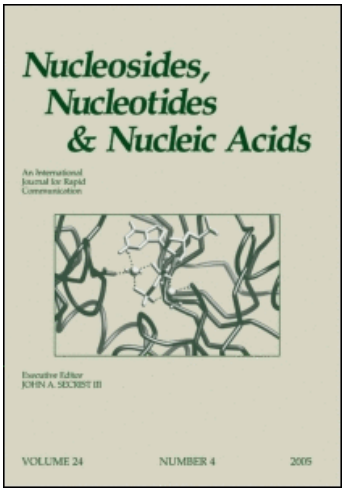

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A Simple Synthesis of 5-Amino-4-imidazolecarboxamide Riboside-5'-Tri-Phosphate: The Proposed Alarmone for 10-Formyl-tetrahydrofolate Deficiency

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A SIMPLE SYNTHESIS OF 5-AMINO-4-IMIDAZOLECARBOXAMIDE RIBOSIDE-5'-TRIPHOSPHATE: THE PROPOSED ALARMONE FOR 10-FORMYL-TETRAHYDROFOLATE DEFICIENCY

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Abstract: A short synthesis of the title compound (1) from the nucleoside, 5-amino-4-imidazolecarboxamide riboside (2), is reported. Treatment of 2 with phosphorus oxychloride ($POCl_3$) in trimethyl phosphate ($PO(OCH_3)_3$), followed by the addition of bis-tri-n-butylammonium pyrophosphate and finally hydrolysis, gave 1 in good overall yield. This synthesis confirms the structure of 1 as proposed and further demonstrates the utility of this triphosphorylation procedure.

Recent investigations of Bochner and Ames indicate that a compound which accumulates in folate-deprived cultures of Salmonella typhimurium acts as an alarmone. Good evidence was given that this compound is 5-amino-4-imidazolecarboxamide riboside-5'-triphosphate (1)[1]. We decided to undertake the synthesis of 1 to: 1) confirm its proposed identity, 2) make this novel triphosphate available in quantity for biochemical studies[2], and 3) test the scope of a triphosphorylation procedure that we have been developing in our laboratory[3-5]. Fortunately all three goals were achieved, we report here the results of this study.

The commercially available nucleoside 2 was allowed to react with $POCl_3$, under conditions which are known to give the 5'-phosphorodichloridate derivative 3 specifically[6]. The reaction mixture containing 3 was then treated with excess bis-tri-n-butylammonium pyrophosphate in dimethylformamide (DMF)[7]. There is evidence that trimetaphosphates (e.g. 4) form[4] under these conditions, these trimetaphosphates then preferentially hydrolyse to linear phosphates[4]. Thus, the reaction mixture, presumably containing 4, is allowed to hydrolyse. Analysis of the crude reaction by HPLC after hydrolysis indicates the presence of small amounts of 2 along with nucleoside mono-and diphosphates, and a major peak corresponding to 1 in a yield of ca. 75%. This mixture is readily separated by ion-exchange chromatography to give 1 containing inorganic salts. The salts are readily removed by passing an aqueous solution of the mixture through a

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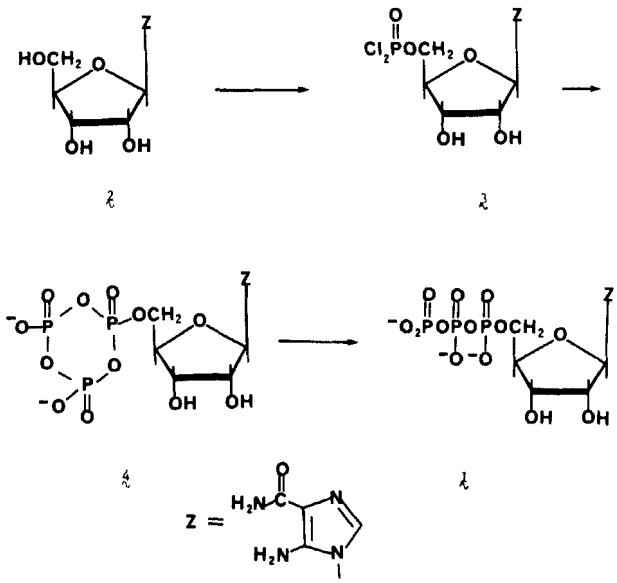
column of activated charcoal and eluting with water. Under these conditions nucleoside triphosphates are slightly retained, while inorganic salts are not. In this way 1 could be obtained nearly free of salts, in an isolated yield of 35% from 2.

