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On the Isomerism of Hydroxyurea

VIII. A Polarographic Study of the Formation Process in Aqueous Solution

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The formation of the isomeride, melting at 72°C, from hydroxylammonium chloride and potassium cyanate in aqueous solution, has been followed at 25°C and at 0°C by a polarographic method. The maximum wave height obtained corresponded to a mole fraction of approximately 0.6, not varying markedly with temperature. The rest of the stoichiometric yield — 0.4 in mole fraction — probably consists of the polarographically inactive higher melting isomeride. It is proposed that the isomerides are formed by a mechanism of concurrent reactions rather than by one of consecutive reactions.

In aqueous solution, buffered at pH 9.2, no formation of the lower melting isomeride was detectable. The preliminary results, reported in this paper, may be of interest in connection with the controversial views on the mechanism of the classical urea synthesis.

In the first paper in this series [1] the ionic reaction

$$HONH_3^+ + Cl^- + K^+ + {}^-OCN = \underset{\text{hydroxyurea isomerides}}{CH_4O_2N_2} + K^+ + Cl^- \qquad (1)$$

was subjected to a preliminary kinetic investigation at 0°C. The advancement of the reaction was followed conductometrically. The data indicated a bimolecular, second order process with a rate constant approximately 8 l mole^{-1} min^{-1}. The ultimate conductivity value, arrived at after 20 h, could be accounted for by the potassium ions alone, from which it may be inferred that the hydroxylammonium ion and the cyanate ion react practically completely to yield products, which are only slightly, if at all, ionized. This method obviously could provide no information on the relative amounts of hydroxyurea isomerides formed.

In a previous publication [2] it was shown that the lower melting hydroxyurea (72°C), which in the following will be denoted OU_{72}, is reduced at the dropping mercury electrode, whereas the higher melting one, OU_{140}, is not.

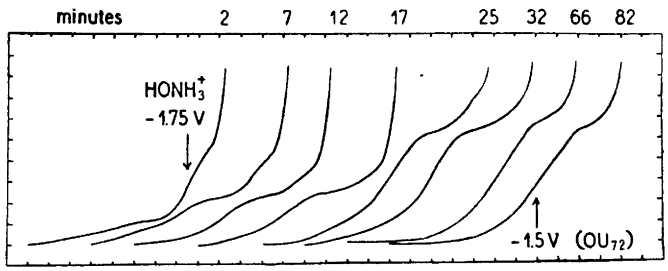

Fig. 1. Formation of hydroxyurea OU_{72} (reaction 1) at 25°C in aqueous solution. Initial concentration of reactants c_0 = 4.0 mM. Dilutions 1:10 with buffer at pH 9.2, polarographed at 25.0°C. Drop time 3.3 sec., height of mercury column 45 cm, rate of mercury outflow 2.66 mg/sec. One unit on the abscissa corresponds to 0.1 volt; the first four curves begin at −1.0 volt *vs.* SCE ("saturated calomel electrode"), one unit on the ordinate corresponds to 0.66 μA. The last four curves begin at −0.8 V *vs.* SCE and were recorded with a sensitivity, increased by a factor 2; one unit on the ordinate corresponds to 0.33 μA.

The polarographic method was worked out in detail for determination of OU_{72} in the presence of OU_{140}.

The present paper is devoted to a further investigation into reaction (1) by following the formation of OU_{72} polarographically.

As a supporting electrolyte potassium borate buffer at pH 9.2 was selected. In a more acid solution the hydroxylammonium wave would interfere severely, and in a more basic solution OU_{72} becomes increasingly unstable [2, Fig. 1].

THE FORMATION OF OU_{72} AT 25°C IN DILUTE AQUEOUS SOLUTION.

A 5 mM aqueous solution of hydroxylammonium chloride and a 20 mM aqueous solution of potassium cyanate, both freshly prepared, were separately equilibrated in a water-thermostat at 25.00±0.02 °C, dissolved oxygen being at the same time removed by passing a stream of moist, oxygen-free nitrogen through the solutions for five minutes. 40.00 ml of the hydroxylammonium solution and 10 ml of the cyanate solution were then rapidly mixed in a flask, which was kept in the thermostat during the reaction. The initial concentration of the reactants was thus 4 mM. 0.5 ml samples were withdrawn at intervals with a pipette, quickly mixed with 4.5 ml of previously deaerated buffer, and polarographed at 25.00°C. The recorded series of current-voltage curves is reproduced in Fig. 1. They clearly show the gradually vanishing hydroxylammonium wave at approximately —1.75 V and the growing hydroxyurea wave at about —1.5 V. The cyanate ion is polarographically inactive in this voltage region. The number appearing above each curve indicates the time in min from the mixing of the reactants and until the recording of the respective polarogram was begun. The wave height, produced by hydroxyurea has previously been demonstrated to be directly proportional to the concentration [2, Fig. 4]. The relation $i = 8.0\ c$ (i being the wave height in μA and c the concentration in mM) was found to apply also at pH 9.2, other conditions being identical. The observed maximum wave height of hydroxyurea OU_{72} is about

1.8 μA, and it corresponds to a concentration of 0.22 mM in the analytical dilution or 2.2 mM in the original reaction mixture. This is slightly more than half the total value, 4.0 mM, expected from stoichiometry, if OU_{72} were the only product. It corresponds to a mole fraction of OU_{72} of *ca.* 0.6; while a mole fraction of *ca.* 0.4 of the polarographically inactive OU_{140} has probably been formed simultaneously.

