One-Step Synthesis of Hypoxanthine from Glycinamide and Diformylurea

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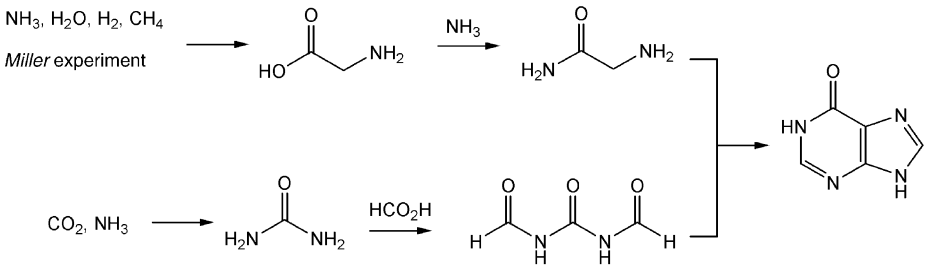
Because of their easy availability and their relative chemical stability, urea, formic acid, and glycine might have played a role in the assembly process of nucleobases. In this paper, a short reaction path is described to prepare hypoxanthine starting from the above mentioned precursors. The formation of hypoxanthine has been verified by high-resolution mass spectrometry with the ^{15}N-labelled urea as starting material, and HPLC analysis. The yield of this condensation reaction has been determined spectrophotometrically.

1. Introduction. – The prebiotic synthesis of purine bases, as proposed by *Oro* [1], and by *Ferris* and *Orgel* [2] starts from HCN [3–5]. Hydrogen cyanide itself is easily hydrolyzed in H_2O to give HCOOH. This work on prebiotic chemistry of purines was preceded by the experiment of *Miller* [6] to obtain amino acids (among them, glycine) by the action of an electric discharge on a mixture of CH_4, NH_3, H_2, and H_2O. A third remarkable experiment in the early phase of organic chemistry (*Wöhler*, 1828) is the synthesis from urea from inorganic material [7]. Because of their easy availability and their relative chemical stability, these molecules (urea, formic acid, and glycine) might have played a role in the assembly process of nucleobases.

2. Results and Discussion. – A question, which may arise, is if the synthesis of the nucleobases and the amino acids may not be intertwined, and if the nucleobases may be obtained by a short reaction path, starting from the above mentioned precursors. Therefore, we have investigated the condensation reaction of glycinamide that may be formed during the *Miller* experiment (*Scheme 1)* and diformylurea (the condensation product of urea and HCOOH).

Scheme 1. *Proposed Reaction Path for the Synthesis of Hypoxanthine from Glycinamide and Diformylurea*



Glycinamide is commercially available, while diformylurea can be synthesized easily from urea and HCOOH. It slowly precipitates from a mixture of both compounds.

Several conditions have been investigated, changing temperature, solvent, and dehydration agent. With DMF as solvent, only in the presence of P_2O_5 product formation could be observed. Best results by the temperature variations could be obtained heating the reaction mixture at 150–160° for 1.5 h. Temperatures above 200° led to a high percentage of insoluble black tar; in case of carrying out the reaction at temperatures between 60° and 100°, either no reaction took place, or a high percentage of polymerization products of glycinamide was obtained (70° over the period of 4–7 d). No formation of hypoxanthine could be monitored by using metal oxides (Na_2O, CaO, BaO) or concentrated H_2SO_4 as dehydration agent. The use of $CaCl_2$, Na_2CO_3, Na_2SO_4, molecular sieves, or silica blue led to the formation of black foam, containing *ca.* 5% of hypoxanthine. Most interestingly, the reaction mixture also yielded *ca.* 5% of hypoxanthine when no dehydration reagent is added. However, best results could be obtained by heating a mixture of diformylurea, glycinamide, and 3 equiv. of P_2O_5. The reaction has been repeated 20 times, and the yield of hypoxanthine fluctuated between 10 and 28% (average yield is 18%). This yield has been determined spectrophotometrically, by using standard solutions of hypoxanthine. *Fig. 1* shows the UV spectrum of the reaction mixture and the hypoxanthine reference.

To further verify the formation of hypoxanthine, the reaction has been repeated with ^{15}N-labeled urea. This experiment allows us to identify the origin of the N-atoms in hypoxanthine formed during this reaction (*Fig. 2*).

