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Biochemistry

Nonenzymatic synthesis of 5-aminoimidazole ribonucleoside and recognition of its facile rearrangement*

(purine biosynthesis/evolution)

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ABSTRACT **5-Amino-1-β-D-ribofuranosylimidazole 5′-monophosphate (AIR, 1) is the ubiquitous precursor to the purine ribonucleotides *in vivo*, and it serves as the biochemical precursor to the pyrimidine portion of thiamin (vitamin B_1) in certain prokaryotic organisms. The corresponding ribonucleoside (AIRs, 5b) was prepared via chemical (nonenzymatic) synthesis from 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide. The tri-O-acetylated derivative of AIRs (5a) was also prepared, and it was shown to undergo a facile ring transformation in aqueous pH 7 buffer to afford *N*-(imidazol-4-yl)-2,3,5-tri-*O*-acetyl-D-ribofuranosylamine as a 1:2 mixture of α and β anomers (6a). Under similar conditions, compound 5b affords the corresponding unprotected β-ribonucleosides 6b. This Dimroth-type ring transformation reaction of 5 to 6, which occurs primarily in neutral aqueous solution, may be responsible for the previously reported lability of AIRs and its derivatives. It may also have relevance to the postulated early biotic pathway to the 9- and 3-substituted purine nucleotide components of an all-purine biopolymer.**

5-Amino-1-β-D-ribofuranosylimidazole 5′-monophosphate (AIR, **1**; see Scheme I) is the ubiquitous precursor to the purine ribonucleotides *in vivo* (see, for example, ref. 1). Recent studies have characterized both the eukaryotic (2) and prokaryotic (3) forms of the enzymes that catalyze the formation of this ribonucleotide along the *de novo* purine ribonucleotide biosynthetic pathway. In addition to serving in this central metabolic capacity, AIR serves as the biosynthetic precursor of the pyrimidine portion of thiamin (vitamin B_1) in certain prokaryotic organisms. This, too, has been the subject of recent studies (4–7). Biochemical investigations have been hindered somewhat by the lack of a reliable and detailed chemical (nonenzymatic) synthesis of **1** or its corresponding ribonucleoside AIRs (**5b**), even though Shaw and coworkers have done extensive work on the synthesis of model 5-aminoimidazole heterocycles and structurally related nucleosides (8–14). Furthermore, the chemistry of AIRs and related compounds (15) is complicated by their often-mentioned lability during routine chromatographic purification procedures, concentration of solutions, or even dry storage at ambient temperature (2, 6, 16). Due to the pivotal roles that AIR and AIRs play in cellular ribonucleotide and deoxyribonucleotide metabolism, there is much interest in understanding the properties and reactions of these elusive compounds. We now report a short chemical (nonenzymatic) synthesis of AIRs based upon the imidazole chemistry reported by Shaw and coworkers. In addition, we describe herein a facile rearrangement of the tri-O-acetylated AIRs, a representative reaction that may be primarily responsible for the previously reported lability of AIRs and its derivatives.

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EXPERIMENTAL PROCEDURES

General Methods. Radial chromatography was performed on a Chromatotron instrument (Harrison Research, Palo Alto, CA), using Merck silica gel-60 with fluorescent indicator as adsorbant.

NMR. ^1H NMR spectra were recorded on a General Electric GN-500 or QE-300 spectrometer at 500 or 300 MHz, respectively, using deuteriochloroform (C^2HCl_3) or deuterium oxide (^2H_2O) solutions. Chemical shifts are reported relative to internal tetramethylsilane (C^2HCl_3 solutions) or sodium 3-(trimethylsilyl)-1-propanesulfonate (^2H_2O solutions) reference at $\delta = 0.0$ ppm. ^{13}C NMR spectra were recorded at 125 or 75 MHz, with chemical shifts reported relative to internal $CHCl_3$ (C^2HCl_3 solutions) at $\delta = 77.0$ ppm, or dioxane (^2H_2O solutions) at $\delta = 66.0$ ppm.

All expected $^1J_{CH}$ short-range correlations were observed for **5a** and **6a**. The $^2J_{CH}$ and $^3J_{CH}$ long-range correlations observed in the 10-Hz-optimized experiments were H4/C5, H2/C4, H4/C2, and CH_3/CH_3CO_2 for both samples. Correlations observed in the 5-Hz-optimized experiments were H2/C5, H4/C5, CH_3/CH_3CO_2 for both samples; H1′/C2′ for **5a** only, and in the 3-Hz-optimized experiments were H2/C5 for both samples; H1′/C2′ and H1′/C2 for **5a** only.

5-Amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxylic acid (4a). This compound was synthesized as described in ref. 17: mp 135–140°C (loses CO_2); UV λ_{max} = 248 nm and $\varepsilon = 10.8 \times 10^3\ M^{-1}\cdot cm^{-1}$ at pH 7.

