of products which was chromatographed on alumina. Elution with petroleum ether (bp 60–80°) gave 80 mg (28%) of 2,4,5-triphenyl-1,2,3-triazole (**18**), mp 124° (mmp). Further elution of the column with a mixture (3:1) of petroleum ether and benzene gave 10 mg (3%) of benzil osazone, mp 234° (mmp). No other product could be isolated from this run.

Registry No.—**4**, 28638-85-9; **7**, 834-27-5; **12**, 17679-79-7; **14**, 28595-91-7; **24**, 18039-45-7; dimethyl acetylenedicarboxylate, 762-42-5; dimethyl fumarate, 624-49-7; dimethyl maleate, 624-48-6.

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A New Synthesis of 5-Amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (AICA Riboside) *via* the Reduction of 1-(β-D-Ribofuranosyl)-5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (DIC Riboside)[1]

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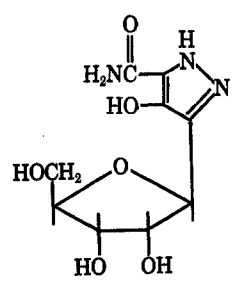
1-(β-D-Ribofuranosyl)-5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (**5**, DIC riboside) and 1-(β-D-ribofuranosyl)-4-(3,3-dimethyl-1-triazeno)imidazole-5-carboxamide (**6**, iso-DIC riboside) have been synthesized by the direct ribosidation of the trimethylsilyl derivative of 5(4)-(3,3-dimethyl-1-triazeno)imidazole-4(5)-carboxamide (DIC). The assignment of anomeric configuration and proof for the site of glycosidation of these nucleosides (**5** and **6**) were achieved by catalytic cleavage of the –N=N– double bond to afford the imidazole nucleosides 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (**7**) and 4-amino-1-(β-D-ribofuranosyl)imidazole-5-carboxamide (**8**) of established structure.

The isolation and characterization[3] of 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (AICA riboside) from the culture medium of sulfonamide-inhibited *E. coli* was followed by the enzymatic conversion of AICA riboside to 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide 5′-phosphate (AICAR).[4] The synthesis of AICA riboside was subsequently accomplished by ring opening of certain purine nucleosides,[5] fermentation,[6] and direct ribosidation of an imidazole derivative followed by functional group transformations.[7] A renewed interest in the chemical synthesis of AICA riboside and related derivatives has been prompted by the report[6] that AICA riboside can function effectively as a substrate for a kinase which results in a facile conversion of AICA riboside to AICAR.[8] Also of considerable interest is the recent isolation and characterization of pyrazomycin[9] as a five-membered heterocyclic riboside which is structurally very similar to AICA riboside. Previous investigations from our laboratory[10–13] on the direct glycosidation of various imidazole derivatives by the fusion procedure have usually resulted in the successful isolation of only one isomer. However, after the appropriate functional group transformations had been accomplished, the actual site of glycosidation was established as being at the ring nitrogen adjacent to the carboxamide group (iso-AICA riboside and derivatives) rather than the ring nitrogen adjacent to the exocyclic amino group (AICA riboside and derivatives) by ring annulation to afford 7-ribosylpurines. We now wish to report a convenient synthesis of both isomers (AICA riboside and iso-AICA riboside).



pyrazomycin

5(4)-(3,3-Dimethyl-1-triazeno)imidazole-4(5)-carboxamide (DIC)[14] was treated with hexamethyldisilazane to afford the trimethylsilyl derivative **1** which was then condensed with 2,3,5-tri-*O*-acetyl-D-ribofuranosyl bromide in acetonitrile. This procedure furnished two major components (**3**, 17.4%; **4**, 32.2%) which were separated by column chromatography. 1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-4-(3,3-dimethyl-

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(2) The recipient of a University of Utah Research Committee Fellowship, 1968–1970.

