Derivatives Of Alloxan As Potential Pancreatotrophic Agents.

DERIVATIVES OF ALLOXAN AS POTENTIAL PANCREATOTROPHIC AGENTS

by

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This thesis is dedicated to the author's parents.

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ABSTRACT

This study entailed the preparation of derivatives of alloxan which were expected to retain the dominant physiological trait of alloxan, namely diabetogenesis. In order to accomplish this task, structure-activity guidelines from the pharmacology literature were adhered to strictly in the synthetic design. The alloxan pyrimidine ring was retained intact and the #5 carbon was readily hydrolyzed or oxidized to a carbonyl.

The structural requirements for diabetogenicity were met with the alloxan anils prepared from alloxan monohydrate and aromatic amines. When the position para to the amino group does not have a substituent then alloxan substitution occurred there to form 5-aryldialuric acids. Alloxan monohydrate and 3,4-dimethylaniline did not produce an anil, but a dioxindolecarboxyureide.

Another class of alloxan derivatives which fulfills the structural requirements for diabetogenicity are the triarylphosphine alloxan adducts. The spectral data of these adducts indicated they had a symmetrical zwitterionic structure. The mechanism of the formation of these adducts was investigated to determine if a C-5 carbonyl is necessary or if a C-4 carbonyl is sufficient. The C-4 carbonyl appeared sufficient since

the adduct was produced from alloxan monohydrate, alloxantin and 5-ethoxy-5-hydroxybarbituric acid in ethanol. However, it was shown that alloxan monohydrate readily forms 5-ethoxy-5-hydroxybarbituric acid in ethanol and alloxantin readily forms alloxan monohydrate in ethanol. By using a non-hydroxylic solvent such as tetrahydrofuran the 5-ethoxy-5-hydroxybarbituric acid very slowly produced the adduct, whereas the alloxan anhydride readily formed the adduct. The dissocation of 5-ethoxy-5-hydroxybarbituric acid into alloxan anhydride and ethanol in refluxing tetrahydrofuran was demonstrated. Thus, a plausible mechanism (in accord with the known reaction data) consists of the conversion of alloxan monohydrate and alloxantin into 5-ethoxy-5-hydroxybarbituric acid followed by transformation into alloxan anhydride which readily reacts with triarylphosphines to produce the adduct.

The reactions of the adducts were also investigated. The adducts are transformed into products in acidic media only. Presumably protonation is necessary before displacement of triphenylphosphine oxide can occur. The study of the reactions of the adduct produced a new synthesis of 5-barbituryl sulfides and 5-chlorobarbituric acid.

The reaction of the adduct and cysteine produced

a material which is very similar in structure to one of the proposed structures for the chromophore ("compound 305") produced from alloxan and glutathione. Since the ultraviolet maximum of "compound 305" is at 305 nm and the material prepared in this study has a maximum at 257 nm, then the proposed structure is not the correct structure for the chromophore.

INTRODUCTION

The 1.5% five-year survival rate of pancreatic carcinoma indicates that the diagnosis of this affliction is usually made past the point when treatment would be effective in a cure. The principal radiopharmaceutical used today in such diagnosis is ^{75}Se-selenomethionine. This agent is able to concentrate sufficiently to permit imaging of the pancreas; however, there are several drawbacks.

Besides the fact that the image obtained when using ^{75}Se-selenomethionine is poor and gives false positives and false negatives in tumor delineation, the patient is exposed to excessive amounts of radiation. The radio half-life of 120 days for ^{75}Se contributes to the high dose which the patient receives. Another factor contributing to give the patient excessive radiation is the extended biological half life of the ^{75}Se-selenomethionine. If ^{75}Se-selenomethionine were readily eliminated from the body, either through the hepato-biliary system or through the kidney, then the danger of the high radiation dose would be drastically reduced. Very possibly a new radiopharmaceutical may be the long sought answer to the problem of early diagnosis of pancreatic carcinoma.[1]

A radiopharmaceutical is composed of two parts, the carrier and the radionuclide. Most biological materials contain C, H, N, O, S, and P and unfortunately these elements do not have useful γ emitting isotopic nuclides. A β emitter which is very useful in the detection of ocular melanomas is ^{32}P-phosphate, because it is possible to place a detector on the surface of the eyeball to detect the emissions of the ^{32}P before they are stopped because of tissue or distance. The nuclides which decay through γ emissions (photons) are necessary for the pancreas because a particle emanating from the pancreas will have to pass through a large amount of tissue and travel a large distance before it can be detected. Thus, radiopharmaceuticals for the pancreas must be labeled with radionuclides of elements which are not present in the parent molecule, a process referred to as external or foreign labeling.[2]

Radiolabeling of the radiopharmaceutical should occur easily and quickly in order to prepare a material with a short-lived nuclide so that there will be sufficient radiation during the diagnostic test to obtain a scan. The chemical stability of the labeled radiopharmaceutical must be checked to ensure the carrier and the radionuclide do not go their separate ways <u>in vivo</u>. If the radiopharmaceutical is unstable, then the

radionuclide will be distributed throughout the animal's (or patient's) body as if only the radionuclide were injected.

Pancreatic carcinoma can occur in any part of the gland and with either the exocrine or endocrine portions of the organ. There are three pathological types of pancreatic carcinoma: adenocarcinoma arising from the exocrine pancreas, islet cell carcinoma and cystadenocarcinoma of the exocrine pancreas.[3] The vast majority (95%) of the pancreatic carcinoma are from the adenocarcinoma of the exocrine pancreas. About 80% of these adenocarcinomas arise from the ductile tissues[4] and the remainder from the acinar tissue. Islet cell carcinomas and cystadenocarcinomas are rare.

The endocrine portion of the pancreas makes up about 1-2% of the total weight of the pancreas and is composed of groups of cells called the islets of Langerhans which are non-uniformly distributed throughout the organ. The three types of islet cells are distinguishable by their function: α cells comprise about 20% of the islets and store glucagon,[5] β cells are the most numerous at 75% of the total and they store insulin,[5] and the remaining 5% of the cells are δ-cells which store gastrin.[6] The islets of the Langerhans are characteristically highly vascular with numerous

capillaries and very few, if any, islet cells are not in contact with a capillary.

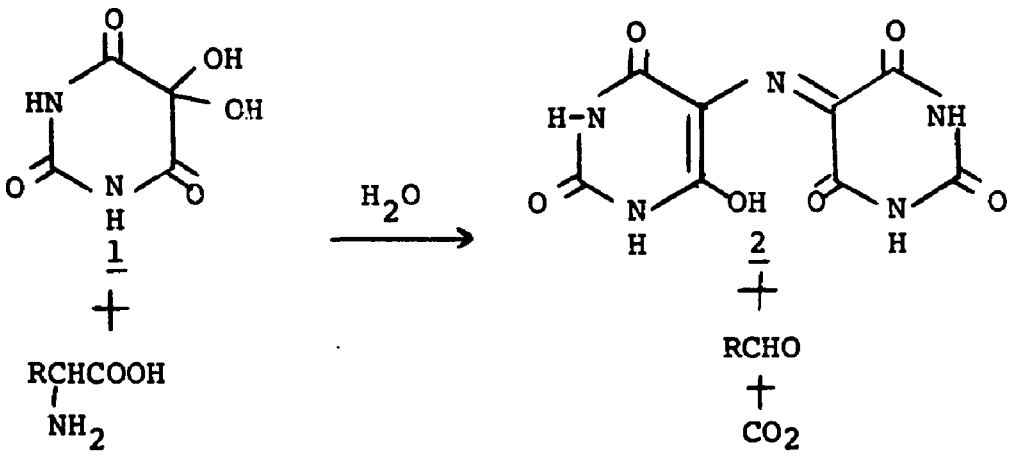
The exocrine pancreas secretes digestive materials into the digestive tract.[7] The proteins secreted are proteases and other enzymes used in cleavage reactions. Also, HCO_3^- is secreted to counteract the acidity of the food as it initially enters the intestine from the stomach. The duct cells release the HCO_3^- and the digestive enzymes are secreted by the acinar cells.[5] The two large pancreatic ducts empty into the duodenum.

I. The Biological Aspect of Alloxan

In 1943 it was reported that alloxan induced diabetes in laboratory animals[8,9,10] with concomitant pancreatic islet necrosis.[11] The following year Dunn and coworkers proved the very specific nature of alloxan in damaging only the ß-cells of the islets and leaving untouched the α-cells.[12] This discovery provided a rapid and easily available method for the induction of diabetes. The recent literature has many articles about studies on diabetes induced by alloxan.

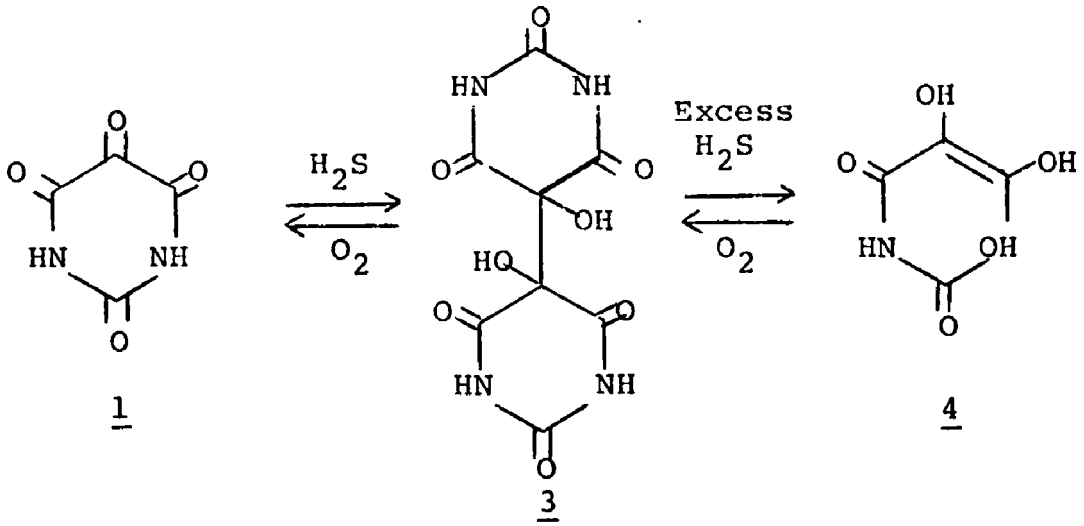
As a chemical compound, alloxan has been known since 1838 when Friedrich Wöhler and Justus Liebig[13] first synthesized it from uric acid, but there were very few reports in the early literature which would indicate that alloxan had any significant biological effects. Wöhler and Liebig did report, however, that when an aqueous solution was placed on the skin, the skin was markedly reddened and a strong smell came from the spot. About 120 years later this early observation was put to practical use when two groups of investigators determined the feasibility of using methanol[14] and ethyl acetate[15] solutions of alloxan to outline fingerprints. Subsequently it was found that ninhydrin solutions were superior.[15]

There were some initial studies which showed alloxan effected enzymes and metabolic systems[16] and the Strecker amino acid degradation was reported in 1862.[17] In this reaction an amino acid is oxidized to the corresponding aldehyde and alloxan(1) is reduced. Stoichiometrically, the alloxan is reduced to uramil (5-aminobarbituric acid) which reacts with another mole of alloxan to form purpuric acid (2) or murexide salts if there is an excess of ammonium ion from the amino acid or if ammonium ion is added to the reaction medium.



