filtrates concentrated by vacuum distillation through a 14" column. The residue is subjected to vacuum distillation through a 5" column. The desired aminoalcohol distils as a viscous oil, b.p. 104–106° (0.1 mm.), yield 56.3 g., (90.8%), $n^{25}D$ 1.5087.

4-Morpholino-2-butynyl N-Methylpipecolinate.—Into a 500-cc. 3-necked round bottom flask equipped with stirrer, reflux condenser, Dean–Stark water separator ($CaCl_2$ tube) and heating mantle is placed a solution of 31.4 g. (0.20 mole) of methyl N-methylpipecolinate and 31.0 g. (0.20 mole) of 4-morpholino-2-butyn-1-ol in 325 cc. of *n*-heptane; 0.5 g. of NaOMe is added and the mixture refluxed. The methanol produced during the transesterification will separate from the heptane in the water separator. Two additional 0.3-g. portions of NaOMe may be required to complete the reaction. The reaction mixture is concentrated by slowly distilling off approximately 50% of the heptane. The residue is chilled, filtered and the balance of the heptane removed by vacuum distillation through a 14" column. The residue is subjected to vacuum distillation through a 3" column. The desired ester boils at 149–151° (0.25 mm.), yield 41.6 g. (74.3%), $n^{25}D$ 1.5012.

4-Morpholino-2-butynyl N-Methylpipecolinate Dimethobromide.—To a solution of 14.0 g. (0.05 mole) of 4-morpholino-2-butynyl-N-methylpipecolinate (0.05 mole) in 80 cc. of isopropyl alcohol is added 19.0 g. (0.20 mole) of methyl bromide. The mixture is refluxed under anhydrous conditions for 3 hr. and then chilled. The solid is filtered off and recrystallized from the minimum amount of hot ethanol, m.p. 208–210° dec., yield 20.8 g. (88.5%).

MILWAUKEE 1, WISCONSIN

[CONTRIBUTION FROM THE PHYSIOLOGY DEPARTMENT, TUFTS UNIVERSITY SCHOOL OF MEDICINE]

A Kinetic Study of the Ultraviolet Decomposition of Biochemical Derivatives of Nucleic Acid. I. Purines[1]

BY M. J. KLAND AND L. A. JOHNSON

RECEIVED APRIL 16, 1956

Irradiation of purines of biological interest with ultraviolet light under oxygen and nitrogen gives decomposition rates which are zero order between 8×10^{-5} and 5×10^{-4} *M* after an initial "induction" period which is a function of the extent and nature of purine substitution. Decomposition during the induction period follows a square law. For adenine (I) and hypoxanthine (II), decomposition is faster under oxygen, but the breakdown of guanine (III) and xanthine (IV) is inhibited by oxygen as compared with nitrogen.

Sensitivity to ultraviolet (uv.) radiation in purines and pyrimidines may be qualitatively correlated with the number[2] and position[3] of C=O groups in the molecule. In conjunction with a chemical examination of the complex mixtures obtained on irradiation of the biologically interesting purines adenine (I), hypoxanthine (II), guanine (III), xanthine (IV) and uric acid (V), more detailed studies of their ultraviolet decomposition rates in the presence of oxygen and the inert gas, nitrogen, have been carried out.

Experimental

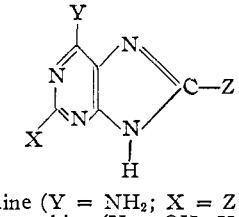
Source and Purity of Purines.—All compounds were recrystallized from distilled water, except guanine which is too insoluble. The recrystallized material was then chromatographed on Whatman No. 1 filter paper in a descending butanol–water–urea system,[4] and in butanol–water–ammonia (1%).[5] The presence of one spot at the end of 72 hours in the former system, and 120 hours in the latter was inter-

TABLE I

PHYSICAL DATA FOR PURINES IRRADIATED

Purine	Source	Purity (chrom.)	*p*H	Spectroscopic purity D^a	D^b	D^c	D^d
Adenine	Schwartz AD-5401	Pure	2.18	0.76	0.76		
			5.94	0.76			
Hypoxanthine	Schwartz HX-5202	Pure	2.02	1.37	1.43		
			5.95	1.35			
Xanthine	Schwartz XA-5201	Pure (BuOH–NH_3–H_2O)	2.13	0.59	0.58		
			5.79	0.59			
Guanine	GN-5402	.....	1.97	1.39	1.37	0.82	0.84
			5.98	1.39			
Uric acid[e]	Fisher, Lot. No. 543969	.....	1.92	0.97	1.0	2.71	2.7
			5.35	1.21			

[a] Ratio of optical densities at 250 and 260 mμ: D_{250}/D_{260}. [b] Literature[6,7] data at *p*H 2 for D_{250}/D_{260}. [c] Ratio of optical densities at 280 and 260 mμ: D_{280}/D_{260}. [d] Literature[6,7] data at *p*H 2 for D_{280}/D_{260}. [e] Uric aid data were obtained from ref. 7 only.