5-Amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole (5a). A solution of **4a** (0.2 g, 0.52 mmol) in 0.25 M aqueous NaOAc/AcOH pH 4.8 buffer was allowed to stand for 1.5 hr at 35–40°C under N_2. The product was extracted into $CHCl_3$ and purified by chromatography on Florisil (15% MeOH/85% $CHCl_3$, vol/vol, as eluent) to afford **5a** as a foam-powder in 65% yield: ^1H NMR (C^2HCl_3) $\delta = 7.34$ (H2), 6.43 (H4, slowly exchanges), 5.77 (d, $J = 5.3$ Hz, 1, H1′); ^{13}C NMR (C^2HCl_3) $\delta = 134.9$ (C5), 130.9 (C2), 114.9 (C4); ^{15}N NMR (C^2HCl_3, 50.68 MHz, referenced to external CH_3NO_2 at $\delta = 380$ ppm) $\delta = 55.1$ (NH_2); UV λ_{max} = 210 nm and $\varepsilon = 3.7 \times 10^3\ M^{-1}\cdot cm^{-1}$ at pH 1, λ_{max} = 213 nm and $\varepsilon = 4.4 \times 10^3\ M^{-1}\cdot cm^{-1}$ at pH 7, and λ_{max} = 236 nm and $\varepsilon = 3.5 \times 10^3\ M^{-1}\cdot cm^{-1}$ at pH 11; low-resolution fast atom bombardment (FAB) mass: 342.2 (MH^+), 259.1; high-resolution FAB mass: 342.1299 observed, $C_{14}H_{20}N_3O_7$ requires 342.1301.

AIRs (5b). A solution of **3** (prepared as described in ref. 17) (1.34 g, 4.8 mmol) in 0.25 M aqueous NaOAc/AcOH buffer

Abbreviations: AIR, 5-aminoimidazole ribonucleotide (**1**); AIRs, 5-aminoimidazole ribonucleoside (**5b**); AICARs, 5-aminoimidazole-4-carboxamide ribonucleoside (**2**); FAB, fast atom bombardment; INEPT, insensitive nuclear enhancement by polarization transfer; INEPTD, INEPT-decoupled.

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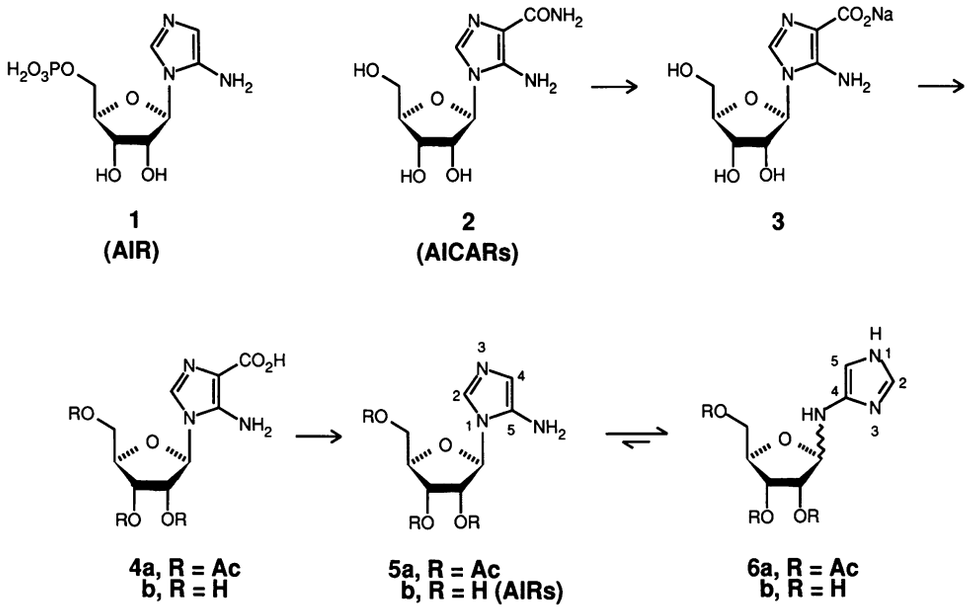

Scheme I. Aminoimidazole nucleoside syntheses. AIR, 5-aminoimidazole ribonucleotide; AIRs, 5-aminoimidazole ribonucleoside; AICARs, 5-aminoimidazole-4-carboxamide ribonucleoside.

