POLAROGRAPHY OF SOME PURINE DERIVATIVES

BY NYDIA G. LUTHY AND BERTHA LAMB

From the Chemistry Department, The University, Manchester 13 *and the Chemistry Laboratory, Evershed and Vignoles, Acton Lane Works, W.*4

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CERTAIN purines and purine derivatives are known to be antitumour agents because of their ability to act either as antagonists or antimetabolites in cell metabolic processes[1–3]. During the syntheses and study of some of these compounds a rapid and sensitive method for the detection of certain functional groups was needed. Because the ultra-violet absorption spectra of the purines lie close together in a region where they are often difficult to distinguish, and because the bands in the infra-red have not yet been definitely assigned, we have been investigating the use of the polarograph as an analytical tool.

The comprehensive review on the subject of the nucleic acids and their chemistry[4], notes only two references to the application of polarography: Heath[5] has shown that of the naturally occurring purine and pyrimidine ribonucleotides, only adenylic acid and its derivatives are reduced in 0·1M perchloric acid at the dropping mercury electrode. Cavalieri and Lowy[6] have studied a series of variously substituted pyrimidines and give a list of twenty-six, ten of which yield polarographic waves. Heath suggests that in adenine, reduction takes place between the nitrogen and the carbon at the 1:6 positions in the pyrimidine portion of the molecule, whereas Cavalieri and Lowy conclude that the system —N=C—C=C— is responsible for the polarographic activity in the pyrimidines they tested.

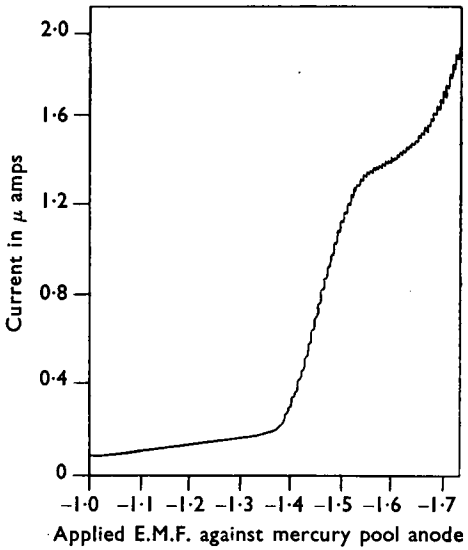


FIG. 1. Polarogram of adenine (*ca* 10^{-4} M) in perchloric acid (0·1 M).

An interesting correlation appears to exist between the presence of an amino group in the six-position of the purine ring and the appearance of a polarographic reduction wave. Besides 6-aminopurine (adenine) the following compounds reduce at the dropping mercury electrode: 2:6-diaminopurine, 6-acetamidopurine (acetyladenine), 2:6-diacetamidopurine. As distinguished from adenine which gives only one step, the acetylated

derivative shows two reduction steps (see Figs. 1 and 2). One of these must be due to the presence of the acetyl group for when that is hydrolysed, the wave at the more positive potential disappears and only the one due to the adenine moiety remains. Futhermore, 2:6-diacetamidopurine shows only two reduction waves although both amino groups have been acetylated

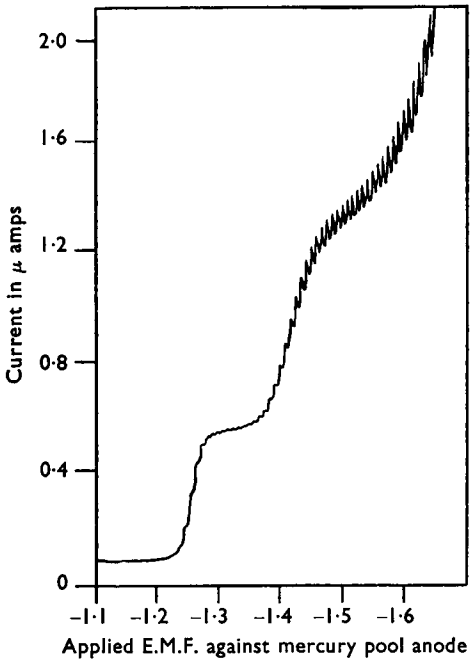


FIG. 2. Polarogram of acetyladenine (*ca* 10^{-4} M) in perchloric acid (0·1 M).

