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NASA Monitors: Dr. Freeman H. Quimby, Office of Life Sciences, Washington, D. C., and Dr. Cyril A. Ponnamperuma, Amés Research Center, Moffett Field, California.

Submitted by: Dr. F. Millich, Director
Department of Chemistry
University of Missouri at Kansas City
Kansas City, Mo. 64110
(816) 333-7400, Ext. 377

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"COMPLETE REACTION STUDIES IN EXOBIOLOGY:

The Chemistry and Photochemistry

of Nucleic Acid Constituents and

Related Compounds, and their Detection,

Characterization, and Isolation."

I. PURPOSE AND NATURE OF THE PROGRAM OF INVESTIGATION.

The vital importance of chemical and physical knowledge of nucleic acids, nucleic acid constituents, and their derivatives needs no special emphasis. The relevance to space science, and man's entry into and sustained existence in space is also quite obviously dependent on knowledge of living processes, and, on a molecular basis, on knowledge of reactions and resistance to reactions of chemical and physical stresses of such key life-sustaining macromolecules as proteins and nucleic acids.

There existed, at the time of the conception of this research investigation in 1962, a body of scientific literature on the effects of radiation, in vivo and in vitro on nucleic acids (1), and on nucleic acid constituents, which delineated various effects derivable by means of irradiation with actinic forms of radiation and ultraviolet light, but which, taken in total, were so obtuse as to provide no recognizable pattern.

For instance, with regard to photochemical degradation of purine derivatives, experimental results existed which, on the one hand, indicated that the imidazole ring was more susceptible to ring opening, while a reasonable body of evidence also led to the conclusion that the pyrimidine ring was the more susceptible (2). Further, photochemical reactions other than ring opening, such as hydration and dehydration of the 5,6-double bond in pyrimidines (3), free radical attack (4), and hydrolysis of amine substituents were also being reported (5). Even for a single compound the chemistry of different ionic forms will differ from the neutral form and among themselves in their stability against photochemical degradation. Clearly, there was a broad variety in the types of photoreactions which could be produced, and many investigations, conducted under comparable conditions, would have to be studied and intimately understood before common chemical patterns could be recognized.

In 1962, it was also apparent to this investigator that photoactivation with ultraviolet and more energetic radiation were capable of effecting such profound changes in purine derivatives that milder photoreactive conditions had to be used in order to effect structural changes with greater finess. Hopefully, one would isolate less complex systems, and effect degradations of purines to structures which are first generation daughter products and have not traveled so far along the degradative path as to make deductions ambiguous concerning the actual chemical path(s) followed. The possibility exists that there is less variety among the primary photochemical stages and that a limited number of patterns will emerge for various purines and pyrimidines, in their various ionic forms.

The studies conducted under this grant and reported herein confirm these lines of thought, at least for the oxidative degradation of guanine. A dye-sensitized system in alkaline solution was chosen such that photons of 40 kcal/mole energy could be employed. The results prove to be in marked contrast with reported studies of sensitization wherein photons of 80 kcal/mole are employed, and where degradation proceeds much beyond the stage of our process. Of course direct ultraviolet irradiation of guanine (energy in excess of 100 kcal/mole) produce results even more divergent than ours. This report describes the isolation of "early" photoproducts which show that guanine has undergone only the loss of one carbon atom and none of its nitrogen content; this stands in contrast with the isolation of urea, carbon dioxide, parabanic acid, and oxaluric acid from luminol sensitized photo-oxidation of guanine (6).

We have found that the dye-sensitized photo-oxidative technique is not restrictive to the dye employed or to guanine, and it is also possible that radiation of lesser energy (near infrared) is sufficient to bring about this reaction.

* *

The above discussion considers part of one of the three projects (Project B) which were incorporated into the original grant proposal. The three projects are identified in the proposal and in the three previous semi-annual progress reports by the following titles:

Project A: The detection and determination of luminescent properties of nucleotide derivatives.

Project B: Detailed kinetic mechanisms of photolysis and photochemical reactions of nucleotide derivatives.

Project C: The isolation and characterization of thermal and photolytic primary products of nucleotide derivatives.

A detailed and intimate study of the kinetics of the primary photochemical processes is required to substantiate precedential origin and sequence of formation among the "early" photochemical products, as well as to compare the photochemistry of one reaction among different purines, and to compare the photochemistry of one reaction with another.

Instrumentation for photometric observation of the reaction has been developed (Figure 1) in the course of this study. The production of "primary" photoproduct can be observed photometrically in our system which may not necessarily correspond to the product which we isolate for structural determination. Knowledge of such coincidence, or lack of it, is vital to a complete understanding of the whole involved process. The chemical literature is replete with segmental studies in which the kinetic studies have been carried out by investigators with training in one discipline, and the structural identifications (what few there are of these) have been carried out by others. The chances for correspondence between results of the various investigators is most frequently seriously impaired. Only in a few studies, e.g. photosynthesis, vision, in which a large pool of investigative talent, time and effort is focused on the same details, may one find knowledgable correlation between the physical processes and the organochemical consequences thereof. This investigator undertook to study and establish both the primary and sequential phenomena of the photochemical oxidations of purines. This theme is embodied in the title of the original grant proposal, i.e. Complete Reaction Studies in Exobiology: The Chemistry and Photochemistry of Nucleic Acid Constituents and Related Compounds, and their Detection, Characterization, and Isolation. For this reason, the three projects were undertaken simultaneously.

* *

Project A was undertaken for several reasons. Detailed study of the kinetics of the primary photochemical processes must include studies of the involved photo-excited states. Fluorometry and phosphorometry provide means of investigating the excited singlet and the triplet states, respectively, and the manner in which these states are affected by substrate complexation, energy transfer, quenching agents, etc.

