Synthesis of Analogues of 5-Aminoimidazole Ribonucleotides and their Effects as Inhibitors and Substrates of the Enzymes, Phosphoribosylaminoimidazole Carboxylase and Phosphoribosylaminoimidazolesuccinocarboxamide Synthetase Involved in the Biosynthesis of Purine Nucleotides *de novo*

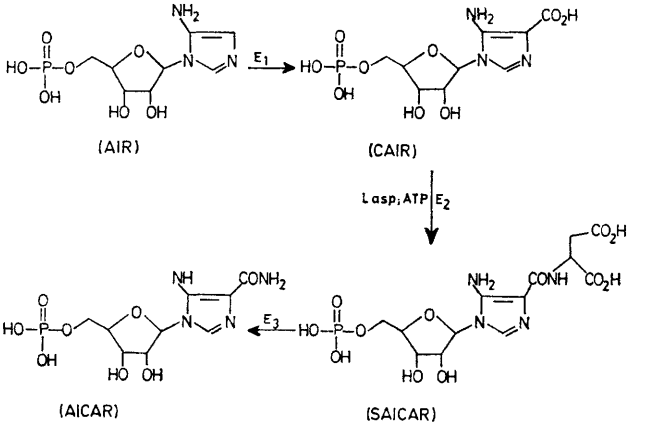
By GRAHAME MACKENZIE, GORDON SHAW, and SUSAN ELIZABETH THOMAS
(*School of Chemistry, University of Bradford, Bradford* BD7 1DP)

Summary α- and β-D-Arabinofuranosyl, α- and β-D-xylofuranosyl nucleotide analogues and the 2-bromo derivatives of 5-amino-1-β-D-ribofuranosylimidazole 4-carboxylic acid 5′-phosphate (CAIR), a central intermediate in the *de novo* biosynthetic pathway to purine nucleotides, and its α-anomer have been synthesised by sequences of reactions from 2,3,5-tri-*O*-benzoyl-D-arabinofuranosyl azide, 3,5-*O*-isopropylidene-D-xylofuranosylamine or ethyl 5-amino-1-α- and β-D-ribofuranosyl-imidazole 4-carboxylates, respectively; the β- but not the α- forms are shown to have inhibitory activity on the enzyme duet AIR-carboxylase and SAICAR-kinosynthetase whilst the arabinose and xylose analogues of CAIR were also converted by a mixture of pathway enzymes through at least two steps into the arabinose and xylose analogues of 5-amino-1-β-D-ribofuranosylimidazole 4-carboxamide 5′-phosphate (AICAR), respectively.

A CENTRAL part of the reaction sequence in the *de novo* biosynthesis of purine nucleotides involves various 5-aminoimidazole ribonucleotides (Scheme).

We have been interested in preparing structural analogues of the aminoimidazole nucleotides and investigating their effects as potential inhibitors of the enzymes involved in their biochemical interconversion.

Reaction of methyl α-D-2,3,5-tri-*O*-benzoylarabinofuranoside[1] with HBr in acetic acid gave an anomeric mixture of glycosyl bromides which with NaN_3 in acetonitrile solution gave the crystalline arabinofuranosyl azide (Ia), m.p. 82 °C, 89% yield. Hydrogenation of the azide in ethyl acetate using Adam's catalyst and condensation of the resulting glycosylamine (Ib) with the formimidate[2] (II) gave, after chromatography on silica gel, the crystalline β-nucleoside (IIIa), m.p. 152 °C, 10·5% yield, and the α-derivative (IIIb), M^+, 599, 19·5% yield, as a solid (pure on t.l.c.). Debenzoylation of the nucleosides with dry



