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Purine *N*-Oxides. XIII. Kinetics of the Photochemical Alteration of Adenine 1-*N*-Oxide*

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Adenine 1-*N*-oxide is decomposed by ultraviolet light with a quantum efficiency of 0.10 and with an action spectrum paralleling the ultraviolet-absorption spectrum. The unique absorption spectrum of adenine 1-*N*-oxide and its relation to the spectra of the products permits it to be proposed as a practical and simple dosimeter for irradiations with the biologically important wavelengths near 260 mμ.

Quantitative measurements of the kinetics of the changes induced (Brown *et al.*, 1964, accompanying manuscript) in adenine 1-*N*-oxide by irradiation with ultraviolet light have been made with monochromatic light at various wavelengths. A large Hilger quartz-prism monochromator (Perry, 1932) and a calibrated photocell were used to obtain monochromatic light of known intensity incident on the absorption cell (Setlow, 1957). Solutions of about 1.51 μg/ml (10^{-5} M) were stirred and irradiated in microquartz cells of 1-cm path-length. They were transferred at various times to a Beckman Model DU Spectrophotometer for measurements of absorbancy at appropriate wavelengths.

Upon irradiation with a broad band of ultraviolet light from a Hanovia sun lamp with a filter transmitting strongly at 253.7 mμ, the major changes in the spectrum (see Fig. 2, Brown *et al.*, 1964) were a sharp decrease in the 230 mμ maximum and minor changes in the 250–270 mμ region. Similar changes were observed when specific wavelengths of monochromatic light were used for the irradiation. The decreases in absorption at 230 mμ for various irradiation times were the measure of decomposition of adenine 1-*N*-oxide.

In Figure 1 is plotted the decrease with time of the absorbancy at 230 mμ, when the solution is irradiated with light of 265 mμ at a level of 10^2 ergs/mm^2 sec^{-1} incident upon the cell. The kinetics of the reaction are first order for well over 50% of its course. The apparent rate then decreases as the absorption of the products becomes a major portion of the total. Similar first-order kinetics for the decrease at 230 mμ were observed when the irradiation was at wavelengths of 230, 237, 248, and 280 mμ.

If Δ_m is the maximum absorbancy decrease at 230 mμ (~0.9 of the initial absorbancy), the decrease, Δ, after an incident dose D, quanta/cm^2, is given by

$$\Delta = \Delta_m(1 - e^{-\sigma D})$$

where σ is the decomposition cross section for the reaction. Measurements of the absorbancies, and of D from the average intensities of the incident light through the samples, allow σ to be calculated (Setlow,

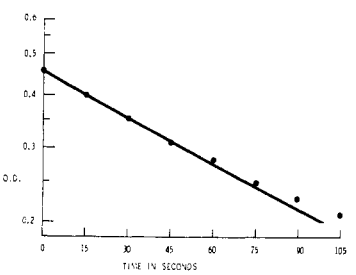

FIG. 1.—Kinetics of the decomposition of adenine 1-*N*-oxide. Decrease in absorption at 230 mμ with irradiation at 265 mμ. Aqueous solution, 10^{-5} M, ~*p*H 6, ε = 40,000. Average incident energy through the cell 200 ergs/mm^2 sec^{-1}.