At the inception of this grant the literature on fluorescence and phosphorescence of nucleotide bases was almost non-existent (7). Indeed, two earlier literature reports stated that fluorescence could not be observed (8). However, fluorescence can be observed from various ionic forms of purines, although fluorescence intensities are generally very weak. A spectrofluorimeter was constructed under this grant (Figure 2) with special design features of high sensitivity in the 300-200 nm. wavelength range. Following a lengthy period of design planning and construction, the instrument has been in operation in our laboratory during the last four months, and this report includes some spectral data which had been gathered to date.

Figure 1. (Right) Mr. Hossein Nanaie and Prof. F. Millich (background) inspect apparatus used in the spectrometric kinetic rate studies of the photochemical oxidation of guanine.

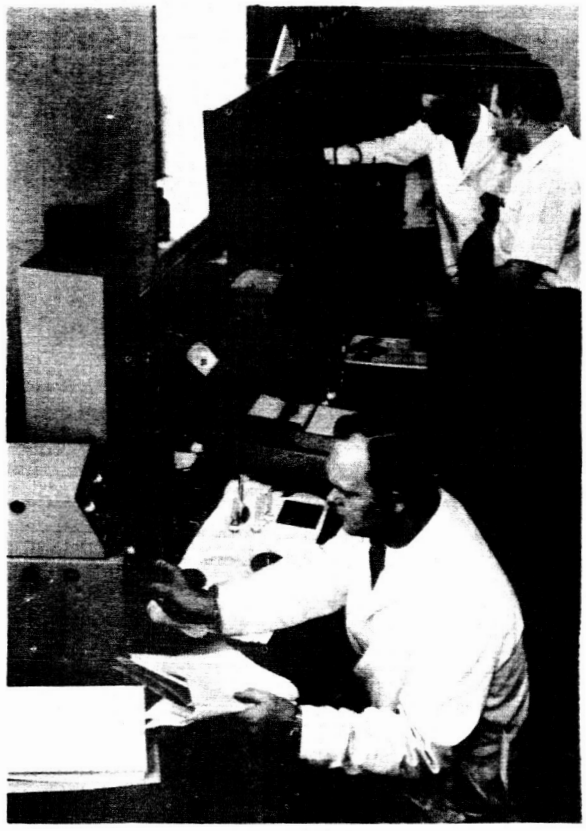


Figure 2. (Below) Miss Akiko Iwata is shown recording fluorescence and excitation spectra of purines. The linear energy-compensated spectrophotofluorimeter, shown in foreground, was constructed, to Dr. Millich's specifications, by the perkin-Elmer Corporation of Norwalk, Conn.

Fluorescence and phosphorescence spectra are valuable for purposes of identification. The value of using fluorescence for quantitative determination of guanine, adenine, and thymine have been pointed out (7). Purines and pyrimidines represent a particularly rich source of compounds for the investigation of the effect of molecular structure on luminescence properties, since so many of these compounds have become available through synthesis. Dr. Millich has sought and gained access to several large sources of supply of purines and pyrimidines.

* *

Project C has to date been concerned with isolation and structural characterization of the primary photoproducts. Most of the research results achieved under this grant has been devoted to this phase. Since this phase was least dependent on the instrument construction. The chemical system generated by the photo-oxidation of guanine has shown good chemical potential, and an intricate sequence of chemical transformations have been detected. While a number of chemical compounds have been isolated and purified, structural identification has not yet been achieved. Currently, some s-triazine derivatives hold the focus of our attention. We will shortly, through chemical synthesis, be able to confirm or deny their relevance. The details of this phase are presented in Part II of this report.

The chemical versatility which we have found in the primary photoproducts promise adaptions to new synthetic routes to interesting heterocyclic compounds.

The implication, if borne out, of s-triazines as degradation products of purines is interesting in consideration of concepts of chemical evolution, since alkyl s-triazines under pressure are thermodynamically unstable, and rearrange, in the presence of hydroxylic solvents, to aminopyrimidines (9).

There is a possibility that our ultimate results will contribute to the understanding of the metabolism of uric acid, a subject which, after 100 years, is still not fully confirmed on a chemical basis (10), and that s-triazines and the problems they might introduce in human metabolic processes need to be considered.

Thus, the identification of degradative products of nucleic acid constituents, and accumulated information concerning the chemical character of such compounds can afford valuable background knowledge for judgements concerning plausible theories of chemo-biological processes.

* *

Some experiments of proflavin-sensitized photoreductions of guanine were conducted without success. These attempts were not exhaustive.

Some experiments of the dye-sensitized photo-oxidation of 8-azaguanine in alkaline solution were also conducted in a manner analogous to the experiments on guanine. These proved very promising in that interesting acid-sensitive photoproducts are produced very rapidly.

The above projects have not been fully developed because the progress being achieved and the complexity of the guanine system has demanded our major preoccupation with that system. The next section, Part II, will not dwell on these two projects.

* *

Dr. Millich has been appointed as a research associate to the Space Sciences Research Center of the University System of Missouri for the 1966-67 year, during which some of his research efforts on chemical evolution studies may have supplementary financial support. Among these continuing studies which will receive support under this new appointment are phases of the projects originally funded under NASA grant NsG-651.

* *

Dr. Millich has received an appointment of visiting scientist, for the summer months of 1966, with Dr. Norman Horowitz's Biosciences research group of the Jet Propulsion Laboratory of California Institute of Technology. He will chiefly be engaged in studies of chemical evolution, and will assist Dr. Joon Rho with the spectrophotofluorimetry which is currently being initiated in Dr. Rho's laboratory, and which is similar to Dr. Millich's in instrumentation.

II. EXPERIMENTAL RESULTS; DETAIL OF PROJECTS A, B, AND C.

Project A. Fluorescence spectra.

The spectrophotofluorimeter (Figure 2), constructed by Perkin-Elmer Corporation, was received on a date near the original termination of this grant. An expected continuation grant, which would have reconstituted the deleted portion of time planned for in the original proposal, did not materialize. An extension of four months, to May 31, 1966, was requested and allowed in order to provide a minimum of time in which to examine the potential of the fluorometric design as applied to purine compounds.

