J. Electroanal. Chem., 122 (1981) 363—365
Elsevier Sequoia S.A., Lausanne — Printed in The Netherlands

Short communication

POLAROGRAPHIC INVESTIGATION OF SOME CYTOKININS

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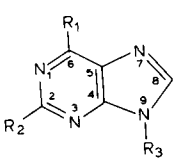
(Received 15th September 1980; in revised form 13th November 1980)

INTRODUCTION

The cytokinins constitute one of the five groups of plant hormones, and are characterised by their ability to promote cell division in cultured plant callus tissue under appropriate conditions [1—3]. With the exception of diphenylurea, all cytokinins that have been unambiguously identified in extracts of higher plants are derivatives of adenine, including 6-amino- and riboside-substituted compounds. While the electrochemical behaviour of other adenine derivatives has been explored quite extensively [4—12], no study has yet been reported on this important class of biologically important adenine derivatives. We have, therefore, carried out a preliminary investigation into the polarographic behaviour of the following cytokinin derivatives as a prelude to more detailed investigations in this topical field of research.



I zeatin; 6-(4-hydroxy-3-methylbut-2-enyl)aminopurine (trans)
R_1 = —NH · CH_2 · CH : $C(CH_2OH)CH_3$; R_2 = H; R_3 = H
II zeatin riboside; 6-(4-hydroxy-3-methylbut-2-enyl)aminopurine riboside (trans)
R_1 = —NH · CH_2 · CH : $C(CH_2OH)CH_3$; R_2 = H; R_3 = ribose
III 2-methylthiozeatin; 2-methylthio-6-(4-hydroxy-3-methylbut-2-enyl)aminopurine (trans)
R_1 = —NH · CH_2 · CH : $C(CH_2OH)CH_3$; R_2 = CH_3S; R_3 = H
IV 6-(3-methylbut-2-enyl)aminopurine riboside
R_1 = —NH · CH_2 · CH : $C(CH_3)_2$; R_2 = H; R_3 = ribose

EXPERIMENTAL

Polarographic measurements were carried out using a P.A.R. Model 303 mercury electrode system in conjunction with a P.A.R. Model 174A polarographic analyser. A sample of 6-(3-methylbut-2-enyl)aminopurine riboside was obtained from Sigma, whereas samples of zeatin, zeatin riboside and 2-methyl-

thiozeatin were synthesised by Dr. Roger Horgan, University College of Wales, according to the methods outlined by Leonard [13]. Stock solutions of these cytokinins (ca. 10^{-3} *M*) were prepared in distilled water and diluted to 10^{-4} *M* using 0.1 *M* HCl or Britton—Robinson (BR) buffers of pH 2—6 prior to polarographic investigation.

RESULTS AND DISCUSSION

The effect of pH on the peak potential, as measured using differential pulse polarography, of the main process exhibited by the four cytokinins studied is shown in Fig. 1. In solutions of pH 1—3, zeatin exhibited a small second peak at more negative potentials (—1.17 V vs. Ag/AgCl) which was independent of pH in this range, but which disappeared on increasing the pH above 3. In the case of the other three compounds, this second peak was exhibited as a shoulder to the main peak and disappeared in solutions of pH >2.

All four compounds exhibited the main process in solutions of pH up to 4.5, at which point the limiting current began to decrease and the process became less diffusion controlled. This indicates that only the protonated forms of these molecules are reduced at the electrode surface since the pK_a values of these compounds are around 4 in aqueous solution [7]. Between pH 1 and 4.5, the electrode process is mainly diffusion controlled but, as can be seen from the normal pulse polarographic behaviour of these compounds (as illustrated for the case of zeatin in Fig. 2a), there is also a degree of reactant adsorption involved in the overall electrode process [14]. A cyclic voltammetric study also showed that the process was irreversible in nature (Fig. 2c). In terms of the ease of reduction of these compounds, addition of a ribose moiety to the adenine ring system makes reduction more difficult, whereas substitution of a methylthio group for a hydrogen atom at position 2 appears to make it a little easier. In addition, substitution of a $-C{=}C(CH_2OH)CH_3$ group in the side-chain at position 6 by a $-C{=}C(CH_3)_2$ group would appear also to make it more difficult.