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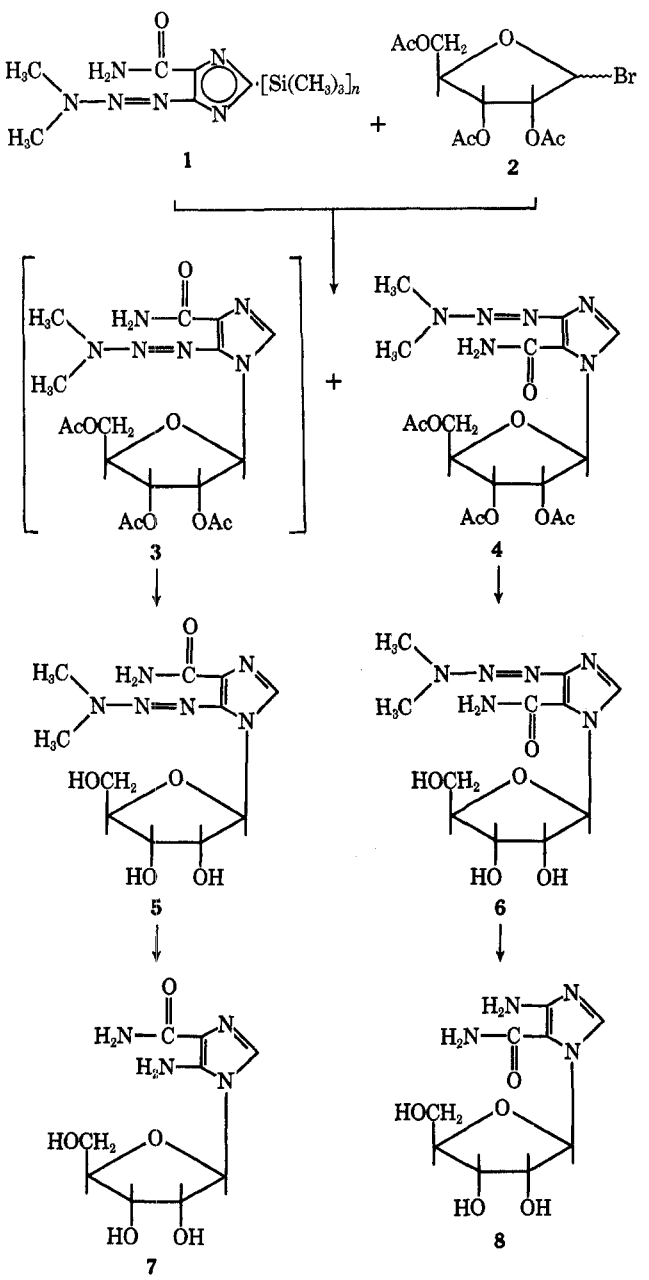
(14) The abbreviations used are DIC, 5(4)-(3,3-dimethyl-1-triazeno)-imidazole-4(5)-carboxamide, NSC-45388; AIC, 4(5)-aminomidazole-5(4)-carboxamide; AICA riboside, 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide; iso-AICA riboside, 4-amino-1-(β-D-ribofuranosyl)imidazole-5-carboxamide; AICAR, 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide 5′-phosphate.

1-triazeno)imidazole-5-carboxamide (4) was crystallized from ethanol, whereas efforts to crystallize 3 (hard foam) from a variety of solvents proved fruitless. Removal of the acetyl groups from 4 by liquid ammonia (24 hr)[15] or methanolic ammonia (16 hr)[13] provided a mixture of 6 and a partially deacetylated product. However, it was found that deacetylation of 4 with methanolic ammonia (4 days) furnished 1-(β-D-ribofuranosyl)-4-(3,3-dimethyl-1-triazeno)imidazole-5-carboxamide (6) in 95% yield as the only product. The same conditions were used to obtain 5 by deacetylation of 3 (Scheme I).