I, Adenine (Y = NH_2; X = Z = H)
II, Hypoxanthine (Y = OH; X = Z = H)
III, Guanine (X = NH_2; Y = OH; Z = H)
IV, Xanthine (X = Y = OH; Z = H)
V, Uric acid (X = Y = Z = OH)

(1) This work was done under the terms of Contract No. AT(30-1)-911 of the Physiology Dept., Tufts University School of Medicine, with the Atomic Energy Commission.

(2) J. R. Loofbourow and M. M. Stimson, *J. Chem. Soc.*, 844 (1940).

(3) A. Canzanelli, R. Guild and D. Rapport, *Am. J. Physiol.*, **167**, No. 2, 364 (1951).

(4) Butanol saturated with 10% aq. urea.

(5) Butanol, saturated with water, and equilibrated with concd. aq. ammonia (99 parts BuOH:1 part NH_4OH).

(6) E. A. Johnson (unpublished) in Chargaff and Davidson, "The Nucleic Acids," Vol. I, Academic Press, Inc., New York, N. Y., 1955, p. 502.

(7) E. Volkin and W. E. Cohn, "Methods of Biochemical Analysis," Vol. I, Interscience Publishers, Inc., New York, N. Y., 1954, p. 304.

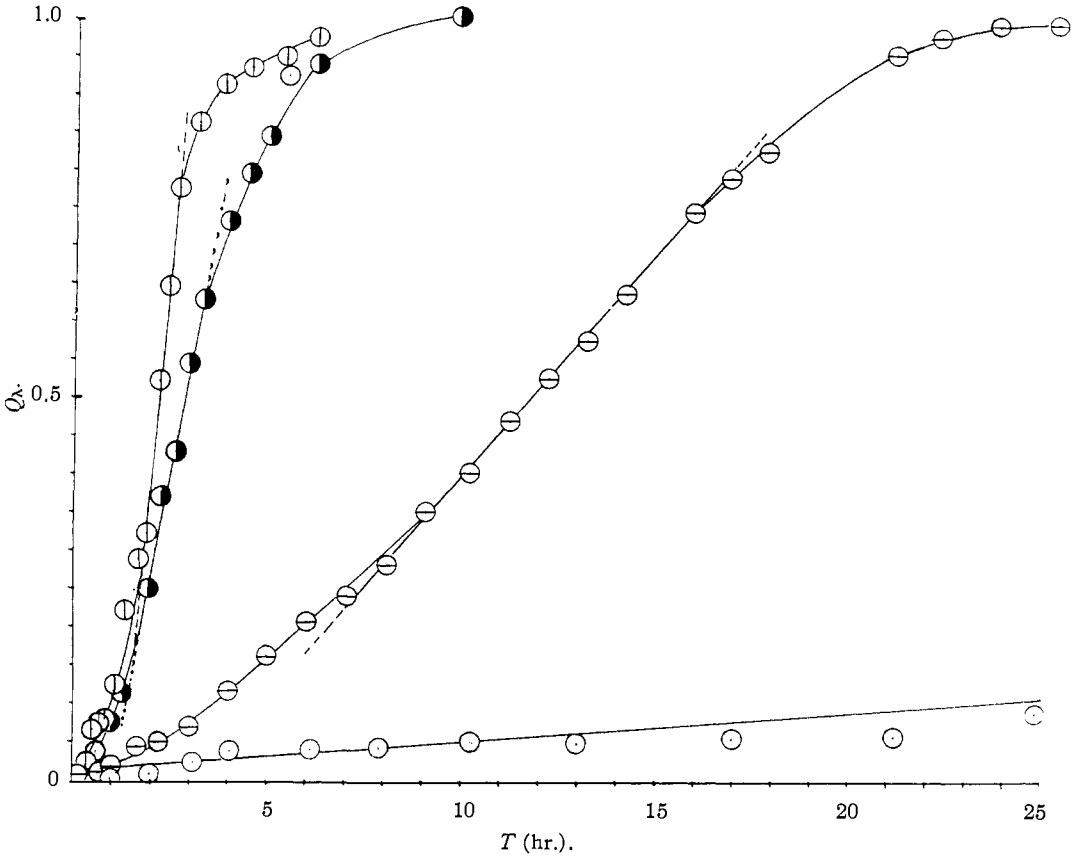


Fig. 1.—Relative ultraviolet decomposition rates of adenine and hypoxanthine under O_2 and N_2.

