β-D-ribofuranosylformamidine 0.28 g (5%).

Ethyl 5-Amino-1-(2,3-O-isopropylidene-α- and -β-D-ribofuranosyl)imidazole-4-carboxylates (11a) *and* (11b).—(*a*) A mixture of *N*-[(dimethylamino)methylene]-2,3-*O*-isopropylidene-α- or -β-D-ribofuranosylamine (2.44 g) in acetonitrile (35 ml), ethyl 2-amino-2-cyanoacetate (1.9 g), and acetic acid (0.6 ml) was refluxed for varying times. The cooled solution was evaporated to a gum which was dissolved in chloroform (20 ml), washed with 2N-sodium hydroxide solution (2 × 6 ml) and saturated sodium chloride solution (6 ml), dried (Na_2SO_4), and evaporated to a gum which was chromatographed on a silica-gel column (2 × 30 cm); the imidazole-β-D-riboside was eluted with ethanol–chloroform (1 : 99) and the imidazole-α-D-riboside

was eluted with ethanol–chloroform (4 : 96); both ribosides crystallised from ethanol (Table 2).

(*b*) *Determination of the* β : α *imidazole–riboside ratio in solution.* To each of the ribosylformamidine anomers (30 mg) was separately added acetonitrile (0.5 ml), ethyl 2-amino-2-cyanoacetate (20 mg), and acetic acid (1 drop) and the mixtures refluxed for 30 min. From the cooled reaction mixtures samples (15 × 2 μl) were pipetted onto a silica-gel t.l.c. plate and developed over 10 cm in solvent system (A). The spots corresponding to the imidazole-α- and -β-D-ribosides were visualised under a u.v. lamp and removed. The samples were extracted with ethanol (3 × 2 ml), the combined extracts evaporated to a gum, and this in ethanol diluted to 5 ml. The absorbances of the solutions at 265 nm were measured: the α-D-formamidine had β : α = 1 : 1 and the β-D-formamidine had β : α = 3 : 1.

Benzyl 5-Amino-1-(2,3-O-isopropylidene-α- and -β-D-ribofuranosyl)imidazole-4-carboxylates (11c) *and* (11d).—(*a*) To benzyl 2-amino-2-cyanoacetate (5.2 g) was added *N*-[(dimethylamino)methylene]-2,3-*O*-isopropylidene-β-D-ribofuranosylamine (5 g), acetic acid (1.2 ml), and acetonitrile (80 ml), and the mixture refluxed for 1.5 h. The cooled mixture was filtered, evaporated to a gum, and dissolved in chloroform (100 ml). The solution was washed with 2N-sodium hydroxide (2 × 30 ml) and saturated sodium chloride solution (30 ml), dried (Na_2SO_4), evaporated to a gum, and dissolved in chloroform (4 ml). The solution was applied to a column of silica gel (2 × 30 cm). The β-D-imidazole riboside was eluted with ethanol–chloroform (1—2 : 99) and the α-D-imidazole riboside with ethanol–chloroform (4 : 96). The eluates were separately reduced to gums by evaporation and crystallised from ethyl acetate–ether (1 : 1); the crystalline D-ribosides were readily recrystallised from ethyl acetate or ethanol–ether. The *imidazole-α-D-riboside* (0.13 g, 1.7%), m.p. 185—186 °C (Found: C, 58.75; H, 5.85; N, 10.70%; M^+, 389. $C_{19}H_{23}N_3O_6$ requires C, 58.60; H, 5.95; N, 10.8%; *M*, 389); $[\alpha]_D^{25}$ −26.9° (*c* 1.0 in CH_2Cl_2); $\lambda_{max.}$ (pH 3) 272 (ε 14 750) and 235 nm (8 700); $\lambda_{max.}$ (pH 10) 272 (ε 15 150) and 235 nm (5 900). The Bratton–Marshall dye had $\lambda_{max.}$ 529 nm (ε 22 580). ^{1}H N.m.r. spectrum in $(CD_3)_2SO$; δ 5.94 (H-1′) ($J_{1'.2'}$ <1 Hz), 7.14 (s, H-2), and 1.34 and 1.28 (CMe_2) (Δδ 0.06). The *imidazole-β-D-riboside* (0.60 g, 7.7%), m.p. 190—192 °C (Found: C, 58.6; H, 6.1; N, 10.7%; M^+, 389. $C_{19}H_{23}N_3O_6$ requires: C, 58.60; H, 5.95; N, 10.8%; *M*, 389); $[\alpha]_D^{25}$ −57.4° (*c* 1.0 in CH_2Cl_2); $\lambda_{max.}$ (pH 3) 271 (ε 14 050) and 248 nm (8 350); $\lambda_{max.}$ (pH 10) 271 (ε 14 450) and 240 nm (6 400). The Bratton–Marshall dyestuff had $\lambda_{max.}$ 524 (ε 18 780). ^{1}H N.m.r. spectrum in $(CD_3)_2SO$; δ 5.74 (H-1′) ($J_{1'\ 2'}$ 4 Hz), 7.4 (H-2), and 1.51 and 1.31 (CMe_2) (Δδ 0.20).