Another reaction with potential implications to a molecular level understanding of how alloxan destroys β-cells is the reaction of alloxan with sulfhydryl groups. While alloxan undergoes reduction the sulfhydryl groups are oxidized to the disulfides. The reaction readily occurs with sulfur containing biomolecules. Cysteine and glutathione have been shown to reduce alloxan in

vitro.[18,19] The reduction of alloxan (1) to alloxantin (3) and finally to dialuric acid (4) has been developed on a general preparative scale using hydrogen sulfide as the reducing agent.[20a,b,c] Dialuric acid and alloxantin are readily oxidized to alloxantin and alloxan, respectively, with the dissolved oxygen in the media of the reaction. Thus if alloxan acts as an oxidizing agent of sulfhydryl groups in a biological system, one mole of alloxan can oxidize several moles of sulfhydryl groups until it is finally metabolized.



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A. The Mechanism of the Diabetogenic Action of Alloxan

Alloxan-induced diabetes causes the characteristic variation of blood glucose levels in mammals, birds, reptiles and certain fish. Initially, there is a 3-4 hour period of hyperglycemia, followed by a 6-12

hour period of hypoglycemia and finally the onset of the permanent hyperglycemic state of diabetes. Five minutes after injection of alloxan there are noticeable changes in the beta cells of the islets. The cells begin to shrink at once and have shrunk still more at 15-30 minutes with tiny vacuoles forming and the nuclei begin to disintegrate. Between five and ten hours the nuclei are almost completely disintegrated and the cells are completely vacuolated. By 24 hours the islets contain cellular debris and within three to five days there are no beta cells visible, but the alpha cells and the acinar cells are normal. The net result is that the beta cells are completely and specifically obliterated within a few days.[16]

Distribution studies with alloxan-2-^{14}C show that alloxan does concentrate in the islets of the Langerhans when tracer doses of the radioactive material are used.[21] However, when large diabetogenic doses of alloxan are used in conjunction with the tracer doses of ^{14}C labeled alloxan, then the ^{14}C is found not to concentrate in the islets put to be distributed throughout the body.[21] Though the ^{14}C labeled alloxan concentrates in islet tissue, studies[22,23] have shown that it does not actually enter the cells but acts instead on the membrane. When D-mannitol-1-^{14}C was incubated with a slice of toadfish islet tissue after treatment of the

slice with a solution of alloxan or concurrently with alloxan, the islet tissue were found to contain a high amount of ^{14}C.[24] In the same study an evaluation was made of the permeability of rat kidney and liver slices with ten times the amount of alloxan employed on the islet tissues and no ^{14}C uptake was observed in these tissues.

After the islet tissue was incubated in the alloxan containing solution, the solution contained a large amount of protein and its insulin-like activity was greater compared to the controls.[24] The islets were found to experience an increase in zinc concentration after administration of small doses of alloxan,[25] but there was an irreversible decrease in zinc after diabetogenic doses of alloxan were given with all the zinc being removed after one to two days.[25,26] Diabetic doses of alloxan also decrease the amount of RNA,[27] glucose-6-phosphate dehydrogenase, glucose-6-phosphatase and ATPase in the islet tissues.[28]

When alloxan is administered there is an abrupt and sudden decline in the glutathione levels of the blood[29,30,31] suggesting that glutathione protects the pancreatic islets from the effects of alloxan. When cysteine or glutathione is administered along with alloxan there is indeed protection against diabetes.[32,33]

Another sulfhydryl compound, British antilewisite (BAL), 2,3-dimercaptopropanol is also effective in inhibiting the effects of alloxan.[34,35] When a special strain of animal with naturally elevated glutathione levels is administered alloxan it is found to be resistant to diabetes.[36,37] Also young puppies up to six months are more resistant to alloxan than are adult dogs.[38] Injection of alloxan in utero in pregnant rats did not effect any change of the islets of the fetuses.[39] A conclusion which can be advanced is that resistance to alloxan-induced diabetes is contingent on the blood glutathione levels (or the presence of other xenobiotic sulfhydryl compounds in the blood) and not to structural features of the beta cells.

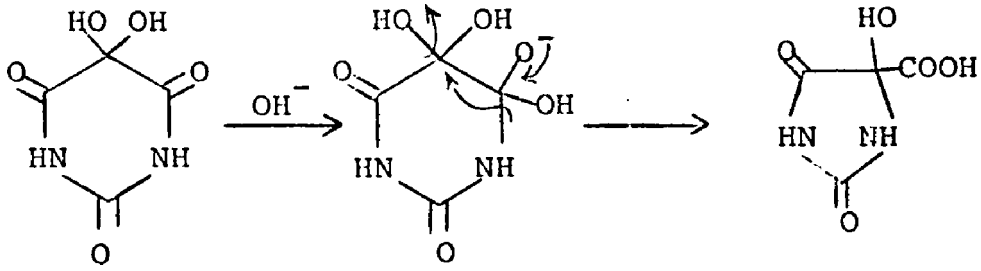
One possible cause of age-onset diabetes in man has been suggested as arising from alloxan production in the body. The body synthesizes uric acid and since uric acid can be oxidized to alloxan in the laboratory an in vivo production has been suggested. Indeed, leukocytes can produce a modest yield of alloxan when there is a dearth of sulfhydryl containing materials.[40,41] Also, the intestines have been implicated as the source of alloxan production in the body.[42] The blood in man, rat, horse, rabbit, and dog has been evaluated

and has been found to contain small but finite amounts of alloxan.[42,43] Oral administration of glucose in rats increased the amount of alloxan in the blood 50 times above the normal alloxan levels.[43] Other tests have shown that alloxan exists in various organs of the body of ducks, hens, guinea pigs, lambs, pigs, rabbits, rats and man, also in small but measurable amounts.[44] The speculation is that over a long period of time the alloxan contributes to the degeneration and destruction of the beta cells of the islets of Langerhans in the pancreas. The beta cells do have some regenerative powers, but if the destruction of the beta cells is faster than their regeneration then diabetes will ensue.

It must be admitted, however, that the studies which found alloxan in the blood and organs are viewed with some skepticism because of the very low concentrations of alloxan (0.001-0.014 mM alloxan) detected. Furthermore, the assay methods employed were not precise and reproducible. Attempts at duplication of these results were not always successful in finding alloxan at all.[45,46] In the blood, alloxan is rapidly rearranged to alloxanic acid via a benzilic acid type of rearrangement. There have been no measurements of alloxanic acid in blood and tissues even though most of the alloxan administered or synthesized <u>in vivo</u> would

be quickly converted to that form.

At 37°C and a pH of 7.4, the half life of alloxan is about 0.9 minute[47] and at pH 7.1 the half life is 1.7 minutes.[22] When the temperature is decreased to 0°C (at pH 7.1) the half life is 201 minutes and at -2°C and at pH 7.1 the half life is extended to 301 minutes.[22]. The benzilic rearrangement of alloxan to alloxanic acid has been described as involving N migration.[48,49,50]



Seligson and Seligson[51] studied the decomposition of alloxan to alloxanic acid in phosphate buffer and in blood plasma at pH 7.35 and 25°C. They found that the half life of alloxan in blood plasma was approximately 4.2 minutes and in phosphate buffer, 3.2 minutes. A Russian group[52] determined that the decomposition of alloxan to alloxanic acid occurred faster in vivo than in vitro when using white rats' blood. The instability of alloxan in polar media has thus been thoroughly demonstrated.

Since alloxan has some effect on enzymes and a definite effect on the beta cells of the pancreatic islets, alloxan has been used in some antineoplastic assays. With the selective destruction of the pancreatic beta cells it was thought that alloxan might be employed successfully against insulinomas. However, it was found that necrosis, or destruction, of the malignant insulin producing cells was not possible with alloxan.[53,54] Since the malignant islet cells were not attacked by alloxan and the insulin levels remained high, the mode of action that alloxan exerts on the beta cells is not linked to the system in the cells responsible for insulin release. The difference between the malignant beta cells and the normal beta cells must be in the membranes of the beta cells.

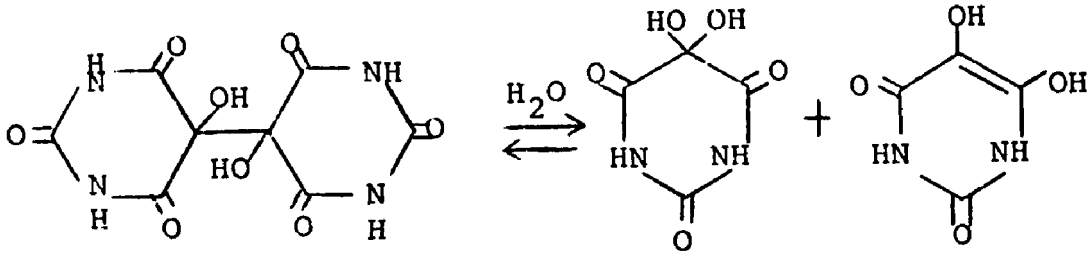
Alloxan has been employed against other tumors with limited success. It slightly enhanced the growth of Walker carcinoma while colchicine inhibited tumor growth.[55] Contrary to this report, a Russian group[56] reported that in rats the weight of Walker's carcinosarcoma was decreased with moderately good results. Another report[57] described how injections of alloxan in humans prevented metastases of cancerous tissue following surgery. The Yoshida ascites tumor in rats has been moderately decreased with alloxan treatments.[58] When

alloxan was used against the SR-61 leukemia in mice there was no anti tumor activity.[59]

Alloxan and dialuric acid have been shown to inhibit and prevent the growth of gram positive and gram negative bacteria and fungi. This being the case, alloxan and dialuric acid were used at the rate of fifty pounds of each, individually, per thousand gallons of paint to test for anti mildew. When these paint mixtures were applied to steel panels and incubated in a room conducive to mildew growth, no mildew grew on the steel panels which were painted with alloxan or dialuric acid containing paint, but the steel panels painted with ordinary paint were covered with mildew.[60]

B. Structure Correlation to Diabetogenic Activity

Alloxan is diabetogenic and any alloxan (1) derivative which is easily hydrolyzed, like alloxantin (3), or oxidized, like dialuric acid (4), to alloxan will also be diabetogenic. Originally, Jacobs[63] noticed that



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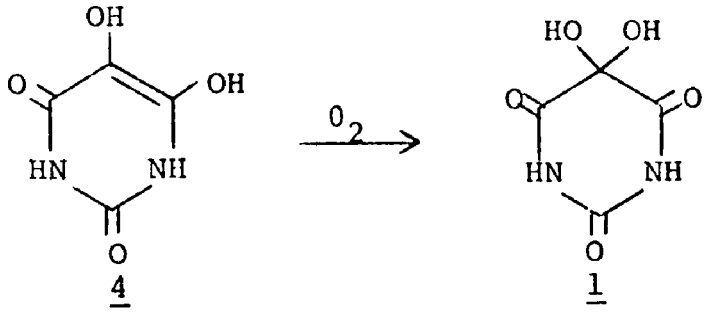