	$Q\lambda$	I_0	Concn. $\times 10^5$
⊙ Adenine–N_2	Q_{260}	58	7.8
⊖ Adenine–O_2	Q_{260}	58	7.8
◑ Hypoxanthine–N_2	Q_{250}	52	7.8
⊕ Hypoxanthine–O_2	Q_{250}	52	7.9

The ordinate, Q_λ, is the fraction decomposed, as defined in the experimental section, and I_0 is the initial radiation intensity in milliwatts per sq. ft. Units for the abscissa: 1 div. = 1 hr.

preted as an indication of chromatographic purity. This test could not be applied to guanine and uric acid, because they failed to move in both systems. Spectrophotometric criteria were applied in the case of these two purines. Optical density ratios D_{250}/D_{260} and D_{280}/D_{260} in acid were satisfactory in both instances (see Table I).

Solutions of the purines were irradiated at 8×10^{-5} and 4×10^{-4} M. Where solubility permitted data were also obtained at 10^{-3} M (hypoxanthine) and 6×10^{-3} M (adenine). All solutions were filtered before irradiation.

Apparatus.—All irradiations were carried out in a quartz tube, diameter 1.5 inches, height 10 inches, fitted with a Pyrex top blown with gas inlet and outlet. Blanks were run in a Pyrex tube of the same dimensions. The irradiation apparatus consisted of six low pressure mercury vapor lamps[8] symmetrically arranged inside a cylindrical reflector, the whole being enclosed in a hinged box of plywood construction so that it could be opened readily for insertion of the tube containing the sample, or for measurement of light intensity. The clamp and bottom support for holding the tube in position were so placed that the center of the irradiated solution was at the geometrical center of the apparatus. The apparatus was cooled by means of a built-in blower in the base, plus additional fans around the apparatus as needed. Holes were drilled in the plywood case to increase ventilation. Temperatures (35 ± 2°) were recorded at each sampling from a thermometer suspended within the apparatus next to the quartz tube. The gas inlet of the irradiation tube was attached to a gas-washing bottle containing water maintained at 35° to replace water evaporated from the irradiated solution during a run. For runs under nitrogen another gas-washing bottle containing "Oxsorbent"[9] was placed between the nitrogen cylinder and the "presaturator." Nitrogen (or oxygen) was bubbled through the solution at 160 bubbles per minute. Breakdown was followed in a Beckman DU ultraviolet spectrophotometer at several suitable wave lengths, including the absorption maximum. Light intensities recorded in Table III were made at a standard distance of 96 inches with a G.E. light meter fitted with a cadmium photo-cell calibrated to give milliwatts per sq. foot at 2537 Å. All intensity measurements are for the apparatus in the open position centered over one of two permanent lines with the meter centered over the second line.

TABLE II

MOLECULAR EXTINCTIONS OF PURINES AT 254 mμ

Purine	pH	E^{254}_{mol}	% T per cm. at 254 mμ for 8×10^{-5} m soln.
Adenine	5.94	12000	10.9
Hypoxanthine	5.82	10000	11.6
Xanthine	5.79	6500	31.0
Guanine	5.88	8650	20.3
Uric acid	5.35	3800	50.0 (30.1 at 4×10^{-4} m)

(8) General Electric 15 watt germicidal lamps, with more than 90% of their emission at the 2537 Å. mercury resonance line, according to L. J. Buttolph, reprint from *Arch. of Physical Therapy*, **25**, 676 (1944).

(9) Burrell Corporation, Fifth Avenue, Pittsburgh 19, Pa.

TABLE III

RELATIVE DECOMPOSITION RATES OF PURINES IRRADIATED WITH ULTRAVIOLET LIGHT[a]

Purine	Max. (mμ)	Energy equiv., kcal./mole[b]	$t_{1/2}$[c] (hr.)	k_{O_2}/k_{N_2}	Relative rates Ad. O_2 = 1	Relative rates G–N_2 = 1	Initial concn. (moles/l. × 10^5)	Light intensity at 254 mμ[d]
Adenine	260	109.8		12.7				
O_2			12.37		1.0		7.8	58
N_2			156[e]		0.08		7.8	58
Hypoxanthine	250	114.3		1.5				
O_2			1.85		6.6		7.96	58
N_2			2.77		4.5		7.88	59
Guanine	245 (275)	116.7 (103.8)						
O_2			1.75	0.46	...	0.46	6.4	53
N_2			0.80			1.0	6.4	53
Xanthine	265	107.7						
O_2			0.39	(.81)	35		9.0	60
			1.25	.45		0.82	8.2	52
N_2			0.57			1.8	8.3	49
			0.32		44		9.0	60–56
Uric acid	287.5 (232.5)	99.3 (123)						
O_2			0.16	(.81)	62		8.0	58
			.25				8.0	58
			1.32	.69		3.7	39.0	50
			1.31				39.5	50–48.5
N_2			0.20		62		8.01	55
			.91			5.5	39.0	49–50