SCHEME

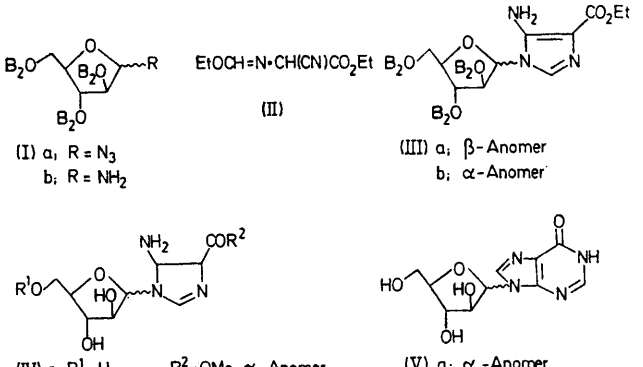
methanolic NaOMe was exceptionally slow and was accompanied by *trans* esterification and *O*-alkyl fission to give the α- (IVa) and β-(IVb) methyl esters, which were separated from corresponding carboxylic acids (IVc) and (IVd) formed in the same reaction, by chromatography on SP-Sephadex (H^+ form). The structures assigned to the two esters were confirmed by n.m.r. spectroscopy (IVa): H-1 (δ; $J_{1',2'}$/Hz) (5·48; 4) and (IVb) (5·76; 4), in $(CD_3)_2SO$ and their con-

TABLE

Activity of CAIR Analogues

Compound	Inhibitory activity: AIR → CAIR Conc. /mM	AIR → CAIR %	Inhibitory activity: CAIR → SAICAR Conc. /mM	CAIR → SAICAR %	Substrate activity as % of that shown by CAIR: AIR-Carboxylase Conc. /mM	AIR-Carboxylase %CAIR	SAICAR Kinosynthetase Conc. /mM	SAICAR Kinosynthetase %CAIR
α-CAIR	1·2	0	—	—	0·12	0	1·2	0
α-Xylo-CAIR (VIIIc)	3·3	0	—	—	0·13	0	3·6	0
β-Xylo-CAIR (VIIId)	0·64	11	0·3	48	0·05	0	0·6	5
	0·8	21	0·6	74				
	1·06	28						
	1·33	37						
α-Ara-CAIR (IVi)	2·6	0	—	—	0·13	0	2·6	0
β-Ara-CAIR (IVj)	1·3—3·9	50	0·7	50	0·13	0	0·7	50
	0·08—1·8	40	1·4	50	—	—	1·4	50
	0·08—1·8	50						
α-2-Br-CAIR (XIc)	0·27	0	—	—	0·08	0	0·27	0
	0·54	0						
β-2-Br-CAIR (XId)	0·35	30	—	—	—	—	0·35	0
	1·0	40	—	—	—	—	1·0	0

version with aqueous ammonia into corresponding α- (IVe), m.p. 207—209 °C, and β- (IVf), m.p. 190 °C[3] carboxyamides. The carboxyamides with HCO_2Et and NaOEt gave the corresponding arabinofuranosyl hypoxanthines α- (Va), m.p. 211 °C (58% yield) and β-(Vb), m.p. 239—241 °C (46% yield). The latter compound was identical with an authentic sample.[4] Phosphorylation of (IVa) and (IVb)



(I) a; R = N_3
b; R = NH_2

(II)

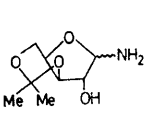
(III) a; β-Anomer
b; α-Anomer

(IV) a; R^1 = H, R^2 = OMe, α-Anomer
b; R^1 = H, R^2 = OMe, β-Anomer
c; R^1 = H, R^2 = OH, α-Anomer
d; R^1 = H, R^2 = OH, β-Anomer
e; R^1 = H, R^2 = NH_2, α-Anomer
f; R^1 = H, R^2 = NH_2, β-Anomer
g; R^1 = $PO(OH)_2$, R^2 = OMe, α-Anomer
h; R^1 = $PO(OH)_2$, R^2 = OMe, β-Anomer
i; R^1 = $PO(OH)_2$, R^2 = OH, α-Anomer
j; R^1 = $PO(OH)_2$, R^2 = OH, β-Anomer