The spectrophotofluorimeter design is based on that of the Slavin-Palumbo prototype (11), but incorporates many design specifications prescribed by Dr. Millich. The instrument features programmed feedback mechanisms which produce spectra that are automatically corrected for wavelength dependencies of optical and electronic components, e.g. the photocathode tubes; such automated corrections permit the production of a large volume of correct spectra, with an enormous saving of time and complicating interpretations.

An additional special design function of the instrument is its sensitivity. Because of the general low fluorescence efficiencies of hetercyclic ring compounds, such as exists with purines and pyrimidines, special provisions were taken in the design of the instrument to insure high sensitivity. Among these are: 1) a mirror arrangement for frontal viewing of the illuminated sample cell, 2) a highly sensitive EMI-9558Q photomultiplier, of SbNaKCs composition and S 20 spectral response, 3) facility to introduce strong light sources for direct excitation of the sample, and 4) facility to convert the monochromator fluorimeter into a filter fluorimeter, which provides a gain in fluorescence intensity at the expense of monochromation. The design has been successful such that even in programmed operation, the instrument has proven sensitive enough to record the corrected fluorescence of adenine at pH 2.5 without pushing the instrument capability in the programmed mode to its limit (the fluorescence efficiency, obtained by indirect means using benzimidazole as an energy-transfer fluorescence indicator, is reported to be 0.003 at prescribed wave lengths of excitation and fluorescence, and prescribed concentration (12)).

The discipline involved in producing true fluorescence spectra is tedious and exacting, but ultimately very rewarding. The routines which we have adopted involve: 1) melting point or chromatographic R_f characteristic, and infrared or ultraviolet spectral determination, to assure identity of the substance; 2) thin layer chromatography and/or (where possible) recrystallization, in order to achieve high purity, an expecially important requirement; 3) ultraviolet spectral determination of absorption bands at one unit increments of pH, over the range of -1 to 14 pH values, in order to specify the various cations, anions and neutral species (all of which have different luminescence properties; and

4) determination of the most effective excitation wavelengths at different concentrations in the range of 10^{-6} to 10^{-4} moles/liter (frequently, these do not correspond to wavelength maximum of absorption; and, simultaneous with operation 4, 5) recording of fluorescence spectra at different concentrations and one unit increments of pH. Occasionally, the fluorescence and absorption spectra, being quite sensitive to pH, show changes at pH values which do not correspond to reported pK_a values given in the literature. Where the absorption spectra do not correspond, a titration should be performed to determine if structural change can be associated with the consumption of acid or base, or, alternatively, should be associated with a tautomeric change. A few such discrepancies with the chemical literature have been found, but titrations although intended with our refined Radiometer (Copenhagen) titration equipment, have not been undertaken in the limited time presently available.

The fluorescence-pH profiles generally correspond with one or another absorption spectral-pH changes, thus, not showing much evidence of data which could be interpretable of large pK_a differences between the ground and excited states. However, this statement should not be taken as an established rule in the light of the limited amount of data collected these past months.

No fluorescence has been observed for imidazole, xanthine, or compound BG-precursor (referred to in a later section of this report). Fluorescence data has been produced for the compounds given in Table I. The fluorescence wavelength maxima which we observe differ at times with values reported by other investigators. This is attributable to the fact that our spectra are automatically corrected for instrumental parameters. An example of a fluorescence-pH profile is presented for guanine and for benzimidazole, in Figures 3 and 4. The actual fluorescence spectra are being accumulated, but a decision for an appropriate publication outlet has not been made at this time, though some probes have been made through correspondence with the Sadtler Research Laboratories of Philadelphia.

The fluorescence spectra are desirable for purposes of quantum mechanical analysis of the ground and excited states of heterocyclic rings. However, not enough data is available at this time to warrant detailed consideration of these aspects in this report.

Dr. Millich plans to continue this massive project beyond the expiration of the grant period.

TABLE I

FLUORESCENCE DATA OF PURINE AND RELATED COMPOUNDS

Compound	Concentration[a] X10^5 (moles/liter)	pH max.	Excitation Max λ (n.m.)	Fluorescence Max. λ (n. m.)
Benzene	100 - 10^6	----	255 - 275	287
Benzimidazole	1.00	3.5	270	362 (360)[b]
	1.00	10	275	292 (290)
Pyrimidine	2.15	4 - 12	255, 285	362 (360)
2-Aminopurine	1.00	5	320	372
6-Aminopurine (adenine)	1.00	2.5	280	367 (380)
6-Hydroxypurine (hypoxanthine)	1.00	3.6	280	395
	1.00	5 - 11	280	346, 355
2-Amino-6-hydroxy-purine (guanine)	1.00	1.5	288	335 (355)
	1.00	10.5	288	335 (340)
BG (see p. 8)	(15 mg/l)	4.8	288	361 weak
		11	288	361 weak

a. Benzene study was performed in cyclohexane solution, all the others, where soluble, were performed in aqueous solution containing an appropriate amount of sodium hydroxide or hydrochloric acid.

b. Figures in parenthesis are values from scientific literature (12).