THE FORMATION OF OU_{72} AT 0°C IN DILUTE AQUEOUS SOLUTION

It was desirable to repeat the above experiment at 0°C. This is the most favourable reaction temperature for preparative work[3], and the data would be directly comparable with the conductometric data, reported earlier for the overall reaction [1], and finally, at the low temperature the slowed-down reaction would be better fit for the polarographic method of analysis.

A rapid withdrawal of well-defined sample-volumes from a reaction mixture at 0°C, followed by dilution and measurement at the standard temperature, 25°C, presented certain experimental difficulties, particularly with the available slow method of analysis. To overcome these obstacles it was first decided to carry out the reaction in the polarographic cell itself, properly thermostated at 0°C, and to work out a concentration-wave-height curve for this particular temperature. Since a pH of about 9.2 is the only region, in which the hydroxylammonium wave and the hydroxyurea wave may be independently and reproducibly measured [2, Fig. 3], the reaction had to be carried out in a buffer having this pH.

Introductory experiments of this kind showed, however, that at pH 9.2 and 0°C the reaction (1) is too slow for practical work. With an initial concentration of 10 mM of the reactants no hydroxyurea-wave was observable and no decrease in the height of the hydroxylammonium-wave was perceptible within 2 h. In unbuffered aqueous solution under otherwise identical conditions this space of time would correspond to more than 90 % reaction, as calculated [1] from $8\ t = \frac{1}{c} - \frac{1}{0.01}$. The temperature of the reaction mixture was raised to 25°C and polarographic measurements were carried out for another hour, still without detection of any hydroxyurea wave.

The failure of reaction (1) in the basic buffer unexpectedly solved the experimental difficulties, met with in the study of the reaction at low temperature, since it provided a convenient means of quenching the reaction in withdrawn samples for subsequent polarographic analysis at the standard temperature, 25°C. It was, therefore, decided to reproduce the experimental technique applied in the first experiment, except that the reaction was carried out at 0°C.

100 ml of an 8.0 mM aqueous solution of hydroxylammonium chloride and 100 ml of an 8.0 mM aqueous solution of potassium cyanate were freshly prepared and cooled separately to 0°C in an ice-water bath. During the temperature equilibration dissolved oxygen was removed in the way previously described. The cyanate solution was poured into the flask containing the hydroxylamine solution, and rapid mixing ensured ($t = 0$). The reaction mixture was maintained at 0°C during the experiment. From time to time 0.5 ml

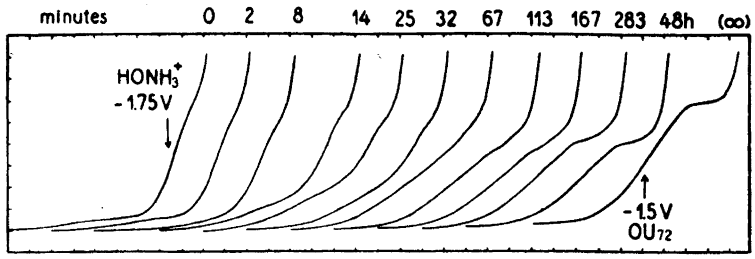

Fig. 2. Formation of hydroxyurea OU_{72} (reaction 1) at 0°C in aqueous solution. Initial concentration of reactants c_0 = 4.0 mM. Dilutions 1:10 with buffer of pH 9.2 polarographed at 25.0°C. Drop time 3.0 sec., height of mercury column 24 cm, rate of mercury outflow 3.02 mg/sec. One unit on the abscissa corresponds to 0.1 V. All curves begin at −1.0 volt *vs.* SCE. One unit on the ordinate represents 0.66 μA.

The first current-voltage curve, marked "0" was obtained with a 4.0 mM aqueous solution of hydroxylammonium chloride, without the addition of cyanate. The last curve ("∞") was obtained with a 4.0 mM aqueous solution of isolated hydroxyurea OU_{72}.

samples were withdrawn with a fast-draining pipette, quickly mixed with 4.5 ml of potassium borate buffer, pH 9.2, previously deaerated and equilibrated at 25.0°C in the polarographic cell. The analytical dilution was further deaerated, and proper mixing and temperature equilibration was ensured by passing a stream of nitrogen through the cell for another minute. The dilutions were then polarographed as previously described in detail [2].