[a] All irradiations in aq. solution, under O_2 or N_2, 160 bubbles per minute. [b] $E = Nh\nu = NhC/\lambda$ ergs/mole = $2.854 \times 10^{-5}/\lambda$ kcal./mole. [c] Half-lives interpolated from data. [d] Measured with a G.E. intensity meter at 96 inches under standard conditions; units: milliwatts per square foot. [e] Extrapolated value.

Method.—Initial volumes of irradiated solutions were 100 cc. except for adenine (120 cc.). The wide range of reaction rates and limited solubility of guanine made it necessary to obtain two sets of data: one at roughly comparable concentrations at high intensity (except for guanine), and another for the three most sensitive purines at lower intensity (and increased concentration for uric acid). At appropriate time intervals (5 minutes to 2 hours) the tube was removed from the irradiation apparatus for about 1.5 minutes for sampling. The aliquot was diluted as necessary, and read at suitable wave lengths in the spectrophotometer. This procedure was continued until there was a distinct falling off in the rate of decomposition, and for at least three samples beyond the linear portion of the breakdown curve.

Data and Calculations.—Table II lists molecular extinctions at 254 mμ for the purines irradiated and the % transmission, T, per cm. of aqueous solutions, calculated from the standard equation for optical density: $D = \log 100/T$. Since our cell is 4 cm. in diameter, absorption of the incident radiation is essentially complete for the first four purines. Uric acid transmits 6% $(0.5)^4$ of the incident radiation at 254 mμ at 8×10^{-5} M, but practically none $(0.3)^4$ at 4×10^{-4} M. pH values are those normally obtained on dissolving the compounds in distilled water.

The function Q plotted in Figs. 1 and 2 is derived from optical density data as follows: $Q = (D_0 - D_t)/(D_0 - D_f)$, where D_0 is the initial optical density, D_t is the optical density at time t and D_f the final density, defined as that optical density at which the slope of Q became negligible compared with its steepest slope. At this time, the value of D_f was less than 10% that of D_0 for all cases except guanine, and further decomposition did not affect the relative kinetic pattern of breakdown. Also, the quantity of initial purine left was negligible, as evidenced by total spectra, the 250/260 and 280/260 optical density ratios, and alkaline shift criteria. Guanine gave a rather high residual density at D_f, indicating probable incomplete destruction of the ring system at this stage.

Since Q is, by definition, a linear function of the optical density, D, and all solutions examined obeyed Beers' law, it follows that Q is also a linear function of the concentration of purine.

The half-life, $t_{1/2}$, of an irradiated purine, is linearly dependent on its initial concentration, C_0. This characteristic could be predicted on the basis of the nature of the Q function alone, and has been experimentally verified over an approximately tenfold concentration range. For purposes of comparison, all curves in Fig. 2 were normalized to 4×10^{-4} M initial concentration, since differences in molecular extinction necessitated irradiation at rather widely different initial concentrations. From the linearity of the function involved, it is apparent that normalization merely entails shifting of the Q curve parallel to itself along the time axis by an amount proportional to the ratio $t(C_0$ exptl./C_0. theor), where the numerator represents the actual working concentration, and the denominator, the theoretical initial concentration—in this case 4×10^{-4} M.

Isolation of Hypoxanthine from Irradiated Adenine. a. Pan-irradiated Adenine.—Preliminary spot chromatograms using pan-irradiated adenine[10] indicated the presence of (1) adenine, (2) hypoxanthine and possibly of (3) xanthine and (4) uric acid. Subsequent elution and ultraviolet spectra confirmed (1) and (2), but not (3) and (4). The possible presence of the latter two components is being further investigated by means of C_{14}-labeled adenine and hypoxanthine irradiated under oxygen. The paper technique appeared sufficiently promising to warrant an attempt at a larger scale isolation of components. We therefore streaked 8-inch strips of Whatman No. 1 paper about 7.5 inches across their width with saturated solutions of irradiated material. About 6 strips per batch were chromatographed in cylindrical tanks set up for descending chromatography. Tank dimensions: 2 ft. high × 1 ft. diameter. Glass troughs each hold two chromatogram strips, 8 in. wide; solvent: butanol saturated with 10% aq. urea. A small beaker of water kept the cylinder saturated with respect to water vapor during the run. For separation of the fastest running components, 60 to 70 hours sufficed. The strips were then dried in air, the fluorescent and absorbing bands marked lightly with pencil under ultraviolet light and numbered, and corresponding strips combined, eluted and lyophilized (freeze dried). The chief contaminant appears to be urea, which is a product of the ultraviolet decomposition as well as one of the components of the solvent system. Table IV summarizes the data from a typical separation.