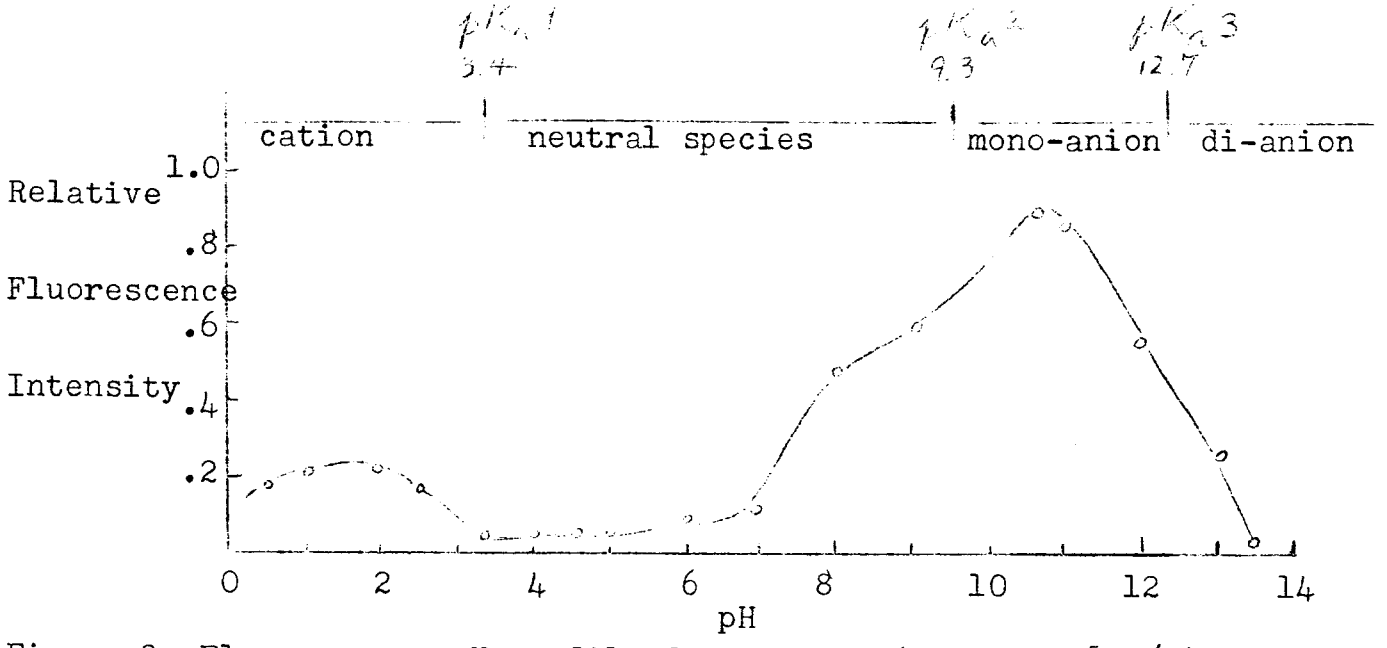


Figure 3. Fluorescence-pH profile for guanine (conc. 10^{-5} m/l): exc. wavelength 288 nm, fluor. wavelength 335 nm.

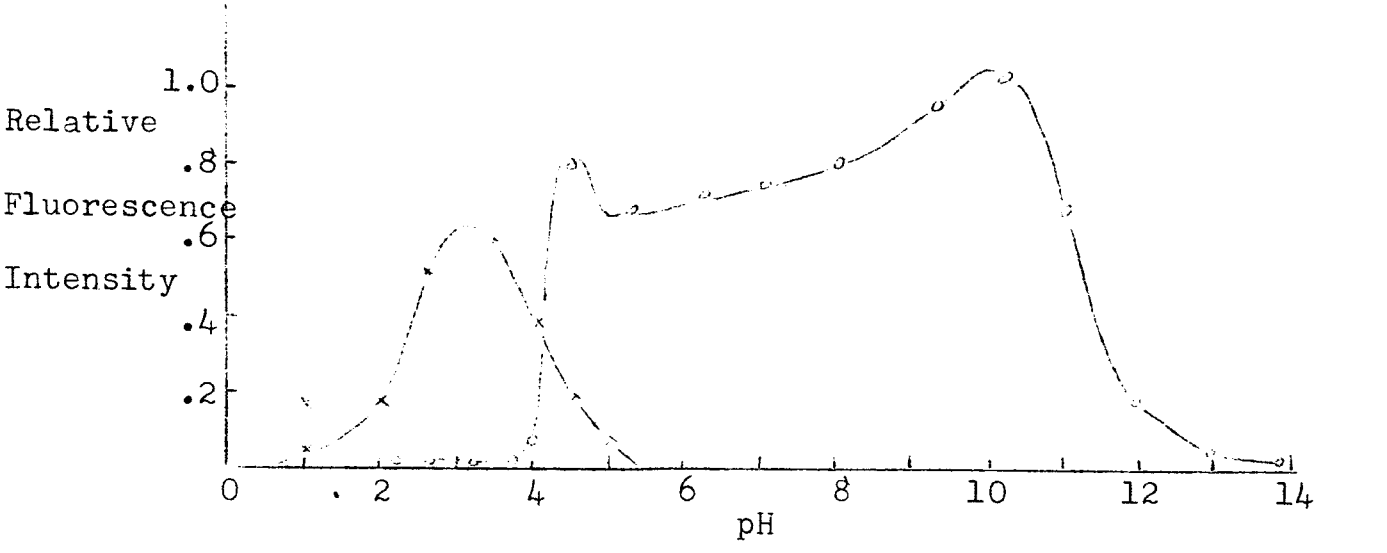


Figure 4. Fluorescence-pH profile for benzimidazole (conc. 10^{-5} m/l):
-x- exc.w. 270 nm, fluor.w. 362 nm,
-o- exc.w. 275 nm, fluor.w. 292 nm.

Project B. Photometric determination of the kinetics of the initial photochemical transformations.

For the purposes of photometric rate studies of the dye-sensitized photo-oxidation of purines an optical bench and auxiliary equipment was assembled according to the schematic shown in Figure 5. The sample-to-phototube area was enclosed in a specially constructed box which permits operation in a lighted room (c.f. Figure 1). Two such units have now been constructed. The main portion of Figure 5 shows the position of the phototube when arranged to observe the intensity of transmitted light of a selected (filtered) wavelength, based on absorption spectra; alternatively, the inset in Figure 5 shows the position of the phototube when arranged to observe a fluorescence maximum of one of the solution components of interest, when the first mode is impractical. The latter case has proved necessary for the direct observation of guanine in our system, since too many of the system components (i.e. dye and primary photoproduct) absorb in the 250-290 nm. spectral region and high transmission filters for this region are not available; we have found that a direct metering of guanine concentration as a function of time is possible by this ploy, affording, at least, initial rates by extrapolation to zero time.

