Photochemically Reversible Dimer Electroreduction Product of 2-Oxopurine

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The electrochemical reduction of 2-oxopurine in buffered aqueous medium was found to proceed *via* two successive one-electron additions. The initial one-electron step involves the addition of a proton, leading to formation of a free radical which rapidly dimerizes. At more negative potentials, reduction leads to formation of 1,6-dihydro-2-oxopurine. The product of reduction on wave I was isolated and identified by various spectroscopic techniques as the dimer 6,6′-*bis*-(1,6-dihydro-2-oxopurine). In neutral aqueous medium this dimer reduction product (but not the product of reduction on wave II, 1,6-dihydro-2-oxopurine) underwent quantitative photochemical dissociation to the parent 2-oxopurine, with a quantum yield at 254 nm of 0.03. The dimer reduction product also quantitatively regenerated the parent monomer in an alkali-catalyzed reaction at room temperature. The proposed mechanism of the reduction reaction, and susceptibility of the reduction product to photodissociation, is compared with the corresponding behaviour of other purine, and pyrimidine, derivatives.

Although the electrochemical reduction properties of 2-oxopurine (**1**) have not hitherto been investigated, it has been inferred that its polarographic behaviour would resemble that of purine[1], largely on the basis of the similarity in $E_{1/2}$ values for pyrimidine and pyrimidone-2[1, 2]. This appears reasonable in the light of observations that electrochemical reduction of purine derivatives proceeds only on the pyrimidine ring, the imidazole moiety being polarographically inert[1, 3, 4].

In view of the previous demonstration that pyrimidone-2 undergoes a one-electron reduction, with formation of a dimer identified as 6,6′(4,4′)-*bis*-(3,6-dihydro-pyrimidone-2) (**3**), which is susceptible to quantitative photochemical dissociation to the parent pyrimidone-2[5], it appeared of interest to examine whether an analogous reaction might occur with 2-oxopurine. As will now be shown, this expectation has been fully realized.

Materials and Methods

Chemicals. 2-oxopurine was a product of Sigma Chemical Co. (St. Louis, Mo., U.S.A.).

Apparatus. D.C. polarograms were recorded on a Radiometer Polariter PO_4 instrument, using a dropping mercury electrode (DME) with the following characteristics: $m = 2.54$ mg s^{-1}, $t = 3.30$ s^{-1} at open circuit in distilled water at a mercury pressure $h = 60$.

Polarography. Test solutions, about 5×10^{-4} M, were prepared by dilution of a stock solution with Britton-Robinson buffers to cover the pH range 2 – 12. The ionic strength was maintained constant at 0.2 by addition of KCl. Solutions were rendered oxygen-free by bubbling nitrogen previously purified by the procedure of Meites[6]. All measurements were at 25° ± 0.2 °C, potential measurements being made *vs* S.C.E.

Macroelectrolysis. Controlled potential electrolysis and coulometry were carried out with the use of a mercury pool electrode (∼ 20 cm^2) at 25° ± 0.1 °C fitted with a magnetic stirrer, with a 2-oxopurine concentration of 1.2×10^{-3} M in Britton-Robinson buffer pH 7 and pH 4.5. A silver coulometer in the circuit was used to determine the number of electrons involved in the reduction process. Nitrogen was continuously passed through the cell during electrolysis. Immediately before and after electrolysis the solution was examined spectrophotometrically after dilution with the buffer used for electrolysis.

Ultraviolet absorption spectra were obtained with the aid of a Zeiss (Jena, GDR) VSU-2 spectrophotometer.

Infrared spectra were recorded on a Zeiss (Jena, GDR) UR-10 spectrophotometer, using samples in KBr matrices.

A Radiometer PHM-4d compensating instrument, with associated glass electrode, was employed for pH measurements.

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Ultraviolet irradiation was with a low-pressure mercury resonance lamp (254 nm) as previously employed. Actinometry, based on photochemical hydration of uridine, was employed for calculation of quantum yields as elsewhere described [7].

Mass spectra were determined on MAT 311 and LKB 9000 instruments at 70 eV (275 °C ion source) and sample temperatures of 150° and 180 °C.

^{1}H NMR spectra were recorded on a Zeiss (Jena, GDR) 60 MHz ZKR-60 spectrometer, fitted with an SPT-1 signal accumulator (Zentrum für Wissenschaftliche Geräte, Berlin, GDR). Solutions were made up to a concentration of 1.5 mg/ml in CD_3COOD, in which both 2-oxopurine and its reduction product were soluble, with TMS as internal standard.