High-resolution mass spectrometry also shows the formation of hypoxanthine. The calculated mass for $^{15}N_2$ labeled hypoxanthine is 139.0021, while the experimentally determined mass was found at 139.0047. The reaction may be visualized as a ring-closure reaction between glycinamide and diformylurea, most probably in a two- or three-step process (*Scheme 2*).

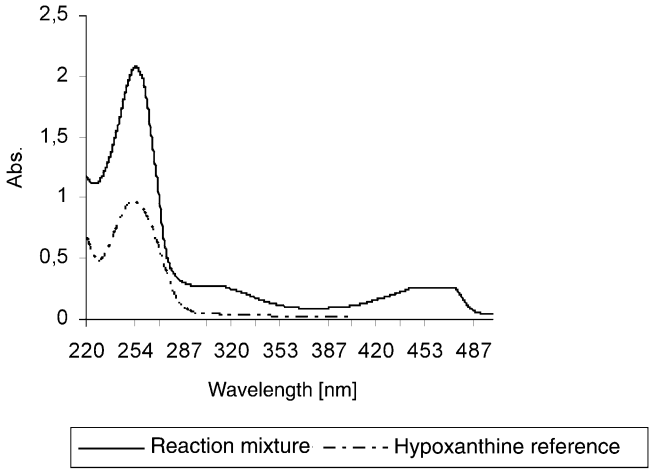


Fig. 1. *UV/VIS Spectrum* (MeOH) *of the reaction mixture and a hypoxanthine reference*

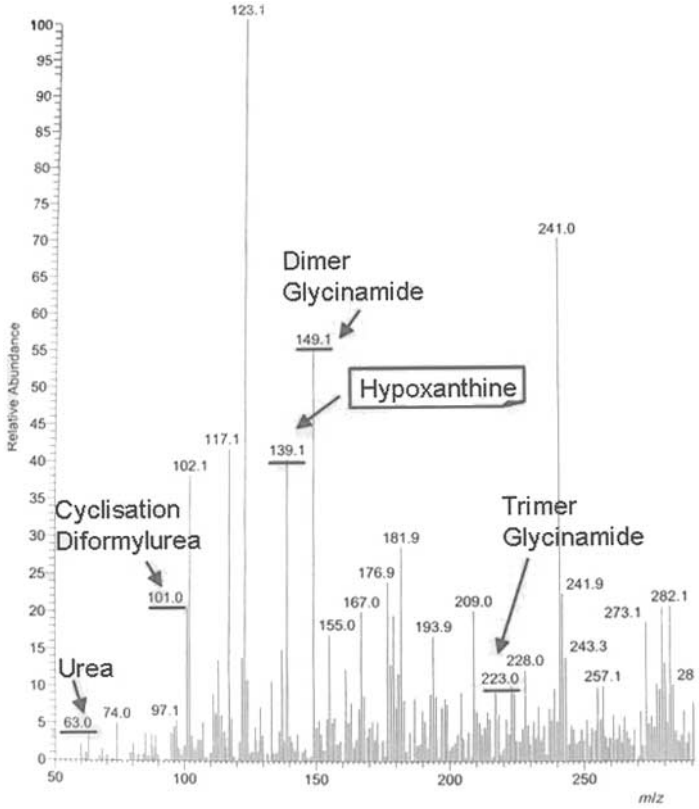
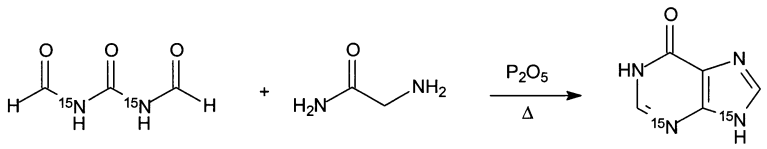


Fig. 2. *Mass spectrum of the crude reaction mixture with labeled diformylurea*

Scheme 2. *The Reaction of Glycinamide and [$^{15}N_2$]Diformylurea in the Presence of P_2O_5 Leading to the Formation of Hypoxanthine*



The ^{1}H-NMR experiment (splitting of the signals for H–C(2) and H–C(8)) demonstrates, that the N(3)- and the N(9)-atom of hypoxanthine are labeled [^{15}N], meaning that these atoms originate from urea. For H–C(2) and H–C(8) a pseudo-*triplett* at δ 8.01 is obtained, whereas, after nitrogen decoupling, two *singlets* at δ 7.97 and 8.10 can be observed. Further confirmation for the formation of hypoxanthine was provided by the HPLC analysis (*Fig. 3*). The reaction mixture has been pre-purified by preparative TLC (silica gel, 0.25 mm; CH_2Cl_2/MeOH/NH_4OH 2 : 1 : 0.5). Starting from 55 mg of labeled diformylurea, 12 mg of crude hypoxanthine (18%) could be obtained;

