

Electroorganic Preparations

XXXIV. Electrolytic Reduction of Some Substituted Purines

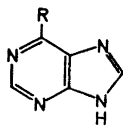
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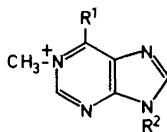
Adenine and some of its methylated derivatives have been investigated by controlled potential electrolysis. During the reduction of adenine, purine could be detected as an intermediate. This suggests that the major reaction route at the mercury pool electrode involves elimination of ammonia after the initial uptake of two electrons and protons.

Purine (Ia), adenine (Ib) and other 6-substituted purines have previously¹⁻⁴ been investigated polarographically and by controlled potential reduction, and a reaction route has been suggested for the reduction at the dropping mercury electrode (DME). Reductions in the purine and quinazoline (III) series, both being derivatives of pyrimidine, often follow similar reaction routes, and, as the reduction paths of certain quinazoline derivatives⁵ in the macroscale reductions were found to be different from that suggested for the corresponding purine derivatives at the DME, a reinvestigation of the macro-scale reduction of adenine and certain derivatives was made.

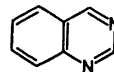
In the investigation were included adenine (Ib), 6-methylaminopurine (Ic), 6-amino-1-methylpurinium iodide (IIb) and its 9-ribose derivative (IIc).



- I a R = H
 b R = NH₂
 c R = NHCH₃



- II a R¹ = R² = H
 b R¹ = NH₂, R² = H
 c R¹ = NH₂, R² = ribosyl



III

RESULTS

According to the investigations by Elving and collaborators¹⁻⁴ purine gives two two-electron waves in acid solution whereas adenine gives a single wave at a potential slightly more negative than the second wave of Ia and with a wave-height corresponding to approximately a four-electron reaction. At $\text{pH} \approx 6$ the waves of both compounds disappear.

In the present study phosphate and borate buffers were used in the slightly alkaline region rather than an ammonia-ammonium chloride buffer; in these buffers purine gave two waves up to $\text{pH} \approx 10$ where the waves merged. At $\text{pH} > 11$ the combined wave merged with the sodium wave.

The reduction of adenine between $\text{pH} 0.1$ and 1.4 consumed $6 F/\text{mol}$; during the reduction a small prewave at the potential of the first two-electron wave of purine could be detected polarographically in the catholyte; the second two-electron wave of purine merged with the foot of the bigger wave of adenine. The identity of the intermediate was further substantiated by polarography of a sample of the catholyte at $\text{pH} 7.5$, where Ib is polarographically inactive whereas purine gives two two-electron waves; purine was further identified by TLC. The product isolated after an exhaustive electrolysis was tetrahydropurine.

A plot of the electron consumption (Fig. 1) against the polarographic wave-height (concentration) of the unreduced adenine during a reduction of adenine in $2 N$ hydrochloric acid containing 50% ethanol at -35°C showed an initial slope of the curve corresponding to $n \sim 2$. By using a low temperature the rate of the chemical step, the elimination reaction, was retarded compared to the electron transfer steps.

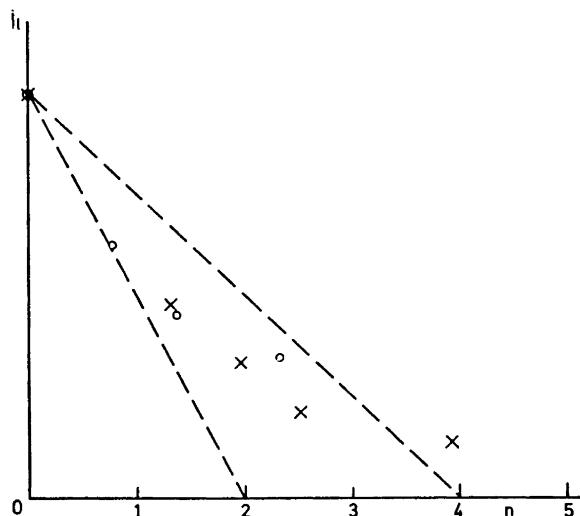


Fig. 1. Dependence of concentration (polarographic limiting current) on electron consumption (in F/mol) during an electrolytic reduction at -35°C in $2 N$ HCl containing 40% ethanol; (\times) adenine (Ib); (\circ) 6-amino-1-methylpurinium iodide (IIb).

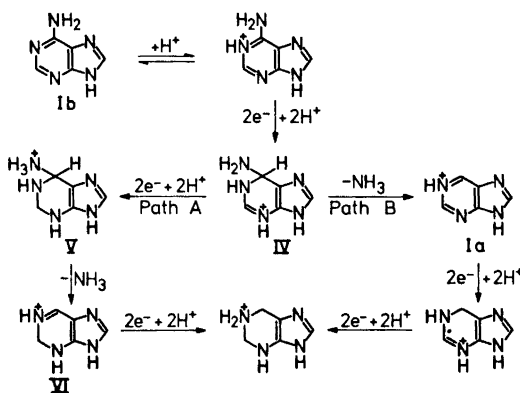
Reductions of adenine at higher pH gave $n=6$ and no prewave could be detected during the electrolysis; this is in accordance with the results from the quinazoline series where the rate of the elimination reaction grows with pH.