(*b*) To a suspension of 2,3-*O*-isopropylidene-D-ribofuranosylammonium toluene-4-sulphonate (11.5 g), in acetonitrile (50 ml) was added triethylamine (3.24 g) and ethyl formimidate hydrochloride (4 g). The mixture was shaken for 20 min and the ammonium chloride which formed was filtered off and washed with acetonitrile (20 ml). The combined filtrates and washings were treated with a solution of benzyl 2-amino-2-cyanoacetate (7 g) in acetonitrile (30 ml) and then set aside at room temperature for 18 h. The mixture was filtered, evaporated to a gum, dissolved in chloroform (100 ml), and the solution was washed, dried, and chromatographed as in (*a*). Yields obtained: the imidazole-α-D-riboside 0.15 g (1.2%), m.p. 184—186 °C, and the imidazole-β-D-riboside 0.65 g (5.2%), m.p. 190—192 °C.

(*c*) To benzyl 2-amino-2-cyanoacetate (7.75 g) was added triethyl orthoformate (6.65 g, 10% molar excess), benzenesulphonic acid (2 mg), and acetonitrile (200 ml), and the mixture refluxed for 1 h. To the cooled solution was added 2,3-*O*-isopropylidene-D-ribofuranosylammonium toluene-4-sulphonate (5.7 g) and triethylamine (4.8 ml) and the reaction set aside at room temperature for 16 h. The red solution was evaporated to a gum, dissolved in chloroform (100 ml), washed, dried, and subjected to chromatography as described in (*a*). Yields obtained were the imidazole-α-D-riboside 0.35 g (2.9%), m.p. 186 °C and the imidazole-β-D-riboside 1.00 g (8.2%), m.p. 190—192 °C.

(*d*) *Large-scale preparation.* Benzyl 2-amino-2-cyanoacetate (43.8 g, 0.23 mol) was divided into five equal portions and to each portion was added triethyl orthoformate (7.3 g, 10% molar excess), benzenesulphonic acid (2 mg), and acetonitrile (100 ml). The mixtures were refluxed for 45 min, cooled, and filtered. To each reaction mixture was added 2,3-*O*-isopropylidene-D-ribofuranosylammonium toluene-4-sulphonate (11.3 g, 0.031 mol), triethylamine (3.2 g, 0.032 mol) and the mixture set aside for 18 h at room temperature. Each mixture was filtered, evaporated to a gum, dissolved in chloroform (100 ml), washed with 2N-sodium hydroxide (2 × 50 ml) and saturated sodium chloride solution (1 × 50 ml), dried (Na_2SO_4), and evaporated to foams. The products were re-evaporated with ethyl acetate (3 × 50 ml), and dissolved in ethyl acetate (15 ml); ether (20—25 ml) was then added and the solution then seeded with the β-anomer and set aside at 4 °C. Crystallisation commenced within 3 d when more ether (5—10 ml) was added. The pure (t.l.c.) benzyl 5-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxylate (9.65 g, 15.5%) was collected by filtration, m.p. 189—190 °C. The imidazole-α-D-riboside (1.8 g, 3%), m.p. 186 °C, was obtained by chromatography of the combined mother-liquor.