The tungsten source provides the energy to excite the dye (and not the purine substrate) and provides energy for the reaction, but may also serve as an analytical beam, which through use of an appropriate filter, may be arranged for the absorption maximum of the dye. The degree of transmission falling on the phototube (RCA 931-A) is amplified and recorded as a function of time. The shapes of the curves determine the kinetic order dependence of the rate on dye concentration. One may also evaluate the influence of other constituents, by measuring the effect of their presence, at differing concentration levels, on the same curves.

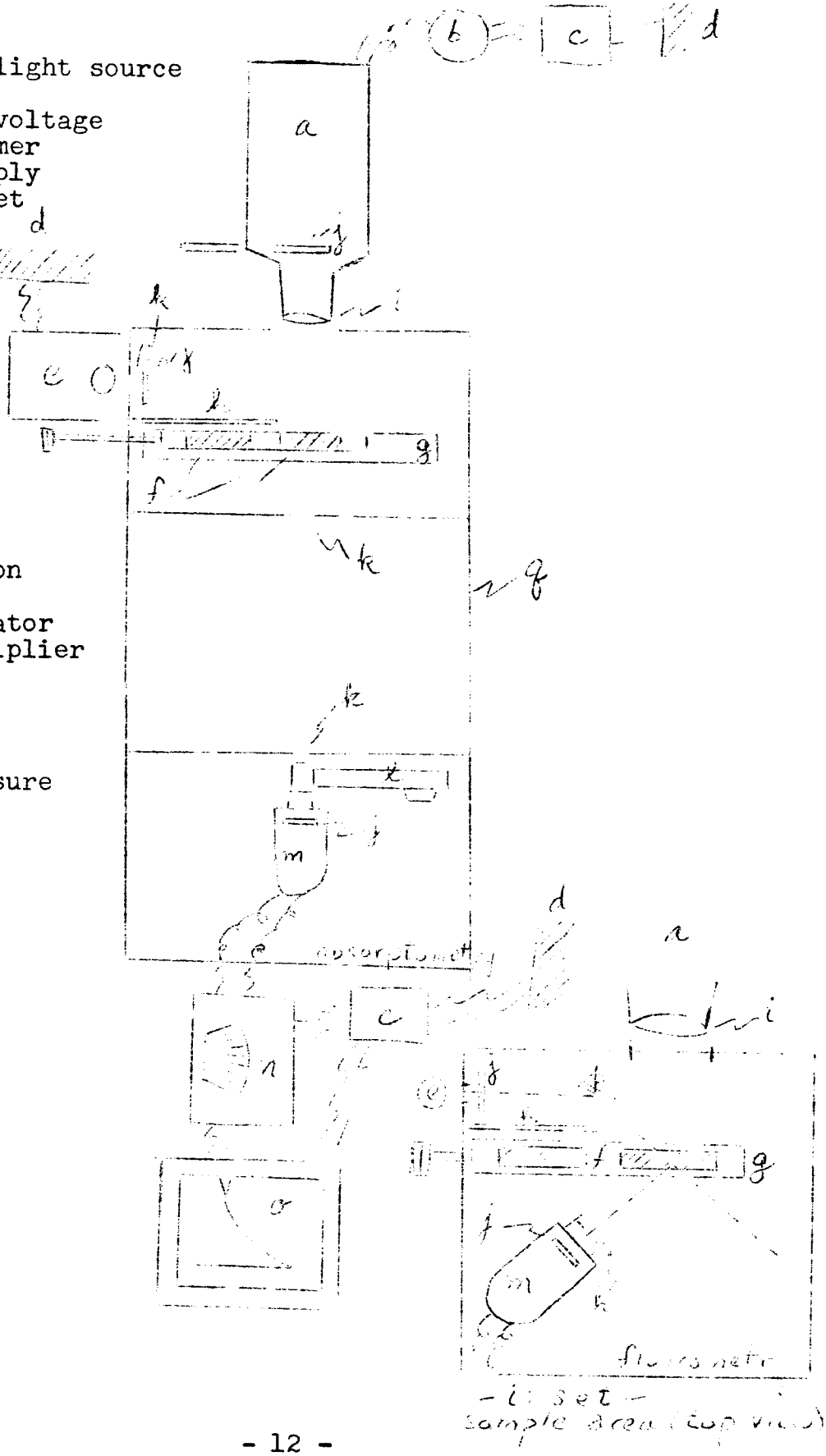
Thus, we have found methylene blue is subject to photo-oxidation, as has been previously reported (13), but that the presence of guanine (and product AG) inhibit and delay the disappearance of the dye until the guanine content is exhausted, giving results such as that sketched in Figure 6, as we believe is the sequence of events; the guanine, methylene blue, and AG-precursor have been observed directly (initial rates), while AG and BG rate of appearance are inferred from semi-quantitative estimates of thin layer chromatograms.

The initial slope of the rate of change of methylene blue curve is linear and slightly negative. The value of this slope is sensitive to the alkalinity of the reaction medium. This is shown in Figure 7. It can be seen that the most rapid change in dye concentration takes place at pH-values greater than 12.0. It may also be noted that the presence of a purine, i.e. 8-azaguanine, displaces the onset of rapid deteriorationof methylene blue by at least one-half pH-unit. The effect is especially strong with the purine, guanine, since the latter undergoes conversion to AG, which we have found to be a reducing agent which tends to temporarily preserve methylene blue, establishing for the latter a level of concentration which approximates a steady-state. The methylene blue concentration, as a result, does not diminish markedly until guanine and AG concentrations begin to diminish. A curve, such as those shown in Figure 7, is sought to identify which anionic forms of the components are most reactive by comparison with known pK_a's of the components.

Figure 5. Schematic arrangement for photometric determination of kinetic rates.