Results

D.C. Polarography. In aqueous buffered medium, 2-oxopurine underwent polarographic reduction with formation of two waves of equal height. The diffusion-like nature of both waves was established from the linear dependence of i_d on $h^{1/2}$, the temperature coefficient (1.8%/°C), and the linear dependence of i_d on concentration over the range 2×10^{-4} M to 2×10^{-3} M.

At pH values below 3, both waves were virtually independent of pH (Fig. 1). At pH values above the pK for protonation of the starting compound, 2.74[8], the half-wave potentials for both waves exhibited linear pH-dependence, as follows:

$$\text{for wave I:} \quad E_{1/2} = -0.46 - 0.086\,\text{pH};$$

$$\text{for wave II:} \quad E_{1/2} = -0.57 - 0.096\,\text{pH}.$$

On the basis of a logarithmic analysis, both waves were found to exhibit non-reversible characteristics. At pH >8 both waves merge to form a single wave with a height equal to the sum of waves I and II.

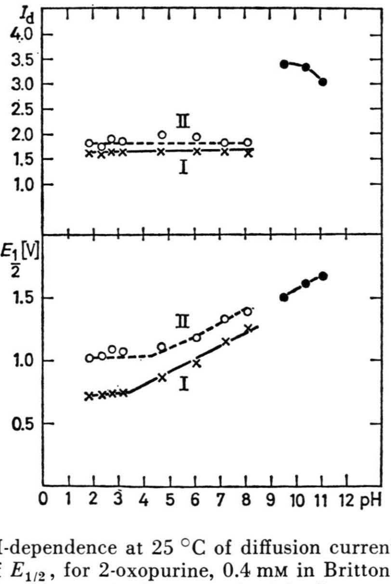


Fig. 1. pH-dependence at 25 °C of diffusion current constant I_d, and of $E_{1/2}$, for 2-oxopurine, 0.4 mM in Britton-Robinson buffers at constant ionic strength 0.2. Roman numerals denote the observed waves.

The appearance on wave II of a "post-wave" pointed to possible partial adsorption characteristics for this wave, which we ascribe to adsorption of the reduction product of wave I. Coulometric determinations, average $n = 0.95$ at pH 7, pointed to both waves as being one-electron.

In aqueous non-buffered solutions of tetraalkylammonium salts, *e.g.*, 0.1 M $(CH_3)_4NCl$, 2-oxopurine displayed two waves, I ($E_{1/2} = -1.25$ V) and II ($E_{1/2} = -1.43$ V), of equal height, similar to those for pyrimidone-2 [5], nicotinamide [9], and NAD$^+$ [10, 11]. The half-wave potential of wave I showed no concentration dependence, whereas that of wave II shifted to more negative values with increasing concentration.

Cyclic voltammetry. The behaviour of 2-oxopurine under cyclic voltammetry conditions at the hanging drop mercury electrode substantiated the D.C. polarographic results. In acid solution, a sweep to more negative potentials gave rise to two cathodic peaks, Ic and IIc. These shifted to more negative potential with increasing pH, and merged to a single peak at pH 8. When the potential sweep was reversed, no anodic peak was discernible at a slow scan rate (8 – 50 mV/sec). This is consistent with the conclusion that the electron transfer processes for both reduction steps of purine are rapid [3]. The irreversibility of peak Ic is due to very fast deactivation of the reduction product by follow-up dimerization (see below), as previously reported for pyrimidone-2 [5], nicotinamide [9] and NAD$^+$ [11]. With increasing pH, peak IIc merged with the subsequent catalytic hydrogen wave.

Macroelectrolysis

Wave I. Electrolysis on wave I of 2-oxopurine at pH 4.5 and pH 7, at potentials corresponding to the initial limiting current, led to the simultaneous disappearance of reduction wave II. The observation that wave II is eliminated upon controlled potential electrolysis of wave I further supports a free radical mechanism. The reduction product no longer exhibited the characteristic absorption bands of 2-oxopurine at 315 nm and 238 nm, but possessed a single new band at 236 nm. As reduction proceeded, the solution became turbid as a result of precipitation of the product. Following electrolysis the latter was isolated by centrifugation or filtration, washed several times with water, and dried under vacuum over P_2O_5. Yields at room temperature were about 60% in different runs, but could be increased considerably by lowering the temperature.