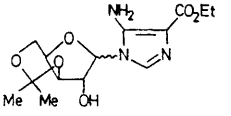
(V) a; α-Anomer
b; β-Anomer

with $POCl_3$ in $(EtO)_3PO$ gave the 5′-phosphates α- (IVg) (31% yield) and β-(IVh) (33%) which were purified by chromatography on Bio-Rad AG1 × 2 (Br^- form) resin. Solutions of the arabinofuranosyl carboxylic acid analogues of CAIR (Scheme) α- (IVi) and β- (IVj) were prepared by alkaline hydrolysis of the corresponding esters using the method used successfully for the analogous ribonucleotides.[4]

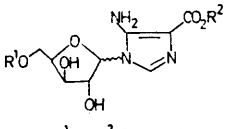
Reaction of 3′,5′-*O*-isopropylidene-D-xylofuranosylamine[6] (VI) toluene-*p*-sulphonate, the formimidate (II), and NaOEt in ethanol–acetonitrile solutions gave, after chromatography on silica gel, the α-xylofuranosyl nucleoside (VIIa), m.p. 178 °C, 15% yield, identical with an authentic specimen (i.r., mixed m.p., n.m.r., t.l.c.) and the β-anomer (VIIb) as a solid (pure on t.l.c.) (M^+ 327, 3% yield). The structures of these nucleosides were also confirmed by n.m.r. spectroscopy



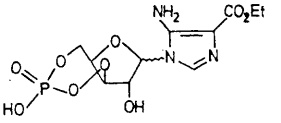
(VI)



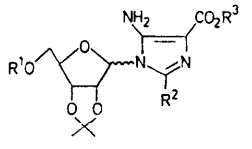
(VII) a; α-Anomer
b; β-Anomer



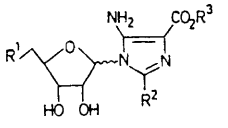
(VIII) a; R^1 = H, R^2 = Et, α-Anomer
b; R^1 = H, R^2 = Et, β-Anomer
c; R^1 = $PO(OH)_2$, R^2 = H, α-Anomer
d; R^1 = $PO(OH)_2$, R^2 = H, β-Anomer



(IX) a; α-Anomer
b; β-Anomer



(X) a; R^1 = R^2 = H, R^3 = Et, α-Anomer
b; R^1 = R^2 = H, R^3 = Et, β-Anomer
c; R^1 = H, R^2 = Br, R^3 = Et, α-Anomer
d; R^1 = H, R^2 = Br, R^3 = Et, β-Anomer



(XI) a; R^1 = $PO(OH)_2$, R^2 = Br, R^3 = Et, α-Anomer
b; R^1 = $PO(OH)_2$, R^2 = Br, R^3 = Et, β-Anomer
c; R^1 = $PO(OH)_2$, R^2 = Br, R^3 = H, α-Anomer
d; R^1 = $PO(OH)_2$, R^2 = Br, R^3 = H, β-Anomer

(VIIa), H-1′ (δ; $J_{1',2'}$/Hz) (5·90; 3) and (VIIb) (5·48; 3) in $(CD_3)_2SO$. The isopropylidene nucleosides with aqueous acetic acid gave the corresponding α- (VIIIa) m.p. 222 °C and β- (VIIIb), solid (pure on t.l.c.) (M^+ 287), nucleosides. Phosphorylation of (VIIIa) and (VIIIb) with $POCl_3$ in $(EtO)_3PO$ and chromatography of the products on Bio-Rad AG1 × 2 (Br^- form) resin gave the 3′,5′-cyclic nucleotides (IXa) (34% yield) and (IXb) (29%), respectively. The cyclic phosphate structures were confirmed by the failure of the nucleotides to react with alkaline phosphatase. A similar conversion of 9-β-D-xylofuranosyl adenine into the corresponding 3′,5′-cyclic phosphate (38% yield) has been recorded.[8] Hydrolysis of each imidazole cyclic phosphate

with aqueous alkali gave the corresponding xylose analogues of α- and β-CAIR (Scheme) namely (VIIIc) and (VIIId) both of which showed positive reactions towards alkaline phosphatase, but probably contained small amounts of the corresponding 3-phosphates.