Legend:

a. tungsten light source
b. rheostat
c. constant voltage transformer
d. power supply
e. ultraviolet source
f. sample and reference cells
g. sample cells slide rack
h. light shield
i. lens
j. filter
k. collimation aperture
l. monochromator
m. photomultiplier tube
n. amplifier
o. recorder
p. mirror
q. box enclosure



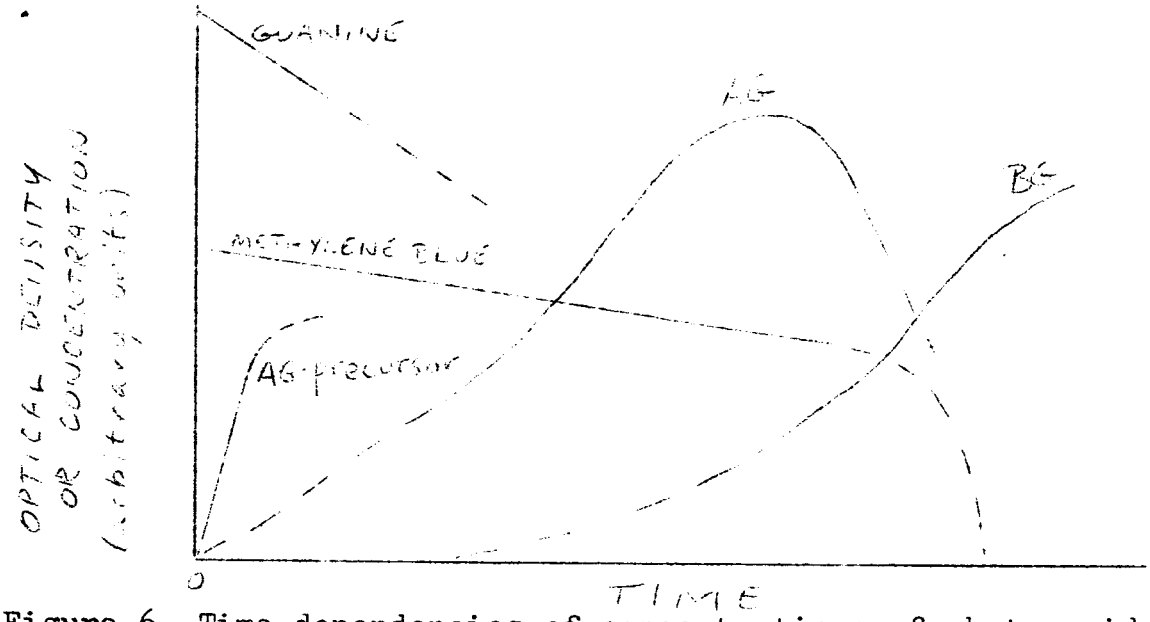
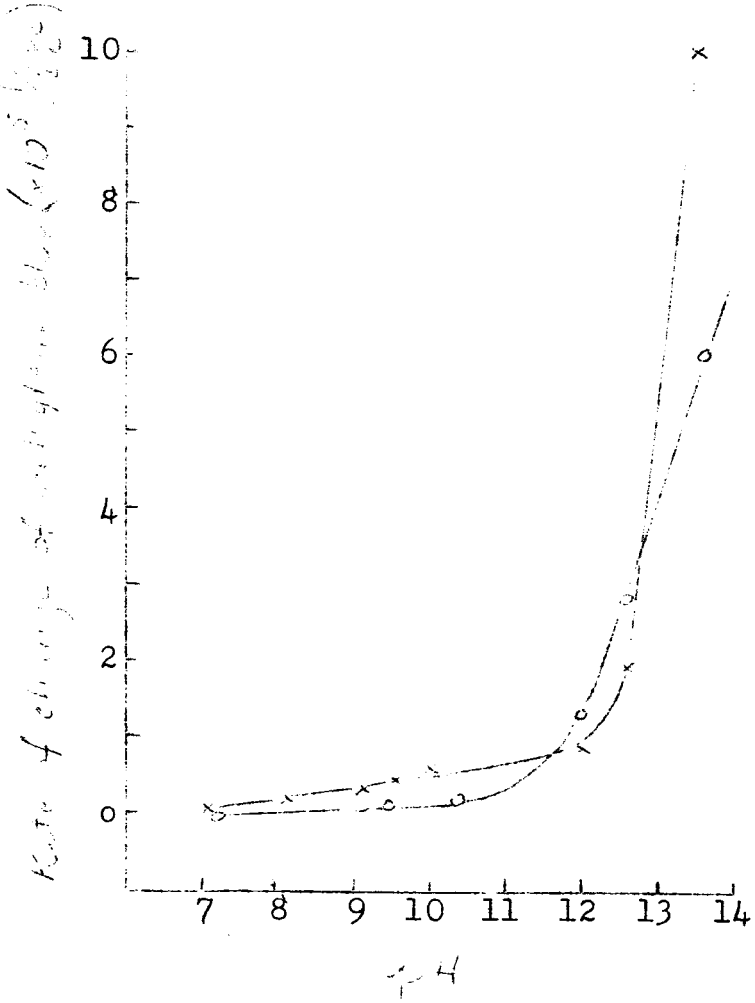


Figure 6. Time dependencies of concentrations of photo-oxidation reactants and products.

Figure 7. Rate dependence on pH of photobleaching of methylene blue (conc. 10^{-5} m/l.)

-x- without any 8-azaguanine

-o- with 10^{-3} m/l 8-azaguanine



It may be pointed out that concern with the isolation, purification and identification of products, the goal of Project C, contributes valuable information to the success of instrumenting properly the photometry of Project B, and indeed delays in the latter have been caused due to a lack of knowledge of the absorption spectra and extinction coefficients of the various intermediates in the very complex guanine system. However, with the current progress of Project C in recognizing and unraveling system components, Project B is showing promise that we may procede beyond the initial rates for accomplishing an intimate understanding of the entire mechanism of the reaction.