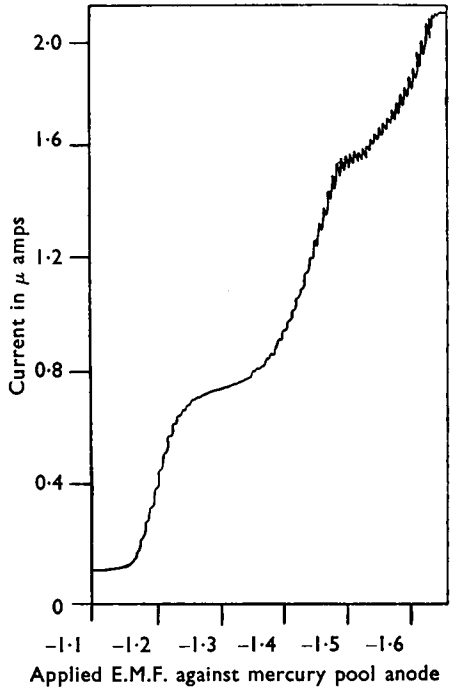
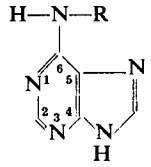


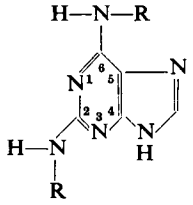
FIG. 3. Polarogram of 2:6-diacetamidopurine (*ca* 10^{-4} M) in perchloric acid (0·1 M).

(see Fig. 3). These steps are very similar in behaviour to acetyladenine and have approximately the same half-wave potentials. This also substantiates the assumption that reduction is associated with the amino group* (or its *N*-acetyl derivative) when it is in the 6-position of the purine ring (see I and II).



R = — H = Adenine
R = — $COCH_3$ = Acetyladenine

I



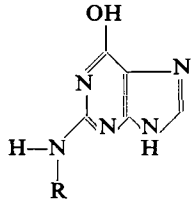
R = H = 2:6-Diaminopurine
R = — $COCH_3$ = 2:6-Diacetamido-purine

II

* The fact, however, that 6-mercaptopurine shows a polarographic wave in alkaline solution indicates that the character of the functional group in the C(6) position of the purine ring is also important.[7]

However, the presence of an acetyl group in *N*-acetylguanine (see III) does not promote reduction, and like the parent compound, guanylic acid and its derivatives, shows no polarographic activity.

A similar behaviour exists in the corresponding pyrimidines: the 6-amino and 2:6-diaminopyrimidine molecules reduce polarographically, whereas 2-amino-6-hydroxypyrimidine (*iso*cytosine) does not[6].



R = — H = Guanine
R = — $COCH_3$ = Acetylguanine

III

Experimental

All measurements were made on a Tinsley pen-recording polarograph Mark 15. Fresh stock solutions of our model compound acetyladenine (0·002M) were prepared in 50 per cent. aqueous ethanol made millimolar with perchloric acid. From this, appropriate dilutions for 2, 4, and 8×10^{-4} molar were made so that the final solution contained 0·001M perchloric acid and 0·01 per cent. starch all made up to volume with 50 per cent. (v/v) aqueous ethanol.

For the first step the instrument setting was 2 microamperes for full scale deflection in all concentrations examined. The work was carried out in the electrolysis stand supplied with the instrument and approximately 10 ml. of each solution was used. Nitrogen was bubbled through the cells to remove dissolved oxygen at which time no mercury was present. A saturated calomel half cell was used as the anode, and the temperature of the system was maintained at 20·8° C. ($\pm$ 0·2°).

All the polarograms were taken at two heights of the mercury reservoir, 36 cm., and 64 cm., drop times 6·3 and 3·6 seconds respectively. Both damped and undamped waves were obtained; the former were used to measure the diffusion current, while on the latter, the half-wave potentials were measured graphically. Derivative waves were also taken to aid the resolution of the two reduction steps produced, and to help in reading the $E_{\frac{1}{2}}$ values.

Results

A quantitative study was made on acetyladenine as a representative compound. Although previous polarographic determinations on adenine and the nucleotides have been made in 0·1M perchloric acid[5], it was found that acetyladenine hydrolysed rapidly in this supporting electrolyte giving non-reproducible wave heights. Additional proof that the changing values were due to the hydrolysis of the acetyl group was obtained from the ultra-violet absorption data which shifted from the known value of acetyladenine ($\lambda_{max.}$ 287 mμ to that of adenine ($\lambda_{max.}$ 262 mμ). Table I.