A complete ultraviolet spectrum of eluate (2) compared with that of standard hypoxanthine, confirmed its identity as hypoxanthine. λ_{max} for both solutions was at 250 mμ,

(10) The large scale irradiation of aqueous solutions in open pans is described in a paper by Conrad, *Radiation Research*, **1**, No. 6, 523 (1954).

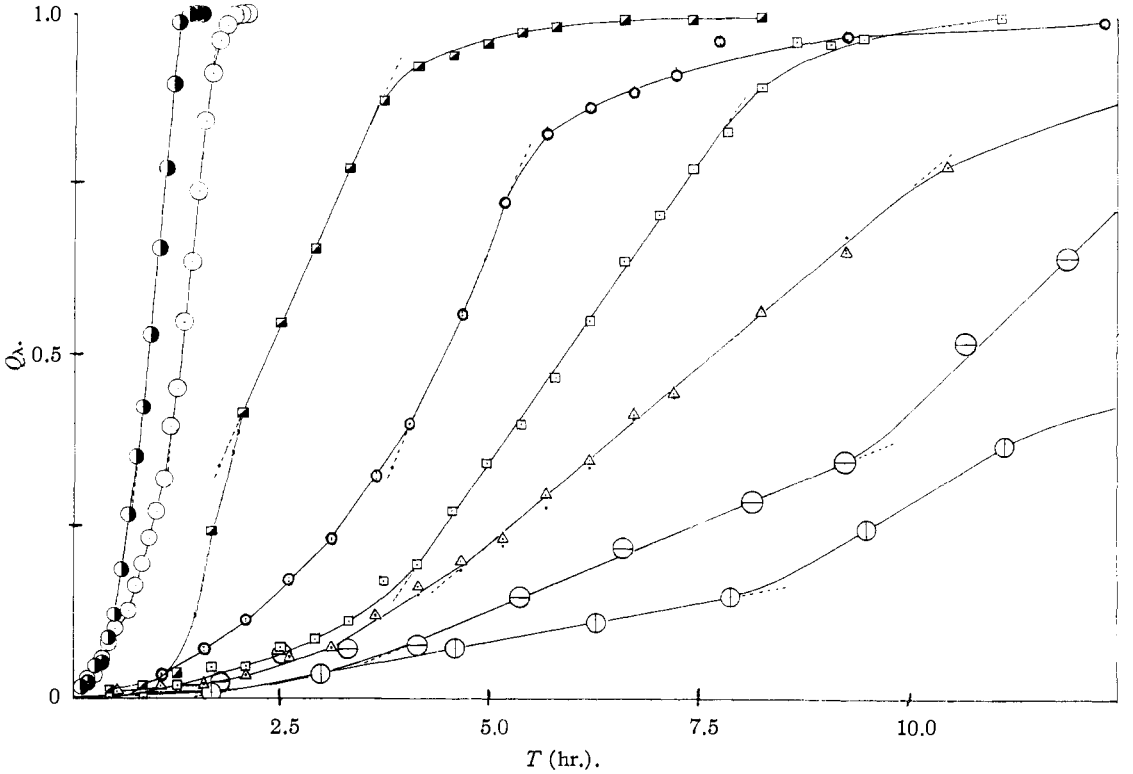


Fig. 2.—Relative decomposition rates of ultraviolet sensitive purines.

◑ Uric acid under N_2	$I = 50$	$Q_{287.5}$
○ Uric acid under O_2	$I = 50$	$Q_{287.5}$
◪ Xanthine under N_2	$I = 50$	Q_{265}
⊡ Xanthine under O_2	$I = 52$	Q_{265}
⊙ Guanine under N_2	$I = 51$	$Q_{245, 275}$
△ Guanine under O_2	$I = 52$	$Q_{245, 275}$
◐ Hypoxanthine under N_2	$I = 52$	Q_{250}
⊖ Hypoxanthine under O_2	$I = 52$	Q_{250}

Ordinate: $Q_\lambda = (D_0 - D_t)/(D_0 - D_f)$, where D_0 is the initial optical density of an aqueous solution, D_t is the optical density at time t, and D_f the final density, as defined in the Experimental section. The subscript λ refers to the wave length at which determinations were made. All curves are normalized to concentrations of 4×10^{-4} M. (For details of normalization, see section on data and calculations, under Experimental.) Dotted lines are extensions of linear portions of curves The abscissa, T, is plotted in hours, with each major division equivalent to 2.5 hours.

