amount of 2-*O*-methylribose in the RNA samples falls into a range of about 0.5–1% of the ribose content. The rather uniform levels in both the microsomal and s-RNA suggest that perhaps there is a statistical distribution of the 2′-*O*-methylribose throughout the RNA. This implies that about one in every 100 ribose residues per se contains the 2-*O*-methyl group and therefore the ratio to each other of the 2′-*O*-methylribonucleosides in the total RNA sample would reflect the ratios of the parent ribonucleosides to each other. The data of Table I, taking into consideration the accuracy of the analyses, permit some general observations in this regard. In the s-RNA of several species there is near equivalence of the molar ratios of adenosine to uridine and guanosine to cytidine, with the latter pair predominating. For example, this is the case in yeast s-RNA (Monier *et al.*, 1960) and in rat liver s-RNA (Lipshitz and Chargaff, 1960). The amounts of 2′-*O*-methylguanosine and of 2′-*O*-methylcytidine are nearly equivalent in the yeast s-RNA and human liver s-RNA, and further, this pair is present in significantly larger amounts than the 2′-*O*-methyladenosine and 2′-*O*-methyluridine pair. Morisawa and Chargaff (1963) also observed a preponderance of 2′-*O*-methylguanosine and 2′-*O*-methylcytidine in the RNA of rat liver and s-RNA of yeast. A statistical distribution of 2′-*O*-methylribose should also be reflected in the occurrence of nucleosides in RNA consisting of the so-called minor bases and this sugar. The anticipated levels would be very small indeed. The isolation of a new nucleoside, tentatively identified as 2′-*O*-methylpseudouridine, lends support to this contention. The isolated amount of this compound is about 1% of the amount of pseudouridine in s-RNA.

ACKNOWLEDGMENT

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Purine *N*-Oxides. XII. Photochemical Changes Induced by Ultraviolet Radiation*

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Adenine 1-*N*-oxide is found to be very sensitive to ultraviolet radiation. For it, and related purine derivatives, two main pathways of decomposition are observed: direct loss of oxygen, and rearrangement of the oxygen to the adjacent carbon.

Since the correlation (Hollander and Emmons, 1941; Stadler and Uber, 1942) of the ultraviolet absorption of nucleic acids with the wavelengths most efficient in producing mutations, there have been studies of the effects of ultraviolet light (Kland and Johnson, 1957) and of other ionizing radiations (Scholes and Weiss, 1952; Barron *et al.*, 1952; Daniels *et al.*, 1955; Hems, 1958, 1960; Ponnamperuma *et al.*, 1961, 1963) on purine derivatives. With high doses, some deamination of adenine to hypoxanthine (Kland and Johnson, 1957; Ponnamperuma *et al.*, 1961), a more extensive opening of the imidazole rings (Hems, 1960; Ponnamperuma *et al.*, 1961), and some formation of 8-hydroxyadenine (Ponnamperuma *et al.*, 1963) are the major alterations observed with the purine bases.

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Because of the ease of oxidation of adenine nucleotides to their 1-*N*-oxides by neutral hydrogen peroxide (Stevens *et al.*, 1959; Cramer and Randerath, 1958; Cramer *et al.*, 1963) and of the secondary production of peroxides by ionizing radiation (Scholes and Weiss, 1952), we first attempted the production of an adenine *N*-oxide derivative by the action of ultraviolet light or X irradiation without success, for reasons now obvious. Subsequently we found that 50% of adenine oxide in an unbuffered aqueous solution may be altered in 45 minutes by an intensity of ultraviolet light which does not measurably alter adenine in 4 days; it is altered with the remarkably high quantum efficiency of 0.1 (Levin *et al.*, 1964). It is also more sensitive to γ irradiation from ^{60}Co than is adenine (Levin *et al.*, 1964).

EXPERIMENTAL

Irradiation.—A Hanovia quartz lamp, Type S-100, with a Corning No. 9863 filter transmitting strongly