Of the two polarographic waves produced by acetyladenine in 0·1M

perchloric acid, the first became almost negligible and the second increased after one hour on the steam bath. This indicated that the first wave came from the acetyl group and the second from the adenine portion of the molecule. When the acetyladenine was completely hydrolysed, both the remaining single polarographic wave and the ultra-violet absorption data were indistinguishable from the parent compound, adenine.

TABLE I

CHANGE WITH TIME IN ULTRA-VIOLET ABSORPTION OF ACETYLADENINE IN 0·1M PERCHLORIC ACID

mμ	Fresh solution*	1 day	2 days	Plus 2 days at 37° C.
λ Maximum ..	287†	274	270	262
λ Minimum ..	235	232	232	228‡

* Concentration: 10 mg./100 ml. † Known value for acetyladenine. ‡ Known value for adenine.

However, acetyladenine could be stabilised in 50 per cent. aqueous ethanol and when adjusted to a concentration of 0·001M in perchloric acid gave two polarographic waves which were reproducible even after the solution had stood for 24 hours at about 20° C. That these reduction waves occurred only in acidic solutions, and in addition were not simply dependent on the perchlorate ion, was shown by the fact that neither in 50 per cent. aqueous ethanol, nor in this medium containing potassium perchlorate did any polarographic steps appear. However, acidification again produced the two reduction waves which improved in appearance upon the gradual addition of perchloric acid to a concentration of 1 millimolar at which they were stabilised.

TABLE II

POLAROGRAPHIC BEHAVIOUR OF ACETYLADENINE IN 0·001M PERCHLORIC ACID

(Reduction Wave at $E_{\frac{1}{2}} = -1{\cdot}03$ V. against the Saturated Calomel Electrode)

Concentration* M × 10^{-4}	Diffusion Current i_d at 36 cm. (μa.)	Diffusion Current i_d at 64 cm. (μa.)	$\frac{i_d \text{ 36 cm.}}{i_d \text{ 64 cm.}}$
2	0·29	0·39	0·75
4	0·56	0·76	0·74
8	1·11	1·47	0·75

* $E_{\frac{1}{2}}$ remained constant in the range of concentration studied. Temperature was maintained at 20·8° C. (± 0.2°)

Although both waves of acetyladenine could be used for identification and quantitative estimation we chose the first, with a half-wave potential of −1·03 volts (against saturated calomel electrode) for investigation, because it was more clearly defined over a wide range of pH. For this reduction step, the wave height was proportional to the concentration (see Table II) and the plots of the diffusion current i_d against concentration for two values of the height of the mercury reservoir were found to be straight lines. In addition, a comparison was made with the reduction of the cadmium ion whose polarographic wave is known to be diffusion controlled[8]. The current readings of a standard solution of cadmium in 0·1M potassium nitrate were found at two heights of the mercury reservoir,

36 cm. and 64 cm. respectively. The ratio of these values was 0·76 which is in good agreement with the calculated figure of 0·75, i.e., the ratio of the square roots of the two heights chosen. From Table II it can be seen that the acetyladenine steps examined gave similar ratios.

Comparison was made with the polarographic behaviour of yeast nucleic acid. A freshly-prepared solution of yeast nucleic acid in 0·2M potassium chloride showed a reduction wave which was for the most part diffusion controlled and whose half-wave potential was — 1·28 volts against the saturated calomel electrode. The step height was nearly linear with concentration in the range between 4 and 16 mg. per cent. during which interval the $E_{\frac{1}{2}}$ value remained constant. Since of the isolated nucleotides only adenylic acid gave a reduction wave, it may be inferred that the adenine portion was probably involved in the polarographic activity of the nucleic acid.

Discussion

The work of Heath[5] was repeated and confirmed.

The second of the two polarographic steps shown by acetyladenine—the one with the half-wave potential at the more negative value—was the more pH sensitive. As distinct from the first step which is relatively unaffected by changes in acidity of the supporting electrolyte, the second step was more clearly defined in 0·1M than in 0·001M perchloric acid. In the absence of ethanol, large maxima occurred which could be suppressed by the addition of starch solution.