λ_{min} at 222.5 mμ, and the optical density ratios in excellent agreement, as shown in Table IV below.

b. **Adenine Irradiated under Nitrogen.**—By the time this work was carried out, it had been found that the butanol–water–ammonia system[5] gave more clear-cut chromatographic separations. The experiments under nitrogen were of necessity small in scale, limited by the size of the apparatus and the very slow breakdown rate. Irradiations were carried at least to half destruction, as indicated by the decrease in optical density at 260 mμ. The irradiated mixture was then freeze-dried, and about half of the product chromatographed along with standard adenine and hypoxanthine as controls. Both hypoxanthine and the corresponding positioned unknown were then eluted with 4 cc. of water for about 10 minutes. The eluates were filtered, and the spectrum of both read between 215 and 290 mμ (pH ~ 6). To 3.5 cc. of each solution was then added 0.03 cc. of 0.02 N NaOH, bringing the pH up to approximately 9, and the spectra of both the control and the eluate again examined. The results indicate that the eluate from adenine irradiated under N_2 is indeed hypoxanthine, as seen from the data in Table V.

TABLE IV

ISOLATION OF COMPONENTS FROM PAN-IRRADIATED ADENINE BY PAPER CHROMATOGRAPHY

Eluate	$D_{250/260}$	$D_{280/260}$	pH
Adenine (Std.)	0.77	0.111	5.9
Eluate (1)	0.77	.108	6.0
Hypoxanthine (Std.)	1.35	.089	6.3
Eluate (2)	1.31–1.37	0.086–0.089	6.0

TABLE V

ISOLATION OF HYPOXANTHINE FROM THE IRRADIATION OF ADENINE UNDER NITROGEN

Sample	pH	$\lambda_{max.}$ mμ	$\lambda_{min.}$ mμ	$D_{250/260}$	$D_{280/260}$
Hypoxanthine Std.	6	250	220	1.36	0.08
Eluate from Ad-N_2	6	250	220	1.37	.08
Hypoxanthine Std.	9	257.5	230	0.88	.11
Eluate from Ad-N_2	9	257.5	230	.89	.11

It should be emphasized that the *amount* of hypoxanthine isolated in these experiments were extremely small, a spot on paper obtained from a single irradiation yielding barely enough for a satisfactory spectrophotometric and chromatographic identification. This is understandable, in view of

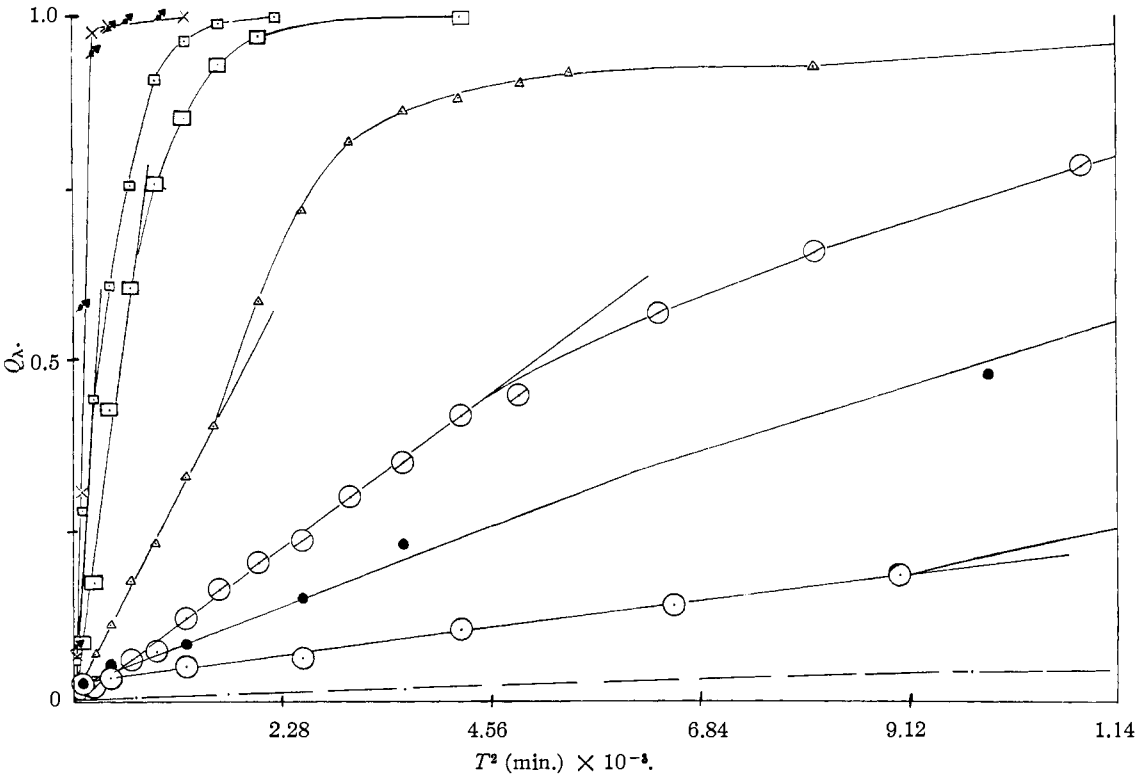


Fig. 3.—Induction periods of ultraviolet irradiated purines.

