Purines, Pyrimidines, and Imidazoles. Part XXVII.[1] The Synthesis of Some Derivatives and Analogues of *N*-(5-Amino-1-β-D-ribofuranosylimidazole-4-carbonyl)-L-aspartic Acid 5′-Phosphate (SAICAR) including a Competitive Enzyme (Adenylosuccinate AMP-Lyase No. 4.3.2.2.) Inhibitor-*N*-(5-Amino-1-β-D-ribofuranosylimidazole-4-carbonyl)-L-threo-β-methyl Aspartic Acid 5′-Phosphate

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Several analogues of *N*-(5-amino-1-β-D-ribofuranosylimidazole-4-carbonyl)-L-aspartic acid 5′-phosphate (SAICAR) have been prepared by reaction of 5-amino-1-β-D-ribofuranosylimidazole-4-carboxylic acid with a number of α-amino-acids and dicyclohexylcarbodi-imide, and phosphorylation of the products. One of the derivatives of SAICAR namely *N*-(5-amino-1-β-D-ribofuranosylimidazole-4-carbonyl)-L-*threo*-β-methylaspartic acid 5′-phosphate was a competitive inhibitor of the enzyme adenylosuccinase. The mechanism of the enzyme-catalysed deamidation of SAICAR is discussed.

THE aminoimidazole carboxyamide ribotide (Ia) (AICAR) and the succino-derivative (Ib) (SAICAR) are important intermediates in the biosynthesis *de novo* of purine nucleotides,[2] and undergo the enzyme-catalysed interconversion SAICAR $\rightleftharpoons$ AICAR + fumarate.[3,4] A similar step occurs later in the same pathway involving the interconversion of the succinoadenylic acid derivative (IIa) (Succino-AMP) and adenylic acid (IIb) (AMP).[5,6]

$$\text{Succino-AMP} \rightleftharpoons \text{AMP} + \text{fumarate}$$

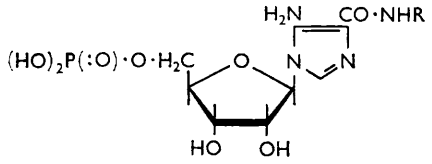
The same enzyme, adenylosuccinase (Adenylosuccinate AMP-lyase No. 4.3.2.2.) has been shown to be involved in each reaction.[7]

An examination of the reverse reactions of fumarate with either AICAR or AMP catalysed by adenylosuccinase in a tritiated aqueous medium has shown that the distribution of tritium in the aspartate grouping [8] of the resulting SAICAR or Succino-AMP was the same as that resulting from the addition of ammonia to fumarate and catalysed by a bacterial aspartase.[9] This last reaction is believed to proceed by a *trans*-mechanism

[1] Part XXVI, I. E. Burrows and G. Shaw, *J. Chem. Soc.* (*C*), 1967, 1088.
[2] S. C. Hartman and J. M. Buchanan, *Adv. Enzymol.*, 1959, **21**, 199.
[3] L. N. Lukens and J. M. Buchanan, *Fed. Proc.*, 1956, **15**, 305.
[4] J. Miller, L. N. Lukens, and J. M. Buchanan, *J. Biol. Chem.*, 1959, **234**, 1806.
[5] G. E. Carter and L. H. Cohen, *J. Biol. Chem.*, 1956, **222**, 17.
[6] G. E. Carter and L. H. Cohen, *J. Amer. Chem. Soc.*, 1955, **77**, 499.
[7] J. S. Gotts and E. G. Gollub, *Proc. Nat. Acad. Sci. U.S.A.*, 1957, **43**, 826.
[8] J. M. Buchanan and J. Miller, *J. Biol. Chem.*, 1962, **237**, 491.
[9] R. A. Alberty, ' The Enzymes,' Academic Press, New York, 1961, vol. 5, ch. 28.

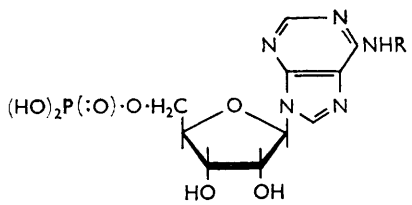
and hence the same mechanism is assigned to the reactions involving the succino-derivatives.

