2-Methyl-4-oxo-3,4-dihydropyrrolo[3,2-d]pyrimidine (VIII). A 1-g sample of acid VII was mixed with 1.5 g of sea sand, and the mixture was heated in vacuo (10-12 mm) at a bath temperature of 320-325°C for 5 min. The reaction product was extracted with 3% HCl solution, and the extract was treated with charcoal and neutralized with dry $NaHCO_3$. Pyrrolopyrimidine VIII was removed by filtration. The yield of product with mp > 330°C (dec.) was 0.4 g (57%). Found, %: C 56.2; H 4.8; N 28.2. $C_7H_7N_3O$. Calculated, %: C 56.3; H 4.7; N 28.2.

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POLAROGRAPHIC REDUCTION OF ADENINE AND ITS 9-(α,ω-DIHYDROXY-2-ALKYL) DERIVATIVES

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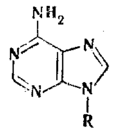
The polarographic reduction of adenine and its 9-(α,ω-dihydroxy-2-alkyl) derivatives on a short-period mercury electrode in strongly acidic media is determined by the rate of diffusion of the corresponding cations and, at pH values that exceed the pK_a values, by the kinetics of surface protonation of the uncharged molecules; this was proved by the dependence of the limiting current on the height of the mercury column, the temperature, and the depolarizer concentration. The polarographic reduction of adenine, adenosine, 9-(α,ω-dihydroxy-2-alkyl)adenines, and the corresponding phosphates in acidic media proceeds with the loss of sic electrons per molecule. The similar trends of the change in the half-wave potentials and the numerical pK_a values is associated with the fact that the site of protonation is simultaneously the site of nucleophilic attack by the electron during electrical reduction.

The electrochemical reduction of purine derivatives, including adenine, on a mercury electrode has already been examined [1-3]. In acidic media adenine is reduced in one overall six-electron step; it has been assumed that the electrode reaction involves only the pyrimidine portion of the molecule, in which the C=N bonds in the 1,6 and 2,3 positions and the deamination product undergo successive reduction to give the 1,2,3,6-tetrahydro derivative of adenine [1]. Adenosine and the corresponding nucleotides also undergo electrical reduction and give one polarographic wave, which with respect to its origin is analogous to the wave of adenine [2].

The aim of the present research was to verify the common character of the phenomena observed in the electrical reduction of the above-indicated natural compounds and their synthetic analogs — (9-α,ω-dihydroxy-2-alkyl)adenines (Ic,d) and the corresponding α,ω-diphosphates



I a-f

I a R=H; b adenosine; c $R=CH(CH_2OH)_2$; d $R=CH(CH_2CH_2OH)CH_2OH$; e $R=CH(CH_2OPO_3H_2)_2$; f $R=CH(CH_2CH_2OPO_3H_2)CH_2OPO_3H_2$

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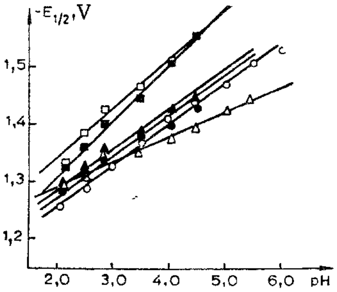

Fig. 1. Dependence of $E_{1/2}$ on the pH for the waves of Ia (-○-), Ib (-●-), Ic (-△-), Id (-▲-), Ie (-□-), and If (-■-) (in an aqueous Britton—Robinson buffer solution at a depolarizer concentration of $5 \cdot 10^{-4}$ mole/liter).

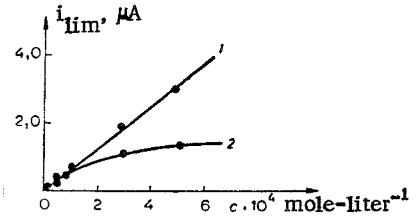

Fig. 2. Dependence of i_{lim} of the wave of adenine on the concentration: 1) pH 2; 2) pH 5 (aqueous Britton—Robinson buffer solution at concentrations from $1 \cdot 10^{-5}$ to $5 \cdot 10^{-4}$ mole/liter).