Benzyl 5-N-Acetylamino-1-(2,3-O-isopropylidene-5-O-acetyl-β-D-ribofuranosyl)imidazole-4-carboxylate (11g).—To benzyl 5-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-imidazole-4-carboxylate (2.1 g) in dry pyridine (10 ml) was added acetyl chloride (0.45 g) and the mixture heated at 50 °C for 3 h, then evaporated to dryness and the residue dissolved in chloroform (100 ml). The solution was washed with 2N-sulphuric acid (50 ml), saturated sodium hydrogencarbonate (40 ml), and water (2 × 20 ml), and dried (Na_2SO_4). The solution was evaporated to a gum and applied to a column of silica gel (2 × 30 cm) and eluted with ethanol–chloroform (1 : 99). The *diacetylimidazole* separated from benzene–diethyl ether as a microcrystalline solid (1.6 g), m.p. 82 °C, homogeneous on t.l.c. [systems (A) and (B)] (Found: C, 58.6; H, 5.85; N, 8.45%; M^+, 473. $C_{23}H_{27}N_3O_8$ requires: C, 58.35; H, 5.75; N, 8.85%; *M*, 473); $[\alpha]_D^{25}$ −28.2° (*c* 1.0 in CH_2Cl_2); $\lambda_{max.}$(MeOH) 240 (ε 9 300) and 218 nm (16 650); it gave no colour with the Bratton–Marshall reagents. ^{1}H n.m.r.; δ($CDCl_3$) 6.02 (H-1′) ($J_{1'.2'}$ 2 Hz), 7.4 (H-2), 1.6 and 1.38 (CMe_2) (Δδ 0.22), and 2.14 and 2.04 (5- and 5′-COMe).

Benzyl 5-Amino-1-(2,3-O-isopropylidene-5-O-acetyl-β-D-ribofuranosyl)imidazole-4-carboxylate (11f).—To benzyl 5-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxylate (2 g) in dry pyridine (10 ml) at 0 °C was added acetic anhydride (2.6 g) in dry pyridine (5 ml) during 45 min. The solution was set aside at room temperature for 2 h, then evaporated to dryness, and the residue dissolved in dichloromethane (100 ml). The solution was

washed with 2N-sulphuric acid (40 ml), saturated sodium hydrogencarbonate (40 ml), and water (2 × 40 ml), dried (Na_2SO_4), and applied to a column of silica gel (2 × 30 cm). The β-D-riboside was eluted with ethanol–chloroform (1 : 99) and evaporated to a gum. The *monoacetylimidazole riboside* crystallised from benzene–ether (1 : 1) as plates (2.9 g), m.p. 107—108 °C (Found: C, 58.65; H, 5.90; N, 9.55%; M^+, 431. $C_{21}H_{25}H_3O_7$ requires: C, 58.45; H, 5.85; N, 9.75%; *M*, 431); $[\alpha]_D^{25}$ −57° (*c* 1.0 in CH_2Cl_2); $\lambda_{max.}$(MeOH) 268 (ε 15 100) and 206 nm (12 650); the Bratton–Marshall dyestuff had $\lambda_{max.}$ 524 nm (ε 16 860); ^{1}H n.m.r.; δ($CDCl_3$) 5.68 (H-1′) ($J_{1',2'}$ 3 Hz), 7.38 (H-2), 1.62 and 1.38 (CMe_2) (Δδ 0.24), and 2.06 (5′-COMe).

Benzyl 5-N-[(*dimethylamino*)*methylene*]-1-(2,3-O-*isopropylidene*-5-O-*acetyl*-β-D-*ribofuranosyl*)*imidazole*-4-*carboxylate* (13b).—To benzyl 5-amino-1-(2,3-*O*-isopropylidene-5-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxylate (2.05 g) in dry acetonitrile (50 ml) was added dimethylformamide dimethyl acetal (1.15 g) and the mixture refluxed for 1 h. The cooled solution was filtered, evaporated to a gum, and re-evaporated with acetonitrile (3 × 30 ml) to yield the β-D-*riboside* as a solid foam (2.1 g), homogeneous on t.l.c. in two solvent systems [(A) and (B)] (Found: C, 59.3; H, 6.45; N, 11.65%; M^+, 486. $C_{24}H_{30}N_4O_7$ requires C, 59.25; H, 6.2; N, 11.5%; *M*, 486); $[\alpha]_D^{25}$ −12.9° (*c* 1.0 in CH_2Cl_2); $\lambda_{max.}$(MeOH) 300 (ε 10 500), 234 (12 800), and 212 nm (20 800); it gave no colour with the Bratton–Marshall reagents; ^{1}H n.m.r.; δ($CDCl_3$) 6.02 (H-1′) ($J_{1',2'}$ 2 Hz), 7.42 (H-2), 1.6 and 1.38 (CMe_2) (Δδ 0.22), 2.08 (5′-COMe), and 3.08 and 2.98 (NMe_2).