SCHEME I



That complete deacetylation had occurred under these conditions was established by elemental analysis and pmr spectroscopy (DMSO-d_6) obtained for both 5 and 6. A feature common to the spectra of both nucleosides is the very broad resonance (singlet, 6 protons) centered at δ 3.36 for the $N(CH_3)_2$ protons. A 100-MHz spectra of 6 (DMSO-d_6–D_2O) revealed that the *N*-methyl resonances could be separated into two broad singlets (one centered at δ 3.58, the other at δ 3.22) which indicates at least some magnetic nonequivalence for the methyl groups.

It has been demonstrated that 5(4)-(3,3-dimethyl-1-triazeno)imidazole-4(5)-carboxamide (DIC) possesses significant antitumor activity.[16-18] However, the potential of this AIC analog has been limited because of its instability toward light and heat.[19,20] Unlike DIC, the ribosides 1-(β-D-ribofuranosyl)-5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (5) (DIC riboside) and 1-(β-D-ribofuranosyl)-4-(3,3-dimethyl-1-triazeno)-imidazole-5-carboxamide (6) (iso-DIC riboside) have shown remarkable stability toward these factors. Solutions of 5 and 6 when exposed to direct sunlight for a period of 4 days exhibited no appreciable degradation or decomposition (tlc, uv).[19] In addition, 6 was also exposed to long-wave uv light (366 nm) for 18 hr without observing any of the effects reported[20] for DIC under similar conditions.

The assignment of anomeric configuration and site of ribosidation was established unequivocally by the reduction and cleavage of the –N=N– double bond. Treatment of an aqueous ammonical solution of 5 with Raney nickel as the catalyst in a hydrogen atmosphere afforded a product in 81% yield which was chromatographically homogeneous.[21] A comparison (ir, uv,[3] and optical rotation)[6] of this product with an authentic sample of AICA riboside[22] showed them to be identical.[23] This same procedure using 6 has provided nucleoside material in 71% yield which was established by ir, uv, and optical rotation comparisons to be iso-AICA riboside (8).[21] This study has furnished a new synthetic route for the preparation of AICA riboside.[5] The preparation of additional triazenoimidazole ribosides *via* the silylation procedure is under active investigation in this laboratory.

R_f values of 4–8 are given Table I.

Experimental Section

Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. The proton magnetic resonance spectra were obtained on Varian A-60 and XL-100 spectrometers utilizing DSS as an internal standard. The infrared spectra were determined in pressed potassium bromide disks with a Beckman IR-8 spectrophotometer. The ultraviolet absorption spectra were recorded on a Beckman DK-2 spectrophotometer. The optical rotations were obtained with a Perkin-Elmer Model 141 automatic digital readout polarimeter. Silica gel suitable for chromatographic use was purchased from J. T.

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(19) Y. F. Shealy, C. A. Krauth, and J. A. Montgomery, *J. Org. Chem.*, **27**, 2150 (1962).

(20) Y. F. Shealy, C. A. Krauth, S. J. Clayton, A. T. Shortnacy, and W. R. Laster, Jr., *J. Pharm. Sci.*, **57**, 1562 (1968).

(21) Other methods such as 5% palladium on charcoal and sodium dithionite failed.

(22) Purchased from Cyclochemical Co., Los Angeles, Calif. [mp 214–215°, $[\alpha]^{27}D$ −63.7 (*c* 0.5, H_2O)].

(23) J. L. Skibba, D. D. Beal, G. Ramirez, and G. T. Bryan, *Cancer Res.*, **30**, 147 (1970), have recently shown that DIC is metabolized to 5-aminoimidazole-4-carboxamide (AIC); see also G. E. Housholder and T. L. Loo, *Life Sci.*, **8**, 533 (1969).

