BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 51 (9), 2739—2740 (1978)

The Polarographic Behavior of 6-Mercaptopurine in Aqueous Solutions

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(Received February 16, 1978)

Synopsis. The polarographic reduction of 6-mercaptopurine at the dropping mercury electrode took place in two steps; they were a two-electron process producing the 1,6-dihydro derivative, followed by a further two-electron process producing the 1,2,3,6-tetrahydro derivative. An anodic wave corresponding to the reaction with the mercury of the electrode material was observed at a relatively low potential, and its analytical utility for the determination of 6-mercaptopurine is suggested.

The electrochemical reduction of purines and pyrimidines, which are important components of nucleic acids, have been investigated in aqueous[1-3] and nonaqueous solutions.[4-6]

Mercaptopurines containing exocyclic sulfur are often anticarcinogens. In particular, 6-mercaptopurine is being used as an anti-leukemia agent. The present work was carried out in order to study the polarographic behavior of 6-mercaptopurine and its applicability to the polarographic determination of this compound.

Experimental

Chemicals. The 6-mercaptopurine(6-MP) was obtained from the Sigma Chemical Company and was recrystallized from water. The other chemicals were of a reagent grade and were used without further purification.

Apparatus. The polarograms were recorded using a Yanagimoto P-8 type Polarograph. The dropping mercury electrode (DME) had the following characteristics: mercury flow rate, *m*, 2.30 mg/s, and drop time, *t*, 3.25 s, with an open circuit under the mercury head of 70 cm, in 1 mol/dm^3 $HClO_4$. A saturated calomel electrode was employed as a reference electrode, and a platinum wire, as a counter electrode, A mercury pool electrode was also used for the controlled potential electrolysis and the coulometric measurements.

Results and Discussion

Polarography and Controlled Potential Electrolysis.
Cathodic Waves: It is known[1)] that purine, which is the model compound of 6-MP, exhibits two pH-dependent waves when in an acidic solution, but none when in a neutral or alkaline solution. Similarly, 6-MP exhibits two cathodic waves at fairly negative potentials. The diffusion-current constant (I_d) and the half-wave potential of 6-MP as a function of the pH values are shown in Fig. 1.

At pH values below 4.5, two relatively close polarographic waves(wave I and II) were observed. The half-wave potentials of the two waves shifted linearly towards a negative potential with an increase in the pH; $E_{1/2}(vs.\ \text{SCE})/\text{V}=-0.680-0.120\ \text{pH}$ for the I wave and $E_{1/2}\ (vs.\ \text{SCE})/\text{V}=-1.016-0.061\ \text{pH}$ for the II wave. The I_d values for both the waves, particularly that for the II wave, were very large in highly acidic solutions (pH 0—3), but were kept constant at pH values around 4. It is known that the first wave of purine is attributable to a two-electron diffusion-controlled process. The I_d value for this wave was 3.60 μA mmol^{-1} dm^3 mg$^{-2/3}$ s$^{1/2}$. At pH 4.02, the I_d values for the I and II waves of 6-MP were *ca.* 4.4 and 3.8 μA mmol^{-1} dm^3 mg$^{-2/3}$ s$^{1/2}$, corresponding to a two-electron reduction process.

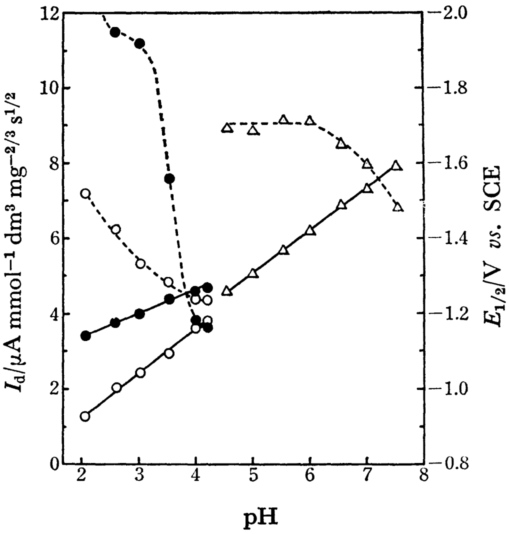

Fig. 1. Effect of pH on the diffusion-current constant (I_d) and half-wave potential of the reduction waves of 0.2×10^{-3} mol/dm^3 6-MP.
○: Wave I, ●: wave II, △: wave III.
(——: $E_{1/2}$, ----: I_d)

At pH values above 4.5 these two waves merged, and the resulting composite wave(wave III) had a pH dependence of $E_{1/2}(vs.\ \text{SCE})/\text{V}=-0.746-0.113\ \text{pH}$. The I_d value of the III wave remained almost constant up to pH *ca.* 6; its value corresponded to a four-electron reduction step. At pH values above 6.0, however, the height of the III wave decreased gradually with an increase in the pH. Since the pK_a of 6-MP was determined to be 7.6 by utilizing the UV absorption method of Shugar and Fox,[7,8)] this decrease in the wave-height seems to imply that 6-MP dissociates into an electroinactive ion in solutions above pH *ca.* 7.