6-Amino-1-methylpurinium iodide (IIb) is polarographically active below pH 11.5; in strongly acid solution (pH < 0.5) two waves are visible, the second wave distorted by a pronounced maximum. The two waves merge at pH ≈ 1 . Between pH 5 and 8 this wave is gradually replaced by another wave at a more negative potential in the usual manner for a transition between the reduction of a protonated species and a species having one proton less. The latter wave disappears gradually between pH 9 and 11.5.

During a controlled potential reduction of IIb in 0.5 N hydrochloric acid a prewave appeared at about the same potential as that of purine at this pH. UV-spectra of samples withdrawn during the reduction showed in the beginning a maximum at about 300 nm due to the 1,6-dihydroderivative of IIb whereas the final reduction product had maxima at 212 nm and 235 nm. Evaporation of the catholyte *in vacuo* at temperatures below 40°C yielded 1-methyltetrahydropurine (VII) together with an equivalent amount of ammonium chloride. Repeated recrystallization from ethanol containing hydrochloric acid/ether gave pure VII.

DISCUSSION

Adenine is reduced in acid solution in a six-electron reduction and tetrahydropurine and ammonia are the products. An elimination of ammonia takes place during the reduction and the reaction could conceivably run along either route A or route B (Scheme 1) or both routes could be followed simultaneously in parallel reactions.



Reaction route A has been favoured^{1,2,4} on the grounds that the polarographic wave-heights of adenine and several other 6-substituted purines correspond approximately to a four-electron reduction and that the half-wave potential of adenine is about 0.05 V more negative than that of 1,6-

dihydropurine. It is, however, difficult to estimate whether 1,6-dihydroadenine (IV) would be just as or more easily reduced than adenine. However, the non-reducibility of 4-amino-3,4-dihydroquinazoline indicates that IV would not be reduced.

No prewave which could be attributed to IV or VI was observed during the reduction, unless the polarographic wave of one of these compounds happened to coincide with the first purine wave. IV is not likely to behave so, whereas it is more difficult to exclude VI, which can be regarded as an azomethine derivative of imidazole-4-carbaldehyde.

If IV were reducible to V at the reduction potential of Ib it is difficult to explain why all IV is not reduced to V as the competing elimination reaction is rather slow in acid solution. It is difficult to visualize a chemical reaction in the reduction of Ib through IV to V which was slow compared to the elimination.

Route B is consistent with the identification of purine as an intermediate and with the finding that the concentration-electron consumption dependence during the first part of the reduction of adenine at low temperatures corresponds to $n \sim 2$ (Fig. 1, \times). Route A would require an initial n -value of 4, as the slow step, the elimination of ammonia from 6-aminotetrahydropurine (V), occurs after the uptake of four electrons. The observed n -value would probably have a tendency to be too high rather than too low, partly because some elimination of ammonia may occur and partly because a concomitant evolution of hydrogen cannot be excluded, as adenine is known to catalyze the cathodic hydrogen evolution. Furthermore, since purine is known to be more easily reduced than adenine, from an energetic point of view route B is allowed. The formation of purine as an intermediate is analogous to the reduction route of 4-substituted quinazolines,⁵ 4-mercaptocinnoline,⁶ and 6-mercaptapurine⁷ in which the parent heterocyclic compound is an intermediate.

The results thus show that at the mercury-pool electrode route B (Scheme 1) is followed at least to a certain extent and possibly exclusively. Whether route A is followed to some extent in a parallel reaction is dependent on the reduction potential of 1,6-dihydroadenine (IV); if the reduction potentials of adenine and IV are not too different a minor part of the reaction may follow route A at the mercury-pool electrode.

When a branching of a reaction occurs, it does not always follow that the proportion of molecules following the one alternative to those following the other alternative is the same at the mercury-pool electrode and at the dropping mercury electrode. Although route B has been shown to be the more important one at the mercury-pool electrode, route A could be the more important one at the dropping mercury electrode which would explain the height of the wave of adenine.

Further experiments may indicate whether 1,6-dihydroadenine is reducible at the required potential; if it is not, route A is improbable even at the dropping mercury electrode.

The predominance of route B in the reduction of 6-amino-1-methylpurinium iodide (IIb) is substantiated by the UV-spectra obtained during the electrolysis. The primarily formed intermediate has a maximum at about 300 nm which is attributed to a dihydropurine derivative rather than a tetra-

hydro derivative. This is also supported by reduction of IIb at very low temperatures, where the same dependence of concentration on electron consumption was found as in the case of adenine (Fig. 1, ○).