Benzyl 5-N-[(*dimethylamino*)*methylene*]-1-(2,3-O-*isopropylidene*-β-D-*ribofuranosyl*)*imidazole*-4-*carboxylate* (13a).—To benzyl 5-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-imidazole-4-carboxylate (2.14 g) in dry acetonitrile (60 ml) was added dimethylformamide dimethyl acetal (1.34 g) and the mixture refluxed for 1.5 h. The cooled solution was filtered, evaporated to a gum, re-evaporated with acetonitrile (2 × 20 ml), dissolved in chloroform (5 ml), and applied to a silica gel column (2 × 30 cm). The β-D-*riboside* was eluted with ethanol–chloroform (1 : 99) and evaporation yielded a solid foam (2.2 g), homogeneous on t.l.c. in two solvent systems [(A) and (B)] (Found: C, 59.3; H, 6.5; N, 12.2%; M^+, 444. $C_{22}H_{28}N_4O_6$ requires C, 59.45; H, 6.35; N, 12.60%; *M*, 444); $[\alpha]_D$ −6.20° (*c* 1.0 in CH_2Cl_2); $\lambda_{max.}$(MeOH) 300 (ε 9 500), 234 (12 050), and 211 nm (19 950); it gave no colour with the Bratton–Marshall reagents; ^{1}H n.m.r.; δ($CDCl_3$) 6.02 (H-1′) ($J_{1',2'}$ 3 Hz), 7.4 (H-2), 1.6 and 1.38 (CMe_2) (Δδ 0.22), and 2.94 and 3.06 (NMe_2).

3-*Methyl*-5-(2,3-O-*isopropylidene*-5-O-*acetyl*-β-D-*ribofuranosyl*)*imidazo*[4,5-d][1,3]*oxazin*-1-*one* (14).—To 5-*N*-acetylamino-1-(2,3-*O*-isopropylidene-5-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxylic acid, from the benzyl ester (0.5 g), in pyridine (30 ml) was added acetic anhydride (0.11 g), and the mixture refluxed for 2 h; the reaction mixture was then evaporated to a gum (0.6 g), which was dissolved in chloroform (80 ml), washed with 2N-sulphuric acid (2 × 40 ml), saturated sodium hydrogencarbonate solution (40 ml), and water (40 ml), dried (Na_2SO_4), and evaporated to a gum. This was dissolved in dry toluene (1 ml), applied to a silica-gel column (2 × 30 cm), and eluted with ethyl acetate–toluene (3 : 7). The *oxazinone ethanolate* was obtained by evaporation of an ethanol solution as a solid (0.27 g), which retained solvent but was homogeneous on t.l.c. in two solvent systems (Found: C, 53.0; H, 5.6; N, 10.3%; M^+, 365. $C_{16}H_{19}N_3O_7{\cdot}C_2H_5OH$ requires C, 52.55; H, 6.1; N, 10.20%; *M*, 365); $\lambda_{max.}$(MeOH) 268 (ε 5 600), 237 (9 300), and 208 nm (11 800); $\nu_{max.}$ 1 370 (CMe_2) and 1 740 cm^{-1} (C=O); ^{1}H n.m.r.; δ($CDCl_3$) 5.98 (H-1′) ($J_{1',2'}$ 4 Hz), 7.76 (H-2), 2.48 (Me-3), 2.04 (MeCO-5′), and 1.6 and 1.38 (CMe_2) (Δδ 0.22). The oxazinone was also formed when 5-amino-1-(2,3-*O*-isopropylidene-5-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxylic acid (0.341 g) was heated in boiling pyridine in the presence of acetic anhydride (0.21 g); the product was isolated and purified as before.