At the same time, in the cleavage of the tritium-labelled succino-derivatives no isotope effect was observed [8] as compared with the unlabelled species suggesting that the β-proton of the aspartate grouping is removed in a non-rate limiting step of the reaction. The analogous systems involving the hydration of fumarate to malate and aconitate to isocitrate and the amination of mesaconate to β-methylaspartate,[9] appear to operate by similar stereospecific mechanisms. In all



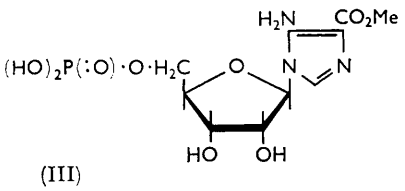
(I)

a; R = H
b; R = HC·CO$_2$H
H$_2$C·CO$_2$H
c; R = HO$_2$C·CH
MeCHCO$_2$H
d; R = HC·CO$_2$H
MeCHCO$_2$H
e; R = HC·CO$_2$H
Me$_2$C·CO$_2$H
; R = HC·CO$_2$H
CH$_2$
H$_2$C·CO$_2$H
g; R = CH$_2$·CO$_2$H



(II)

a; R = CH·CO$_2$H
CH$_2$CO$_2$H
b; R = H



(III)

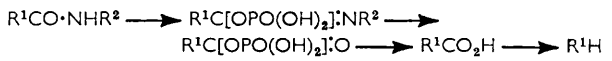
these systems therefore, the stereospecificity involves the configuration of the α-carbon atom and the mechanism of the removal of the β-proton.

If the mechanism ascribed to the cleavage of SAICAR is correct, then we consider it of interest to examine a variety of derivatives of the nucleotide but especially those in which the L-aspartate moiety was substituted in the β-position in the two possible ways. We hoped that such studies might lead to useful information about the stereochemical requirements of adenylosuccinase, the mechanism of its action, and also to the formation of compounds with competitive enzyme-inhibitory properties.

[10] G. Shaw and D. V. Wilson, *J. Chem. Soc.*, 1963, 1077.
[11] G. Shaw, D. V. Wilson, and C. P. Green, *J. Chem. Soc.*, 1964, 2650.

Earlier,[10-12] we outlined various methods for the preparation of derivatives and analogues of AICAR (Ia) and SAICAR (Ib) in which the aminoimidazole ester (III) was a common starting material.

We now report the preparation of analogues of SAICAR (Ib) in which the L-aspartate part of the molecule is replaced by D-, L-, and DL-*threo*-β-methylaspartate (Ic), DL-*erythro*-β-methylaspartate (Id), D-aspartate, DL-ββ-dimethyl aspartate (Ie), L-glutamate (If), and glycine (Ig), by hydrolysis of the aminoimidazole ester riboside (III)[13] with alkali and condensation of the resulting carboxylic acid with the appropriate amino-acid ester and dicyclohexylcarbodi-imide in pyridine, phosphorylation of the intermediate products with 2-cyanoethyl-phosphate and dicylohexyl carbodi-imide,[14] and finally hydrolysis with acid and alkali to remove protecting groups. The method which is analogous to one recorded by us in an earlier publication [10] has been considerably improved and much better yields are now obtainable. SAICAR (Ib) itself has also been resynthesised in this manner and in better yield than previously reported by us. Although losses in this reaction sequence are in part due to decarboxylation of the aminoimidazole-4-carboxylic acid we have noted that phosphorylation of the corresponding amides under various conditions equally appears to lead to some decarboxylation suggesting that *O*-phosphorylation is occurring as follows:



In a forthcoming publication we hope to present further detailed work related to this series of reactions.

TABLE 1

Absorption Spectra (λ in mμ) of SAICAR and some derivatives and analogues*

Compound	λ_{max} (1)	$\varepsilon_{max.}$(1)	$\lambda_{max.}$(2)	$\varepsilon_{max.}$(2)	Optical density ratios 250:260	540:600
Ib [a]	269	11,850	244	9580	0·96	1·23
Ic [b]	269	10,750	244	8500	0·98	1·32
Ic [a]	269	12,680	244	9100	0·96	1·30
Ic [c]	269	11,300	244	8550	0·98	1·28
Id [b]	269	11,650	244	7900	1·10	1·26
Ie	269—270	11,800	244	9000	0·96	1·30
If	270	12,200	245	8500	1·00	1·23
Ig	270	11,500	245	8100	0·98	1·26
Ib [c]	269	11,000	245	8800	0·96	1·25

* Measurements of ultraviolet absorption spectra carried out at pH 1.