TABLE I

R_f VALUES OF CERTAIN IMIDAZOLE NUCLEOSIDES[a,b]

No.	Compd	Chromatographic solvent systems[c] A	B	C	D	E
4	1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-4-(3,3-dimethyl-1-triazeno)imidazole-5-carboxamide	0.71	0.84	0.76	0.87	0.90
5	1-(β-D-Ribofuranosyl)-5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide	0.77	0.05	0.62	0.59	0.04
6	1-(β-D-Ribofuranosyl)-4-(3,3-dimethyl-1-triazeno)imidazole-5-carboxamide	0.64	0.17	0.72	0.70	0.21
7	5-Amino-1-(β-D-ribofuranosyl)-imidazole-4-carboxamide (AICA riboside)	0.67	0.07	0.54	0.54	0.10
	5-Amino-1-(β-D-ribofuranosyl)-imidazole-4-carboxamide[d]	0.66	0.07	0.54	0.54	0.10
8	4-Amino-1-(β-D-ribofuranosyl)-imidazole-5-carboxamide (iso-AICA riboside)	0.74	0.06	0.48	0.55	0.07
	4-Amino-1-(β-D-ribofuranosyl)-imidazole-5-carboxamide[e]	0.73	0.06	0.49	0.55	0.07

[a] All compounds were run on Whatman No. 1 chromatographic paper and the descending technique was used. [b] Short-wave ultraviolet light (254 nm) was used to detect the spots. [c] Chromatographic solvent systems: A, 5% aqueous ammonium bicarbonate (w/w); B, 1-butanol saturated with water; C, 1-propanol–ammonium hydroxide (sp gr 0.90)–water, 6:3:1 (v/v); D, ethanol–water, 7:3 (v/v); E, ethyl acetate–1-propanol–water, 4:1:2 (v/v) upper phase. [d] See ref 22. [e] See ref 10.

Baker Chemical Co. Elemental analyses were performed by Heterocyclic Chemical Corp., Harrisonville, Mo.

The trimethylsilyl derivative of 5(4)-(3,3-dimethyl-1-triazeno)imidazole-4(5)-carboxamide (DIC)[24] was prepared using the general procedure of Wittenburg.[25] DIC was added to an excess of hexamethyldisilazane containing a catalytic quantity (10 mg) of ammonium sulfate and the reaction mixture heated at reflux temperature (130°) under anhydrous conditions for 7 hr. During this period a clear solution was effected and the reaction was protected from light. The excess hexamethyldisilazane was then removed under reduced pressure and the oily residue was used in the condensation procedure without further purification.

1-(2,3,5-Tri-*O*-acetyl-β-ribofuranosyl)-5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (3) and 1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-4-(3,3-dimethyl-1-triazeno)imidazole-5-carboxamide (4).—A solution of 2,3,5-Tri-*O*-acetyl-D-ribofuranosyl bromide,[26] prepared from 9.5 g (29.8 mmol) of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose, in dry acetonitrile (40 ml) was added to the trimethylsilyl derivative of DIC prepared from 5.0 g (27.4 mmol) of DIC. The reaction mixture was then stirred at room temperature in a sealed vessel in the dark for 4 days. To this solution was added methanol (15 ml), water (10 ml), and a slight excess of sodium bicarbonate. The mixture was warmed gently on a steam bath for 15 min and then evaporated to dryness under reduced pressure (water bath 40°). The remaining traces of water were removed by coevaporation with absolute ethanol and the residual syrup was extracted with chloroform (three 100-ml portions). The chloroform extract was washed with cold water (three 100-ml portions) and the chloroform phase was dried over anhydrous magnesium sulfate. The chloroform layer was evaporated *in vacuo* to provide a light tan syrup. This syrup was dissolved in ethanol (30 ml) and then allowed to stand at 4° for 12 hr. The crystalline solid (2.49 g) which had separated from solution was collected by filtration and washed with a small amount (10 ml) of cold ethanol. An additional quantity (0.68 g) of **4** was obtained when the above procedure was repeated. The ethanol filtrates were evaporated to a syrup, dissolved in a minimal amount of chloroform, and applied to a silica gel column (2.2 × 70 cm). The column was eluted with chloroform (300 ml) and chloroform–methanol (49:1, v/v, 1.5 l.), with 100-ml fractions being collected. Fractions 6–8 were combined and evaporated to yield 0.71 g of **4** (32.2%). Fractions 10–15 were pooled and evaporated to provide **3** (2.1 g, 17.4%) as a hard foam (homogeneous on tlc). Total yield of nucleoside material was 49.6%.