Wave II. Electrolysis at the potential of wave II was accompanied by slight turbidity, shown to be due to partial formation of the same reduction product as for electrolysis on wave I. Coulometric measurements, however, yielded an average $n = 1.75$ for electrolysis at a potential corresponding to the crest of wave II, at pH 7. It follows that the major reduction product of 2-oxopurine at the potential corresponding to wave II is the dihydroderivative, 1,6-dihydro-2-oxopurine. In contrast to the reduction product on wave I, it was soluble in water. Although no attempt was made to isolate this product, its absorption spectrum exhibited a single band at 235 nm at neutral pH, identical with that for the reduction product of wave I (see below). This is in agreement with the coulometric measurements, pointing to 2-electron reduction, obviously of the 1,6 bond, since the imidazole ring is non-reactive.

Properties of reduction product of wave I. The isolated reduction product of wave I was a pale yellow amorphous powder, m.p. 300 °C (decomp.), relatively insoluble in aqueous medium and in a wide variety of organic solvents, and attempts at crystallizationwere unsuccessful. Elementary analysis gave: C, 39.01%; H, 4.14%; N, 36.48%. Calculated, for a dihydrated dimer of reduced 2-oxopurine, *i.e.* $C_{10}H_8N_8O_2 \cdot 2\,H_2O$: C, 38.71%; H, 4.19%; N, 36.13%. Similar strong binding of water has been observed in the crystals of a tetramer photoproduct of thymine [12, 13] and the electrochemical dimer reduction product of pyrimidone-2 [5].

In contrast to the parent 2-oxopurine, the UV absorption spectrum of which, for the neutral form, exhibits $\lambda_{\max}$ 315 nm ($\varepsilon_{\max}$ 4.9×10^3) and $\lambda_{\max}$ 238 nm ($\varepsilon_{\max}$ 2.9×10^3), the reduction product exhibited a single short-wavelength band, $\lambda_{\max}$ 235 nm ($\varepsilon_{\max}$ 4.5×10^3). This is consistent with elimination of one of the double bonds in the pyrimidine ring and, in fact, the absorption spectrum of the reduction product is that of a substituted imidazole [14].

In strongly alkaline medium, there was a time-dependent disappearance of the 235 nm band of the reduction product, with the simultaneous appearance of two new bands at 260 nm and 315 nm, characteristic of the absorption spectrum of the parent 2-oxopurine in alkaline medium. The alkali-catalyzed conversion of the reduction product to the parent 2-oxopurine, verified spectrally and polarographically, was quantitative. The reaction was pseudo first-order at pH 13 and proceeded at room temperature with a $t_{1/2}$ of about 3 h. At pH 12 the rate was reduced about 10 fold, and was almost imperceptible at pH 11. By contrast, an increase in pH to 14 was without effect on the reaction rate. It follows that the conversion reaction in alkali is due to the instability of the anionic form of the reduction product, in agreement with spectral observations that it possessed a pK somewhat higher than 12, which could not be accurately measured because of the inherent instability of the anion.

Infrared spectroscopy. Due to solubility limitations (see above), infrared spectra were recorded on KBr matrices. The spectrum of 2-oxopurine exhibited an intense band centred at 1660 cm^{-1}, corresponding to a carbonyl frequency [15], in agreement with the accepted 2-keto structure of this compound. The reduction product exhibited a similar intense band centred at 1652 cm^{-1}, showing that the 2-keto structure is maintained [5, 15]. Furthermore the reduction product also possessed a series of bands in the region 1100 – 1500 cm^{-1}, strikingly similar to that of the dimer reduction product of pyrimidone-2, and ascribed to characteristic frequencies of reduced pyrimidone-2 [5]. The existence of this series of bands in the reduction product of 2-oxopurine provides further evidence that reduction occurred in the pyrimidine ring.