[a] Product derived from the L-amino-acid. [b] Product derived from the DL-amino-acid. [c] Product derived from the D-amino-acid. [d] Wavelength measurements of Bratton–Marshall spectra; $\lambda_{max.}$ for all compounds in this Table after the text was 550—560 mμ.

The structures assigned to the SAICAR analogues were confirmed in the following ways. (*a*) The similarity of their ultraviolet absorption spectra to that of SAICAR (Table 1). (*b*) The formation in each case of an unstable

[12] R. Carrington, G. Shaw, and D. V. Wilson, *J. Chem. Soc.*, 1965, 6864.
[13] G. Shaw and D. V. Wilson, *J. Chem. Soc.*, 1962, 2937.
[14] G. M. Tener, *J. Amer. Chem. Soc.*, 1957, **79**, 1002.

TABLE 2

Electrophoretic, thin-layer, and paper chromatographic data on SAICAR and some derivatives and analogues

Compound	R_F in solvent systems * A	B	C	D	E	F	G	Electrophoresis-position of spot from origin in cm.[d]
Ib [a]	0·15, 0·18	0·25, 0·28	0·18, 0·20	0·95, —	0·58, 0·71	0·28, 0·32	0, 0·02	14·2
Ic [b]	0·15, 0·18	0·25, 0·28	0·18, 0·20	0·95, —	0·58, 0·71	0·28, 0·32	0, 0·02	14·2
Ic [a]	0·15, 0·18	0·25, 0·28	0·18, 0·20	0·95, —	0·58, 0·71	0·28, 0·32	0, 0·02	14·2
Ic [c]	0·15, 0·18	0·25, 0·28	0·18, 0·20	0·95, —	0·58, 0·71	0·28, 0·32	0, 0·02	14·2
Id [d]	0·15, 0·18	0·25, 0·28	0·18, 0·20	0·95, —	0·58, 0·71	0·28, 0·32	0, 0·02	12·3
Ie	0·16, 0·18	0·25, 0·28	0·19, 0·20	0·95, —	0·58, 0·71	0·28, 0·32	0, 0·02	13·8
If	0·16, 0·20	0·25, 0·28	0·19, 0·22	0·95, —	0·60, 0·71	0·28, 0·32	0, 0·02	14·0
Ig	0·16, 0·21	0·28, 0·29	0·20, 0·23	0·95, —	0·61, 0·71	0·28, 0·34	0, 0·02	13·6
Ib [e]	0·15, 0·18	0·25, 0·28	0·18, 0·20	0·95, —	0·58, 0·71	0·28, 0·32	0, 0·02	14·2

* The first figure in each case refers to paper chromatographic results and the second to results from ascending thin-layer chromatography. *a*, *b*, and *c* as in Table 1. [d] Paper electrophoresis was carried out in the Shandon High Voltage equipment on Whatman No. 1 chromatographic grade paper saturated with a citrate buffer, pH 4·5, over 40 min. at 5° with 6000 v and 18 ma.

diazonium salt, reportedly characteristic of SAICAR [7,10] but now extended by these related compounds, which in the Bratton–Marshall assay under the usual condition at room temperature fails to give a colour if coupling is delayed for 5 min.; in order to obtain a reasonable colour yield, diazotisation and coupling must be carried out quickly at low temperature and under these conditions the spectra of the dyestuff formed in each case were very similar (Table 1); (*c*) Hydrolysis of each compound with 5N-hydrochloric acid at 120° when in each case the appropriate amino-acid and in all cases glycine was formed. (*d*) Their similar behaviour on paper and thin-layer chromatograms and on paper electrophoresis (Table 2). (*e*) Phosphate or other microanalysis (Table 4). The compounds also had similar specific optical rotations (Table 4).