Typical initial concentrations used for the photometric studies are as follows: 10^{-5} moles/liter of methylene blue, 10^{-3} moles/liter purine substrate, in alkaline aqueous solution containing 0.2 moles/liter phosphate buffer, stirring with a vigorous stream of humidified oxygen gas, and illuminated with a 500 watt projector tungsten lamp employing Corning glass filters. The initial rates achieved in such a mixture may be seen in Figure 7.

* *

Project C. Isolation, Characterization, and Identification of photoproducts and sequential chemical products from the dye-sensitized photo-oxidation of guanine in alkaline solution.

Guanine may be oxidatively photo-degraded to give two major photoproducts, according to abridged scheme I:

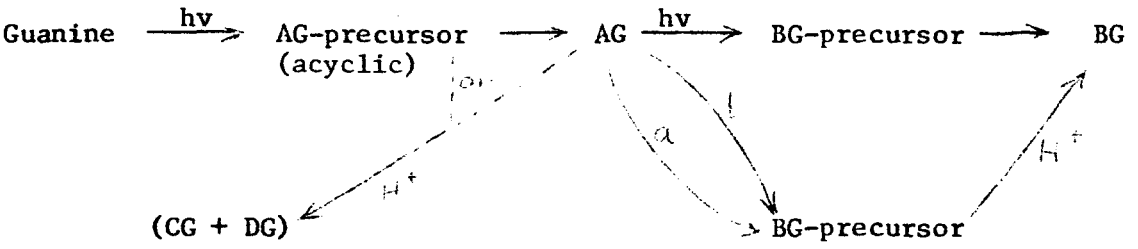
$$\text{Guanine} + O_2 \xrightarrow[\text{aq. NaOH}]{\text{red light, dye}} \underset{\text{(slow)}}{\text{AG}} \xrightarrow[\text{aq. NaOH}]{O_2,\ \text{red light, dye}} \underset{\text{(fast)}}{\text{BG}}$$

Compounds AG and BG are produced when either the conditions of methylene blue and 570-690 nm. light (40 kcal/mole), or Rose Bengal and 500-570 nm light (50 kcal/mole) are used, showing that below some energy limit (in the near ultraviolet region) the reaction is not sensibly dependent on the specific nature of dye and the energy of the excited state of the dye.

Compound AG has been isolated and photoconverted to BG in a separate reaction. Later experiments have shown that AG may also be oxidized, rather easily, to BG without the agency of light, but at a slower rate. Aliquots of the photoreaction mixture, removed at different periods of time, and separated by means of thin layer chromatography indicate that the appearance of BG is sequential to that of AG.

Total, current experience with this system shows that the above scheme must be expanded to include other intermediates and derivatives

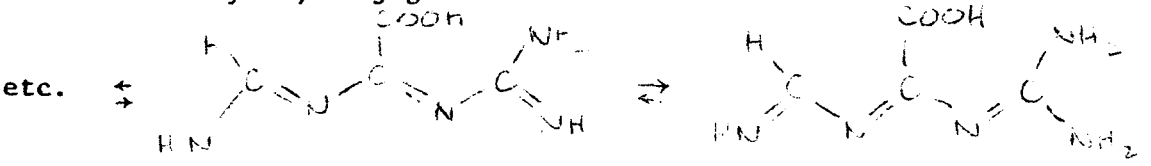
which are indicated to be present. Thus, Scheme II is:



a. with O_2 and a trace of methylene blue as indicator;
b. with stoichiometric equivalent of methylene blue as oxidizing agent; both a and b conducted in alkaline solution.

We feel that shortly we will be able to establish the structures of most of the seven indicated compounds, on the basis of our present activities involving independent synthesis of selected sym-triazine and hydantoin derivatives. A reasonably consistent relation of sym-triazines has occurred to us which is compatible with many details of our experimental evidence. The following proposed structures will be offered in this light, with the reservation that proof has not established beyond doubt.

Photometric evidence from Project B shows that a photoproduct is initially generated from guanine which is more strongly absorbing at 280 nm. than are any of the original solution components. This absorption is tentatively assigned to a substance we designate as AG-precursor and which we formulate as the acylic, conjugated structure I:

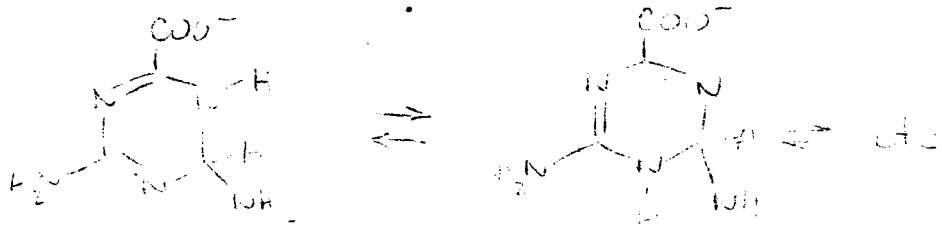


(I) AG-precursor

Such an open-chain structure would tend to resolve the dilemma of which ring of a purine, the imidazole or the pyrimidine, undergoes a ring opening in photochemical processes. This investigator believes that both rings are open in this and a number of systems reported in the literature. The particular formulat ion of structure I implies that both rings are open at one time. Such acyclic compounds have been proposed in the past (10,14), especially of note are the chemical sequences proposed to explain the oxidation of uric acid.