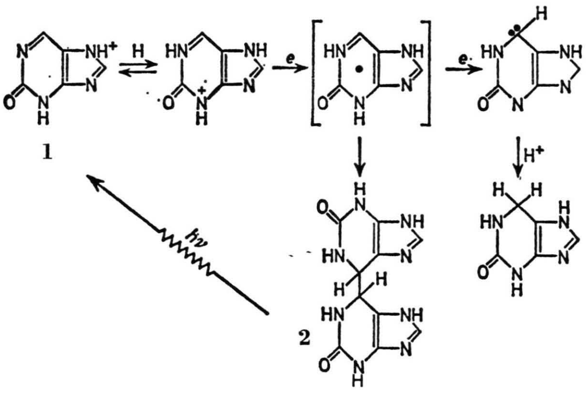
Mass spectrum analysis: The mass spectrum of 2-oxopurine is similar to that of hypoxanthine (6-oxopurine) [8, 16], with peaks at *m/e* 136, 109, 81, 54, 44, 28. A similar spectrum is exhibited by xanthine (2,6-dioxopurine) [8].

The mass spectrum of the reduction product of 2-oxopurine exhibited the peaks characteristic for 2-oxopurine, plus additional intense peaks at 231 (6.8), 203 (8.0), 194 (8.5), 149 (81), 137 (12.3), 136 (72.3), 135 (17.9), 109 (24.3), 82 (15.7), 82 (19.1), 55 (24.3), 43 (100). A very faint peak appeared at *m/e* 274, corresponding to a dimer of reduced 2-oxopurine; the very low intensity of this peak is readily accounted for by the high melting point of the reduction product.

The appearance of intense peaks at $m/e > 200$, plus the intense peak at 136 corresponding to the parent 2-oxopurine, suggested that the reduction product is a dimer. This was further reinforced by the appearance of a relatively strong peak at $231 = 274 - 43$, corresponding to the expected elimination of HCNO from the dimeric reduction product.

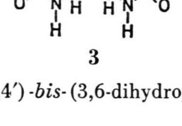
^{1}H NMR spectroscopy. The ^{1}H NMR spectrum of 2-oxopurine (**1**) in CD_3COOD exhibits two singlets, at 8.43 ppm (H-6) and 8.75 ppm (H-8). The reduction product exhibits two singlets at 4.63 ppm and 8.09 ppm.

Since the UV absorption spectrum of the reduction product is that of a substituted imidazole, the IR spectrum shows that it is 2-keto, while the mass spectrum indicates that it is a dimer, a reasonable structure for the product is **2** (Scheme I). The signal at 8.09 ppm would then correspond to H-8 and that at 4.63 ppm to H-6.



Scheme 1. Proposed reaction pathway for the reduction of 2-oxopurine in aqueous medium.

This assignment is supported by a comparison with the dimer reduction product of pyrimidone-2 (**3**) (Scheme II)[5]. The reduced pyrimidine rings in **2** and **3** are similar, so that the chemical shift of H-6 in **2** should be similar to that of H-6 in **3**. The imidazole ring in the former would be expected to lead to a moderate downfield shift, in agreement with the value of 4.63 ppm for H-6 in **1** as compared to 4.15 ppm in **3**. One would also expect H-8 in 2-oxopurine to undergo an upfield shift following reduction of the $N_1 - C_6$ bond, in accord with the observed change in chemical shift of H-8 from 8.75 ppm in 2-oxopurine to 8.09 ppm in **2**.



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Scheme 2. 6,6′(4,4′)-*bis*-(3,6-dihydropyrimidone-2).

The foregoing interpretation is confirmed by the results of earlier studies on 1 : 1 photoadducts of alcohols to purine[17, 18]. The products were identified as substituted 1,6-dihydropurines[17], and the site of addition of the α-hydroxyalkyl groups was readily established as C-6 on the basis of the ^{1}H NMR spectra prior to, and following, selective deuteration at C-6 or C-8. In this way the singlets at 4.89 ppm and 7.25 ppm for the photoproducts in DMSO were assigned to H-6 and H-8, respectively. These values confirm our assignments for the 2-oxopurine reduction product II of 4.63 ppm for H-6 and 8.05 ppm for H-8.

Photodissociation of reduction product. Irradiation at 254 nm of a neutral aqueous solution of the reduction product of 2-oxopurine led to the stepwise disappearance of the absorption band at 235 nm, and the simultaneous appearance of a new band with λ_{max} 315 nm (Fig. 2). It will be noted from the figure that the modified absorption spectra accompanying the course of the photochemical conversion reaction pass through two isosbestic points

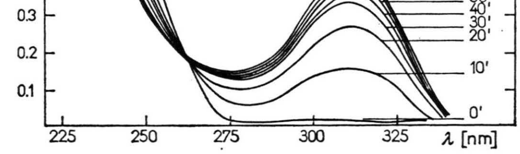

Fig. 2. Photochemical transformation, on irradiation at 254 nm, of the dimer reduction product of 2-oxopurine, 0.8×10^{-4} M in 0.01 M phosphate buffer, leading to quantitative regeneration of the parent 2-oxopurine as shown by the isosbestic points at 222 and 263 nm. Figures beside each curve correspond to the time of irradiation in minutes.

at 222 and 262 nm, consistent with formation of a single ultraviolet absorbing photoproduct. Chromatography, the absorption spectrum of the product and its pH-dependence, as well as polarographic analysis, showed the product to be the parent 2-oxopurine.