TABLE 3

Paper chromatography of products of hydrolysis of SAICAR and some derivatives and analogues *

Compound Hydrolysed	Amino-acid produced	R_F of amino-acids [d] solvent systems A	H	J
Ib [a]	L-Aspartic acid	0·33	0·20	0·20
Ic [b]	DL-*threo*-β-Methylaspartic acid	0·30	0·18	0·12
Ic [a]	L-*threo*-β-Methylaspartic acid	0·30	0·18	0·12
Ic [c]	D-*threo*-β-Methylaspartic acid	0·30	0·18	0·12
Id [b]	DL-*erythro*-β-Methylaspartic acid	0·30	0·18	0·12
Ie	DL-di-β-Methylaspartic acid	0·32	0·23	0·11
If	L-Glutamic acid	0·33	0·25	0·15
Ig	Glycine	0·35	0·25	0·15
Ib [e]	D-Aspartic acid	0·33	0·20	0·20

* The hydrolysate from each nucleotide contained glycine R_F 0·33, 0·23, and 0·15 in solvent systems A, H, and J.

a, *b*, and *c* as in Table 1. [d] Samples of the authentic amino-acids were run on the same paper as the hydrolysates.

Each of the compounds and the nucleoside corresponding to SAICAR was examined both as a possible substrate for the enzyme adenylosuccinase and as an inhibitor of the SAICAR $\rightleftharpoons$ AICAR reaction step. The results revealed that none of the above-mentioned analogues of SAICAR, not excluding the nucleoside, was able to act as a substrate for adenylosuccinase, but L- and to a lesser extent D-*threo*-β-methyl SAICAR (Ic) inhibited the conversion SAICAR ⟶ AICAR. From Lineweaver–Burk curves (Figure 1) obtained from experiments carried out in the absence of inhibitor, the Michaelis constant for the latter reaction was found to be $1·4 \times 10^{-4}$ mole/l. compared with figures of $1·9 \times 10^{-4}$ and $1·1 \times 10^{-4}$ mole/l. quoted at different times by

TABLE 4

Analytical results on SAICAR derivatives and analogues

Compound	Yield (%)	Found (%) P	Formula	Reqd. (%) P	$[\alpha]_D^{21}$ [d]
Ib [a]		4·15	$C_{13}H_{15}Ba_2N_4O_{12}P$	4·25	−26·07
Ic [b]	26·4	4·05	$C_{14}H_{17}Ba_2N_4O_{12}P$	4·2	
Ic [a]	11·3	4·10	$C_{14}H_{17}Ba_2N_4O_{12}P$	4·2	−24·20 [f]
Ic [c]	10·7	4·05	$C_{14}H_{17}Ba_2N_4O_{12}P$	4·2	−27·78 [g]
Id [b]	9·8	4·0	$C_{14}H_{17}Ba_2N_4O_{12}P$	4·2	−26·17
Ie	8·5	4·15	$C_{15}H_{19}Ba_2N_4O_{12}P$	4·2	−26·50
If	21·8	4·1	$C_{14}H_{17}Ba_2N_4O_{12}P$	4·2	−26·30
Ig	9·1	5·2	$C_{11}H_{13}Ba_{1\frac{1}{2}}N_4O_{10}P$	5·2	−25·25
Ib [e]	28·2	4·2	$C_{13}H_{15}Ba_2N_4O_{12}P$	4·25	−22·13

a, *b*, and *c* As in Table 1. [d] Determinations were carried out on a Perkin-Elmer model 141 photoelectric polarimeter at *c* 1·5 in 0·1N-hydrochloric acid. [e] Recorded value $[\alpha]_D^{26}$ −26·3° (*c* 1 in 0·1M-sodium hydrogen carbonate).[16] [f] L-*threo*-β-Methylaspartic acid had $[\alpha]_D^{21}$ +12·9°; the recorded value is +13·4°.[15] [g] D-*threo*-β-Methylaspartic acid had $[\alpha]_D^{21}$ −12·3°; the recorded value is −13·4°.[15]

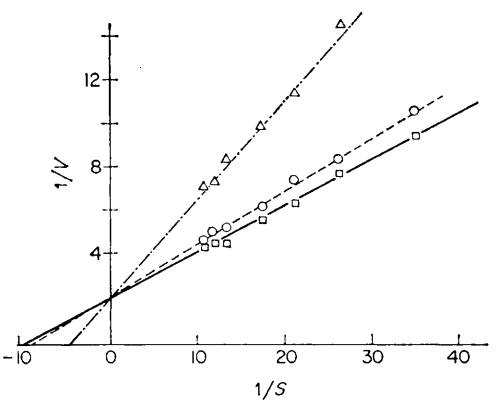


FIGURE 1 The effect of D- and L-β-methyl SAICAR on the enzyme-catalysed conversion SAICAR ⟶ AICAR (△ = SAICAR + L-*threo*-β-methyl SAICAR, ○ = SAICAR + D-*threo*-methyl SAICAR, □ = SAICAR; *v* = velocity measured as the absorbance at 540 mμ in the modified Bratton–Marshall assay after 15 min, *s* = volume of SAICAR solution)