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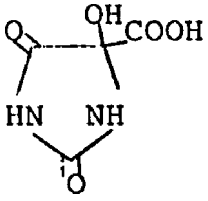
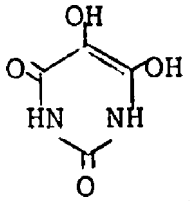
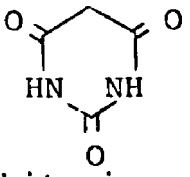
alloxan caused a change in blood sugar levels and tested several derivatives and degradation products of alloxan for hypo and/or hyperglycemia. All of the following were without effect on blood sugar levels:



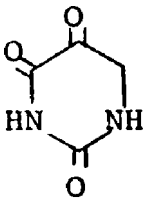
alloxanic acid



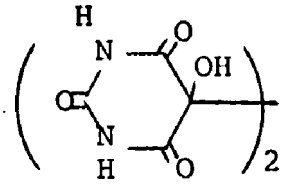
dialuric acid



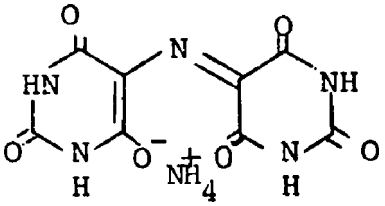
barbituric acid



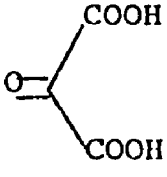
isobarbituric acid



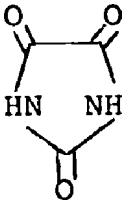
alloxantin



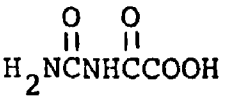
murexide



mesoxalic acid

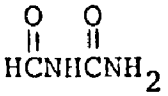


parabanic acid



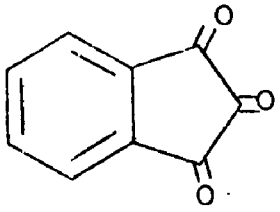
oxaluric acid

formyloxaluric acid

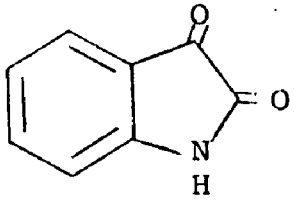


formylurea

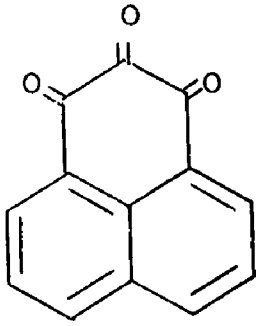
One hypothesis is that alloxan's ability to degrade amino acids to the corresponding aldehydes (Strecker degradation) constitutes the main mechanism for diabetes induction.[61] However, the following do not cause diabetes but have been used to bring about the Strecker degradation:



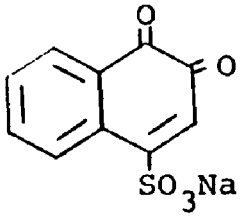
ninhydrin[61,62,64]



isatin[61,62,64]



1,8-mesoxalyl-naphthalene[61]

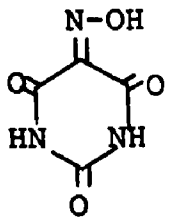


Sodium 1,2-naphthoquinone-4-sulfonate[61]

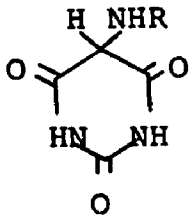
Apparently, the presence of the urea grouping in a molecule is necessary but not sufficient for this biological effect of β-cell destruction. Contrary to Jacobs'[63] report that dialuric acid does not alter blood glucose levels, Brückmann and Wertheiner[61,64] have found dialuric acid and 1-methyldialuric acid to be diabetogenic materials.

When the urea part of alloxan, dialuric acid and alloxantin is changed by substitution on nitrogen, then the toxicity of the material increases. Alloxan derivatives which are diabetogenic are the mono-methyl,[62] ethyl and n-propyl alloxan derivatives.[61] However, the mono n-butyl, iso-butyl, phenyl and benzyl alloxan derivatives are non-diabetogenic and are toxic at levels below the expected diabetogenic dose (except the phenyl derivative which is non-toxic and non-diabetogenic). Similarly, the monomethyl derivative of dialuric acid and the dimethyl (and diethyl) derivative of alloxantin did cause diabetes.[61,64] The disubstituted alloxans do not cause diabetes and are toxic. 1,3-Dimethyl, 1-methyl-3-ethyl- and 1-methyl-3-n-propylalloxan were tested and the most toxic material was the n-propyl derivative.

Other derivatives which have shown no diabetogenic action are violuric[64] acid and uramil.[61]



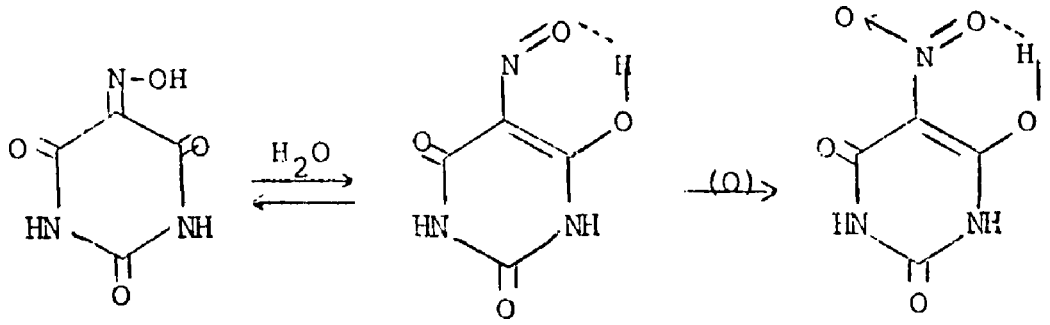
Violuric acid



Uramil (R = H, CH_3)

Violuric acid has a #5 carbon in the same oxidation state as alloxan, but it may tautomerize to the 5-nitroso-barbituric acid in solution. If so, the nitroso

would oxidize before the #5 carbon.



Uramil is in the oxidation state of dialuric acid but when it is oxidized the 5-amino nitrogen is probably oxidized first.

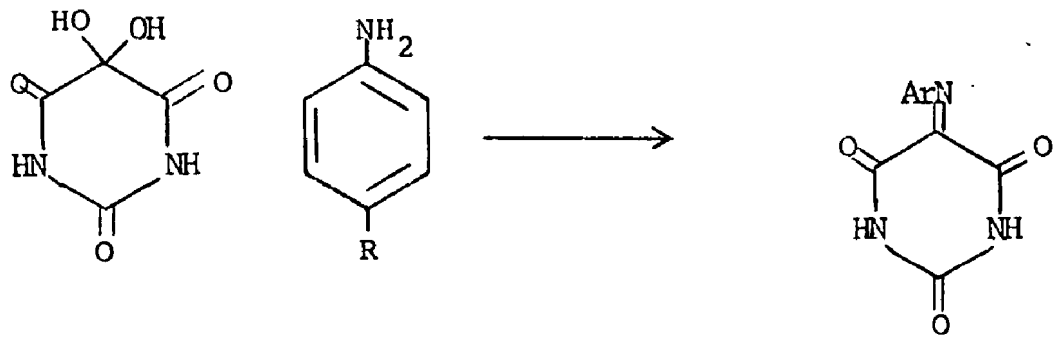
It is necessary to have the pyrimidine ring in an oxidation state such as in alloxan or in a reduced oxidation state as with dialuric acid or alloxantin. Since alloxan is readily reduced to alloxantin or dialuric acid in the blood by glutathione or other sulfhydryl containing materials, then they may be the materials that are diabetogenic. Recently, hydrogen peroxide, hydroxy radical (·OH) and superoxide radical (O_2^-) have been detected during the oxidation of dialuric acid with oxygen. These three materials were proposed as being cytotoxic towards the pancreatic β-cells.[65]

II. The Chemistry of Alloxan

A. Amine Chemistry of Alloxan

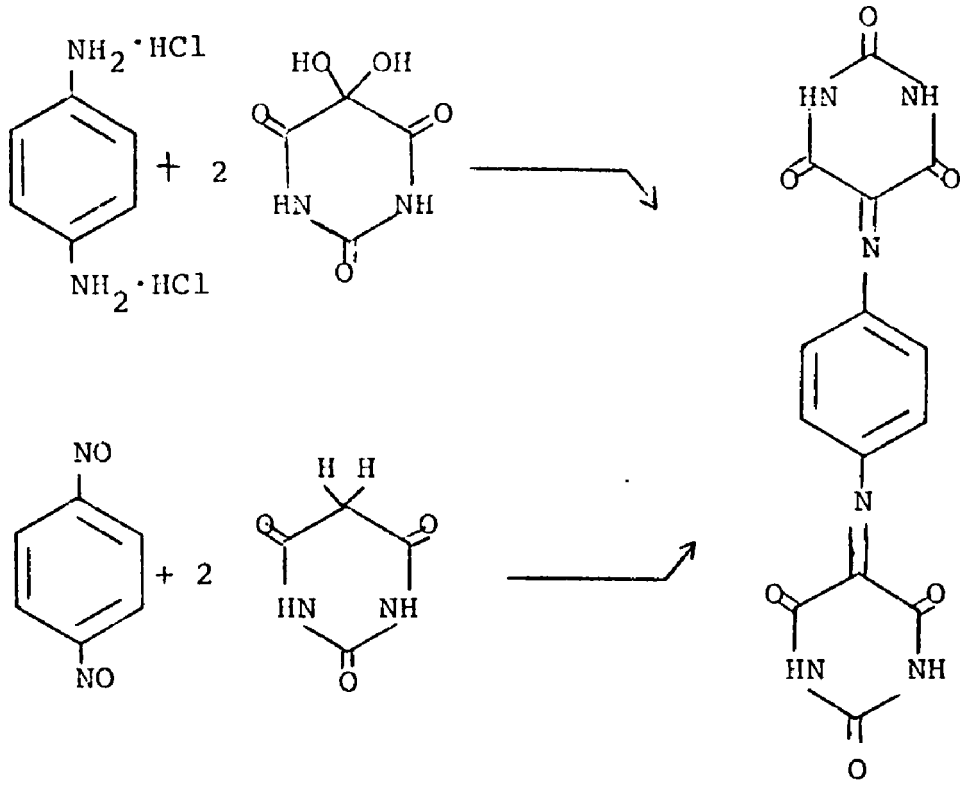
As noted earlier, alloxan reacts with the $-NH_2$ portion of an amino acid at the #5 carbon. The #5 carbon is the most susceptible site for nucleophilic attack because of activation by the two adjacent carbonyls. The monohydrate of alloxan is really covalent, 5,5-dihydroxybarbituric acid, as determined by X-ray crystal analysis,[66] just as the monohydrate of chloral exists as the dihydroxy material.