The aminoimidazole ester nucleosides (Xa) and (Xb)[5] with bromine in aqueous dioxan containing Na_2HPO_4 gave the α- (Xc) as a solid (pure on t.l.c.) and β- (Xd), m.p. 221 °C, 2-bromo nucleosides, respectively. N.m.r. spectroscopy of (Xc): H-1′, (δ; $J_{1',2'}$/Hz) (5·88; 4) and (Xd) (6·30; 4) in $CDCl_3$ confirmed the anomeric configurations and 2-bromo substitution on the imidazole ring [as indicated by the absence of the H-2 singlets (Xa), δ 7·16, and (Xb), δ 7·42]. The bromo nucleosides were phosphorylated with $P_2O_3Cl_4$ in $(EtO)_3PO$ to produce, after acid hydrolysis, the corresponding bromo nucleotides (XIa) and (XIb), respectively. Hydrolysis of each of these with aqueous NaOH gave the corresponding acids (XIc) and (XId). Under the conditions of hydrolysis there was no formation of Br^-.

The direct assay of the enzymes phosphoribosylaminoimidazole carboxylase (E.C. 4.1.1.21, AIR-carboxylase) (E_1) and phosphoribosylaminoimidazolesuccinocarboxyamide synthetase (E.C. 6.3.2.6-SAICAR-kinosynthetase) (E_2) has been recorded elsewhere.[9] These assay procedures have been used to examine the inhibitory and substrate properties on these enzymes of the arabinose, xylose and 2-bromoribose nucleotides described above. The results (Table) may be summarised as follows. (i) The α-analogues including the α-anomer of CAIR (Scheme) were all without inhibitory or substrate activity. (ii) The three β-analogues (IVj), (VIIId), and (Xh) had inhibitory activity against E_1, E_2, and E_2. (iii) Inhibition of E_1, E_2, or of E_2 by the arabinose analogue (IVj) reaches a maximum of 50% and additional amounts of inhibitor failed to increase this figure. (iv) In addition the arabinose analogue of AIR (Ara-AIR) (Scheme) [prepared by chemical decarboxylation of (IVj)] was not a substrate for E_1 but (IVj) is converted with a mixture of enzymes into the arabinose analogue of AICAR (Ara-AICAR) (Scheme). Clearly therefore Ara-CAIR and Ara-SAICAR can both act as substrates for the appropriate enzymes. The xylose analogue (VIIId) of CAIR (Xylo-CAIR) was similarly converted into the xylose analogue of AICAR (Xylo-AICAR) in contrast to 2-bromo-CAIR (XId) which was not a substrate for E_2.†

(*Received, 5th April* 1976; *Com.* 363.)

† Satisfactory analytical, t.l.c. and spectral data were obtained for all new compounds.

[1] H. G. Fletcher, Jr., *Methods in Carbohyd. Chem.*, 1963, **2**, 228.
[2] D. H. Robinson and G. Shaw, *J.C.S. Perkin I*, 1972, 1715.
[3] J. A. Montgomery and H. J. Thomas, *J. Medicin Chem.*, 1972, **15**, 1334.
[4] E. J. Reist, A. Benitez, L. Goodman, B. R. Baker, and W. W. Lee, *J. Org. Chem.*, 1962, **27**, 3274.
[5] N. J. Cusack, B. J. Hildick, D. H. Robinson, P. W. Rugg, and G. Shaw, *J.C.S. Perkin I*, 1973, 1720.
[6] N. J. Cusack, D. H. Robinson, P. W. Rugg, G. Shaw, and R. Lofthouse, *J.C.S. Perkin I*, 1974, 73.
[7] D. H. Robinson and G. Shaw, *J.C.S. Perkin I*, 1974, 774.
[8] M. Hubert-Habart and L. Goodman, *Chem. Comm.*, 1969, 740.
[9] C. A. H. Patey and G. Shaw, *Biochem. J.*, 1973, **135**, 543; G. Shaw and S. E. Thomas, *J. Neurochem.*, in the press.