× Uric acid (8 × 10^{-5} *M*) under O_2	I_0 = 58
Uric acid (8 × 10^{-5} *M*) under N_2	I_0 = 58
□ Xanthine (9 × 10^{-5} *M*) under O_2	I_0 = 60
⊡ Xanthine (9 × 10^{-5} *M*) under N_2	I_0 = 60
△ Guanine (6.4 × 10^{-5} *M*) under N_2	I_0 = 52
⊖ Guanine (6.4 × 10^{-5} *M*) under O_2	I_0 = 52
● Hypoxanthine (8 × 10^{-5} *M*) under O_2	I_0 = 58
⊙ Hypoxanthine (8 × 10^{-5} *M*) under N_2	I_0 = 59
• Adenine (8 × 10^{-5} *M*) under O_2	I_0 = 58

Q_λ has the same meaning as in Figs. 2 and 3. The decomposition of adenine under nitrogen is negligible in the time range covered by this plot. All induction periods plot linearly with respect to the square of the exposure time (T^2), within the limits of experimental error. Guanine, irradiated at 6.4 × 10^{-5} *M* because of its low solubility, exhibits this linear *Q vs.* T^2 relationship up to about 40% breakdown. Dilute uric acid solutions at high intensity are linear to 96%. However, at lower intensity and higher concentrations (Fig. 3), uric acid follows the normal S-shaped *Q vs. T* curve, with a linear portion following the induction period.

the appreciable sensitivity of this compound to ultraviolet irradiation. This would be even more true of subsequent irradiation breakdown products, such as xanthine and uric acid which are even more ultraviolet-sensitive.

Results and Discussion

A typical plot of *Q vs.* time of exposure to ultraviolet radiation (Fig. 1) shows an initial slow rate (missing in adenine irradiated under N_2), the time of this "induction" period depending on the purine—it is longest for adenine irradiated under O_2 and barely observable in uric acid at high radiation intensities. This is followed by a linear portion of relatively rapid breakdown, finally levelling off with the slope of the *Q* function approaching zero at infinite exposure. At high radiation intensities, dilute solutions of all the purines except adenine appear to exhibit decompositions proportional to the square of the exposure time, T^2 rather than the first power. This T^2 dependence is also shown by adenine under O_2 during the initial induction period and appears to be generally characteristic of this stage of the breakdown for all of the purines examined (Fig. 3).

Examination of Figs. 1 and 2 shows that the purines I through V fall into two distinct classes with respect to their behavior on irradiation under oxygen and nitrogen. Those purines substituted only in the 6 position (adenine and hypoxanthine) decompose more slowly under nitrogen ($k_{O_2}/k_{N_2} > 1$), whereas the decomposition rates of the disubstituted purines (guanine, xanthine), and of uric acid are slower under oxygen. In either system, the *relative* rates for the series are: V > IV > III > II >> I. The relative rate constants k_{O_2}/k_{N_2} differ little for the three most reactive purines ($k_{O_2}/k_{N_2} < 1$), but a great deal for adenine (12.7) and hypoxanthine (1.5) (Table III, columns 6 and 7).

The data in columns 6 and 7 of Table III compare rates of ultraviolet decomposition of the purines at high intensity with adenine under oxygen and at a lower intensity with guanine under nitrogen. Here the inhibiting effect of the amino group is apparent from the relative decomposition rates of adenine *vs.* hypoxanthine and of guanine *vs.* xanthine under both oxygen and nitrogen. The inversion of k_{O_2}/k_{N_2} which occurs at guanine is shown in column 5 of the table.