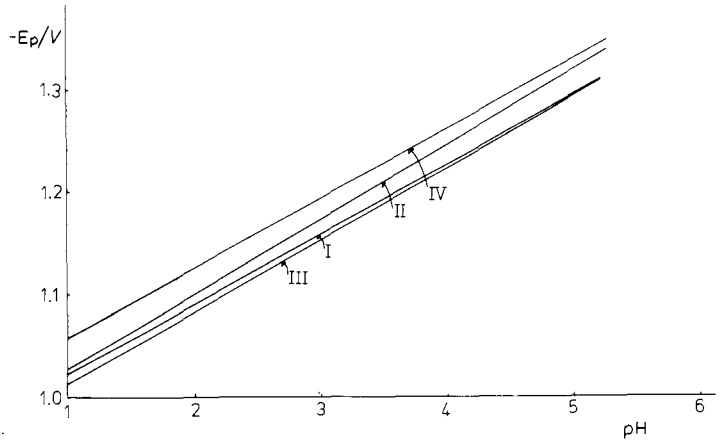


Fig. 1. Plots of E_p vs. pH for zeatin (I), zeatin riboside (II), 2-methylthiozeatin (III) and 6-(3-methylbut-2-enyl)aminopurine riboside (IV) (v = 5 mV s^{-1}, ΔE = 50 mV).

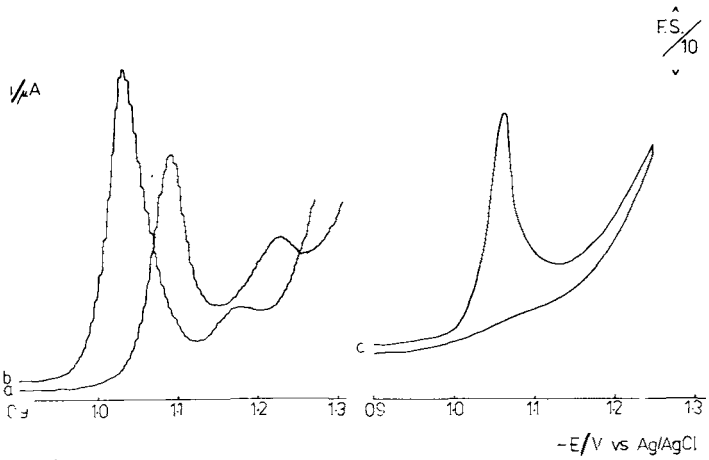


Fig. 2. Voltammetric behaviour of zeatin in 0.1 *M* HCl as measured using: (a) normal pulse polarography (v = 5 mV s^{-1}, ΔE = 50 mV, F.S. = 20 μA); (b) differential pulse polarography (v = 5 mV s^{-1}, ΔE = 50 mV, F.S. = 5 μA); (c) cyclic voltammetry (v = 50 mV s^{-1}, F.S. = 10 μA).

If one assumes that the reduction process involves successive reduction of $N_1{=}C_6$ and $C_2{=}N_3$ double bonds in the adenine nucleus, as reported by other authors [6,11], then it is clear that the side-chain in position 6 must have an indirect effect on the reduction process, probably due to its adsorption properties. This is probably also the cause of the second peak exhibited by these compounds, since compounds containing only an $-NH_2$ group in position 6, e.g. adenosine, do not show this behaviour. The reason why this adsorption is most pronounced in the case of zeatin, as compared with the other compounds studied is not yet clear, and it is obvious that more detailed experiments using fast scan linear sweep and alternating current techniques should be carried out to investigate this more fully.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. R. Horgan and Prof. Dr. H.W. Nürnberg for valuable discussions. D.B.K. is indebted to the Science Research Council, London, for financial assistance.

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