(pH 4.8, 50 ml) maintained at 27°C for 15 hr under N_2 afforded, after chromatography (Dowex 50W-X8, NH_4^+-form, 500 ml of water, then 500 ml of 1.0 M NH_4OH as eluent), lyophilization, dissolution in water (50 ml), and relyophilization, **5b** (AIRs, 861 mg, 88% yield) as a gray-white, hygroscopic, fluffy solid: mp 92–94°C. ^1H NMR (^2H_2O) δ = 7.51 (H2), 6.34 (H4, slowly exchanges); ^{13}C NMR (^2H_2O) δ = 135.4 (C5), 131.0 (C2), 112.1 (C4); UV λ_{max} = 210 nm and $\varepsilon = 4.6 \times 10^3$ $M^{-1}\cdot cm^{-1}$ at pH 1, λ_{max} = 214 nm and $\varepsilon = 4.4 \times 10^3$ $M^{-1}\cdot cm^{-1}$ at pH 7, and λ_{max} = 234 nm and $\varepsilon = 3.7 \times 10^3$ $M^{-1}\cdot cm^{-1}$ at pH 11; low-resolution FAB mass: 216.1 (MH^+); high-resolution FAB mass: 216.0977 observed, $C_8H_{14}N_3O_4$ requires 216.0984.

***N*-(Imidazol-4-yl)-2,3,5-tri-*O*-acetyl-α- and β-D-ribofuranosylamines (6a).** ^1H NMR (C^2HCl_3) δ = 9.23, 9.16, 8.04, 7.68 (four s, each exchanges, four NH), 7.61 (H2α), 7.54 (H2β), 6.95 (H5α), 6.85 (H5β), 5.58 (d, J = 1.5 Hz, 1, α-H1′), 5.52 (d, J = 5.5 Hz, 1, β-H1′); ^{13}C NMR (C^2HCl_3) δ = 132.5 (C2β), 131.6 (C2α), 126.3 (C4β), 126.0 (C4α), 122.1 (C5β), 121.2 (C5α); ^{15}N NMR (C^2HCl_3) δ = 111.7, 111.2, 108.1, 107.9 (four d, J = 90 Hz, four NH); UV λ_{max} = 212 nm and $\varepsilon = 5.0 \times 10^3$ $M^{-1}\cdot cm^{-1}$ at pH 1, λ_{max} = 214 nm and $\varepsilon = 5.5 \times 10^3$ $M^{-1}\cdot cm^{-1}$ at pH 7, and λ_{max} = 255 nm and $\varepsilon = 3.3 \times 10^3$ $M^{-1}\cdot cm^{-1}$ at pH 11; low-resolution FAB mass: 342.2 (MH^+); high-resolution FAB mass: 342.1293 observed, $C_{14}H_{20}N_3O_7$ requires 342.1301. The decarboxylation of **4a** in 0.5 M pH 7 sodium phosphate buffer at 27°C for 48 hr affords **6a** in 74% yield. The thermal decarboxylation of **4a** at 155–160°C (neat, for 10 min under N_2) affords a mixture of **5a** and **6a**, isolated by radial chromatography (5% MeOH/95% CH_2Cl_2, vol/vol, as eluent) in 67% and 29% yield, respectively.

RESULTS AND DISCUSSION

The synthesis of AIRs and its tri-O-protected derivative from 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (AICARs, **2**), is shown in Scheme I. AICARs was saponified in 6 M NaOH according to the method of Srivastava *et al.* (17, 18) to afford sodium 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxylate (**3**), which was then acetylated with excess Ac_2O in pyridine below 0°C to give the tri-O-acetylated 5-aminoimidazole-4-carboxylic acid **4a** (17). Compound **3** was converted to the unprotected enamino acid **4b** in a pH 4.8 aqueous NaOAc/HOAc buffer. Compound **4b** is sensitive to decarboxylation (17–20) and readily loses CO_2 to afford the 5-aminoimidazole ribonucleoside **5b** (AIRs). Under similar conditions, **4a** afforded the corresponding tri-O-acetylated 5-aminoimidazole ribonucleoside **5a**. The O-acetylated ribonucleoside **5a** and the unprotected ribonucleoside **5b** exhibit a characteristic high-field H4 proton resonance (6.0–6.5 ppm) in the ^1H NMR spectrum and high-field C4 resonance (112–115 ppm) in the ^{13}C NMR spectrum. The presence of these unusually shielded nuclei is interpreted to suggest that nucleosides **5** exist in solution as species possessing a significant contribution from the imino tautomeric form; thus the C4 ring atoms of nucleosides **5** have more sp^3 character than the single enamino structural representation suggests. Indeed, the presence of the putative imino tautomeric contributor is further indicated by the observation that the H4 protons of **5a** and **5b** undergo a slow exchange with deuterium in neutral ^2H_2O solution at ambient temperature.