Recrystallization of **4** from ethanol provided an analytical sample: mp 159–160°; $[\alpha]^{27}D$ −39.2° (*c* 0.98, EtOH); uv λ_{max}^{MeOH} 322 nm (ε 18,277), 237 (12,992); uv λ_{min}^{MeOH} 256 nm (ε 5373); pmr (DMSO-d_6) δ 8.05 (s, 1, 2 H), 7.61 (vbd,[27] 2, $CONH_2$), 3.38 (vbd, 6, NCH_3), 6.80 (d, 1, $J_{1',2'}$ = 4 Hz, 1′ H), 2.11 (s, 9, $COCH_3$).

Anal. Calcd for $C_{17}H_{24}N_6O_8$: C, 46.36; H, 5.49; N, 19.08. Found: C, 46.41; H, 5.44; N, 18.85.

1-(β-D-Ribofuranosyl)-5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (5).—A solution of **3** (2.1 g, 4.76 mmol) in methanol (50 ml, previously saturated at −5° with ammonia) was allowed to stand at room temperature for 4 days in a sealed pressure bottle. The solvent was then removed *in vacuo*, and the residual solid was dissolved in methanol (20 ml) and allowed to stand at room temperature for 12 hr. The crystalline solid which had precipitated was collected by filtration to provide 1.13 g (75.5%) of **5**. An analytical sample was prepared by recrystallization from methanol: mp 215–217°; $[\alpha]^{27}D$ −290.6° (*c* 0.5, H_2O); uv $\lambda_{max}^{pH\ 1}$ 324 nm (ε 16,186), sh 270 (7543), 223 (11,943); $\lambda_{min}^{pH\ 1}$ 249.5 nm (ε 6097); $\lambda_{max}^{pH\ 11}$ 333 nm (ε 13,389), 236.5 (17,537); $\lambda_{min}^{pH\ 11}$ 273 nm (ε 6254); λ_{max}^{MeOH} 336 nm (ε 14,143), 235.5 (15,275); λ_{min}^{MeOH} 267 nm (ε 4777); pmr (DMSO-d_6) δ 7.67 (s, 1, 2 H), 7.00 (vbd, 2, $CONH_2$), 3.35 (vbs, 6, NCH_3), 6.03 (d, 1, $J_{1',2'}$ = 4 Hz, 1′ H).

Anal. Calcd for $C_{11}H_{18}N_6O_5$: C, 42.08; H, 5.78; N, 26.76. Found: C, 42.03; H, 5.73; N, 26.62.

5-Amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (7).—1-(β-D-Ribofuranosyl)-5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (**5**) (0.295 g, 0.93 mmol) was dissolved in 50 ml of water. Raney nickel[28] (1.2 g, wet weight) was added to this solution followed by 5 drops of ammonium hydroxide (sp gr 0.90) and the mixture hydrogenated, with shaking, in a Paar hydrogenator at 40 psi for 6 hr. The catalyst was removed by filtration and washed with water (three 25-ml portions), and the filtrate and washings were combined and evaporated *in vacuo* to afford a pink glass. The glass was dissolved in a small amount of boiling ethanol (15 ml) and allowed to stand at room temperature for 12 hr to yield clusters of pink rosettes (0.195 g, 81%): mp 215–216°[29] (lit.[8] 213–214°); $[\alpha]^{27}D$ −62.4° (*c* 0.495, H_2O) [lit.[6] $[\alpha]^{26}D$ −63.0° (*c* 1.0, H_2O)]; uv, ir, and chromatographic mobilities were identical with those of an authentic sample.[22]