[15] J. P. Greenstein, *Biochem. Prep.*, 1961, **8**, 96.
[16] H. T. Huang, *Biochemistry*, 1965, **4**, 58.

Buchanan.[8] The inhibition constant for the L-*threo*-isomer (Ic) was found to be $13{\cdot}0 \times 10^{-4}$ mole/l. and that for the D-isomer $0{\cdot}9 \times 10^{-4}$ mole/l. We feel fairly confident however that most, if not all, of the latter's activity is due to contamination with the L-isomer. The method of preparation of the D- and L-*threo*-β-methyl aspartic acids by preferential hydrolysis of the *N*-acetyl derivatives with pig renal acylase II makes impurities in the D-isomer most likely since it is recovered from a mother-liquor; this is confirmed by measurements of optical rotations of the amino-acids (see footnotes to Table 4).

DISCUSSION

It would appear that the enzymic deamidation of SAICAR involves the fully *transoid* staggered structure for the substrate (Figure 2) and elimination occurs by an *E*2 reaction in which the four centres concerned are coplanar. Inspection of the *threo*- and *erythro*-β-methyl SAICARs (Figures 3—6) shows that when they are in the required conformation for the elimination reaction only the *threo*-isomers have the two leaving groups in the favourable *trans* position. In the *erythro*-series (Figures 5 and 6) the β-methyl group becomes *trans* to the amide group. As might be expected, therefore, the *erythro*-isomers are neither metabolised by the enzyme nor do they act as competitive inhibitors. However, the marked competitive inhibition of adenylosuccinase exhibited by the L-*threo*-isomer (Figure 3) suggests that

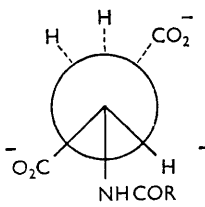


FIGURE 2

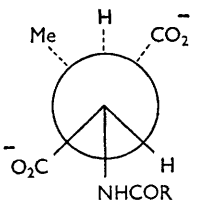


FIGURE 3

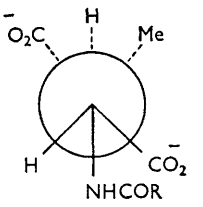


FIGURE 4

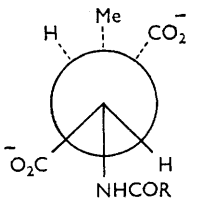


FIGURE 5

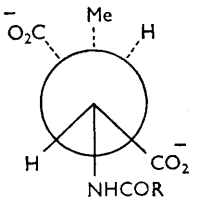


FIGURE 6

a *trans* arrangement of the amide group and β-hydrogen atom is essential if the substrate is to fit on the enzyme surface in the correct manner. Subsequent metabolism of substrate is inhibited, however, by the adjacent methyl group (Figure 3) and is clearly a highly specific process. We suggest that the mechanistic course of the reaction involves controlled acid–base catalysis by the enzyme in which protonation of the amide group from a suitable proton-donating site on the enzyme surface is accompanied by concomitant release of the β-proton and this is enhanced by a proton-acceptor suitably orientated on a different part of the enzyme surface (Figure 7).