1. Aromatic Amines. Aromatic amines can react in several different ways with alloxan. The reaction can proceed to form a dark, almost black, anil. The anil

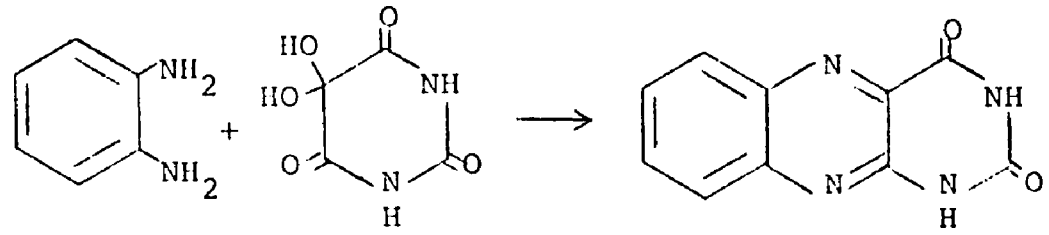


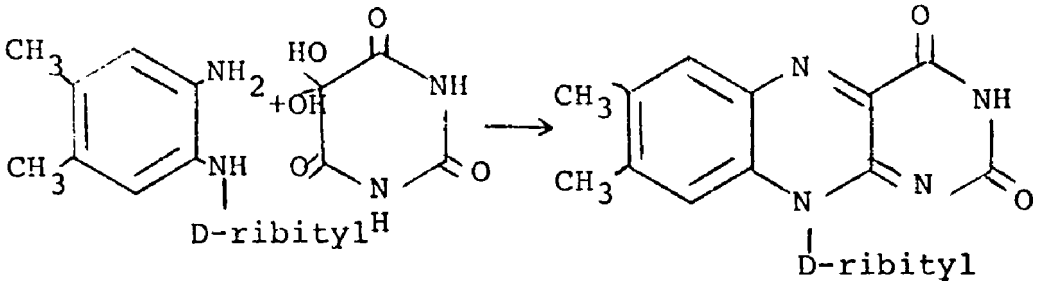
R	Reaction Conditions	Reference
Me_2N-	Δ, EtOH	67
HO-	Δ, EtOH	67
CH_3-	AcOH	68

formed from the hydrochloride salt of p-phenylenediamine[69] and alloxan has also been made from p-dinitrosobenzene and barbituric acid.[70]

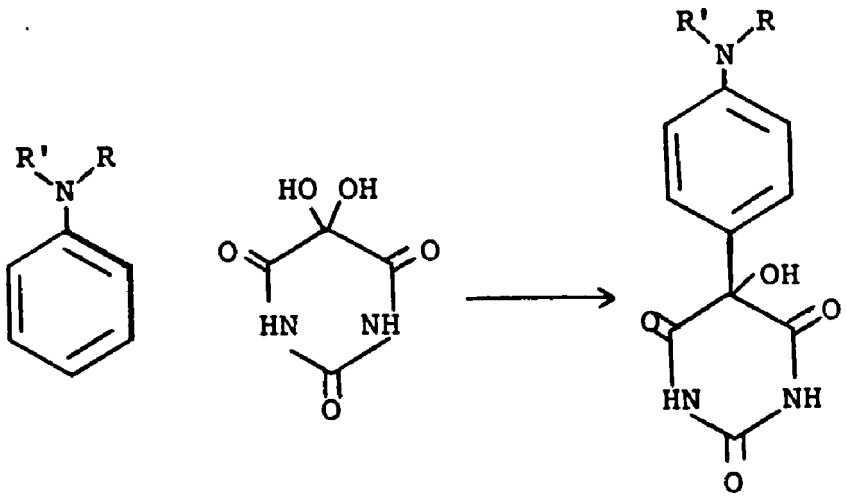


When o-phenylenediamines are condensed with alloxan under acidic conditions, alloxazines and isoalloxazines are formed. When the diamine has two methyl groups in the 4 and 5 position of the diamine ring and one of the amino groups is attached to the D-ribityl group, then the resulting isoalloxazine is riboflavin.



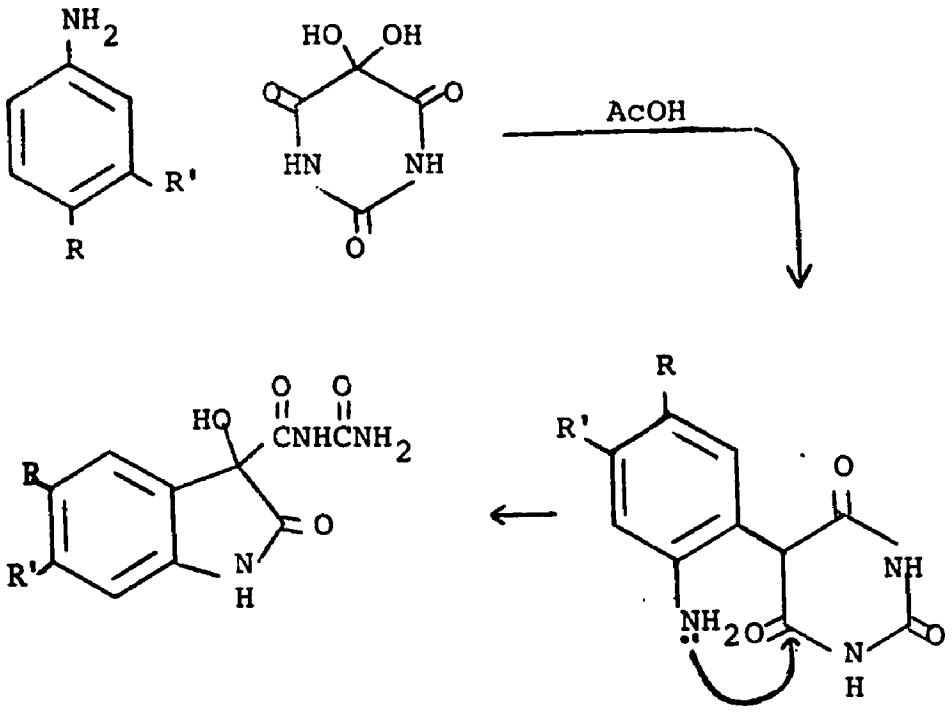


5-Aryldialuric acids are produced when alloxan reacts with aromatic amines which do not have a group <u>para</u> to the amino group.



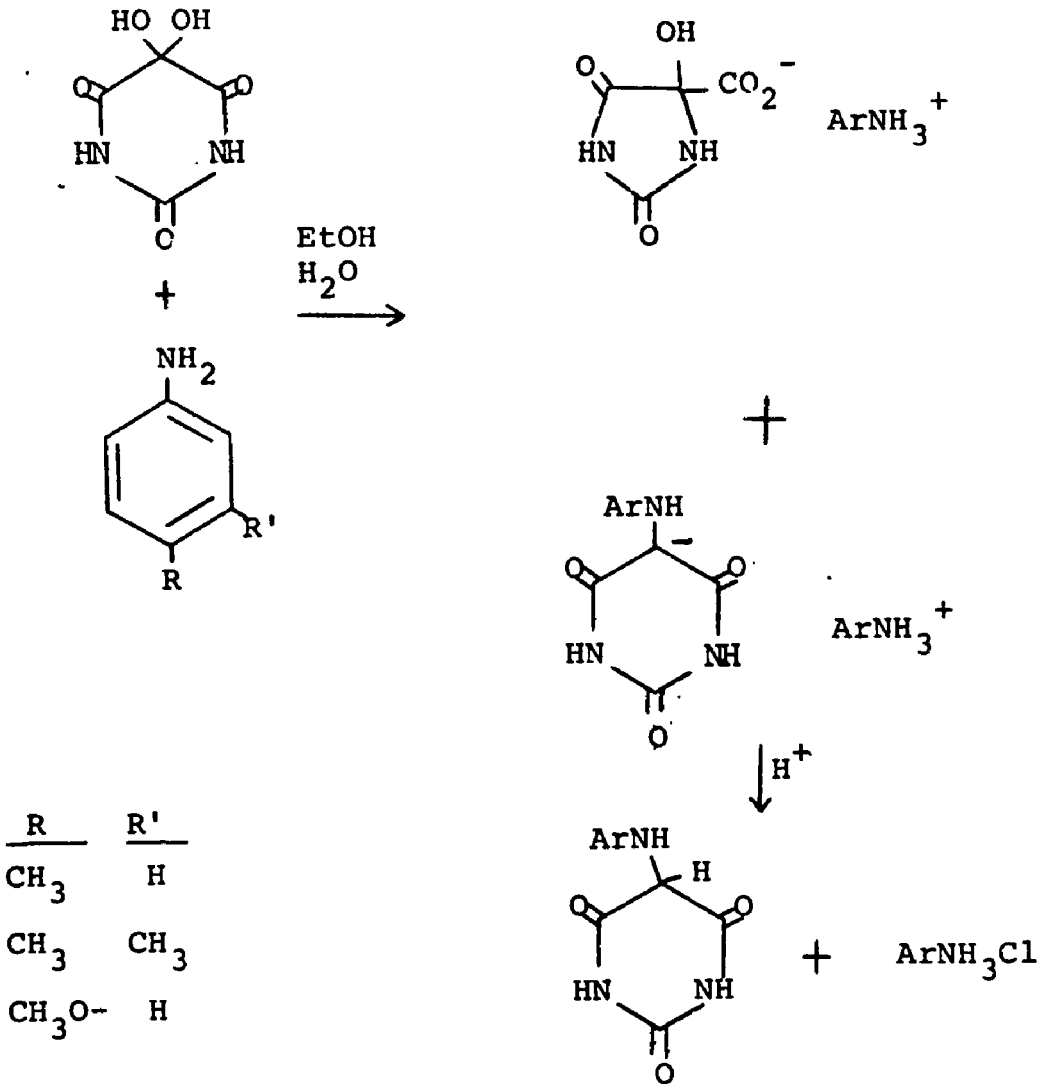
R	R'	Reference
H	H	71
CH_3	CH_3	71
CH_3CH_2-	CH_3CH_2-	72

However, when the <u>para</u> position is blocked and there are strong electron donating groups on the ring besides the unsubstituted amino group, then alloxan will electrophilically substitute at the position (if free) ortho to the amino group.[68] The 5-aryldialuric acid is an intermediate whose pyrimidine ring is opened by the aromatic amino group to form a dioxindole carboxyureide (see below).

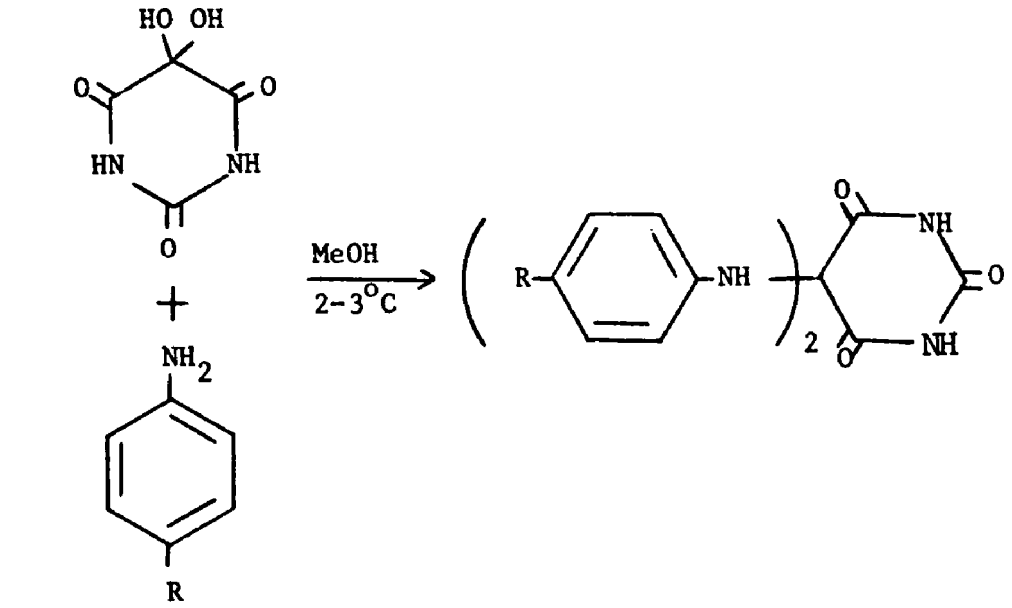


R	R'
CH_3	H
CH_3	CH_3
CH_3O-	CH_3O-

Recently it has been found that aromatic amines react with alloxan in ethanol-water solutions to form uramils and aromatic amine salts of alloxanic acid.[73] Less vigorous reaction conditions brought about the formation of 5,5-dianilinobarbituric acid.