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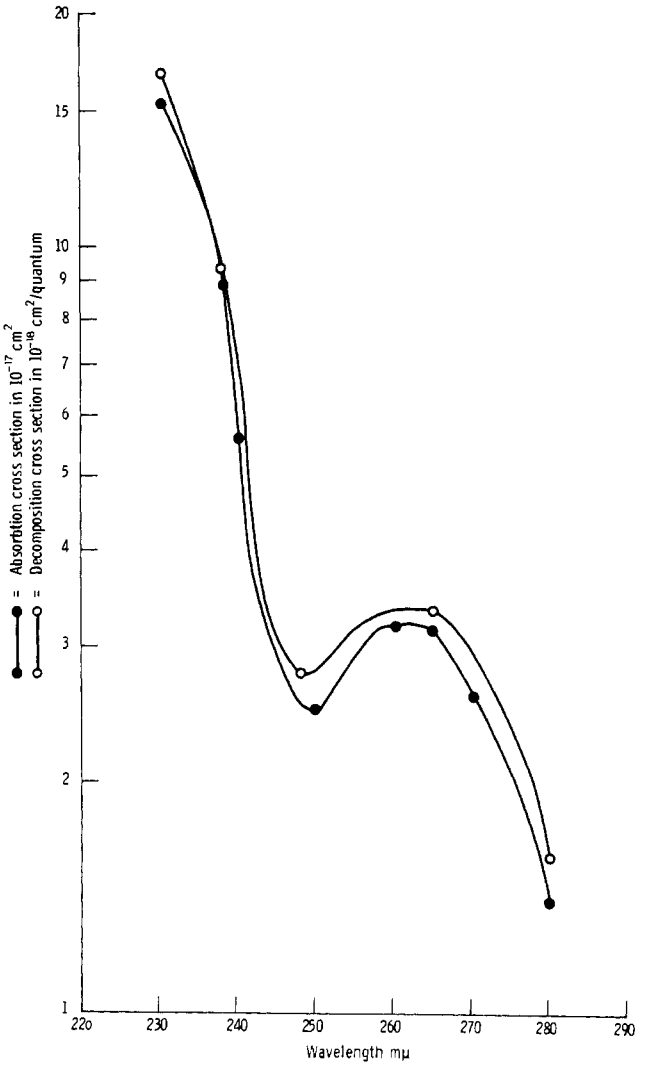

FIG. 2.—Absorption and decomposition cross sections of adenine 1-*N*-oxide. The absorption cross section equals 3.83×10^{-21} ϵ.

1957). The action spectrum (σ versus wavelength) is plotted in Figure 2 and parallels the absorption spectrum. The quantum yield of the photochemical reaction (Φ) at the various wavelengths was found from the ratio of the decomposition to absorption cross-sections (Setlow, 1957). It averaged 0.107, with a minimum of 0.094 and a maximum of 0.114. This high value of 0.10 is to be compared with Φ of 0.00006–0.02 for constituents of nucleic acids (Shugar, 1960) and of approximately 0.01 for thymine dimer formation (Johns *et al.*, 1962; Deering *et al.*, 1963).

With γ-irradiation from a cobalt-60 source,[1] adenine 1-*N*-oxide was also decomposed more rapidly than was adenine, although the differential was much less. With 150 r/minute the $t_{1/2}$ for a 4.6-μg/ml solution of adenine 1-*N*-oxide was about 15 hours, and was first order for more than one-half of the course. With an adenine solution of 4.2 μg/ml, the $t_{1/2}$ was approximately 45 hours and was first order for over one-third of the course.

The sensitivity to ultraviolet light coupled with the observation that the absorption of the irradiated solution does not change appreciably over the range 250–270 mμ, and that it is constant at 255 or 267 mμ (Fig. 2, Brown *et al.*, 1964), suggests that adenine 1-*N*-oxide may be used as a practical and simple reference standard in actinometry for irradiations with light of a wavelength of 253.7 mμ, or with filtered ultraviolet sources yielding the bulk of their radiation near that wavelength. The decomposition may be followed easily by plotting the decrease with time at 230 mμ. Since the absorption of the resulting mixture remains essentially constant over the wavelengths of irradiation no correction need be made for a changing absorption by the solution, as is necessary with the more sensitive ferrioxalate (Lee and Seliger, 1964) or uranyl oxalate (Leighton and Forbes, 1930) actinometers. Calculation of the number of molecules decomposed, as measured by the decrease in absorption at 230 mμ (ϵ = 40,000) and multiplication by 10, yields a direct measure of the number of quanta absorbed by the solution, and thus a measure of the quanta available per unit time to any similar solution placed in the same position.

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