1-(β-D-Ribofuranosyl)-4-(3,3-dimethyl-1-triazeno)imidazole-5-carboxamide (6).—A solution of **4** (2.23 g, 5.06 mmol) in methanol (60 ml, previously saturated at −5° with ammonia) was allowed to stand at room temperature for 4 days in a sealed pressure bottle. The crystalline white solid (1.51 g, 94.6%) which had precipitated was collected by filtration and washed with a small amount of methanol. Recrystallization of the product from water provided an analytical sample as needles: mp (softens at 125° and resolidifies) 218–220°; $[\alpha]^{27}D$ +9.0° (*c* 0.5, H_2O); uv $\lambda_{max}^{pH\ 1}$ 322 nm (ε 21,687), sh 270 (8077); $\lambda_{min}^{pH\ 1}$ 247 (ε 4652); $\lambda_{max}^{pH\ 11}$ 326 nm (ε 18,669), 239 (11,315); $\lambda_{min}^{pH\ 11}$ 258 nm (ε 4872); λ_{max}^{MeOH} 324 nm (ε 18,229), 238.5 (12,949); λ_{min}^{MeOH} 259 nm (ε 5343); pmr (DMSO-d_6) δ 8.18 (s, 1, 2 H), 7.53 (vbd, 2, $CONH_2$), 3.36 (vbd, 6, NCH_3), 6.56 (d, 1, $J_{1',2'}$ = 2 Hz, 1′ H).

Anal. Calcd for $C_{11}H_{18}N_6O_5 \cdot {}^1/_2H_2O$: C, 40.86; H, 5.92; N, 26.00. Found: C, 40.78; H, 6.14; N, 26.03.

4-Amino-1-(β-D-ribofuranosyl)imidazole-5-carboxamide (8).—1-(β-D-Ribofuranosyl)-4-(3,3-dimethyl-1-triazeno)imidazole-5-carboxamide (**6**) (0.200 g, 0.64 mmol) was dissolved in 50 ml of water. Raney nickel[28] (0.80 g, wet weight) was added to this solution followed by 4 drops of ammonium hydroxide (sp gr 0.90) and the mixture hydrogenated, with shaking, in a Paar hydrogenator at 40 psi for 6 hr. The catalyst was removed by filtration and washed with water (three 25-ml portions), and the filtrate and washings were then combined and evaporated *in vacuo* to afford a colorless powder. The powder was dissolved in methanol (10 ml) and allowed to stand at 5° for 18 hr to yield white crystals (0.118 g, 71%): mp 187–189°[29] (lit.[7] 187–189°); $[\alpha]^{27}D$ −29.9 (*c* 0.975, H_2O) [lit.[10] $[\alpha]^{26}D$ −30.9° (*c* 1.0, H_2O)]; uv, ir, and chromatographic mobilities were found to be identical with those of an authentic sample.[10]

Registry No.—**4**, 28405-60-9; **5**, 28405-61-0; **6**, 28455-56-3; **7**, 2627-69-2; **8**, 7132-71-0.

(24) The authors wish to thank Dr. Robert E. Engle, Cancer Chemotherapy National Service Center, National Cancer Institute, for the generous gift of 5(3)-(3,3-dimethyl-1-triazeno)imidazole-4(5)-carboxamide (DIC) and Dr. R. A. Long for helpful suggestions on the synthesis of **7** and **8**.

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(26) H. Zimmer, A. Koine, and H. Nimz, *Chem. Ber.*, **98**, 2705 (1960).

(27) vbd = very broad doublet; vbs = very broad singlet; s = singlet.

(28) Purchased from W. R. Grace and Co., South Pittsburgh, Tenn.

(29) No change on taking mixture melting point with authentic sample.