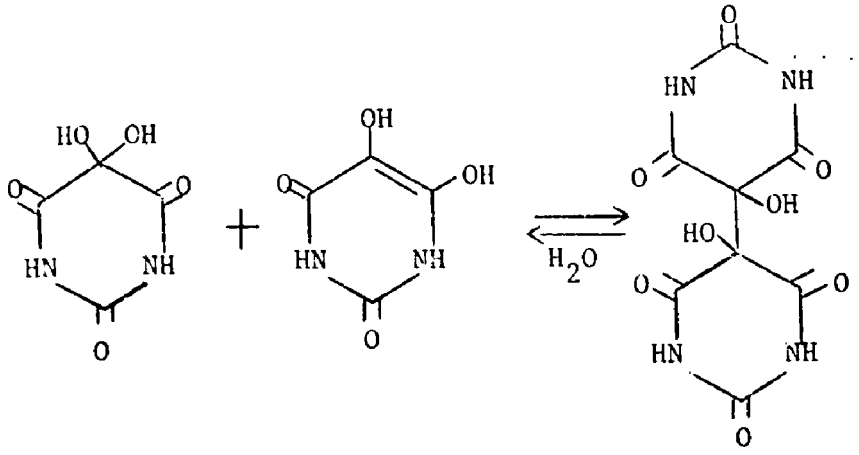
R	R'
CH_3	H
CH_3	CH_3
CH_3O-	H



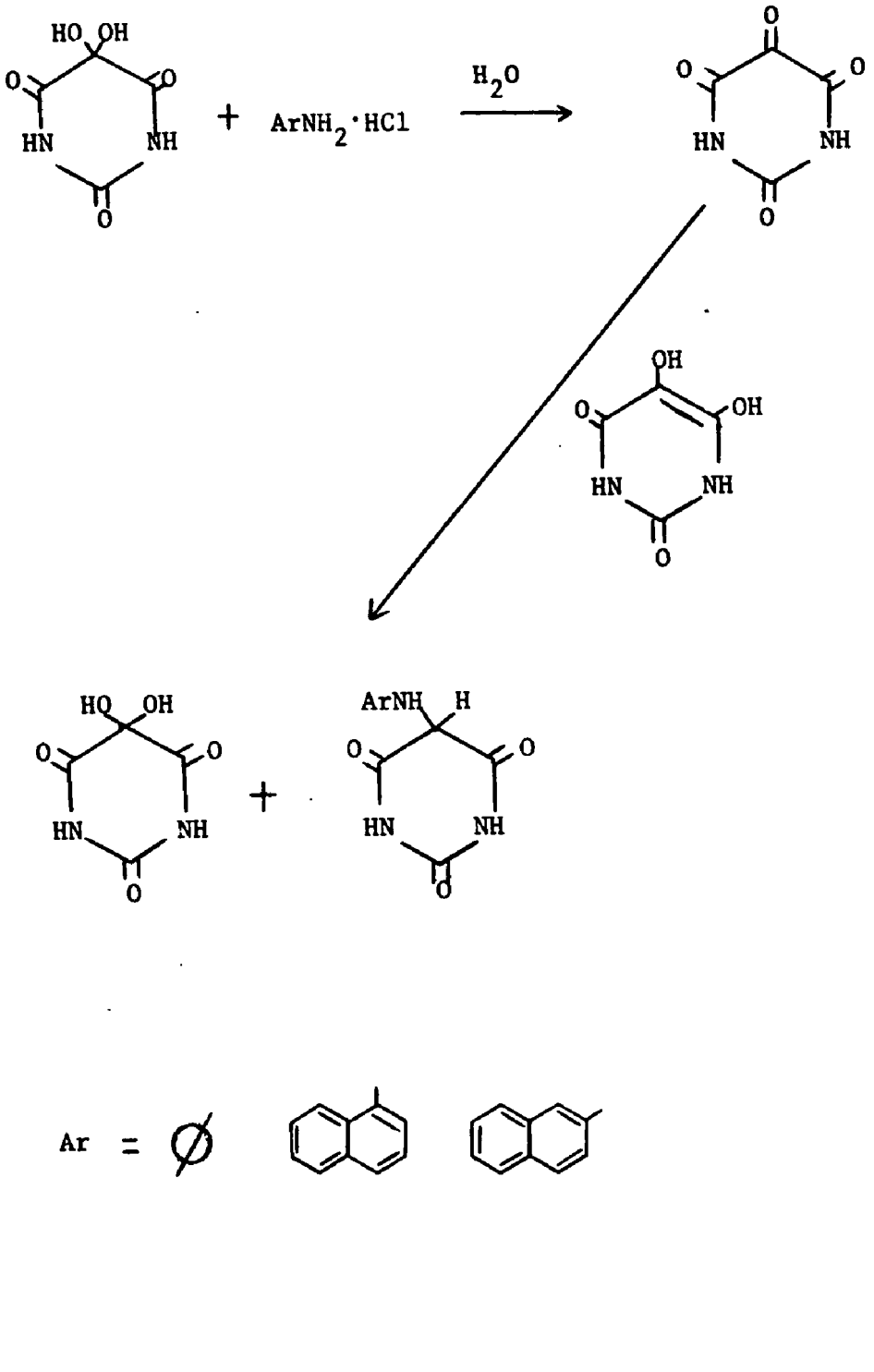
R = CH_3 or CH_3O-

The uramils were formed via a reduction of alloxan or the aromatic anil of alloxan with the ethanol in the system acting as the reducing agent. In the ethanol-water system it was possible to have the alloxan-alloxanic acid rearrangement take place because of the non-acidic (pH ≥ 5)[47] conditions.

Alloxantin is readily formed from alloxan and dialuric acid and in water an equilibrium exists among the components. When an aqueous solution of alloxantin

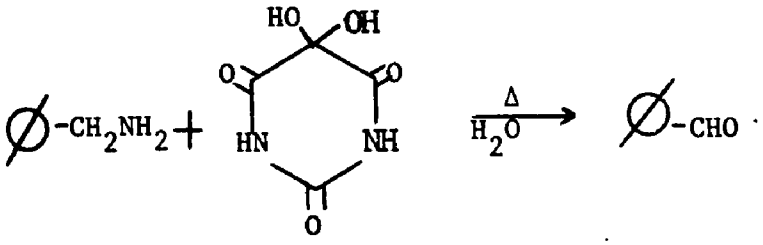


and an aromatic amine hydrochloride salt is refluxed, the product is the N-aryl uramil.[69] Initially, the reaction probably proceeds via the well-known dissociation of alloxantin to alloxan and dialuric acid, followed by condensation of alloxan with the aromatic amine to form the anil. This anil can then be reduced to the uramil by the dialuric acid and the dialuric acid is oxidized to alloxan.

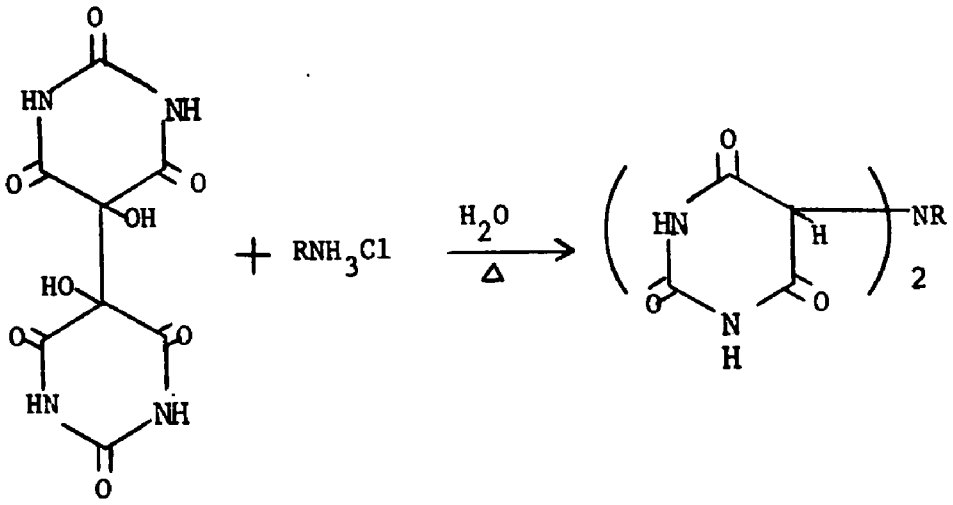

HO OH
O
O
HN
NH
O
+ ArNH2·HCl
H2O
O
O
HN
NH
O
OH
OH
O
HN
NH
O
HO OH
O
O
HN
NH
O
+
ArNH H
O
O
HN
NH
O

Ar = ∅

2. Aliphatic Amines. As mentioned before, alloxan will be converted to derivatives of purpuric acid in the presence of amino acids at the same time amino acids are degraded to aldehydes. Similarly, simple aliphatic primary amines can be oxidized to aldehydes.[74]

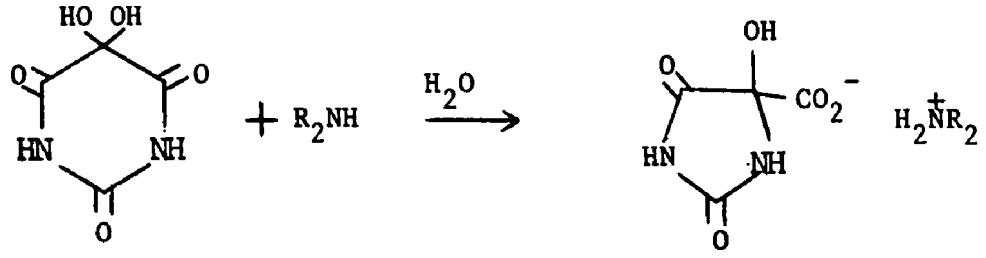


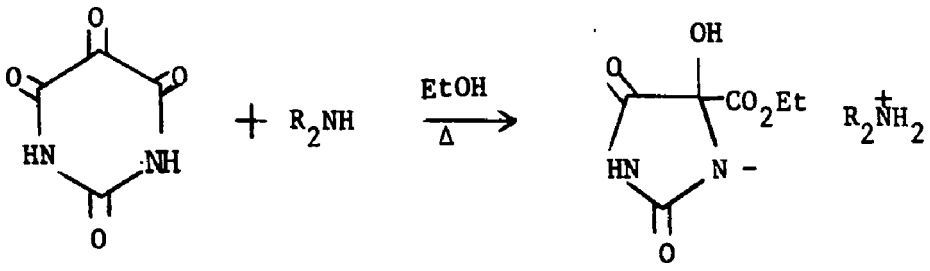
When methyl or ethyl amine hydrochloride and alloxantin are refluxed the dibarbituryl alkyl amine is formed.[69]