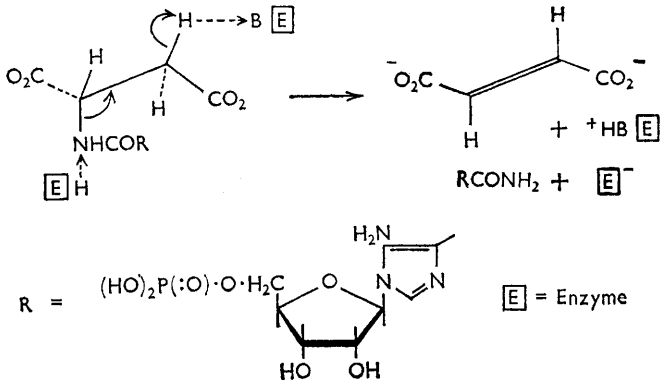


FIGURE 7

EXPERIMENTAL

Unless otherwise stated, evaporations were carried out in a Büchi rotary evaporator, under water-pump vacuum, with a flask temperature of 38° or less. Paper chromatograms were run on Whatman No. 1 paper in the solvents (A) n-butanol–acetic acid–water (12 : 3 : 5), (B) isobutyric acid–ammonia (*d* 0·88)–water (66 : 1 : 33), (C) butan-2-one–acetic acid–water (9 : 2·5 : 3), (D) 5% aqueous potassium hydrogen phosphate–isoamyl alcohol (1 : 1), (E) ethanol–0·1M-acetic acid (1 : 1), (F) n-butanol–acetic acid–water (50 : 25 : 25), (G) isopropanol–water–ammonia (*d* 0·88) (70 : 20 : 10), (H) acetone–urea–water (60 : 0·5 : 40), (J) phenol–water (80 : 20) [all in volume proportions except for (J) w/w and urea in (H)]. Thin-layer chromatograms were run on Cellulose (Whatman CC 41) coated glass plates (20 × 20 cm.) in the above solvent systems. Spots were detected by an ultraviolet lamp or by a modified Bratton–Marshall test. Ion-exchange separations were performed in an apparatus, all Teflon or glass, equipped with Buchler micropump for accurate control of flow rates, 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. All resins used for ion-exchange chromatography were an analytical grade of Dowex prepared and marketed by Bio-Rad Laboratories, Richmond, California. Pyridine used was British Drug Houses anhydrous grade. Fresh pig kidneys required for the preparation of renal acylase II were obtained from the Shelf Bacon Factory Ltd., and yeast for the preparation of adenylosuccinase from the United Yeast Co., Brighouse. Ultraviolet spectra were measured on a Perkin-Elmer 137 UV or Unicam SP 500 spectrophotometers.

DL-ββ-*Dimethyl Aspartic Acid.*—Diethyl acetamidomalonate (33 g.) in ethanol (80 ml.) with sodium (4 g.) in ethanol (75 ml.) was set aside for 20 min.; the sodio-derivative was precipitated. Ethyl 2-bromoisobutyrate (40 g.) was slowly added to the mixture which was heated on a steam-bath for 7 hr. and then evaporated and the residue mixed with water (50 ml.). The suspension was extracted with ether (6 × 25 ml.), and the combined extracts were washed with water and then evaporated; the residue was heated on a steam-bath with water (50 ml.) and 10N-hydrochloric acid (150 ml.) for 8 hr. The solution was evaporated to small

volume and then evaporated several times with water and then finally taken to dryness. The residue was dissolved in ethanol (100 ml.) and aniline was added to the solution until Congo Red paper no longer turned blue; a white crystalline solid separated. The amino-acid (5·8 g.) recrystallised from water as prisms, m. p. 265—273° (decomp.) (Found: C, 44·7; H, 6·85; N, 8·7. Calc. for $C_6H_{11}NO_4$: C, 44·65; H, 6·85; N, 8·7%).