EXPERIMENTAL

Materials. Purine and adenine were obtained from Fluka AG, Switzerland. 6-Amino-1-methylpurinium iodide and 6-methylaminopurine were prepared according to Jones and Robins.³

Reduction of Ib. Ib (0.200 g) was reduced in 1 N hydrochloric acid solution (pH 0.10) at -0.95 V *vs.* SCE at 0°C . The reduction consumed 6F/mol. Polarograms taken during the reduction showed a small wave at -0.65 V, corresponding to the first wave of Ia. A sample of the catholyte brought to pH 7.0 showed 2 waves at -1.20 V and -1.45 V for Ia. The reduction completed, the catholyte was evaporated to dryness *in vacuo* at a bath temperature not exceeding 40°C . The 1,2,3,6-tetrahydropurine was isolated as its dihydrochloride together with an equivalent amount of ammonium chloride. NMR in D_2O : $\delta = 4.82$, $\sum\text{H} = 2$, singlet (H-6); $\delta = 5.35$, masked by H_2O peak (H-2); $\delta = 8.92$, $\sum\text{H} = 1$, singlet (H-8). (Found: C 22.86; H 5.14; N 26.33; Cl 45.54. Calc. for $\text{C}_5\text{H}_{15}\text{N}_4\text{Cl}_3$: C 23.85; H 5.58; N 27.94; Cl 42.51.)

Reduction of Ib in a 0.04 N hydrochloric acid solution at -1.10 V *vs.* SCE (pH 1.40) proceeded in the same way. In the reduction of Ib in a citric acid buffer (pH 2.65) at -1.20 V *vs.* SCE, the intermediate formation of Ia could not be shown polarographically.

From a reduction of Ib in 1 N HCl at -15°C , samples were withdrawn in the course of the reduction and brought to pH 8.5. The waves of Ia gradually grew at room temperature until about 3 times the original height. By TLC on silica with a 3:1 mixture of chloroform and isopropyl alcohol as eluent the samples were shown to contain purine (R_F -value 0.865) and adenine (R_F -value 0.92).

Similarly Ib was reduced in 2 N HCl containing 50 % ethanol at -35°C . In the first part of the reduction the concentration of Ib was followed polarographically and the wave height of Ib plotted against the electricity consumption (Fig. 1, ×).

Reduction of Ic. 0.200 g of Ic was reduced in a 1 N HCl solution (final pH 0.60) at room temperature at -1.15 V *vs.* SCE. The intermediate formation of Ia could be shown polarographically.

Reduction of IIb. IIb (0.200 g) was reduced in 1 N HCl solution (pH 0.30) at -0.98 V *vs.* SCE at 0°C . The deamination could be detected by a small polarographic wave appearing at -0.72 V during the reduction. In a phosphate buffer of pH 6.65 a sample of the catholyte also gave a second wave for IIb at -1.35 V. UV-spectra taken during reduction showed a steady decrease in the peak height of IIb (λ_{max} 260 nm), while a peak at 300 nm due to the dihydrocompound was increasing. Later, another peak at 235 nm due to the tetrahydrocompound began to increase, while the one at 300 nm decreased. The reduction consumed 6F/mol. The catholyte was then evaporated to dryness *in vacuo* at a bath temperature below 40°C and the tetrahydroderivative of IIa was isolated together with an equivalent amount of ammonium chloride. Repeated crystallization from acidified ethanol/ether and methanol/ether yielded the pure 1-methyltetrahydropurine dihydrochloride. UV in 1 N HCl (20°C): λ_{max} 210 nm and 235 nm. NMR in D_2O : $\delta = 3.55$, $\sum\text{H} = 3$, singlet (assigned to $-\text{CH}_3$); $\delta = 5.13$, $\sum\text{H} = 2$, singlet (H-6); $\delta = 5.27$, singlet (assigned to H_2O and H-2); $\delta = 9.13$, $\sum\text{H} = 1$, singlet (H-8). (Found: C 33.86; H 5.98; N 26.68; Cl 33.57. Calc. for $\text{C}_6\text{H}_{12}\text{N}_4\text{Cl}_2$: C 34.12; H 5.68; N 26.54; Cl 33.64.)

In the reduction of IIb in a phosphate buffer (pH 6.65) at -1.45 V *vs.* SCE the intermediate formation of IIa could not be followed, but the UV-spectra showed the same course as in the acid reduction.

A reduction of IIa in a borate buffer of pH 10.12 also consumed 6F/mol and yielded the tetrahydroderivative. UV λ_{max} 225 nm at pH 10.12.

IIc was reduced in the same way as Ib in 2 N HCl containing 40 % ethanol at -35°C (Fig. 1, ○) with a total consumption of 6F/mol.

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