N-*Succinimidyl*-5-*amino*-1-(2,3-O-*isopropylidene*-5-O-*acetyl*-β-D-*ribofuranosyl*)*imidazole*-4-*carboxylate* (11k).—Benzyl 5-amino-1-(2,3-*O*-isopropylidene-5-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxylate (6.1 g) in dry ethyl acetate (200 ml) was hydrogenated over 10% palladium–charcoal (350 mg). The solution gelled after 2 h and examination by t.l.c. [system (A)] revealed a single spot, R_F 0.0—0.05. The imidazolecarboxylic acid gave a Bratton–Marshall dyestuff and also released carbon dioxide from carbonates.

The mixture was cooled to 0 °C, and DCC (3.4 g) was added followed by *N*-hydroxysuccinimide (1.8 g); the reaction was then stirred at room temperature for 60 h. The mixture was filtered, precipitate was washed with ethyl acetate, and the combined filtrates and washings were evaporated to *ca.* 30 ml; acetic acid (0.7 g) was then added and the mixture stirred for 1 h. The solid was filtered off, washed with ethyl acetate, and the filtrate and washings were combined and evaporated to a gum, which was dissolved in dichloromethane, washed with water (2 × 50 ml), and dried (Na_2SO_4). Evaporation of the solution gave a gum, from which a solid was obtained by adding dry ether to a solution of the gum in dichloromethane–benzene (1 : 1) (30 ml). The *succinimidyl ester* (5.5 g), m.p. 110—120 °C, was purified by precipitation from the same solvent mixture and obtained as a solid, homogeneous on t.l.c. in two solvent systems [(A) and (B)] (Found: C, 49.05; H, 5.1; N, 12.45%; M^+, 438. $C_{18}H_{22}N_4O_9$ requires C, 49.3; H, 5.05; N, 12.75%; *M*, 438); $[\alpha]_D^{25}$ −58.4° (*c* 1.0 in CH_2Cl_2); $\lambda_{max.}$(MeOH) 277 (ε 15 100) and 235 nm (4 400); ^{1}H n.m.r.; δ($CDCl_3$) 5.7 (H-1′) ($J_{1',2'}$ 4 Hz), 7.4 (H-2), 1.62 and 1.4 (CMe_2) (Δδ 0.22), 2.88 (CH_2), and 2.06 (5′-MeCO).

Dimethyl N-[5-*amino*-1-(2,3-O-*isopropylidene*-5-O-*acetyl*-β-D-*ribofuranosyl*)*imidazole*-4-*carbonyl*]-L-*aspartate* (17a).—To *N*-succinimidyl-5-amino-1-(2,3-*O*-isopropylidene-5-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxylate (2.5 g) in dry dichloromethane (25 ml) was added dimethyl L-aspartate (1.15 g) and the mixture stirred for 48 h. The volume was adjusted to 50 ml with dichloromethane, and the solution successively washed with water (35 ml), 5% acetic acid (50 ml), 5% sodium carbonate (50 ml), and water (2 × 35 ml). The dried (Na_2SO_4) solution was evaporated to a gum, applied to a column of silica gel (2 × 30 cm), and the *imidazole riboside* (2.75 g) eluted with ethanol–chloroform (1 : 99) and was evaporated with ethyl acetate to give a solid foam [homogeneous on t.l.c. in the solvent systems (A) and (B)] which retained ethyl acetate (Found: C, 50.25; H, 6.15; N, 10.25%; M^+, 484. $C_{20}H_{28}N_4O_{10}{\cdot}CH_3CO_2C_2H_5$ requires C, 50.35; H, 6.35; N, 9.8%; *M*, 484); $[\alpha]_D^{25}$ −17.3° (*c* 1.0 in CH_2Cl_2); $\lambda_{max.}$(MeOH) 268 (ε 8 500) and 206 nm (7 650); the Bratton–Marshall dyestuff had $\lambda_{max.}$ 550 nm; ^{1}H n.m.r.; δ($CDCl_3$) 5.68 (H-1′) ($J_{1',2'}$ 4 Hz), 7.38 (H-2), 1.62 and 1.40 (CMe_2) (Δδ 0.22), and 2.1 (5′-MeCO).