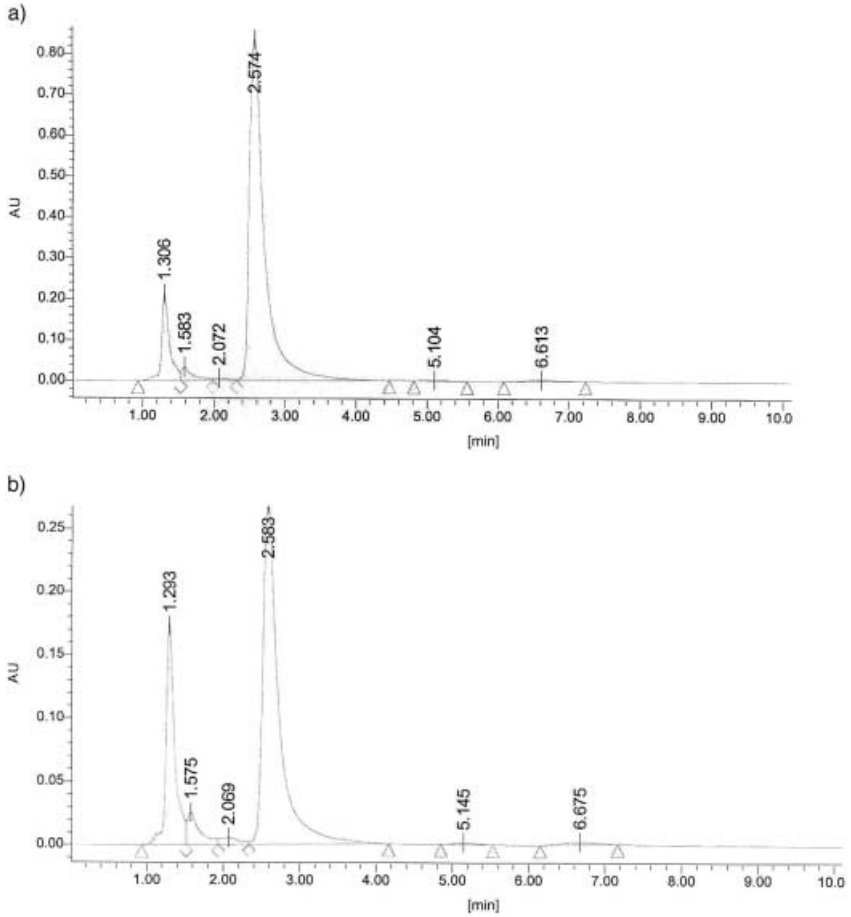


Fig. 3. *HPLC Analysis:* a) *the HPLC profile of the reaction mixture after purification by preparative TLC*, b) *after the addition of hypoxanthine as reference material to the pre-purified reaction mixture*

HPLC analysis (25 mM NH_4HCO_3, *C-18* column, sample dissolved in 10% DMSO/H_2O) indicated 68% of pure compound; therefore, the total isolated yield of hypoxanthine is 12.6%. In a second HPLC run, unlabeled hypoxanthine was added to the sample, showing an increase of the product peak and, therefore, establishing the identity of the two samples.

Previous experiments have demonstrated that adenine can be obtained by heating ammonium formate and HCN tetramer, or by heating formamide in a sealed tube [3–5]. We demonstrated here that hypoxanthine can be formed by heating glycinamide and diformylurea. The yield is low (5%), but can be increased considerably by adding a dehydration reagent, of which P_2O_5 is the best (yield up to 28%). Although it is hardly acceptable that a reagent like P_2O_5 could have existed on a primitive earth, the meaning of this experiment should be interpreted in function of the possibility of increasing the efficiency of this reaction by using a dehydrating catalyst in general. While glycinamide

and urea are plausible prebiotic reagents, HCOOH (which we need to synthesize diformylurea in the test tube) should better be presented under its salt form (ammonium formate).

However, we were able to obtain neither diformylurea by heating urea with ammonium formate, nor hypoxanthine by heating a mixture of glycinamide, urea, and ammonium formate. Therefore, these experiments must be rather considered as a generic approach (to obtain a purine base from a single amino acid derivative and an ureum derivative). A prebiotic equivalent is not implausible, but will ask for new experimental setups.