(Ie,f) [4, 5]. Since we studied Ic-f in Britton—Robinson universal buffer solutions on a short-period mercury electrode, we felt it was necessary to make a comparative polarographic study under the same conditions of adenine and adenosine in order to obtain comparable results. Their polarographic study on a brief-period mercury electrode also is of independent significance, for it makes it possible to ascertain the role of protolytic and adsorption processes in the electrical reduction of these compounds.

Polarographic Behavior of Adenine and Adenosine

In strongly acidic media (pH 2) adenine gives one partially reversible polarographic reduction wave at $E_{1/2} = -1.26$ V. The recording of the oscillographic polarograms and the dependence of the strength of the limiting current (i_{lim}) on the frequency of the alternating voltage indicate partial reversibility of the wave. As the pH increases, the limiting current of adenine decreases in the form of a dissociation curve (the polarographic value of which is designated by pK_a^{pol}), and the wave vanishes completely when pH > 6. As the pH increases, the $E_{1/2}$ values are shifted to more negative potentials, and the $\Delta E_{1/2}/\Delta pH$ coefficient is —70 mV/pH unit (Fig. 1).

When the polarographic waves of adenine recorded at pH 2 and 5 are compared, it is noted that the character of the wave changes markedly as the pH changes (Fig. 2). At pH 2 the i_{lim} value is directly proportional to the square root of the height of the mercury column, whereas at pH 5 it is directly proportional to the height of the mercury column. The i_{lim} values of the wave of adenine increase at pH 2 as the temperature rises, whereas at pH 5 they decrease (Fig. 3).

Considering the above peculiarities of the wave of adenine at pH 2 and the fact that the limiting current of adenine has low temperature coefficients (1.6% per degree) under these conditions, decreases with the amount of ethanol added to the solution in conformity with the Stokes—Einstein equation, and increases in proportion to the drop period of the

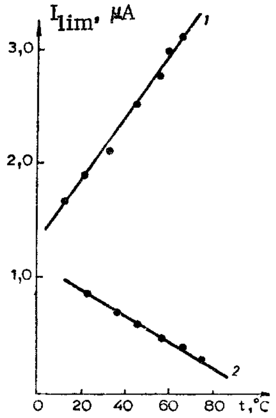

Fig. 3. Dependence of i_{lim} of the wave of adenine on the temperature: 1) pH 2; 2) pH 5 (aqueous Britton—Robinson buffer solution at a depolarizer concentration of $5 \cdot 10^{-4}$ mole/liter).

TABLE 1. True (pK_a^{true}) and Polarographic (pK_a^{pol}) Dissociation Constants, Half-Wave Potentials ($E_{1/2}$), and Limiting Currents (i_{lim}) at pH 2 for Adenine, Adenosine, and 9-(α,ω-Dihydroxy-2-alkyl) Derivatives

Compound	pK_atrue	pK_apol	pK_apol − pK_a true	$-E_{1/2}$, V	i_{lim}, μA
Ia	4,18	5,32	1,14	1,260	4,24
Ib	3,52	5,07	1,55	1,285	4,12
Ic	3,97	4,15	0,18	1,285	3,20
Id	4,00	4,25	0,25	1,300	3,44
Ie	4,14	4,43	0,29	1,335	3,56
If	4,24	4,83	0,59	1,325	3,96

mercury electrode (the slope of the line in coordinates of log i_{lim} and log t is 0.2 [6]), it may be concluded that in acidic media up to the point at which the true (thermodynamic) pK_a value of protolysis of adenine is reached the wave of the electrical reduction of this compound is a diffusion wave. As the pH increases, the wave of adenine takes on kinetic character, i.e., the limiting current is determined by the rate of protonation of the neutral electrochemically inactive adenine molecule to give the electrochemically active cation.

The protonation of the adenine molecule when pH > pK_a is also confirmed by the difference in the "apparent" and true values of the protonation constants of adenine and related compounds (Table 1). This effect cannot be the result of self-association of the molecules on the electrode surface, since self-association should have caused a decrease in the effective pK_a value [7].