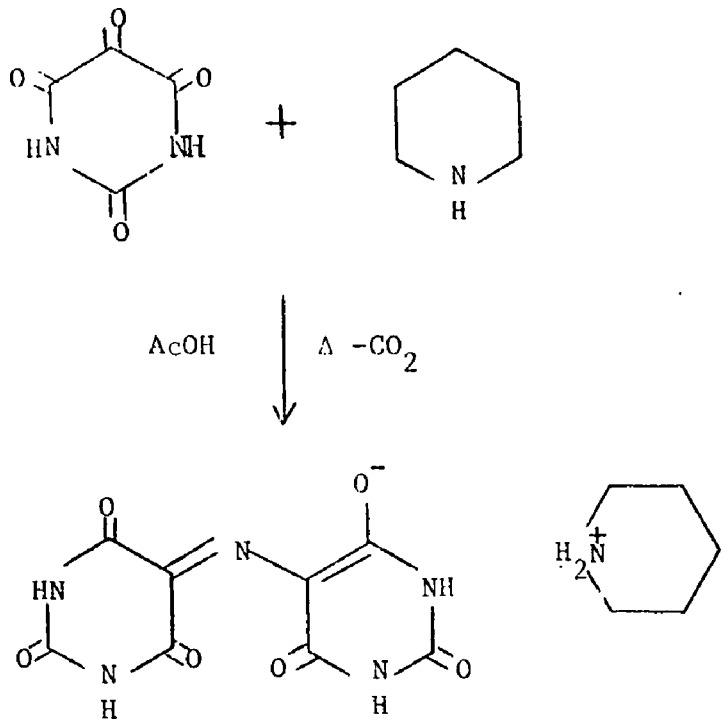
The reaction of secondary amines and alloxan in water produces the amine salts of alloxanic acid and in

absolute ethanol with anhydrous alloxan the amine salts of ethyl alloxanate are formed.[75] However, when anhy-

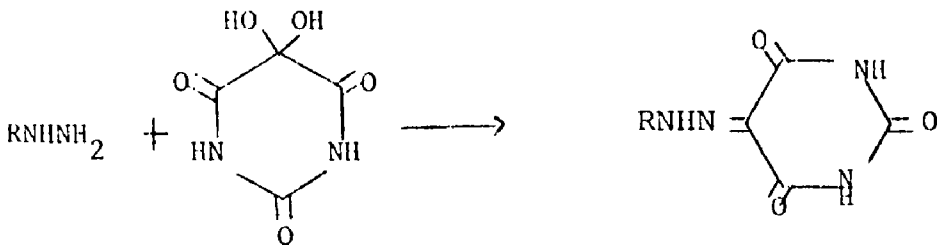




drous alloxan and alicylic amines are combined in dry acetic acid, the reaction proceeds with evolution of carbon dioxide to give the amine salts of purpuric acid.[76] It appears that alloxan decomposes in hot acetic acid to yield ammonia and CO_2 and that the liberated ammonia is the source of the nitrogen in the murexide analogues.[76]



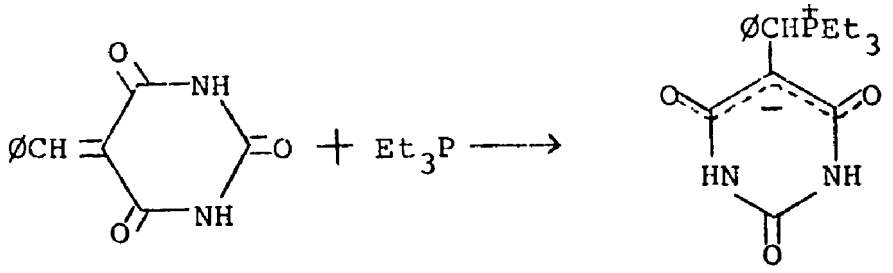
When alloxan and hydrazine are combined, alloxan is reduced to dialuric acid and hydrazine is oxidized to N_2.[78] Aromatic hydrazines form hydrazones[78] with alloxan and carboxylic acid hydrazides form carbazones.[79] At least in these reactions a typically ketonic behavior is observed for the C#5 carbonyl.



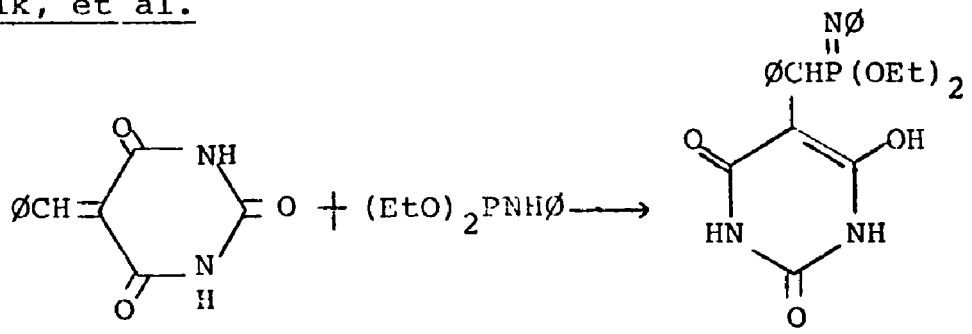
B. Phosphorus and Related Chemistry of Alloxan

In 1955 Leopold Horner[80] synthesized an adduct of 5-benzylidenebarbituric acid and triethylphosphine. Since then two groups have obtained adducts with 5-arylidenebarbituric acid and trivalent phosphorus derivative. Several examples are shown below:

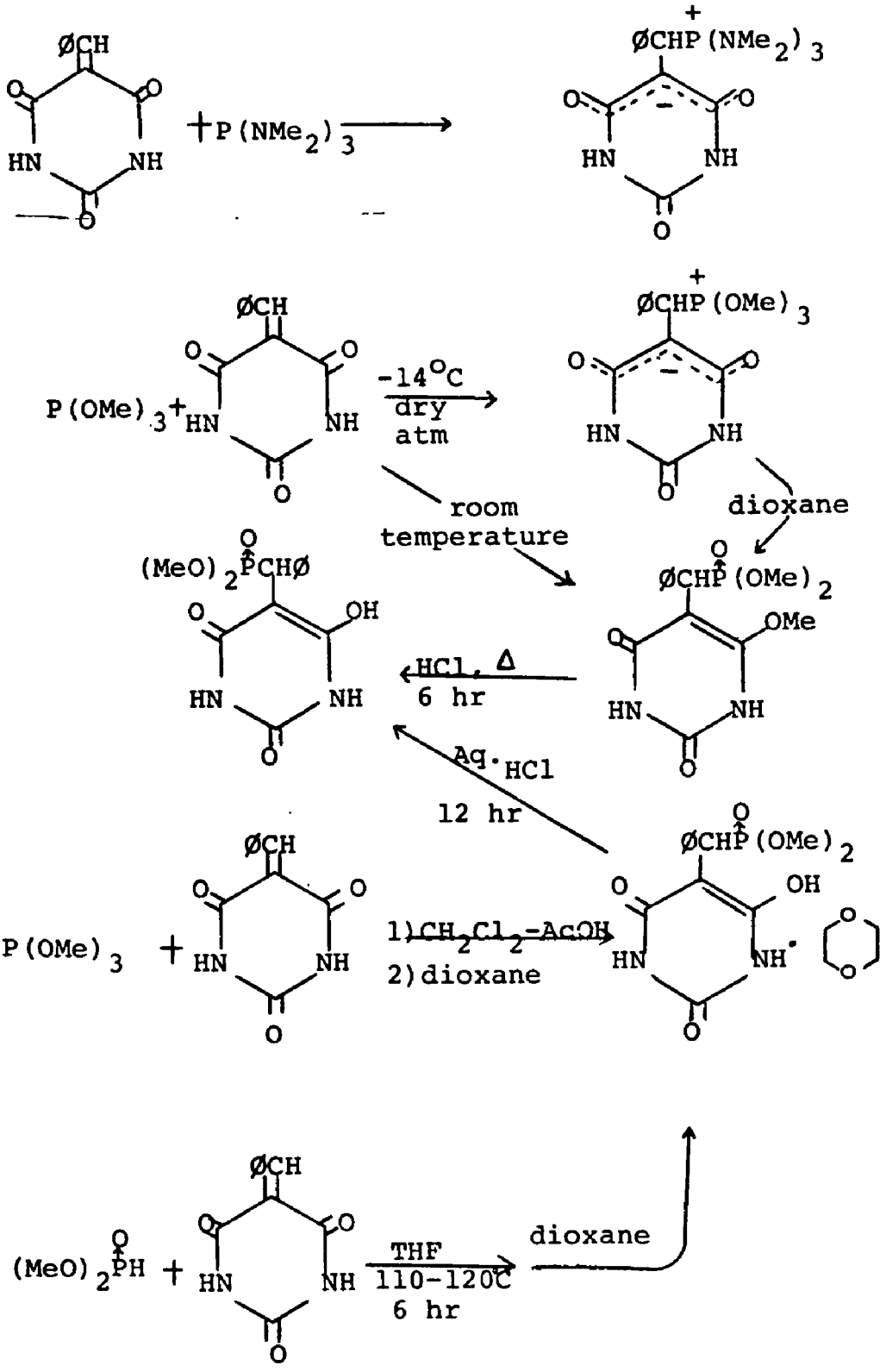
Horner[80]



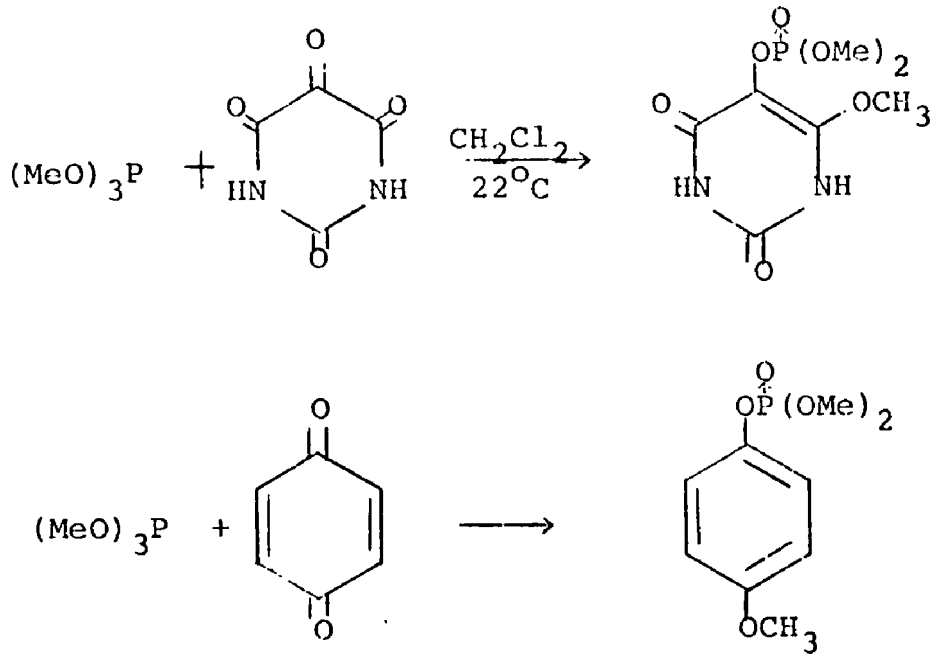
Pudovik, et al.[81]



Arbuzov, et al.[82]