TABLE I
CHROMATOGRAPHY OF PURINE N-OXIDES AND REFERENCE COMPOUNDS

Substance	R_F in Solvents A[a]	B	C
Adenine 1-N-oxide	0.48	0.48	0.42
Adenine	0.61	0.40	0.50
Isoguanine	0.18	0.47	0.30
Hypoxanthine	0.62	0.58	0.36
4,5,6-Triaminopyrimidine	0.17	0.48	0.33
4,6-Diamino-5-formamidopyrimidine	0.19	0.60	0.20
8-Hydroxyadenine	0.60	0.38	0.41
4-Aminoimidazole-5-carboxamidoxime	0.48[b]	0.57	0.35
Unknown(s)	(0–0.1)[c]		0.63
Unknown "C-24"	(0.63)	(0.65)	(0.24)
Unknown "B-60"	(0.05)	0.60	(0.45)
Adenosine 1-N-oxide	0.44	0.71	0.27
Adenosine	0.61	0.52	0.40
Crotonoside	0.31	0.67	0.18
Unknown	0.70		
6-Methylpurine 1-N-oxide	0.60	0.66[d]	0.56
6-Methylpurine	0.77	0.60[d]	0.76
2-Hydroxy-6-methylpurine	0.50	0.66	0.36
Isoguanine 1-N-oxide	0.26	0.50	0.18[e]

[a] R_F values are variable due to weak buffering capacity. [b] 0.31 in Stevens and Brown (1958). [c] R_F values in parentheses were located by radioactivity. [d] 0.57 and 0.55 in Stevens *et al.* (1962), but these compounds migrate further when the alcohol layer is thicker. [e] 0.11 in Cresswell *et al.* (1963).

at 253.7 mμ and later a Spectroline lamp R-51 with 90% emission at 253.7 were used at distances of 10–50 cm from the quartz vessel. To follow the kinetics of the photodecomposition, a 1-cm quartz cuvet, directly transferable to a spectrophotometer, was used with a solution of 2.75 μg/ml (about 1.5×10^{-5} M). For larger-scale experiments 25- or 50-ml quartz Florence flasks containing magnetically stirred unbuffered aqueous solutions of 10–20 μg/ml were used. The change was followed on diluted aliquots and was usually about 50% in 1 hour. The solutions were then concentrated *in vacuo* and the concentrates were studied chromatographically. In an experiment with ^{14}C-labeled adenine oxide, 25 ml of a solution of 5 μg/ml (initial OD 1.33 at 230 mμ) at a distance of 30 cm from the Spectroline lamp was altered 48% in 33 minutes.

Chromatography.—Chromatographic separations were performed, ascending, on Whatman No. 1 or Schleicher and Schuell No. 597 paper, with the development solvents as follows: A, 1% ammonium sulfate–isopropyl alcohol, 1:2, with paper presoaked in 1% ammonium sulfate and dried (Anand *et al.*, 1952); B, 5% disodium phosphate–isoamyl alcohol, two-phase (Carter, 1950); and C, 1-butanol–acetic acid–water, 4:1:1. Reference compounds were always included. The chromatograms were inspected under ultraviolet light. Selected areas were eluted with water, with parallel clear areas for blanks, and the spectra were determined at neutral, acid, and alkaline *p*H values. The R_F values for the pertinent compounds are given in Table I.

Radioactivity Determinations.—These were performed in an Atomic Accessories strip-flow counter, Model SA-160, with slit widths of 1.5 or 3.0 mm. Speeds and scales, which accumulated integrated totals of 25,000–50,000 counts per strip, were used when ratios of radioactivities were determined. Conditions which made the major peaks excessively large were used to detect the minor radioactivity areas. Eluates of the major spots, planchetted and measured in a Tracerlab flow counter, confirmed the ratios of activities in the major products.

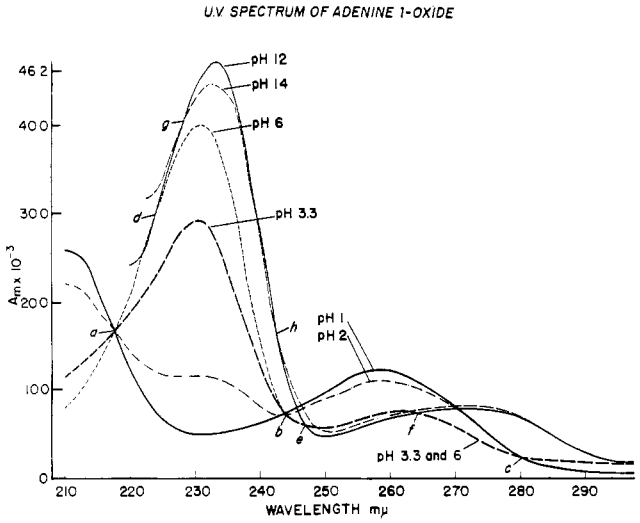

FIG. 1.—Spectrum of adenine 1-N-oxide (Stevens and Brown, 1958). The letters designate isosbestic points associated with the *pK* values involved.