The experimental data also showed that the wave observed at pH 5, i.e., in the kinetic region, has adsorption character. This follows from a calculation of tan α (the slope of the line in coordinates of log i_{lim} and log H_{Hg}, where H_{Hg} is the height of the mercury column), which for the diffusion current should be half the value for the adsorption current [8]. At pH 2, tan α for adenine was found to be about 0.62, as compared with about 1.0 at pH 5. The character of the dependence of i_{lim} on the concentration also indicates the adsorption nature of the wave in this case (Fig. 2). The absence of a rectilinear section parallel to the axis of abscissas constitutes evidence that adsorption equilibrium of adenine on the electrode surface is still not reached over the investigated concentration range; this is probably a consequence of the use of a fast-dropping electrode [9].

The adsorption character of the wave of adenine at pH values that exceed the pK_a^{true} value provides evidence that the rate of protonation of the neutral adenine molecules is determined by their rate of adsorption on the electrode, and only previously adsorbed adenine molecules, which have time to undergo protonation during the lifetime of the mercury drop, are consequently reduced on the electrode.

In principle, similar phenomena are observed during a study of the electrical reduction of adenosine under the same conditions.

Polarographic Behavior of 9-(α,ω-Dihydroxy-2-alkyl)adenines (Ic-f)

In strongly acidic media Ic-f give one polarographic wave, which, according to calculations with the Ilkovic equation, is a six-electron wave. The limiting current values of these compounds remain practically constant up to pH 3.5, whereas they decrease sharply in the form of the dissociation curve as the pH is increased durther; the wave vanishes completely when pH > 5.5. The pK_a^{pol} values are always higher than the true pK_a values determined in [5] for the reasons set forth in the preceding section.

The increase in the $E_{1/2}$ values as the corresponding pK_a^{true} values of Ia-d increase (Table 1) is associated with the fact that the pK_a values are proportional to the electron density at the site of protonation [the $N_{(1)}$ atom], whereas in this case the site of protonation is simultaneously the site of nucleophilic attack by the electron during electrical reduction.

The dependences of i_{lim} and $E_{1/2}$ on the depolarizer concentration, temperature, percentage of ethanol in the mixed aqueous alcohol solution, and other experimental factors for Ic–f are generally identical in character and in turn do not differ fundamentally from the dependences for adenine. However, it should be noted that the limiting currents of Ib–f are lower than i_{lim} of adenine at identical pH values; this is associated with the larger size of the molecules and consequently with the decrease in their diffusion coefficients.

On the basis of the data obtained it can be concluded that the character of the electrical reduction of the synthetic analogs of adenosine is similar to the electrical reduction of adenine and natural adenosine. The introduction of sugar, sugar phosphate, and diol residues does not change the mechanism of electrical reduction fundamentally, but these groups do have a certain effect on the adsorbability, the diffusion coefficients, and other polarographic characteristics of the compounds. The experimental results constitute evidence in favor of the assumption that all of the investigated compounds are adsorbed via the purine ring on a mercury electrode.

The shift of the $E_{1/2}$ values to the more negative region in the case of Ie,f as compared with Ia–d (Fig. 1) is explained by ionization of the phosphate groups. The negatively charged phosphate groups are repulsed from the like-charged electrode surface; this is in agreement with the data obtained by comparison of natural adenine nucleosides and nucleotides [3].

The less pronounced change in the $pK_a^{pol} - pK_a^{true}$ differences in the case of the synthetic analogs of adenosine as compared with adenine and adenosine should possibly be associated with a certain degree of self-association of the molecules of these compounds on the electrode surface in the adsorbed state.

EXPERIMENTAL

The polarographic studies of Ia–f were made in aqueous Britton–Robinson universal buffer solutions [10]; the depolarizer concentration in the polarographic solution was $5 \cdot 10^{-4}$ mole/liter, and the ionic strength was 0.25. Ethanol was added to the aqueous solutions in individual cases.

The polarograms were recorded with a Radiometer PO-4 recording polarograph with a two-electrode thermostatted cell at 25.0 ± 0.2°C. A dropping mercury electrode with forced detachment of the drops [11], and characteristics m = 0.31 mg/sec and t = 0.25 sec was used as the cathode; a saturated calomel electrode served as the anode.

The number of electrons consumed in the electrical reduction of one depolarizer particle was calculated for Id from the Ilkovic equation. The diffusion coefficient of Id was estimated from pycnometric data from the Stokes–Einstein equation ($D = 6.4 \cdot 10^{-6}$ cm^2/sec) [11]. The number of electrons was found to be 5.89.

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