Diethyl Esters of Substituted Aspartic Acids.—Diethyl ester hydrochlorides of D- and L-aspartic acid, D- and L-*threo*-β-methylaspartic acid, DL-*erythro*-β-methylaspartic acid and DL-ββ-dimethylaspartic acid were prepared by the following general procedure. Hydrogen chloride was passed through a mixture of the amino-acid (1 g.) in ethanol (20 ml.) for 1 hr. which was then set aside for 1 hr. The solution was evaporated and re-evaporated several times with ethanol. The residue was washed with ether, and crystallised from methanol–ether (13 : 7). Crystallisation was generally slow and in some cases (*e.g.*, the *erythro*-derivatives) took several weeks. New compounds prepared include diethyl D-threo-ββ-*methylaspartate hydrochloride*, prisms, m. p. 118° (Found: C, 45·15; H, 7·55; Cl, 14·85; N, 5·9. $C_9H_{18}NO_4Cl$ requires C, 45·1; H, 7·55; Cl, 14·8; N, 5·85%); diethyl L-threo-β-*methylaspartate hydrochloride*, prisms, m. p. 118° C, 45·15; H, 7·55; Cl, 14·85; N, 5·85%); diethyl DL-erythro-β-*methylaspartate hydrochloride*, prisms, m. p. 114° (Found: C, 45·15; H, 7·6; Cl, 14·9; N, 5·85%) and *diethyl* DL-ββ-*dimethylaspartate hydrochloride* (Found: C, 47·5; H, 7·9; Cl, 14·05; N, 5·6. $C_{10}H_{20}ClNO_4$ requires C, 47·45; H, 7·9; Cl, 14·05; N, 5·55%). The free amino-esters were obtained by neutralisation of ethanolic solutions of the hydrochloride with solid silver carbonate. The filtered solutions were evaporated and the residues were distilled under reduced pressure to give colourless oils, b. p.s at 0·1 mm., 100° (L-diethylaspartate), 102° (D-diethylaspartate), 114° (diethyl DL-*threo*-β-methylaspartate), and 120° (diethyl DL-di-ββ-methylaspartate); they were stable at −18° over a 3—5 month period. When kept for longer times, the esters gradually solidified to white solids.

N-(5-*Amino*-1-β-D-*ribofuranosylimidazole*-4-*carbonyl*)-DL-threo-β-*methylaspartic Acid* 5′-*Phosphate*.—A solution of methyl 5-amino-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-imidazole-4-carboxylate [13] (0·98 g.) in ethanol (12 ml.) and N-sodium hydroxide (12 ml.) was boiled under reflux for 2 hr. and then concentrated to half volume; an equal volume of pyridine was then added. The solution was placed on a jacketed column (20 × 1·7 cm.) of Zeo-Karb 225 (pyridine form) resin which was maintained at 2°. The column was eluted with 50% aqueous pyridine at 2° and diazotisable amine (detected by the Bratton–Marshall test) was collected in fractions (20 ml.) 2—6. These were combined and evaporated at room temperature four times, with addition of dry pyridine, to give a final volume of 10 ml. (A). To this cooled solution (5 ml.) was added diethyl DL-*threo*-β-methylaspartate (0·4 g.) and dicyclohexylcarbodi-imide (0·45 g.) and the mixture was set aside for 60 hr. at room temperature. The solution was again cooled to 0° and treated with 2-cyanoethyl phosphate [4 mmole in dry pyridine (10 ml.)] followed by dicyclohexylcarbodi-imide (4 g.), and was then set aside at room temperature for 48 hr.

The solution was diluted with water (5 ml.) and kept for 1 hr.; it was then evaporated several times with water until free from pyridine. The residue was heated with 10% aqueous acetic acid (40 ml.) on a water-bath for 1 hr.; the solution was then evaporated to dryness and the residue was heated with 0·5N-lithium hydroxide for a further hour on a water-bath. The cooled solution was filtered and the residue was washed with water (5 × 20 ml.); the combined filtrate and washings were adjusted to pH 7·2 with N-hydrobromic acid and the solution was evaporated to *ca.* 50 ml. Barium bromide (1 g.) was added and the clarified solution was treated with ethanol (200 ml.) and set aside overnight at 4°. The resulting solid precipitate (1·13 g.) was collected at the centrifuge, washed with ethanol and ether, and dried. The solid was mixed with water (200 ml.), and the solution was filtered free from precipitate; it was then added to a column (12 × 0·5 cm.) of Dowex analytical grade 1 × 8 (200—400 mesh) resin in the Br-form. The column was washed with water (480 ml.) and then eluted with 0·008N-hydrobromic acid at a flow rate of 40 ml./hr. Ultraviolet-absorbing material was obtained after 1·4—1·52 l. of eluent had been used; the material had the same Bratton–Marshall test behaviour as SAICAR. The solution was adjusted to pH 7·2 with barium hydroxide solution, and was then concentrated to 40 ml.; it was clarified and diluted with ethanol (200 ml.) and then set aside overnight at 4°. The resultant precipitate was collected by centrifugation, washed with ethanol and ether, and dried. The purified barium salt (0·088 g.) was free from ultraviolet-absorbing and ninhydrin-reacting impurities, and had a very similar spectra to that of SAICAR (Table 1). Furthermore, it gave a purple colour in the Bratton–Marshall assay analogous to that of SAICAR and had similar thin-layer and paper chromatographic and electrophoretic behaviour (Table 2). It retained barium carbonate (Found: C, 18·8; H, 3·0; Ba, 43·0; N, 5·85; P, 3·2. $C_{14}H_{17}Ba_2N_4O_{12}P,BaCO_3,4H_2O$ requires C, 18·75; H, 2·85; Ba, 43·0; N, 5·85; P, 3·25%).