The current-voltage curves are reproduced in Fig. 2. They present the same general picture as Fig. 1, deviating only in the reaction velocity. The initial concentration of the reactants is c_0 = 4.0 mM (0.4 mM in the dilutions). For comparison a 4.0 mM aqueous solution of hydroxylammonium chloride, denoted 0, has been recorded first in the same dilution 1:10. The hydroxylamine wave at −1.75 V is seen to decrease in height as the reaction proceeds, being replaced by a hydroxyurea wave at −1.5 V. The time for the maximum wave-height attained (curve "48 h") corresponds to almost 99 % reaction, as calculated from the conductometric rate constant k = 8 l · mole^{-1}min^{-1}, *vide* Ref. [1]. For comparison a 4.0 mM aqueous solution of authentic OU_{72} has been recorded (last curve in Fig. 2) under identical conditions (dilution 1:10). This curve represents the stoichiometric wave-height for 100 % reaction, if the lower melting hydroxyurea OU_{72} were the only product. We thus arrive at the same conclusion as from the experiment performed at 25°C, *i.e.* that somewhat more than half the reaction product is OU_{72}, mole fraction *ca.* 0.6, the rest being probably the polarographically inactive OU_{140}, in other words the ratio OU_{72}/OU_{140} is well over one. This agreement was to be expected. If follows from simple kinetic arguments that the ratio of isomeric products formed in a process should not vary markedly with temperature, provided the same reaction mechanism applies.

DISCUSSION

The above experimental material is not of sufficient accuracy to justify a detailed kinetic treatment. The individual wave heights, particularly those recorded for degrees of advancements round 50 %, can only be roughly estimated. In the experiment (Fig. 2) there has inevitably been a short rise of temperature during the transfer of samples to the arresting buffer solution. This error grows smaller as the reaction proceeds and becomes insignificant in the last curves. Frequent re-recording showed that the error due to undesired further reaction in the polarographic cell, during the recording of a curve (about 2 min), was negligible. The withdrawal of 0.5 ml samples was made with a fast-draining pipette, which was calibrated at 20°C as usual, and obviously no temperature equilibration could be permitted. The volume mixed with 4.5 ml buffer (measured out at 20°C), therefore, corresponded to slightly more than 0.5 ml at the standard temperature of measurement. Since, however, the same procedure was strictly adhered to, also in preparing the dilutions, recorded in curve 0 and ∞, this error does not invalidate the qualitative conclusions drawn in the following.

The actual mechanism of reaction (1) will be considered in a forthcoming publication, but it may be briefly mentioned here that *a priori* there seems to exist two possibilities, either a mechanism of concurrent reactions (Fig. 3 a) or one of consecutive reactions (Fig. 3b). In the figures 1 denotes the starting materials in reaction (1), 2 and 3 the isomeric hydroxyureas. According to the mechanism depicted in Fig. 3a the isomerides are formed simultaneously and side by side, whereas in Fig. 3b OU_{72} is the primary product, which is then partly isomerized to OU_{140}. Preliminary experiments [2, Fig. 1] have shown that in neutral aqueous solution at room temp. the isomerization 23 is incomparably slower than the formation 12, and this has been confirmed in unpublished work on the kinetics and mechanism of reaction 23 under various conditions. Under such circumstances the mechanism b would manifest itself as a polarographic hydroxyurea wave rapidly rising to almost the stoichiometric height and subsequently falling off very slowly. What actually was observed, however, (Figs. 1 and 2) was a maximum wave height slightly more than half the stoichiometric one, which seems to be in favour of mechanism a.

The apparent failure of reaction 12 in alkaline buffer pH 9.2 (*vide* p. 7) raises the question, as to whether the actual mechanism of the overall reaction (1) is ionic, as indicated in the stoichiometric equation, or rather involves free hydroxylamine and free cyanic acid. For the analogous process leading to

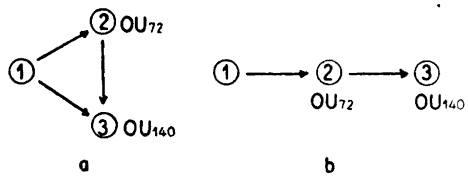

Fig. 3. Possible mechanisms of the formation of the hydroxyurea isomerides (reaction 1). 1 stands for the reactants; a. concurrent formation, b consecutive formation.

urea a non-ionic mechanism has recently been given priority [4] for steric reasons. Weil and Morris [5] have emphasized that the ionic and non-ionic mechanisms are kinetically indistinguishable, owing to the mobile equilibrium $NH_3 + HOCN \rightleftharpoons NH_4^+ + {}^-OCN$, and the same would probably apply to the reaction (1). It is, therefore, highly improbable that any useful information can be obtained from an examination of the pH-dependency of the rate constant of (1), and apart from that such measurements are impracticable with the polarographic method, because the hydroxylammonium wave and the hydrogen wave would interfere with the hydroxyurea wave in neutral and acid solution.

Materials, apparatus and general technique have been described in detail in previous publications [1,2].

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