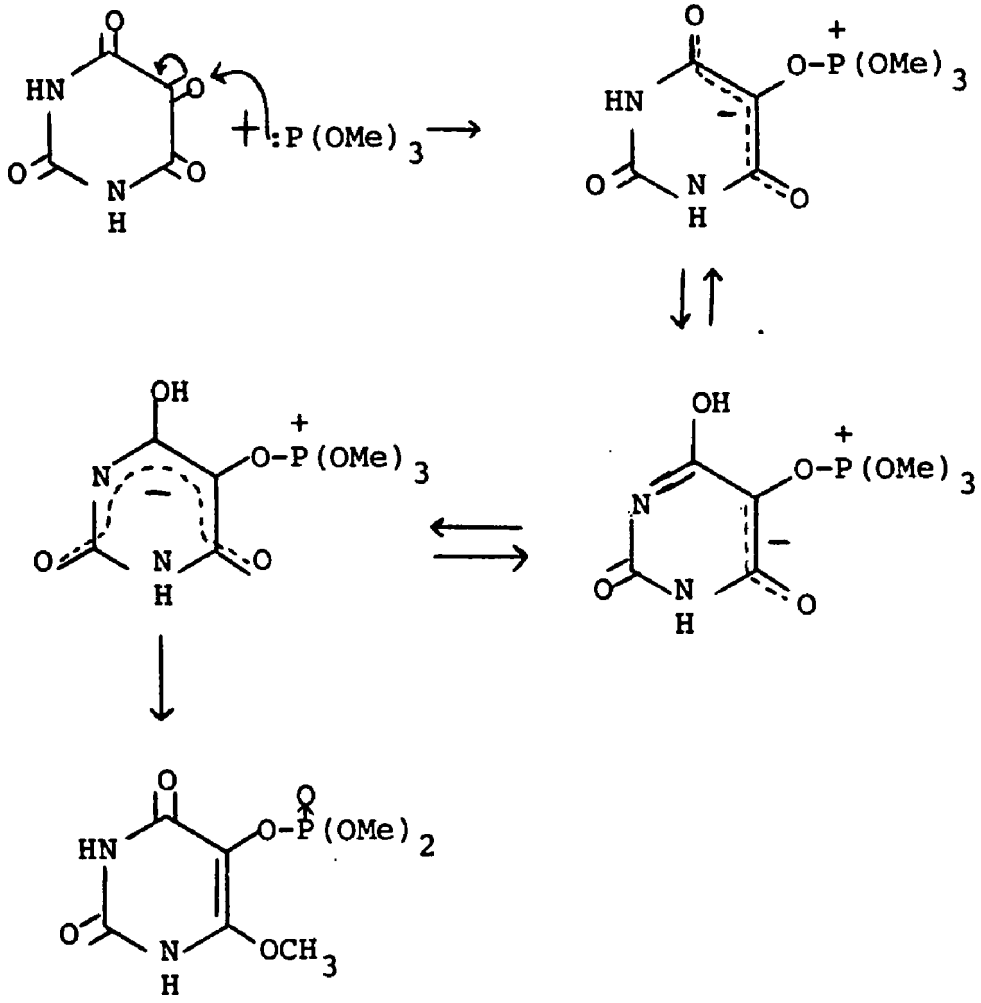


Ramirez and coworkers[83] have elucidated the products obtained from alloxan and trimethyl phosphite. The reaction involved a very similar rearrangement of a methyl group from the trimethyl phosphite to its coreactants as occurred in Arbuzev's work and when p-benzoquinone and trimethyl phosphite were condensed.[84]

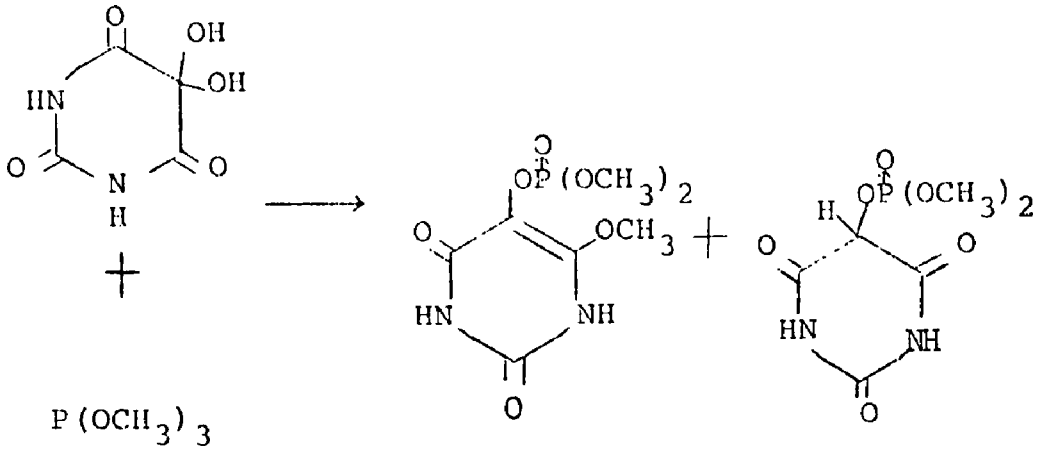


The 5-hydroxy-6-methoxyuracil dimethyl phosphate was treated with triethylamine and diazomethane to give the symmetrical 5-hydroxy-2,4,6-trimethoxypyrimidine-5-dimethyl phosphate which was proved by its ^{1}H nmr spectrum. The mechanism proposed by Ramirez[83] involves

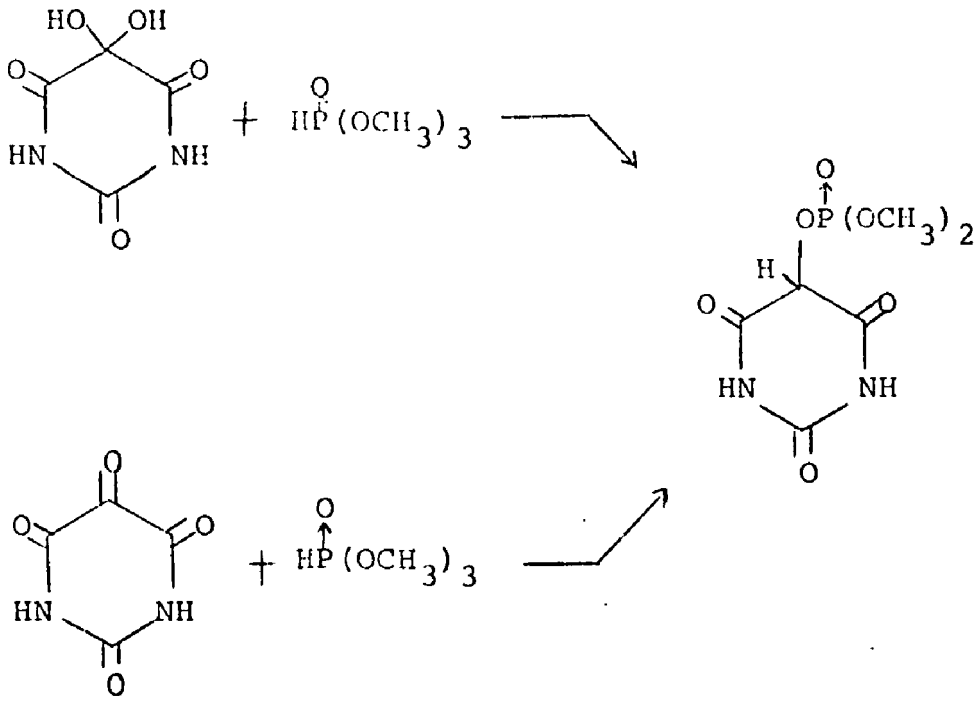
the phosphorus attacking the #5 carbonyl oxygen atom.



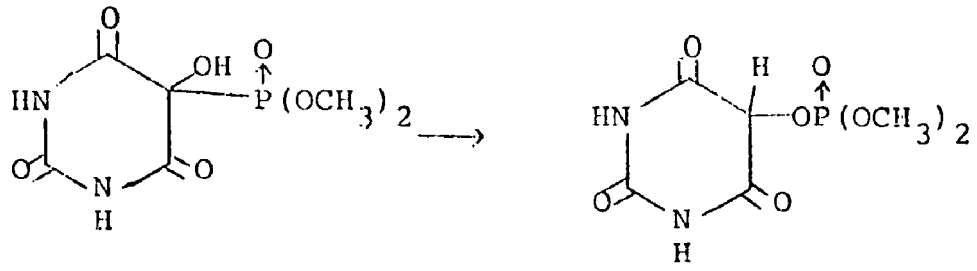
When alloxan menohydrate was used instead of the anhydrous form of alloxan, the same product was formed, as from alloxan anhydride, but an additional product was formed, 5-hydroxybarbituric acid 5-dimethylphosphate.



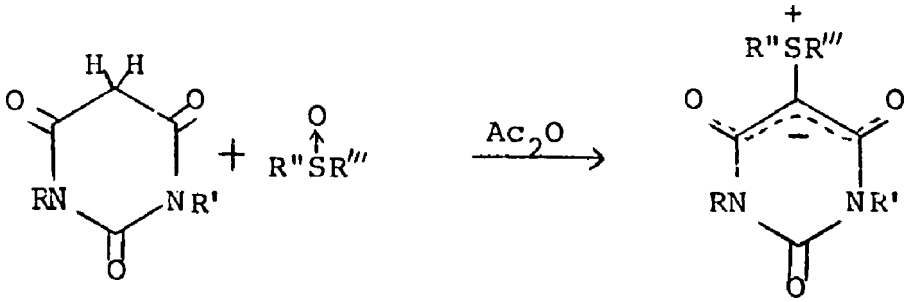
This new product probably arises from the hydrolysis of the $-O-\overset{+}{P}(OCH_3)_3$ group during the reaction. The alloxan monohydrate was converted to the 5-hydroxybarbituric acid 5-dimethylphosphate in 77% yield when dimethylphosphite was used instead of trimethylphosphite. Similarly, alloxan anhydride was converted to the same



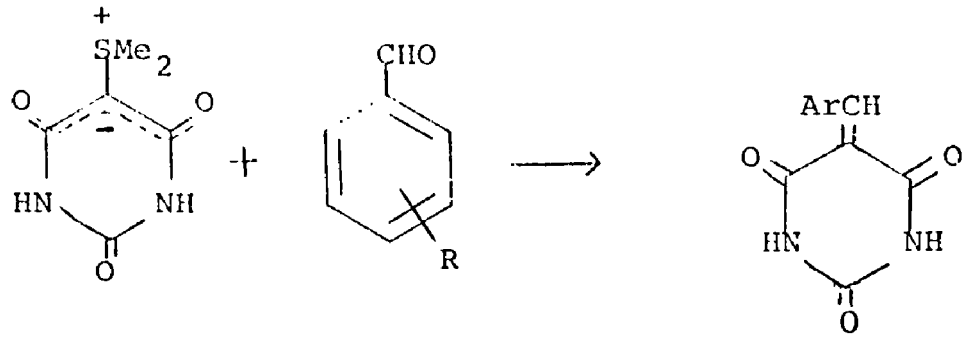
dimethylphosphate derivative when it was treated with dimethylphosphite. It has been suggested that the two reactions proceed through a common alpha-hydroxy phosphonate intermediate.[83] This intermediate could then undergo isomerization to the phosphate.