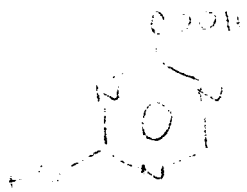
Compound AG is a reducing agent. This is an interesting and apparent paradox that a reducing agent should be generated under oxidizing conditions. A previous research progress report (No. 2, p. 8, April 30, 1965) called attention to the chance observation that, when AG is mixed with methylene blue, the dye is bleached; the dye can be regenerated from the leuco-form by shaking with air. This phenomenon and the structures being considered gain credence when one imagines AG-precursor to undergo a cyclization, by agency of the very basic, guanine-amino group, nucleophilic attack upon the imine carbon of the amidine group at the other end of the molecule, to give one or another

of the tautomeric pair of structures,II:

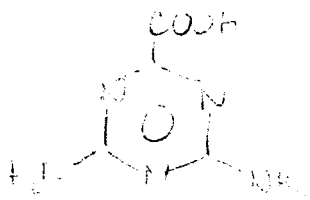


(II) AG

Because of the large resonance energy gain upon aromatization of the dihydrotriazine (much larger than benzene) one can expect a strong driving force for dehydrogenation or deamination of AG to yield a sym-triazine derivative. Ammonia has been detected effusing from the solution, but in all probability both deamination and dehydrogenation may be expected to occur, to structures such as III and IV, respectively:



III



IV

Compound AG has been isolated, investigated spectrally, and subjected to elemental analysis which confirm an empirical molecular formula $C_{4.0}H_{6.9}N_{4.9}O_{2.0}$ supporting structure IV. Compound AG has been oxidized with a stoichiometric amount of methylene blue, and, alternatively, with oxygen in alkaline solution containing a trace quantity of methylene to act as an end point-indicator. Either procedure yields compound BG-precursor, which has been isolated, but not yet characterized.

Compound BG-precursor is sensitive to aqueous acid solutions and upon treatment yields compound BG (confirmed by identical infrared spectrum with the photoproduced sample). The latter chemical conversion of AG to BG is considerably slower than the photochemical conversion. Elemental analysis is approximately consistent with the structures of the tautomeric pair shown below, structures V:

Structures V

The essential change from structure IV is that of hydrolysis of an amino-group substituent. However, osmometry, elemental analysis and titrimetric data, as given in a previous research report (No. 2, p. 7, April 30, 1965) gives evidence that BG may be at least a tetramer of the structure shown. (Progress is somewhat slowed in these determinations because of the poor solubilities of derivatives such as these, both in aqeous and organic media, and complete purification of each substance, even through dire efforts, is not always assured.)

Compounds CG and DG, with the analytical empirical formulae $C_4H_9N_5O_2$ and $C_4H_8N_4O_3$, respectively, are derived by strong acid treatment of the solution containing AG and/or AG-precursor. A molecular weight of 158± 8 has been determined for CG, a basic substance; no confirmative work has been done on DG, though this is just beginning.

Thus, at least five compounds have been isolated, and the structures contemplated above have not been reported in the chemical literature. Decarboxylation and/or hydrolysis of structure IV would yield compounds which can be related to compounds reported in the literature---literature mostly related to uric acid degradation schemes. For the "new" compounds which we report elemental analysis has been achieved for three of these, molecular weight determinations for two, titration data for two, and infrared and ultraviolet data have been collected.

The chemical potential of a compound such as the presumed-linear AG-precursor, and of the acid-catalyzed transformations of subsequent derivatives provides a versatile and variable synthetic pathway to interesting new compounds. Thus, also, similar changes which we have brought about with 8-azaguanine and other purines invite our continued attention in this line of endeavor.

A preparative procedure for the dye-sensitized photo-oxidation of guanine is given below:

Guanine (2.0 g) is suspended in 50 ml. of distilled water. Sodium hydroxide (3.5 g) is then added. The mixture is swirled with cooling to bring about complete solution. The resulting clear and colorless solution is then diluted to 90 ml. with distilled water, and 10 ml. of 10^{-3} M methylene blue solution is added. (The pH of this resulting solution is greater than 13.)

Oxygen is bubbled through the solution at a rate of about 100 bubbles per minute through a disposable pipette tube. The solution is illuminated with a light beam from a 500 watt tungsten filament bulb in a slide projector situated 7-8 cm from the reaction vessel. The beam is filtered with a Corning #2424 CS 2-63 (2.5 mm.) red filter. Temperatures of the reaction mixture were noted to range between 25 and 30°C.

After a period of 21 to 24 hours, the blue color of the sensitizer has disappeared and a light yellow solution remains.

During the reaction 5 -drops may be spotted on cellulose-fluorescein T.L.C. plates, and then developed with a solvent composed of saturated aqueous ammonium sulfate: water: isopropanol (80:12:2). Three components can be detected with short wave-length ultraviolet light. Characteristic spots can be identified as AG, BG, and guanine by their chromatographic Rf values. When guanine disappears from the chromatogram, the entire reaction mixture is worked up as follows:

The reaction mixture is extracted with n-butanol to remove all dye materials. The reaction mixture is then poured into a large beaker and neutralized with 6 ml. of glacial acetic acid and stirred until CO_2 evolution is completed (pH is 5). The solution is then immediately filtered to remove BG precursor. (The BG precursor is converted to BG by evaporating it to dryness with 1N HCl). The clear filtrate is allowed to chill in the refrigerator for about an hour; the resulting slow-forming crystals of AG are then removed by filtration. An average run will give 800 mg. of AG and 500 mg. of BG. Continued evaporation of the filtrate will yield more AG.

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III. Personnel

A. Dr. Frank Millich, Associate Professor,
Department of Chemistry, U.M.K.C.....Project Director

B. Mr. Hossein Nanaie, Graduate Assistant,
Department of Chemistry, U.M.K.C.....Appointee (Project B,C)
9/1/1964 - 1/31/1966

C. Mr. Marion F. Botts, Graduate Student
Department of Chemistry, U.M.K.C.....Not Appointed (Project C)
2/1/1966 - Present

D. Mrs. Karen E. Kugler, Chemist
Department of Chemistry, U.M.K.C.....Technician (Project A)
2/1/1965 - 6/11/1965

E. Miss Akiko Iwata, Chemist
Department of Chemistry, U.M.K.C.....Technician (Project A)
(part time) 6/12/1965 - present

F. Miss Beatrice M. Oakes, Undergraduate.....Laboratory Assistant
(part time) 6/17/1965 - 9/20/1965

G. Mr. Kenneth F. Scott, Undergraduate.....Laboratory Assistant
(part time) 11/1/1965 - 1/31/1965