Experimental Part

General. Samples were infused in a i-PrOH/H_2O 1:1 mixture at 3 µl/min. TLC: TLC aluminum sheets (silica gel *60 F_{254}* (*Merck*)). Prep. TLC: with precoated TLC Plates (0.25 mm, silica gel *60 F_{254}* (*Merck*)), with CH_2Cl_2/EtOH/NH_4OH 2:1:0.5 (R_f (hypoxanthine) *ca.* 0.6). HPLC: *C-18* column, with 25 mM NH_4HCO_3. NMR Spectra: *Varian Gemini-200* spectrometer (^1H: 200 MHz) and a *Varian Unity-500* spectrometer (^1H: 500 MHz). UV Spectra: *Varian Cary-Bio-300* spectrometer. Exact mass measurements were performed on a quadrupole time of flight (TOF) mass spectrometer (*Q-Tof-2*, *Micromass*, Manchester, UK) equipped with a standard electrospray ionization (ESI) interface.

1. *Preparation of the Staring Materials. Glycine Amide.* Commercially available glycine amide hydrochloride (3.0 g) was suspended in CH_2Cl_2 (30 ml). After addition of Et_3N (3 ml), stirring was continued overnight. After filtering off the precipitate, it was dried at 70° for 2 h. Yield 1.9 g (94%). The product was verified by NMR spectroscopy (^{13}C-NMR (50 MHz, (D_6)DMSO): 40.0 (CH_2); 168.0 (C=O)).

Diformylurea. Urea (3.0 g, 50 mmol) was dissolved in HCOOH (5.0 g, 110 mmol). The mixture is kept at r.t. for 2 weeks. A white precipitate formed and was filtered off. First, the precipitate was washed with cold H_2O (10 ml), followed by sat. aq. $NaHCO_3$ soln. (10 ml), the pH of the filtrate was controlled (pH 8), and the white precipitate was dried at 70° for 2 h. Yield: 2.02 g, (35%). The product was verified by NMR spectroscopy (^{13}C-NMR (50 MHz, (D_6)DMSO): 154.4 (C=O); 163.7 (2 CH)).

[$^{15}N_2$]Diformylurea. [$^{15}N_2$]Urea (100.0 mg, 1.6 mmol) was dissolved in HCOOH (160.0 mg, 3.4 mmol). The mixture was kept at r.t. for 2 weeks. A white precipitate formed. After addition of sat. aq. $NaHCO_3$ soln. (0.5 ml), the pH was controlled (pH 8), and the white precipitate was filtered off and dried at 70° for 2 h. Yield: 60.1 mg (30%).

Condensation Reaction to Hypoxanthine. In a glass vial, glycineamide (13.0 mg, 17.5 mmol), diformylurea (20.4 mg, 17.5 mmol), and P_2O_5 (50 mg, 35.2 mmol) were carefully mixed together and put under N_2. The vial was sealed and heated in an oil bath (bath temp. 175°) for 2.0 h. The resulting black foam was treated with NH_3 conc. (0.1 ml) and MeOH (1.9 ml). The mixture was kept in an ultrasonic bath to dissolve for 20 min.

2. *Preparation of the Hypoxanthine- and Baseline Correction-Standards.* Commercially available hypoxanthine (8.6 mg, 6.3 mmol) and P_2O_5 (20 mg) were dissolved in conc. NH_3 (0.1 ml) and MeOH (1.9 ml). P_2O_5 (20 mg) was dissolved in conc. NH_3 (0.1 ml) and MeOH (1.9 ml). For the baseline correction, 2 µl of the baseline standard soln. was diluted with 998 µl MeOH.

3. *Quantification of the Reaction Mixture by UV Spectroscopy.* The reaction mixture/standard soln. (2 µl) was diluted with 998 µl of MeOH. All samples were measured twice. Five different hypoxanthine standards were prepared, and the average extinction coefficient was calculated ($\lambda_{max} = 254.15$ nm, $\varepsilon = 11220.184$, $\log \varepsilon = 4.05$) [8]. The condensation reaction was carried out 14 times unlabeled and 6 times labeled. The average yield was 18%, the minimum yield 10%, and the maximum yield 28%, $\lambda_{max} = 254.4$ nm. HR-MS: 139.0047 ($C_5H_5{}^{15}N_2N_2O$; calc. 139.0021).

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