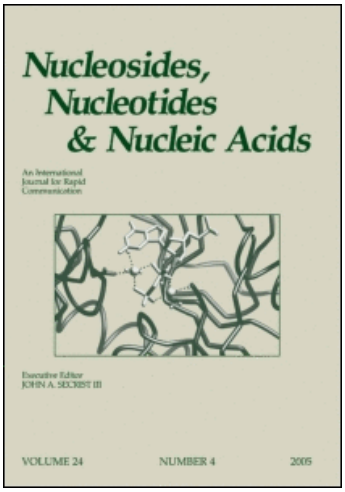

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Electrochemical Analysis of 3'-Azidothymidine (AZT)

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ELECTROCHEMICAL ANALYSIS OF 3'-AZIDOTHYMIDINE (AZT)

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Abstract: 3'-Azido-3'-deoxythymidine (AZT) exhibits a two-electron diffusion-controlled polarographic reduction wave, with conversion to 3'-amino-3'-deoxythymidine. The mechanism of reduction, analytical and clinical applications, and its use for one-step synthesis of amino from azido nucleosides, are described.

The thymidine analogue 3'-azido-3'-deoxythymidine (AZT), a potent inhibitor of retroviruses and human immunodeficiency virus (HIV), is currently clinically approved for treatment of selected patients with AIDS and AIDS-related complex (1). However, the source(s) of its toxic side-effects remains to be clarified, and lends interest to studies of its chemical and biochemical reactivities. Thiol reduction of alkyl and aryl azides, long known, has been shown, in the case of AZT, to lead to conversion to the 3'-amino-3'-deoxy congener (2).

In a continuation of studies on electrochemical reduction of purine and pyrimidine analogues (3), we have found that AZT is readily reduced at the dropping mercury electrode. With both d.c. polarography and d.p.p. (differential pulse polarography), AZT exhibits a diffusion-controlled two-electron reduction wave over the pH range 2 - 12. Dependence of the half-wave potential ($E_{1/2}$) and limiting current (I_g) of the reduction wave of AZT is shown in Fig.1. The height of the wave is linearly dependent on the AZT concentration, permitting of its analytical determination in aqueous medium by d.p.p. in the range 1×10^{-3} to 2×10^{-7} M.

Electrolysis of AZT (as well as AZdU) conducted on 10^{-4} M solutions buffered at pH 7 at a potential of -1.1 V, was accompanied by disappearance of the cathodic wave at $E_{1/2}$ = -1.05 V, with simultaneous appearance of an anodic wave at $E_{1/2}$ = -0.15 V, identical to that exhibited by 3'-deoxy-3'-aminothymidine. The initial UV absorption maximum of the solution at 266.5 nm shifted from 266.5 nm to 265 nm, the minimum from 234.5 nm to 233 nm, and the ratio of maximum to minimum from 4.5 to 4.0, the final spectrum corresponding to 3'-deoxy-3'-aminothymidine, further confirmed by appearance chromatographically of a single product with the mobility of authentic 3'-deoxy-3'-aminothymidine.

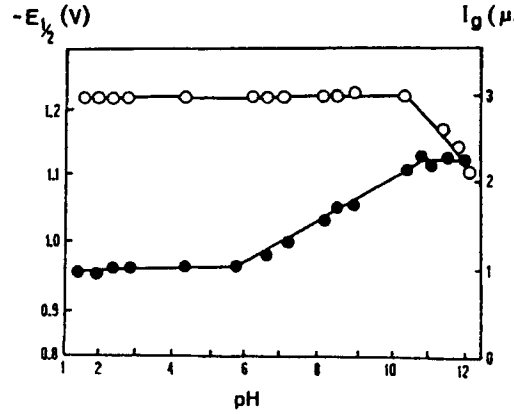

Fig. 1: pH-dependence of half-wave potential ($E_{1/2}$, -●-●-) and limiting current (I_g, -o-o-) of (2 x 10^{-4} M) AZT.

From the height of the anodic wave $E_{1/2}$ = -0.15 V for 3'-deoxy-3'-aminothymidine, and the spectral changes accompanying formation of this product from AZT, the reaction was virtually quantitative (96-98%). Furthermore, electrolysis of AZT in acid medium (pH 2) turns out to be a convenient procedure for the large-scale preparation of 3'-deoxy-3'-aminothymidine, probably applicable more generally to one-step conversion of various azido nucleosides to their amino derivatives, as in the case of reduction of azido derivatives of cinnamic acids (4).

Analytical determination. Levels of AZT were determined by d.p.p. in 0.1 M phosphorate buffer pH 7. In aqueous medium the height of the reduction peak was linearly dependent on AZT concentration over the range 10^{-3} M - 10^{-7} M. With serum samples, following deproteination with trichloracetic acid, neutralization with 1 M NaOH, and 10-fold dilution with 0.1 M phosphate buffer, the lower detection limit was 5 x 10^{-6} M. Samples of urine were diluted 10-fold with 0.1 M KCl prior to polarography, the lower limit of detection being then also 5 x 10^{-6} M. Attempts to improve sensitivity are under way.

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