Adenine (7.8×10^{-5} *M*) is essentially completely destroyed at the end of 24 hours irradiation under aerobic conditions, whereas under N_2, more than 90% remains at the end of this period. This suggests oxidative breakdown with the amino group a likely point of attack

$$RNH_2 \xrightarrow[h\nu]{[O]} [R\cdot NH:OH] \xrightarrow[h\nu]{H_2O} ROH$$

The isolation of hypoxanthine in the photochemical oxidation of adenine is further evidence in favor of this step.[11]

The following sequence of steps in the photochemical decomposition of adenine is suggested by the isolation of hypoxanthine—namely, (I) → (II) → (IV) → (V). Preliminary work on paper chromatograms indicates a complex mixture, with broad bands occurring at all the expected positions. However, only hypoxanthine has been eluted and sufficiently purified for both spectrophotometric and chromatographic identification. Since the other expected intermediates are increasingly sensitive to ultraviolet radiation, their presence would be difficult to establish by ordinary chemical means. We are therefore irradiating C_{14}-labeled adenine and hypoxanthine for chromatographic examination.

One possible explanation of the inhibiting effect of oxygen on the ultraviolet decomposition of guanine and xanthine, is organic radical and peroxide formation, since oxygen is known to function frequently as an inhibitor in photochemical reactions involving radicals[12] by peroxide formation. Complex dependence on radiation intensity, such as the more reactive purines III, IV, V demonstrate, is also characteristic of photochemical decompositions involving radicals.

While chemical information is yet too fragmentary to provide a detailed mechanism of the ultraviolet decomposition of purines, any working hypothesis must be consistent with the following experimental observations. (1) The purines examined, except adenine under nitrogen, exhibit an initial induction period. (2) The second stage of breakdown is independent of purine concentration. (3) Hypoxanthine has been isolated from adenine irradiated both under oxygen and nitrogen.

Acknowledgment.—We are indebted to Prof. L. J. Heidt of the Massachusetts Institute of Technology for criticism of this manuscript and many helpful suggestions in connection with photochemical techniques, to Prof. E. Heinz of the Biochemistry Department, and to Dr. A. C. English of General Electric Company, for suggestions connected with the interpretation of kinetic data.

(11) The isolation of hypoxanthine from adenine irradiated under N_2 indicates that I → II may be a hydrolytic step, but the great difference in rates under O_2 and N_2 favors the oxidative sequence suggested above.

(12) E. J. Bowen, "The Chemical Aspects of Light," 2nd ed. Clarendon Press, Oxford, 1946, Chap. VI, pp. 205 ff. See also G. K. Rollefson and M. Burton, "Photochemistry and the Mechanism of Chemical Reactions," Prentice-Hall Inc., New York, N. Y., 1946, for scattered references to the role of oxygen in photochemical reactions—for example, pp. 165, 307–310, 381.

BOSTON, MASS.

[CONTRIBUTION FROM THE LABORATORY OF BIOCHEMISTRY, NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH

Studies on Diastereomeric α-Amino Acids and Corresponding α-Hydroxy Acids. IX. Configuration of the Isomeric γ-Hydroxyglutamic Acids

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An epimeric mixture of the two racemic modifications of γ-hydroxyglutamic acid was prepared *via* the sodium ethoxide-catalyzed condensation of ethyl acetamidocyanoacetate with ethyl α-acetoxy-β-chloropropionate, followed by acid hydrolysis of the condensation product, so derived. Treatment of an aqueous solution of the epimeric mixture with hydrogen chloride gas to saturation led to the quantitative deposition of one racemate (A-form) as its lactone hydrochloride, whilst the other racemate (B-form) was isolated as the free amino acid upon adjustment of the filtrate to *p*H 3. That the A- and B-forms, so secured, were free from contamination of one by the other was evidenced by column chromatography on Dowex-1. After chloroacetylation of the A-form, as its lactone hydrochloride, and the B-form, as the free amino acid, the appropriate chloroacetyl-amino acid was subjected to asymmetric enzymic cleavage with cobalt-activated hog renal acylase I at *p*H 7.5. Such procedure permitted both the eventual isolation of each of the four optically pure stereomers and the establishment of the configuration of the α-asymmetric center of each, with $[M]^{26}D$ values in water as follows: L-A, −22.3°; D-A, +21.5°; L-B, +31.8°; D-B, −31.2°. Conversion of each of the optical isomers to its corresponding α.α′-dihydroxyglutaric acid was effected upon treatment with nitrous acid. The D- and L-enantiomorphs of A, so treated, and subsequently isolated as their respective barium salts, revealed $[M]^{26}D$ values of +31.4° and −30.9° (in water), respectively, whereas comparable treatment of the D- and L-forms of B led, in both instances, to the formation of *meso*-α,α′-dihydroxyglutaric acid which, by virtue of internal compensation, was devoid of optical activity. Such data established the stereochemical relationships between the α- and γ-asymmetric centers of each stereomer and permitted the assignment of a configurational designation to the γ-center of asymmetry of each.

γ-Hydroxyglutamic acid is a monoaminodicarboxylic acid which, by virtue of its two asymmetric centers, may exist as four optically active stereomers or two racemic modifications. Isolation of