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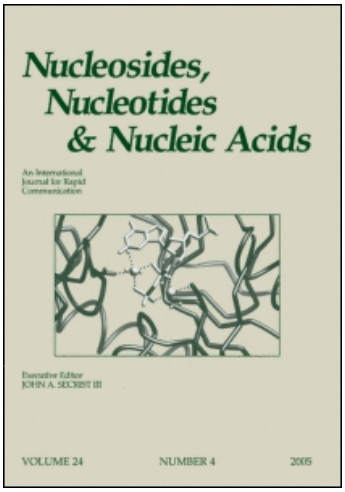

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Synthesis and Mass Spectrometric Fragmentation Characteristics of Imidazole Ribosides-Analogs of Intermediates of Purine De Novo Synthetic Pathway

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SYNTHESIS AND MASS SPECTROMETRIC FRAGMENTATION CHARACTERISTICS OF IMIDAZOLE RIBOSIDES-ANALOGS OF INTERMEDIATES OF PURINE DE NOVO SYNTHETIC PATHWAY

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□ *Two inherited deficiencies have been described in purine de novo synthesis pathway. Both the defects are diagnosed by detecting ribosides—dephosphorylated substrates of the enzymes—in patient's urine. We describe here a synthesis and mass spectrometric fragmentation of ribosides potentially of diagnostic importance for defects in the second part of the pathway. All the species, except 5-amino-4-imidazolesuccinocarboxamideriboside can be synthesized from the commercially available 5-amino-4-imidazolecarboxamideriboside by chemical methods. Fragmentation spectra of the compounds were obtained by the ion trap mass spectrometry. During fragmentation an opening of the imidazole ring was not observed for any of the compounds but loss of its substituents in the form of small molecules (NH_3, CO_2, CO) is the major route of fragmentation. The ribose moiety cleaves off molecule(s) of water, undergoes a cross-ring cleavage or breaks away as a whole.*

Keywords Purine de novo synthesis; Inherited defects; Metabolism

INTRODUCTION

Two inherited enzyme deficiencies have been recognized in purine de novo synthesis (PDNS) pathway—adenylosuccinate lyase (ADSL, EC 4.3.2.2)

Authors contributed equally to the work.

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deficiency and AICA-ribosiduria (defect of bifunctional aminoimidazolecarboxamide ribonucleotide formyltransferase—IMP cyclohydrolase; ATIC, EC 2.1.2.3, 3.5.4.10). Both the defects are diagnosed by detecting ribosides analogous to the substrates of the enzymes. Nucleosides corresponding to substrates of other enzymes of the pathway are not commercially available. The presented communication reports on synthesis and mass spectrometric fragmentation of ribosides related to the second part of the pathway.

MATERIALS AND METHODS

Synthesis and Purification of Imidazoleribosides

5-Amino-4-imidazoleriboside (AIr, Mr 215) is dephosphorylated metabolite of the sixth intermediate of PDNS. We prepared AIr from the commercially available 5-amino-4-imidazolecarboxamideriboside (AICAr, Toronto Research Chemicals Inc., North York, Canada, Mr 258) by alkaline hydrolysis and subsequent decarboxylation.[1] The solution of AICAr (1 g) in NaOH (8 ml, 6 mol/l) was refluxed on water bath (90°C, 24 hours) with the use of KOH pellets as a CO_2 barrier. The mixture was cooled, pH adjusted to 4.7 with chilled acetic acid while shaking, bubbled with argon for 18 hours and evaporated at 90°C using a rotary vacuum vaporizer.

We prepared 5-amino-4-imidazolecarboxyriboside (CAIr, Mr 259) by alkaline hydrolysis of AICAr in NaOH (6 mol/l) as above. After cooling ethanol (8 ml) was added to the mixture and a brown syrup was obtained.[2] The product was triturated with ethanol (3 × 2 ml and 3 × 1 ml), placed in a vacuum dessicator for 24 hours and then triturated with methanol (2 ml). The resulting solid brown matter was dessicated.

5-amino-4-imidazolesuccinocarboxamideriboside (SAICAr, Mr 374) prepared by recombinant adenylosuccinate lyase[3] was kindly provided by Dr. J. Krijt (Prague, Czech Republic).