Dimethyl N-[5-*Amino*-1-(2,3-O-*isopropylidene*-β-D-*ribofuranosyl*)*imidazole*-4-*carbonyl*]-L-*aspartate* (17b).—To the

foregoing acetylated ester (2.2 g) in dry methanol (75 ml) was added sodium (30 mg) and the solution stirred for 2 h. The solution was then stirred with Amberlite CG-120 (H^+ form) resin (3 g) for 30 min, after which a flame test for sodium proved negative. The solution was evaporated to a gum and re-evaporated with ethyl acetate (50 ml) to give the *peptide nucleoside* (1.45 g) as a solid which retained ethyl acetate but was homogeneous on t.l.c. in two solvent systems [(A) and (B)] (Found: C, 49.7; H, 6.05; N, 10.95%; M^+, 442. $C_{18}H_{26}N_4O_9 \cdot CH_3CO_2C_2H_5$ requires C, 49.80; H, 6.45; N, 10.55%; *M*, 442); $[\alpha]_D^{25}$ −7.7° (*c* 1.0 in CH_2Cl_2); $\lambda_{max.}$(MeOH) 268 (ε 7 000) and 232 nm (ε 5 900); the Bratton–Marshall dyestuff had $\lambda_{max.}$ 550 nm; ^{1}H n.m.r.; δ($CDCl_3$) 5.64 (H-1′) ($J_{1',2'}$ 2 Hz), 7.34 (H-2), and 1.58 and 1.36 (CMe_2) (Δδ 0.22).

Diethyl N-[5-*Amino*-1-(2,3-O-*isopropylidene*-5-O-*acetyl*-β-D-*ribofuranosyl*)*imidazole*-4-*carbonyl*]-threo-DL-β-*methyl*-*aspartate* (17c).—The succinimidyl ester (5.7 mmol) and diethyl *threo*-DL-β-methylaspartate,[3] similarly gave the *peptide nucleoside* (75%) as a solid foam (3.75 g) which retained solvent but was homogeneous on t.l.c. in two solvent systems [(A) and (B)] (Found: C, 52.9; H, 6.8; N, 8.9%; M^+, 526. $C_{23}H_{34}N_4O_{10} \cdot CH_3CO_2C_2H_5$ requires C, 52.75; H, 6.9; N, 9.1%; *M*, 526); $[\alpha]_D^{25}$ 0° (*c* 1.0 in CH_2Cl_2); $\lambda_{max.}$(MeOH) 268 (ε 7 000) and 207 nm (6 850); the Bratton–Marshall dyestuff had $\lambda_{max.}$ 550 nm; ^{1}H n.m.r.; δ($CDCl_3$) 5.72 (H-1′) ($J_{1',2'}$ 4Hz), 7.42 (H-2), 1.6 and 1.38 (CMe_2) (Δδ 0.22), 2.1 (5′-MeCO), and 1.25 (C-Me).

Diethyl N-[5-*Amino*-1-(2,3-O-*isopropylidene*-β-D-*ribofuranosyl*)*imidazole*-4-*carbonyl*]-threo-DL-β-*methylaspartate* (17d).—The foregoing 5′-*O*-acetyl derivative with sodium ethoxide in absolute ethanol gave the *peptide riboside* (1.7 g) as a solid, homogeneous on t.l.c. in two solvent systems [(A) and (B)] but retaining ethyl acetate (Found: C, 51.95; H, 6.4; N, 9.80%; M^+, 484. $C_{21}H_{32}N_4O_9 \cdot CH_3CO_2C_2H_5$ requires C, 52.45; H, 7.1; N, 9.8%; *M*, 484); $[\alpha]_D$ −7.04° (*c* 1.0 in CH_2Cl_2); $\lambda_{max.}$(MeOH) 268 (ε 9 400) and 230 nm (7 950); the Bratton–Marshall dyestuff had $\lambda_{max.}$ 550 nm; ^{1}H n.m.r.; δ($CDCl_3$) 5.66 (H-1′) ($J_{1',2'}$ 4 Hz), 7.36 (H-2), 1.58 and 1.36 (CMe_2) (Δδ 0.22), and 1.25 (C-Me).