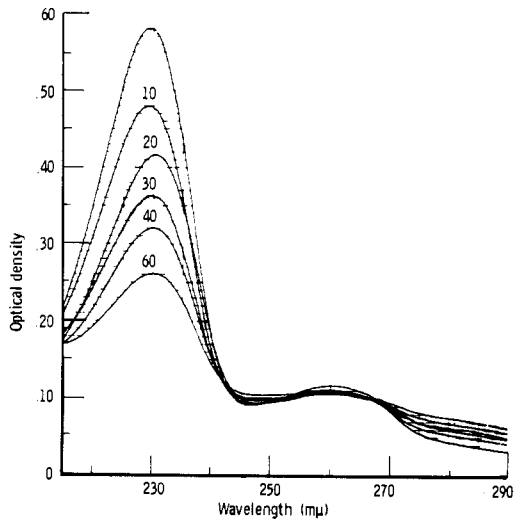

FIG. 2.—Changes in absorption of adenine 1-N-oxide during irradiation. The decomposition is followed through the decrease with time (minutes) of the 230 mμ absorption maximum. Note the small changes of absorption between 250 and 270 mμ.

The 5-year-old adenine 1-N-oxide-[8-^{14}C] (Dunn *et al.*, 1959) was chromatographed in solvent C, with over 99.5% of the radioactivity at the expected R_F, 0.42, and no detectable radioactive impurity elsewhere.

Irradiation of Adenine 1-N-Oxide.—The distinctive absorption spectrum of adenine oxide (Fig. 1) (Stevens and Brown, 1958) permits its decomposition to be followed by the decrease in absorption of an intense band of ϵ = 40,000 at 230 mμ, although there is little change in absorption of the solution at wavelengths near 260 mμ (Fig. 2). The *pK* values of adenine oxide are 2.6 and 9.0 and between *p*H 5 and 7 the absorption is independent of *p*H. From samples that had been 50% altered, chromatography in solvents A and C indicated two major products in addition to unchanged adenine oxide. These were identified as adenine and isoguanine by spectra at different *p*H values and by R_F values in other solvents. An additional spot of R_F 0.63 in solvent C, although slightly fluorescent under ultraviolet light, contained but a trace of material, as confirmed by the subsequent experiment with ^{14}C. In solvent B a discrete adenine spot, a combined isoguanine and adenine oxide spot, and a third dark spot at R_F 0.60 were observed.

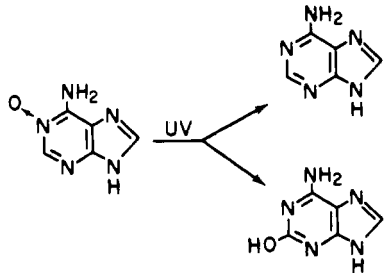

FIG. 3.—Major products of irradiation of adenine 1-*N*-oxide: adenine and isoguanine (2-hydroxyadenine).

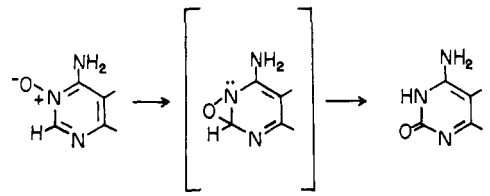

FIG. 4.—The oxaziridine intermediate possible in the transfer of the oxygen from the nitrogen to the adjacent carbon.

From adenine oxide-[8-^{14}C] irradiated to 50% decomposition and chromatographed in solvent A, three well-separated areas of radioactivity were measured. These were in the ratio adenine oxide–isoguanine–adenine, 10:5:4. An additional one part of radioactivity was present near the origin, although no distinct spot was detected there in ultraviolet light. In solvent C, the three peaks were not as well resolved but those of adenine and isoguanine were approximately equal. The fluorescent unknown at R_F 0.63 in solvent C represented about 1 or 2% of the total radioactivity. Another area at R_F 0.24 (unknown C-24) possessed a few per cent of the activity but was less well defined because it was on the tail of the isoguanine radioactivity peak. A discrete spot could not be seen under ultraviolet light. In solvent B the unknown at R_F 0.60 represents up to 15% of the total activity.