The synthesis of 5-formylamino-4-imidazolecarboxamideriboside (FAICAr, Mr 286) was similar[4] to phosphorylated penultimate intermediate of PDNS. We added the mixture of formic acid (13.6 ml), NaOH (1.1 g) and acetanhydride (25 ml) to AICAr (250 mg). The solution was heated up (60°C, 1 minute) to initiate reaction, cooled to 25°C and after 10 minutes heated again (50°C, 20 minutes). The product was freeze-dried.

All synthesized products were analyzed by capillary electrophoresis[5] during purification procedures. CAIr and SAICAr contained no by-products. FAICAr (contaminated with AICAr) and AIr (several by-products) were purified using cation exchanger (DOWEX 50WX8).

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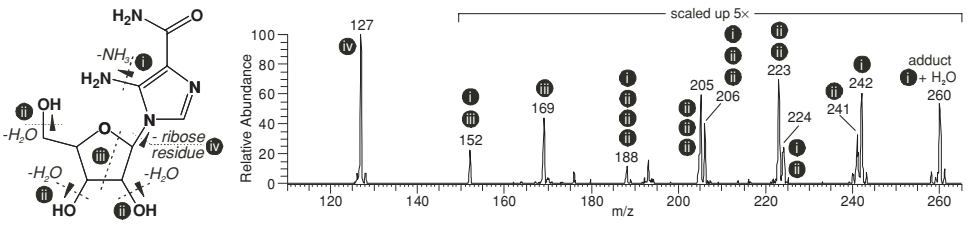

FIGURE 1 Mass spectra and fragmentation of 5-amino-4-imidazolecarboxamideriboside. Wedge-like arrows indicate hydrogen transfer.

Mass Spectrometric Fragmentation of Synthesized Compounds

MS analysis was carried out using an LCQ ion trap mass spectrometer (Finnigan MAT, San Jose, CA, USA). Atmospheric pressure chemical ionization source operated in the positive mode was employed. The discharge current in the ion source was set to 7 μA, vaporizer temperature to 300°C, flow rate of the nebulizing gas to 40 arbitrary units and heated capillary temperature to 150°C.

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RESULTS

Synthesis and purification of the compounds yielded compounds with more than 90% purity. Fragmentation spectra of protonated molecules of the studied compounds were obtained by collision induced dissociation in the ion trap in MS/MS experiment (AIr, AICAr) or by in-source fragmentation in MS experiment (CAIr, SAICAr, FAICAr). The in-source fragmentation may result either from collisions with molecules of gas present in the ion optics adjacent to the ion source or from the effect of heat in the vaporizer of the source.

Mass spectra and fragmentation of AICAr are shown in Figure 1. Fragment ion masses and relative intensity of all compounds studied are summarized in Table 1.

TABLE 1 Fragmentation of Imidazole Ribosides-Analogs of Intermediates of Purine De Novo Synthetic Pathway

Compound	Parent → fragment ion masses and relative intensity
AIr	198 (55%), 180 (51%), 126 (100%), 108 (8%)
CAIr	232 (17%), 216 (100%), 128 (8%), 84 (46%)
SAICAr	357 (46%), 339 (7%), 321 (27%), 303 (24%), 243 (100%), 225 (45%), 216 (14%), 84 (31%)
AICAr	242 (12%), 241 (7%), 224 (5%), 223 (14%), 206 (8%), 205 (12%), 188 (3%), 169 (9%), 152 (5%),127 (100%)
FAICAr	155 (100%), 138 (77%), 127 (12%), 110 (23%)

DISCUSSION

All the structures are related closely to each other and dissociate in similar ways. The imidazole moiety may lose its substituents in the form of small molecules (NH_3–AIr, AICAr, FAICAr; CO_2–CAIr; CO–CAIr, FAICAr). The N-succinnocarboxamide group of SAICAr either loses water from the free carboxyl groups or breaks away as a whole. Opening of the imidazole ring was not observed for any compound. The ribose moiety cleaves off one, 2, or even 3 molecules of water. An alternative to the loss of water molecules is a cross ring cleavage (AIr, AICAr). In all compounds, another frequent way of dissociation is a cleavage of the glycosidic C–N bond with charge retention at the imidazole moiety. While transferring one hydrogen atom to the offspring ion, neutral ribose residue breaks away.

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