For exact measurement of the second wave carefully controlled conditions were required to prevent the hydrolysis of the acetyl group, a process which caused the observed fluctuation in step heights, and the $E_{\frac{1}{2}}$ values. However, if a water solution was acidified immediately before polarographing, both waves could be determined quantitatively.

The half-wave potential for each wave remained constant with concentration. However, the $E_{\frac{1}{2}}$ values varied with pH and were found to be more negative with decreasing acidity: in 0·001M perchloric acid the value was — 1·03 volts for the first step and — 1·26 volts for the second step, but in 0·1M perchloric acid the corresponding potentials were — 0·85 and — 1·0 volts against the saturated calomel electrode respectively. It was also observed that in less acid solutions, better resolution between the two waves was obtained when the concentration of the acetyladenine was ca. 1×10^{-4}M, a fact which might be of some importance in biological investigations. Table III gives a list of purine derivatives which show polarographic waves in 0·1M perchloric acid.

The polarograph was also used to decide quickly between various methods of acetylation, since the acetylated compounds show the characteristic step with a half-wave potential in the region of —1·2 volts against the anode pool in addition to the purine reduction step in the region of — 1·5 volts. Yet a further application of this method was its use in

identifying adenine as an impurity in a commercial sample of a non-reducible nucleotide. The presence of adenine was later confirmed by R_F values obtained from paper chromatography of the sample.

TABLE III

POLAROGRAPHIC BEHAVIOUR OF SOME PURINE DERIVATIVES IN 0·1M PERCHLORIC ACID

Compound*	Analyses		$E_{\frac{1}{2}}$† volts
	Found	Calc'd.	
Adenylic acid			− 1·34
Adenosine			− 1·42
Adenine	C, 44·35 H, 3·85	C, 44·44 H, 3·73	− 1·46
Acetyladenine‡	C, 47·1 H, 4·0	C, 47·45 H, 3·95	− 1·22, − 1·43
2:6 Diaminopurine ..	N, 56·1	N, 55·97	− 1·4
2:6-Diacetamidopurine ..	N, 35·95	N, 35·9	− 1·27, − 1·5
Nucleic acid§			− 1·32

* All compounds were determined at a concentration of ca. 1×10^{-4} molar, capillary drop time 3·4 sec.
† Against the mercury pool anode, ± 0·02 volts.
‡ $E_{\frac{1}{2}}$ against the saturated calomel electrode = − 0·85, − 1·0 volts.
§ $E_{\frac{1}{2}}$ against the saturated calomel electrode = − 1·28 volts.

SUMMARY

1. The acetylation of 6-aminopurine derivatives were followed polarographically since two reduction waves were produced in distinction to the parent compound which gave but one. That the first of the two waves is due to the presence of the acetyl group was shown by its disappearance when this group was hydrolysed. Confirmation was also obtained by following the changes in ultra-violet absorption.

2. A quantitative study was made on acetyladenine and the reduction step with the more positive half-wave potential was determined in 50 per cent. aqueous ethanol made millimolar with perchloric acid, in which medium it was stabilised.

3. The position and nature of a functional group on the purine ring was found to be a critical factor for polarographic activity. Examples are given which show that the reduction waves obtained were associated with the amino group of its *N*-acetyl derivative in the 6-position of the purine ring.

4. Nucleic acid gave a well-defined polarographic step in 0·2M potassium chloride. Since of the isolated nucleotides only adenylic acid (or its derivatives) were reducible at the dropping mercury electrode, it was inferred that the adenine moiety was involved, a fact which may prove useful in biological investigations.

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References

1. Bergel, *J. Pharm. Pharmacol.*, 1955, **7**, 297.
2. Barker, Gulland, Parsons, *Nature, Lond.*, 1946, **157**, 482.
3. Barker, Luthy, and (in part) Dhar, *J. chem. Soc.*, 1954, 4206.
4. Chargaff and Davidson, Editors, *The Nucleic Acids*, Vol. I, Academic Press, Inc., New York, 1955.
5. Heath, *Nature, Lond.*, 1946, **158**, 23.
6. Cavalieri and Lowy, *Arch. Biochem. Biophys.*, 1952, **35**, 83.
7. Luthy and Lamb, *Analyst*, 1956, in the press.
8. Kolthoff and Lingane, *Polarography*, 2nd Ed., Vol. II, Interscience Publishers, Inc., New York, 1952, p. 509.