When a sample of the adenine oxide-[8-^{14}C] was irradiated until 80% was decomposed, the radioactivity of unknown C-24 rose to 20% of the total, and that which remained near the origin in solvent A rose to 15%. That of C-63 was not greatly increased. Radioactivity eluted from the area of unknown C-24 of this sample migrated to an R_F of 0.63 in solvent A, coincident with the adenine area, and in solvent B to R_F 0.65, near the unknown B-60. The small amount of isoguanine present was of assistance as a reference marker. The unknown B-60 remained near the origin in solvent A, and in solvent C it migrated to about R_F 0.45. None of the minor products could be identified (Table I) as an opened imidazole derivative, hypoxanthine, or 8-hydroxyadenine, all known to arise from adenine under much more vigorous radiation conditions. Neither was the opened-pyrimidine derivative, 4-aminoimidazole-5-carboxamidoxime, which can arise from adenine oxide upon gentle hydrolysis (Stevens and Brown, 1958), found.

Irradiations of Other Purine Derivatives.—Similar irradiation of adenosine 1-*N*-oxide solutions resulted in an equally facile photochemical reaction, with only two major products formed, crotonoside (Davoll, 1951) and adenosine, which were readily distinguishable in solvents A and C. There was a trace of ultraviolet light–absorbing material at R_F 0.70 in solvent A.

6-Methylpurine-1-*N*-oxide (Stevens *et al.*, 1959) similarly yielded 2-hydroxy-6-methylpurine (Bergmann *et al.*, 1961) and some 6-methylpurine, also most readily identified in solvents A and C.

Isoguanine 1-*N*-oxide (Cresswell *et al.*, 1963) was also irradiated. The decomposition proceeded with approximately the same ease and yielded one product, isoguanine, readily identified in solvents A and C, and distinguishable in solvent B through its fluorescence. Isoguanine is not decomposed under the conditions of irradiation.

Results and Discussion

Adenine 1-*N*-oxide is well over 1000-fold more sensitive to ultraviolet radiation than is adenine (Shugar, 1960). The loss of the distinctive 230 mμ absorption (Fig. 1) permits the change to be followed (Fig. 2). Two major products result, isoguanine (2-hydroxyadenine) and adenine (Fig. 3) (Levin and Brown, 1962). After 50% alteration of the adenine oxide the rearrangement product, isoguanine, predominates by a ratio of about 5:4. The reaction becomes more complex as the decomposition approaches 100%, but these two products remain in the majority.

Adenosine 1-*N*-oxide similarly yields crotonoside (isoguanosine) and adenosine, and 6-methylpurine 1-*N*-oxide yields 2-hydroxy-6-methylpurine and 6-methylpurine.

With isoguanine 1-*N*-oxide, in which the carbon adjacent to the *N*-oxide function is already occupied by a hydroxyl, the decomposition proceeds with approximately the same ease and yields but one product, isoguanine.

Photolysis of gaseous pyridine *N*-oxide to pyridine (Hata and Tanaka, 1962) has been reported, as have photochemical rearrangements of quinoxaline (Landquist, 1953) and quinoline *N*-oxides (Buchardt, 1963) to the corresponding 2-hydroxy compounds.

In the photochemical alterations of purine *N*-oxides both mechanisms are apparent: one involves direct removal of the oxygen, possibly as peroxide, from the *N*-oxide function; the second involves the transfer of the oxygen to the adjacent carbon, and may proceed through a three-membered oxaziridine intermediate (Fig. 4), as in a mechanism suggested for the photolysis of nitrones (Kamlet and Kaplan, 1956; Splitter and Calvin, 1958). With isoguanine oxide, the second type is obviously blocked, and only the first can operate.

The extreme ultraviolet-light sensitivity of adenine oxide in comparison to other purine or pyrimidine derivatives may be of biological interest. The possibility that hydroxyl radicals produced by the indirect action of ionizing radiation might induce *N*-oxidation of adenine and thereby sensitize a nucleic acid to the primary effects of radiation is to be considered. The isoguanine derivative which would be one result of such events could still pair with thymine, but with a third H-bond possible.

Added in Proof

From X-ray-irradiated adenine J. J. Conlay (1963), (*Nature 197*, 555) noted the production of a trace of isoguanine but not of adenine 1-*N*-oxide, as might be expected if the latter were formed and decomposed as described here.

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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,

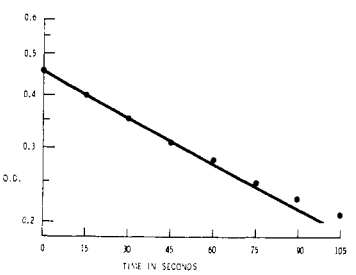

FIG. 1.—Kinetics of the decomposition of adenine 1-*N*-oxide. Decrease in absorption at 230 mμ with irradiation at 265 mμ. Aqueous solution, 10^{-5} M, ~*p*H 6, ε = 40,000. Average incident energy through the cell 200 ergs/mm^2 sec^{-1}.

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