From Fig. 2 it is clear that the photochemical conversion reaction does not go to completion. This result, at first sight puzzling, becomes clear when it is recalled that the irradiation wavelength is 254 nm. At this wavelength the conversion reaction is accompanied by a marked decrease in absorbance of the reduction product and an increase in that of the photoproduct, the parent 2-oxopurine. The resulting increased absorption at 254 nm due to the photoproduct consequently diminishes the relative absorption (internal shielding) of the reduction product, hence its rate of photochemical transformation.

By contrast, the changes in absorption spectrum during the initial stages of the reaction show that the conversion reaction itself is *quantitative*. Furthermore, since under these conditions, 2-oxopurine itself was relatively resistant to irradiation, the rate of appearance of the 315 nm band of 2-oxopurine with irradiation dose at 254 nm could be used to calculate the quantum yield for photodissociation of the reduction product. This was 0.03 mol/Einstein, hence somewhat lower than the quantum yield for photodissociation of the dimer reduction product of pyrimidone-2, about 0.1 [5].

In contrast to the foregoing, the reduction product of wave II, 1,6-dihydro-2-oxopurine, the absorption of which at 254 nm is similar to that for the dimer reduction product, was unaffected by irradiation at 254 nm. It is of interest that, whereas 1,6-dihydro-2-oxopurine is stable at neutral pH, the corresponding product of reduction on wave I of purine, *viz.* 1,6-dihydropurine, is slowly reoxidized on exposure to air. A solution of 1,6-dihydropurine was therefore prepared by electrolysis of purine at pH 5.3 as described by Smith and Elving [19]. The solution was then brought to pH 7 and irradiated at 254 nm. Under these conditions the characteristic absorption spectrum of 1,6-dihydropurine (with $\lambda_{\max}$ 240 nm and 292 nm) [1, 19] was gradually replaced by a single band at 263 nm, characteristic of the parent purine. Direct photochemically induced conversion of 1,6-dihydropurine to purine was testified to by the existence of three isosbestic points at 222, 240, and 282 nm during the entire course of the reaction. The conversion reaction was fully quantitative and proceeded with a quantum yield of 0.03; while, during the 40 min irradiation required for completion of the reaction, the extent of spontaneous reoxidation of 1,6-dihydropurine to purine was barely discernible. Furthermore, from the foregoing, it appears highly probable that the 1 : 1 photoadducts of alcohols to purine reported by Connolly and Linschitz [18] (see above), and the structures of which are formally identical to 1,6-dihydropurine, will equally readily photodissociate to the parent purine and alcohol.

Discussion

The foregoing results show that, in buffered aqueous medium, 2-oxopurine undergoes electrochemical reduction in two steps. Both the diffusion current constant and the coulometric data show that both reduction steps are one-electron. Polarographic analysis and the pH-dependence of $E_{1/2}$ point to the involvement of protons in both electrode processes. The simplest scheme consistent with this behaviour is a rapid acid-base equilibrium preceding uptake of the first electron [20].

With establishment of the reduction product of wave I of 2-oxopurine as the dimer 6,6′-*bis*-(1,6-dihydro-2-oxopurine) (**2**), the resultant reduction pathway, based on the polarographic and coulometric results, may be formulated as in Scheme 1. The 6,6′ linkage is in agreement with findings for electrochemical reduction of other purine derivatives [1, 4, 21], where the initial attack of the electron is at C-6 of the purine ring.

At the potential of wave II, 2-oxopurine is reduced to an apparent 1,6-dihydro-2-oxopurine species, which is equivalent to further 1e reduction of the free radical. The dimer product itself does not undergo further reducion at the mercury electrode.