Diammonium N-[-5-*Amino*-1-β-D-*ribofuranosylimidazole*-4-*carbonyl*]-threo-DL-β-*methylaspartate*.—The foregoing isopropylidene derivative in aqueous acetic acid (100 ml, pH 3) was heated at 100 °C for 1.5 h. The cooled solution was evaporated to a gum and re-evaporated with water (3 × 50 ml). The residue was dissolved by shaking with barium hydroxide solution (150 ml, 0.015M) and then shaken for a further 2 h. Sulphuric acid (0.5M, 30 ml) was added, the barium sulphate centrifuged down and the cake extracted with water (2 × 10 ml). The combined supernatants were adjusted to pH 7 with ammonia solution (*d* 0.88), diluted to 3 l, and the solution applied to a column of Bio-Rad AG1X2 resin (OH^- form, 0.9 × 15 cm). The column was washed with water (1 l) and elution commenced with 0.008N-hydrochloric acid. The riboside was eluted between 2.06 and 2.53 l, the solution adjusted to pH 7.0 with barium hydroxide, evaporated to *ca.* 5 ml, ammonium sulphate solution added, the barium sulphate centrifuged down, and the cake extracted with water (2 × 5 ml). The combined supernatants were evaporated to *ca.* 4 ml and the *riboside peptide hydrate* was obtained as a freeze-dried solid (0.304 g), homogeneous on t.l.c. in two solvent systems [(A) and (B)] (Found: C, 30.55; H, 6.15; N, 15.35. $C_{14}H_{26}N_6O_9 \cdot 7H_2O$ requires C, 30.65; H, 7.35; N, 15.3%); $[\alpha]_D^{25}$ −11.7° (*c* 1.0 in H_2O); $\lambda_{max.}$ (pH 3) 270 nm (ε 6 900); $\lambda_{max.}$ (pH 10) 268 nm (ε 7 850); the Bratton–Marshall dyestuff had $\lambda_{max.}$ 552 nm; ^{1}H n.m.r.; δ($CD_3)_2SO$ 5.54 (H-1′) ($J_{1',2'}$ <1 Hz) and 1.25 (C–Me).

5-*Amino*-1-β-D-*ribofuranosylimidazole*-4-*carbonyl*-L-*aspartic Acid* 5′-*Phosphate* (1a).—To dimethyl *N*-[5-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carbonyl]-L-aspartate (1 g) in acetonitrile (20 ml) at −40 °C, was slowly added pyrophosphoryl chloride (0.9 ml) in acetonitrile (1.1 ml). The reaction was set aside for 3 h at room temperature then cooled to −20 °C and added dropwise to ice-cold barium acetate (3.6 g) in water (60 ml). The solution was adjusted to pH 6.5 with saturated barium hydroxide solution and boiled for 5 min, then filtered through Celite and the residue extracted with hot water (3 × 50 ml). The combined filtrates were acidified to pH 3 with acetic acid and the solution heated at 100 °C for 1.75 h. The cooled solution was evaporated to dryness and re-evaporated with water (3 × 50 ml). The solids formed were dissolved in barium hydroxide solution (150 ml) and stirred for 2 h at room temperature. Sulphuric acid (0.5M) was added and the precipitated barium sulphate removed by centrifugation. The combined supernatants were adjusted to pH 7.5 with ammonia solution (*d* 0.88), filtered, and diluted to 2 l. The solution was applied to a column of Bio-Rad AG-1X2 resin (Br^- form, 2 × 30 cm). The column was washed with water (1 l) and elution commenced with 0.02N-hydrogen bromide. The SAICAR, contaminated with some CAIR, was eluted between 220 and 670 ml. The solution was adjusted to pH 2 with 1N-hydrogen bromide, boiled for 5 min, cooled, adjusted to pH 7.5 with ammonia solution (*d* 0.88), diluted to 1 l, and applied to a column of Bio-Rad AG-1X2 resin (Br^- form, 0.9 × 15 cm). The column was washed with water (500 ml) and the product eluted with 0.008N-hydrogen bromide. The purified SAICAR was isolated as the barium salt (0.55 g) and purified by re-precipitation. The physical properties of the product were identical to an authentic sample.

N-[5-*Amino*-1-β-D-*ribofuranosylimidazole*-4-*carbonyl*]-threo-DL-β-*methylaspartic Acid* 5′-*Phosphate* (1c).—The product was prepared by phosphorylation of dimethyl *N*-[5-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carbonyl]-*threo*-DL-β-methylaspartate with pyrophosphoryl chloride using the method described for SAICAR. The β-D-riboside obtained after the first chromatographic purification, when examined in the t.l.c. system (D), revealed only a trace of β-CAIR to be present. The further purified β-D-riboside was isolated as the barium salt (0.42 g). The product showed physical properties analogous to the L-isomer sample synthesised earlier.[3]

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