The decarboxylation of **4a** to **5a** in aqueous pyridinium acetate buffer solution (pH 7–8) was accompanied by the formation of another nucleoside material that showed the same MH^+ peak at m/z 342 in the low-resolution FAB mass spectrum as in that of **5a**, but (unlike **5a**) had no accompanying peak at m/z 259 ascribable to the tri-O-acetylated ribosyl radical cation. Analysis by high-resolution FAB mass spectrometry confirmed that the new material was isomeric with **5a**, molecular formula $C_{14}H_{19}N_3O_7$. With the aid of a comparison of the high-field ^1H, ^{13}C, short- and long-range two-dimensional ^1H–^{13}C heteronuclear shift correlation, and ^1H-coupled and decoupled (INEPT and INEPTD) natural-abundance ^{15}N NMR spectra of this isomer with those of **5a**, this nucleoside material was identified as a 1:2 mixture of *N*-(imidazol-4-yl)-2,3,5-tri-*O*-acetyl-α-and-β-D-ribofuranosylamines (**6a**). While the three-bond correlation of the anomeric proton (H1′) with the C2 carbon atom of the imidazole ring of **5a** was observed under conditions designed to optimize for intensities of 3-Hz ^1H–^{23}C correlations, no such H1′/C2 correlation was evident in the spectrum of **6a** determined under identical conditions. This comparison indicated a greater than three-bond separation of H1′ and C2 in **6a**. In addition, neither of the imidazole CH protons of **6a** was found to exchange with deuterium in neutral ^2H_2O solution, even at

elevated temperatures. The anomeric assignments (α,β) were made on the basis of the $J_{H1'-H2'}$ values.

The α- and β-ribonucleosides **6a** are formed predominantly upon concentration and/or warming of aqueous solutions of **5a** buffered near neutral pH, even at temperatures as low as 30°C. The use of elevated temperatures results in the rapid and complete conversion of **5a** to **6a**, although **5a** and **5b** isolated as dry powders are stable when stored cold under N_2. The reaction path envisaged for the conversion of **5a** to **6a** contains a formylglycinamidine ribonucleoside intermediate, and is similar to that proposed for the Dimroth rearrangement observed in the case of 1-alkyl-5-amino-1,2,3-triazoles to 4-alkylamino-1,2,3-triazoles (21, 22). The photolytic ring-opening of related imidazoles and their nucleoside derivatives has been shown to proceed via hydration of the C2–N3 double bond (23–25). Unlike the base-promoted Dimroth rearrangement of 1,2,3-triazoles, however, the conversion **5a** to **6a** was found to occur readily under aqueous neutral conditions ($t_{1/2} \approx 15$ hr at pH 6.75, by TLC) and more slowly under aqueous basic (pH 11) and acidic (pH 4) conditions. Upon standing in pH 7 sodium phosphate buffer for 48 hr, AIRs (**5b**) afforded *N*-(imidazol-4-yl)-β-D-ribofuranosylamine (**6b**).

CONCLUSIONS

We describe herein the detailed chemical synthesis of 5-aminoimidazole ribonucleoside (**5b**) and its tri-O-acetylated derivative (**5a**), compounds of interest due to their relation to AIR, the biosynthetic precursor to the 9-substituted purine ribonucleotides. In addition, we have demonstrated that **5a** (and also **5b**) undergoes a facile Dimroth-type rearrangement in pH 7 aqueous phosphate buffer at room temperature. This facile rearrangement, which we believe to be primarily responsible for the previously reported lability of the biologically important AIRs and its derivatives, also has the relevance to the postulated early biotic pathway to the 9- and 3-substituted purine nucleotide components of an all-purine biopolymer (ref. 26; note that the structures in figure 1 of this reference are incorrectly drawn). In this hypothesis, 9-substituted purine nucleotide components base-pair with 3-substituted purine counterparts. The Dimroth-type rearrangement described herein illustrates a potential branch-point in the early biotic pathway to 9- and 3-substituted purine nucleotides, providing that the subsequent biosynthetic steps could generate the 3-substituted purine nucleotides from the ribonucleotide corresponding to our compound **6a**. The great stability of the base pairings of poly(3-isoadenylate) with poly(U) and with poly(I) has been demonstrated (27). In addition, a template-directed oligomerization of an activated form of 3-isoadenosine 5′-monophosphate on a poly(U) template has recently been described (28) as part of an ongoing examination of the properties of 3-ribofuranosylpurine derivatives. This oligomerization (28) was found to be much more efficient than that of the analogously activated form of adenosine 5′-monophosphate (29). Thus, it appears that the 3-substituted purine ribonucleotides are chemically well suited to participate in hydrogen-bonding with appropriate ribonucleotide partners. Finally, the experimental discovery of a Dimroth rearrangement among the precursors of enzymatic purine 9-ribonucleotide synthesis suggests that an aberrant chemical process, albeit slow, may supervene in an *in vivo* system that becomes deficient in the normal enzymatic conversion process.

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