The proposed mechanism of electroreduction of 2-oxopurine is similar to that for pyrimidone-2 [5], which appears reasonable in light of the fact that the imidazole ring does not undergo polarographic reduction [1, 3, 19], so that 2-oxopurine appears to behave formally as a 5,6-substituted derivative of pyrimidone-2. This is further underlined by the fact that for pyrimidone-2, $E_{1/2} = -0.53 - 0.078$

pH; whereas for 2-oxopurine, $E_{1/2} = -0.46 - 0.086$ pH, again pointing to the similarity in the first reduction step for both compounds.

For pyrimidone-2, a second reduction step is observed only in the presence of tetraalkylammonium salts. Purine itself was initially reported to undergo reduction *via* two 2-electron steps [1, 3, 19]; but more recent investigations by means of A.C phase selective polarography indicated that the first irreversible 2e reduction step should be ascribed to two sucsessive rapid 1-electron reduction stages [3]. The fact that only one 2-electron cathodic wave is observed for reduction of each purine bond implies that the initially produced free radical is as, or more, readily reduced than protonated purine. Comparison of the reduction potentials for the two stages of reduction of 2-oxopurine and purine shows that those for the former are about 300 mV more positive. Consequently conditions are more favourable for the appearance of two well-resolved 1-electron reduction waves with 2-oxopurine; in the case of purine, the reduction potential of wave I is sufficiently negative to result in transport of the 2nd electron.

Relevant to the foregoing are the earlier findings of Dryhurst [21] on the polarographic reduction of 2-thiopurine, which was also found to occur *via* two 1-electron processes. At pH values above 5, the first of these was postulated as leading to dimerization of the resulting free radical, the proposed product being 6,6′-*bis*-(1,6-dihydro-2-thiopurine) [21]. At pH values below 5 the radical species resulting from the first reduction step underwent further reduction to a product proposed to the 1,6-dihydro-2-thiopurine. Although no attempts were made to isolate and identify the reduction products, these proposals are fully supported by the results of the present investigation. The consequent similarities in polarographic reduction between 2-oxopurine and 2-thiopurine are, in fact, analogous to the corresponding similarities in reduction behaviour between 4,6-dimethylpyrimidone-2 [22] and 4,6-dimethylpyrimidonethione-2 [23]. It should be noted that, although Dryhurst [21] erroneously regarded 2-thiopurine as being in the thiol form, this does not affect the validity of his results. Furthermore, by analogy with the ability of the dimer reduction products of both 4,6-dimethylpyrimidone-2 [22] and 4,6-dimethylpyrimidonethione 2 [23] to undergo photochemical dissociation to the parent monomers, it is most likely that 6,6′-*bis*-(1,6-dihydro-2-thiopurine) will also photodissociate to the parent 2-thiopurine as readily as 6,6′-*bis*-(1,6-dihydro-2-oxopurine) to the parent 2-oxopurine.

The free radical mechanism of electroreduction of 2-oxopurine is also supported by EPR measurements of other purine derivatives [24, 25]. At low temperature, purine and its oxo analogues form radical hydrogen atom adducts, with a lifetime of several days under vacuum or in a hydrogen atmosphere. In the presence of air or oxygen, these are rapidly liquidated [26].

As regards the alkaline lability of the dimer reduction product, which was shown above to proceed *via* the ionized or anionic form, this may be interpreted as follows. The ionized form of the dimer is formally similar to hydrogen adducts of two radicals generated by ionizing radiations by addition of an electron and proton to pyrimidines [27] or purines [25] ionized in alkaline medium. It was shown by Rao and Hayon [28] that ketone radicals in strongly alkaline medium are in the "enol" form, and are strong reducers, *i.e.*, they may undergo rapid oxidation. In the case of the dimer of reduced 2-oxopurine, this may lead to transfer of 2 electrons to some electron acceptor, with concomitant transformation of the dimer to monomer, as follows:

$$\mathrm{RH-RH} + 2\,\mathrm{OH^-} \rightarrow \mathrm{R^- - R^-} + 2\,\mathrm{H_2O}$$
$$\mathrm{R^- - R^-} \leftrightharpoons 2\,\mathrm{R^{\cdot-}}$$
$$2\,\mathrm{R^{\cdot-}} + 2\,\mathrm{A} \rightarrow 2\,\mathrm{A\cdot} + 2\,\mathrm{R^-}$$

where A is the electron acceptor.