The material prepared by this procedure co-migrates precisely with 1 from the natural source[8], in the two-dimensional thin-layer chromatographic system of Bochner and Ames[1]. The synthetic material was also converted to the corresponding 5'-monophosphate when treated with snake venom phosphodiesterase at pH 8[8]. These facts when taken together with the elemental analysis and ultraviolet absorbtion properties of synthetic 1 (see experimental) firmly establish the identity of 1 as the proposed alarmone from S. typhimurium.



2 3 4 1

Experimental

DMF, tri-n-butylamine, $POCl_3$ and $PO(OCH_3)_3$ were purchased from Aldrich. $POCl_3$ and $PO(CH_3)_3$ were distilled. All other reagents were stored over 4 Å molecular sieves for 24h before use. Pyrophosphoric acid was purchased from Fluka. 5-Amino-4-imidazolecarboxamide riboside was obtained from Calbiochem-Behring. QAE sephadex A25 was purchased from Pharmacia. All reactions were performed with protection from moisture in an air atmosphere. For HPLC, the following system was

employed: column; Whatman Partisil PXS 10/25 SAX; solvent 0.25 M KH_2PO_4 + 0.50 M KCl, flow rate = 3 ml/min; detector set at 267 nm. Elemental analysis was performed by Galbraith, except for phosphorus analysis which was done at Calbiochem-Behring.

Preparation of 1; a suspension of 2 (1.0g, 3.9 mmoles) in 10 ml of $PO(OCH_3)_3$ was treated with 0.7 ml of $POCl_3$ (7.8 mmoles). After stirring for 2 h at 25°C the solution became homogenous. This solution was treated with a solution of 3.5 g (19.5 mmoles) of pyrophosphoric acid in a mixture of 17 ml tri-n-butylamine and 15 ml anhydrous DMF. This mixture was allowed to stir for 4 h at 25°C, then diluted with 1000 ml of deionized water and left at 5°C for 16 h. The solution was adjusted to pH 7.5-8.0 with a 0.1 M NaOH solution, and applied to a column of QAE sephadex A25 resin. The column was eluted with a linear gradient of 8 liters of NH_4HCO_3 (0 to 0.4 M) in water. The fractions (500 ml) were analyzed by HPLC. Fractions containing pure 1 were combined and concentrated (bath temp. ca. 40°C), and then lyophilized to remove the bulk of NH_4HCO_3. This material, containing 1 and inorganic salts, was dissolved in 100 ml water and applied to a column (4 X 30 cm.) of a 1:1 mixture of Darco G-60 and John-Mansville celite 360. The column was eluted with water. Fractions (10 ml) were collected and monitored by UV at 267 nm. The fractions containing 1 were combined and lyophilized to give 810 mg (35% yield) of 1 as the triammonium salt dihydrate containing one half equivalent of ammonia[9]. The ammonia is retained by solid 1 even after extended pumping at high vacuum. Theory for $C_9H_{26}N_7P_3O_{14}\cdot 2H_2O\cdot\frac{1}{2}\ NH_3$; %C 18.17, H 5.50, N 17.66, P 15.62. Found; %C 18.07, H 5.48, N. 17.89, P 15.10. Theory % H_2O 6.0%, found 7.3%. λmax at pH 7.0 = 267 nm., ε = 12,700.

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REFERENCES

(1) Bochner, B.R.; Ames, B.N., Cell 1982, 29, 929.

(2) Compound 1 is now available from Calbiochem-Behring (catalog # 158228).

(3) The triphosphorylation procedure that we have been developing is based on the original report of reference 6 and unpublished work of R.A. Sanchez (of this laboratory). Independent publications have recently described procedures similar to ours; see references 4 and 5.

(4) Ludwig, J., Acta Biochim. et Biophys. Acad. Sci. Hung. 1981, 16 (3-4) 131.

(5) Ruth, J.L.; Cheng, Y.C., Mol. Pharmacol. 1981, 20, 415.

(6) Yoshikawa, M.; Kato, T.; Takenishi, T., Bull. Chem. Soc. Japan 1969, 42, 3505.

(7) Moffatt, J.G., Can. J. Chem. 1964, 42, 599.

(8) Lee, P. and Ames, B.N.; unpublished results 1983.

(9) A referee has commented that the isolated material is better formulated as a 1:1 mixture of tri and tetraammonium salts.

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