The coulometric measurements at the controlled potentials were performed in order to determine the number of electrons involved in the overall electron-transfer reactions. The controlled potential electrolysis at a potential on the crest of the I wave in the Britton-Robinson buffer (pH 4.02) indicated that 2.1±0.2 electrons were transferred per molecule of 6-MP. A coulometric *n* value for the II wave gave an unusually high value. Also, the electrolytic current remained at a high value throughout the electrolysis and the pH of the solution increased. This behavior indicates that a major part of the current is a catalytic reduction of hydrogen ions, rather than being due to the reduction of 6-MP itself. Also, the electrolysis at a potential on the crest of the II wave resulted in the liberation of H_2S (detected by lead acetate paper), but no such liberation was detected in the case of the electrolysis at a potential on the crest of the I wave. At pH 6.00 the coulometric *n* value of the III wave was 5.8 ± 0.4, and the liberation of H_2S was detected just as in the case of the electrolysis at a potential on the crest of the II wave. This discrepancy between the polarographic and coulometric *n* values may be explained by a ECE mechanism comprised of a slow chemical reaction (liberation of H_2S) which does not occur during the lifetime of a mercury drop, but which becomes important under conditions of prolonged electrolysis.

It is known[1] that purine is reduced in two steps at the DME; the first step is a two-electron reduction to 1,6-dihydropurine, while the second is a further two-electron reduction to 1,2,3,6-tetrahydropurine, which is then hydrolyzed by water to [(5-aminoimidazol-4-ylmethyl)amino]methanol. The mechanisms for the reduction of 6-MP were also proposed. The values of αn_a and of P (the number of hydrogen ions involved in the rate-determining step) were calcualted for the I wave on the basis of the wave-slope($E_{1/4}$-$E_{3/4}$) and the slope of the $E_{1/2}$-pH plots.[9] An average αn_a value of 1.05 and an average P value of 2.13 were determined. Also, the I wave was a two-electron process judging from its I_d values. These facts suggest the hydrogenation of a double bond. Thus by analogy with the first step of purine, the I wave of 6-MP is probably a two-electron process, producing the 1,6-dihydro derivative(**1**). The II wave would then seem to result from a further two-electron reduction to the 1,2,3,6-tetrahydro derivative(**2**) of 6-MP. The UV absorption spectrum of a solution obtained by controlled-potential electrolysis on the crest of the II wave is essentially superimposed on that of the product of the complete electrolysis at a potential on the crest of the second wave of purine, indicating the identity of the two products. The formation of identical products in the reduction processes of 6-MP and purine requires the elimination of the 6-mercapto group from the **2** of 6-MP; this can be explained from the facts that the *n* values obtained coulometrically at a potential on the crests of the II and III waves were both *ca.* 6 and the liberation of H_2S was detected during the electrolysis. These experimental results seem to indicate that the **2** produced by a four-electron reduction of 6-MP evolves H_2S by means of a relatively slow chemical reaction to produce 2,3-dihydropurine(**3**), which then undergoes a further two-electron reduction to produce 1,2,3,6-tetrahydropurine(**4**), which is probably hydrolyzed to [(5-aminoimidazol-4-ylmethyl)-amino]methanol (**5**) as an end product.

Anodic Waves: An anodic wave of 6-MP was also observed at the DME over the pH range of 2—12. The relationship between the half-wave potentials and the pH was composed of two straight lines. At pH values below 7.6, the anodic wave shifted linearly towards negative potentials with an increase in the pH; $E_{1/2}(vs.\ \mathrm{SCE})/\mathrm{V} = 0.140 - 0.060\,\mathrm{pH}$. At pH values above 7.6, however, the half-wave potential of the anodic wave was independent of the pH; $E_{1/2} = -0.324$ V *vs.* SCE. The pH values at the intersection of these two lines was 7.66, which agreed very closely with the pK_a value (7.60) determined by the UV absorption method,

The height of the anodic wave remained almost constant at the I_d value corresponding to a one-electron faradaic process. The anodic current was proportional to the square root of the height of the mercury head, and the temperature coefficient was 1.3% per degree, indicating that the anodic current was controlled by diffusion.

However, this anodic wave was not observed in this potential range when a platinum electrode was used as the working electrode.

From these experimental results, it seems that the anodic wave of 6-MP does not correspond to the oxidation of 6-MP itself, but to the following reaction of 6-MP with the DME:

$$\mathrm{pH} < pK_a \qquad \mathrm{RSH} + \mathrm{Hg} \longrightarrow \mathrm{RSHg} + \mathrm{H}^+ + e,$$

$$\mathrm{pH} > pK_a \qquad \mathrm{RS}^- + \mathrm{Hg} \longrightarrow \mathrm{RSHg} + e$$

(RSH : 6-MP).

Analytical Application. Compared with the cathodic waves of 6-MP, the anodic wave of this compound was observed at a relatively low potential and was well-defined over the wide pH range of 2—12. The height of this wave in the Britton-Robinson buffer was proportional to the concentrations ranging (5×10^{-5}—10^{-3})mol/dm^3. The satisfactorily wide range of this linear relation suggests that the polarographic method may have analytical utility for the determination of 6-MP, which is being used as an anti-leukemia agent.

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