It should be noted that both the photodissociation properties and lability in alkali of the dimer of 2-oxopurine resemble these for the ***trans-anti*** thymine cyclobutane photodimer [29]. It was shown by Helene [30] that the mechanism of monomerization of thymine photodimers is accompanied by acceptance of an electron, so that electrochemical studies on purines and pyrimidines may be of value in the clarification of the mechanism of photochemical formation and dissociation of cyclobutane photodimers, which are of considerable significance in relation to radiation damage and repair.

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[1] P. J. Elving, J. E. O'Reilly, and C. O. Schmackel, Methods of Biochemical Analysis (David Glick, ed.), **Vol. 21,** pp. 287–465, John Wiley, Inc., 1973.

[2] D. L. Smith and P. J. Elving, J. Amer. Chem. Soc. **84,** 2741–2747 [1962].

[3] P. J. Elving, Salvatore P. Pace, and James E. O'Reilly, J. Amer. Chem. Soc. **95,** 647–656 [1973].

[4] G. Dryhurst and P. J. Elving, Talanta **16,** 855–874 [1969].

[5] B. Czochralska and D. Shugar, Biochim. Biophys. Acta **282,** 1–10 [1972].

[6] L. Meites, Polarographic Techniques, pp. 54–62, Interscience, New York 1965.

[7] D. Shugar, The Nucleic Acids, **Vol. III,** Academic Press, New York 1960.

[8] Brown Lister, Fused Pyrimidines. Part II. Purines. J. H. Lister (D. J. Brown, ed.), p. 481, Wiley-Interscience, New York 1971.

[9] C. O. Schmackel, K. S. V. Santhanam, and P. J. Elving, J. Electrochem. Soc. **221,** 345–353 [1974].

[10] J. N. Burnett and A. L. Underwood, Biochemistry **4,** 2060–2065 [1965].

[11] C. O. Schmackel, K. S. V. Santhanam, and P. J. Elving, J. Amer. Chem. Soc. **97,** 5083–5092 [1975].

[12] S. Y. Wang and D. E. Rhoades, J. Amer. Chem. Soc. **93,** 2554–2556 [1971].

[13] J. L. Flippen, R. D. Gilardi, I. L. Karle, D. F. Roades, and S. Y. Wang, J. Amer. Chem. Soc. **93,** 2556–2557 [1971].

[14] A. Albert, Heterocyclic Chemistry, 2nd ed. Oxford University Press, New York, N. Y. 1968.

[15] N. B. Colthup, H. Daly, and S. E. Wiberely, Introduction to Infrared and Raman Spectroscopy, Academic Press, New York 1964.

[16] S. Craver and M. Zoni, Chimica **33,** 473–482 [1957].

[17] H. Linchitz and J. S. Connolly, J. Amer. Chem. Soc. **90,** 2979–2980 [1968].

[18] J. S. Connoly and H. Linschitz, Photochem. Photobiol. **7,** 791–806 [1968].

[19] D. L. Smith and P. J. Elving, J. Amer. Chem. Soc. **84,** 1412–1418 [1962].

[20] P. Zuman, The Elucidation of Organic Electrode Processes, Academic Press, New York 1969.

[21] G. Dryhurst, J. Electroanal. Chem. **28,** 33–44 [1970].

[22] B. Czochralska, D. Shugar, R. Cutler, K. Arora, and R. Bates, J. Amer. Chem. Soc. **99,** 2583–2587 [1977].

[23] M. Wrona, J. Giziewicz, and D. Shugar, Nucleic Acids Res. **2,** 2209–2222 [1975].

[24] M. Lacroix and A. Van der Vorst, Photochem. Photobiol. **7,** 477–490 [1968].

[25] A. Van der Vorst and I. Lion, Biochim. Biophys. Acta **238,** 417–422 [1971].

[26] A. Van der Vorst and I. Lion, The Purines — Theory and Experiment, The Jerusalem Symposia on Quantum Chemistry and Biochemistry IV. The Israel Academy of Sciences and Humanities, Jerusalem 1972, pp. 362–369.

[27] P. S. Rao and E. Hayon, J. Amer. Chem. Soc. **96,** 1287–1293 [1974].

[28] P. S. Rao and E. Hayon, J. Amer. Chem. Soc. **97,** 2986–2989 [1975].

[29] M. A. Herbert, J. C. Le Blanc, D. Weinblaum, and H. E. Johns, Photochem. Photobiol. **9,** 33–45 [1969].

[30] C. Helene and M. Charlier, Biochem. Biophys. Res. Commun. **43,** 252–257 [1971].