Following exactly the same general procedure as outlined above, and using the same molar quantities of materials the following compounds were prepared: *N*-(5-amino-1-β-D-ribofuranosylimidazole-4-carbonyl)-D- and L-*threo*- and DL-*erythro*-β-methylaspartic acid, DL-ββ-dimethylaspartic acid, L-glutamic acid, glycine, and D-aspartic acid 5′-phosphates. Phosphate analyses, yields, and optical rotations are recorded in Table (4), paper chromatographic and electrophoretic data in Table (2), and ultraviolet absorption spectra in Table (1).

The compounds were further characterised by hydrolysis with 5N-hydrochloric acid at 120° for 2 hr. when in addition to glycine the appropriate aspartic acid derivative or relative were identified by paper chromatography (Table 3).

Measurement of the Effect on the Enzymic Conversion of SAICAR to AICAR by Various Analogues of SAICAR.—Into each of two tubes was pipetted the following solutions in the order given: (*a*) phosphate buffer pH 7·2 (0·5 ml.), (*b*) 7·5% aqueous trichloroacetic acid (0·1 ml.) (second tube only), (*c*) water to a final volume of 1·4 ml., (*d*) stock solution of SAICAR (0·1 ml., $1·6 \times 10^{-6}$M), and (*e*) a stock solution of adenylosuccinase (0·1 ml.). Into a further two tubes was placed as before, (*a*) phosphate buffer pH 7·2 (0·5 ml.), (*b*) 7·5% aqueous trichloroacetic acid (0·1 ml.) (second tube only), (*c*) water to a final volume of 1·4 ml., (*d*) stock solution of SAICAR (0·1 ml.), (*e*) stock solution of SAICAR analogue (0·1 ml.) of concentrations ranging from $1·5 \times 10^{-7}$ to $1·9 \times 10^{-7}$M, and (*f*) stock solution of adenylosuccinase (0·1 ml.). All four tubes were incubated at 38° for 15 min. The enzyme in tubes 1 and 3 was destroyed by addition to each of 7·5% aqueous trichloroacetic acid solution (0·1 ml.) The amount of AICAR present in the tubes was determined by the following assay procedure. Precipitates were

removed by centrifugation and to the supernatant phase (0·5 ml.) was added 0·1% sodium nitrite solution (0·1 ml.), then after 8 min. 0·5% ammonium sulphamate solution (0·1 ml.), and after 3 min. 0·1% aqueous α-naphthylethylene diamine dihydrochloride solution (0·1 ml.). After a further 1 min. the solutions were accurately diluted to 5 ml. with water and the concentration of AICAR calculated from the optical density of the resulting purple dyestuff was measured at its maximum absorption (540 mμ) assuming a molecular extinction coefficient of 26,000. The results of these experiments are described in the Discussion section.

Measurement of the Inhibition Constant for N-(5-*Amino*-1-β-D-*ribofuranosylimidazole*-4-*carbonyl*)-L-threo-β-*methyl-aspartic Acid* 5′-*Phosphate and the Michaelis Constant for the Conversion of SAICAR to AICAR*.—Into a series of paired tubes was pipetted the stock SAICAR solution (0·01 to 0·1 ml. in increments of 0·01 ml.). To each tube was also added a solution of the L-*threo*-β-methyl SAICAR (0·1 ml. $1{\cdot}7 \times 10^{-7}$M). The tubes were then incubated with adenylosuccinase as in the foregoing experiment with identical solvents, reagents, and pipetting sequences. The experiments were repeated but without addition of the methyl SAICAR to the incubation tubes. All sets of experiments with attendant blanks were performed under identical conditions and for the same periods of time.

The results are recorded as Lineweaver–Burk plots (Figure 1 and Discussion section) and indicate that inhibition of adenylosuccinase by the L-*threo*-β-methylaspartic acid analogue of SAICAR is of a competitive nature.

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