Gompper and Euchner[85] prepared several analogues of a sulfur ylid at the #5 position of barbituric acid. They used barbituric acid, the N-methyl, and N,N'-dimethylbarbituric acids in their condensation with sulfoxides in acetic anhydride. The sulfoxides they employed were dimethyl, diethyl, methylethyl, and tetramethylene sulfoxide. No nmr or ir data were reported to confirm product structures. Ivin, et al.[86], reported the formation of similar ylids.

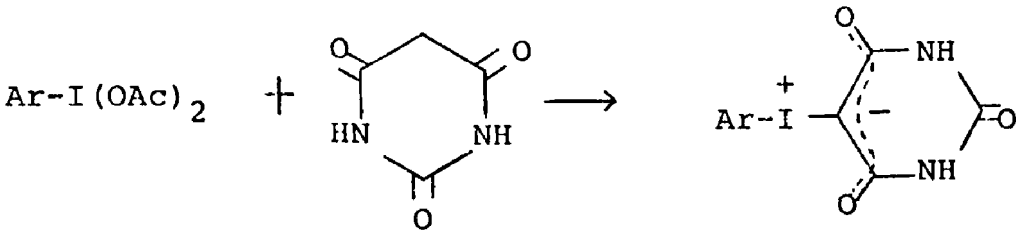


Vladzimirskaya, et al.,[87] reported the formation of 5-arylidenebarbituric acid from the sulfur ylid of barbituric acid and an aromatic aldehyde.

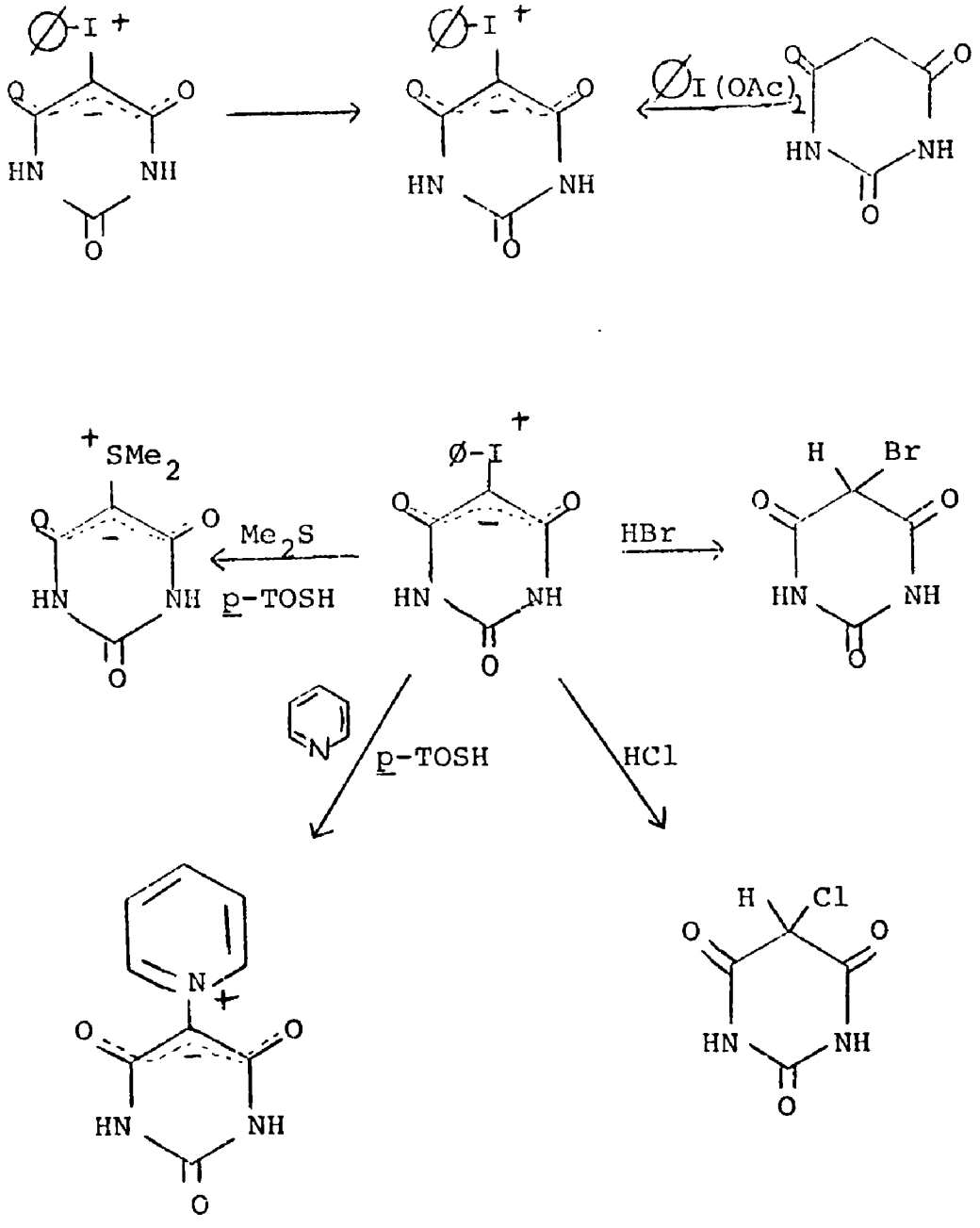


R = p-Me_2N-, p-Et_2N-, o-O_2N-

Neilands and Neimanis[88] prepared 5-iodonium barbiturates from phenyliodo diacetate or p-tolyliodo diacetate and barbituric acid. They found that methylation



with dimethylsulfate gave the same dimethyl product as the reaction of the iodo diacetate materials and 1,3-dimethyl barbituric acid. The aryl iodides were displaced with various nucleophiles.



The 5-N-pyridinium barbiturate synthesized by this displacement route had previously been prepared by Taylor and co-workers[89] from 5,5-dibromobarbituric acid, 5,5-dichlorobarbituric acid, and 5-chloro-5-nitrobarbituric acid in condensation with pyridine.

RESULTS AND DISCUSSION

The aim of this project was to alter the alloxan molecule in such a way so that the derivative remained diabetogenic and acquired the ability to be labeled with a radioisotope. In order to retain the diabetogenicity attributed to alloxan, the derivatives must have the pyrimidine ring with the ureide group and the #5 carbon in the oxidation state of a carbonyl. Alternatively, the derivative must be readily hydrolyzed or oxidized so that the #5 carbon attains the oxidation state of a carbonyl. Several of the materials, which will be discussed later, are undergoing testing at Hahnemann Medical College. The initial test involves determining the blood insulin changes in dosed animals by a radioimmunoassay. If any of these candidate radiopharmaceuticals are considered promising after the insulin radioimmunoassays they will be labeled with a radioisotope and animal distribution studies will be performed for their efficacy in localizing pancreatic tumors.

The first section deals with the preparation of derivatives from alloxan and aromatic amines. The intent of this investigation was to obtain anils of alloxan which could presumably be radiolabeled by electrophilic iodination on the aryl ring. The radiolabeling,

as was previously indicated, will only be performed on those anils which still retain a physiological action on the pancreas. Literature precedent from prior pharmacological studies is ambiguous as to whether one should expect diabetogenic behavior in such anils. Furthermore, as will be indicated herein, not every aromatic amine yields an alloxan anil. Under some reaction conditions appropriately substituted anilines gave dialuric acids instead of anils.

Obviously, addition of a bulky anil substituent at C-5 may be expected to alter the alloxan-like biological behavior. However, anils do retain the essential features required for diabetogenic activity: intact alloxan ring, C-5 at carbonyl oxidation state. The essential question of whether a C=N behaves physiologically like a C=O cannot be answered from literature precedent. No appropriate models have been studied.

The oxime of alloxan (violuric acid, as it is commonly called) is non-diabetogenic. However, this molecule does not possess a true C=N linkage and is a tautomeric situation between a nitroso form and an oximino form. Animal experiments to be performed on the products of this synthetic study will provide considerable help in understanding structure-activity relationships in diabetes induction by alloxan analogs.

The second section deals with the triarylphosphine alloxan adducts and derivatives prepared from them. Since it was reported that some triarylphosphoranes and the corresponding phosphonium salts (prepared from triarylphosphines and substituted phenacyl halides) had hypoglycemic activity,[90] it seemed appropriate to combine the effects of alloxan and triarylphosphines by forming the triarylphosphine adducts. The adducts, though speculation of combining the effects of alloxan and the triphenylphosphine in one molecule may seem impressive, might have the same limitations as the anils. The bulkiness of the triarylphosphine group may limit the alloxan-like diabetogenic action and the pyrimidyl portion of the molecule may limit the effectiveness inherent in the derivatives of triarylphosphine which cause hypoglycemic reactions.

I. AROMATIC AMINES AND ALLOXAN

Clark-Lewis and Edgar prepared one alloxan anil from p-toluidine and alloxan anhydride in glacial acid.[68] Apparently they believed that alloxan had to have a free carbonyl in the #5 position in order for the anil to form. Biltz was the first to prepare alloxan anhydride by sublimation of the monohydrate at reduced pressure and at a temperature of around 200-220°C and to study the compound.[91] Biltz observed that alloxan anhydride was a yellow material and that alloxan monohydrate developed a yellow coloration upon dissolution in glacial acetic acid. He concluded that dehydration of the mono-hydrate may be taking place. He then tried to crystallize alloxan anhydride out of glacial acetic acid, and, indeed, he did form a mixture of alloxan anhydride and alloxan monohydrate.

The anils prepared for this study were synthesized from alloxan monohydrate and substituted anilines in glacial acetic acid. No attempt was made to dry the commercial glacial acetic acid. Thus, the reaction mixtures definitely contained water but this did not interfere with the formation of the anils.

Since alloxan monohydrate can be a precursor for anils and since solutions of alloxan monohydrate in glacial acetic acid are yellow (presumably due to

equilibrium formation of free alloxan) then what is (or are) the reactive alloxan specie(s) in glacial acetic acid? Mechanistically, anils can be rationalized from either the mono-hydrate or the anhydrous alloxan. Figure 1 graphically illustrates the ultraviolet spectra of alloxan anhydride (prepared by the method of Biltz), alloxan monohydrate and 5-ethoxy-5-hydroxybarbituric acid. It was hoped the 5-ethoxy-5-hydroxybarbituric acid would be unaffected by the glacial acetic acid and thus give an ultraviolet spectrum very similar to that of alloxan monohydrate and dissimilar to that of alloxan anhydride. Unfortunately, the spectra are all so similar that it cannot be deduced whether there is only one species or two species present in glacial acetic acid.

The uv of alloxan monohydrate and the anhydride should theoretically be dissimilar since the anhydride has a carbonyl where the monohydrate has two hydroxy groups. Inspection of figure 2 reveals the differences in spectra of the two forms of alloxan in absolute ethanol. Since 1) the molar extinction coefficients of the two forms of alloxan in glacial acetic acid and of alloxan anhydride in ethanol are very similar, 2) the spectrum of 5-ethoxy-5-hydroxybarbituric acid indicates that it forms a very similar species in glacial acetic acid to the two forms of alloxan and 3) alloxan itself produces

Figure 1. UV Spectra of Alloxan Monohydrate, Alloxan Anhydride and 5-Ethoxy-5-hydroxy Barbituric Acid in AcOH

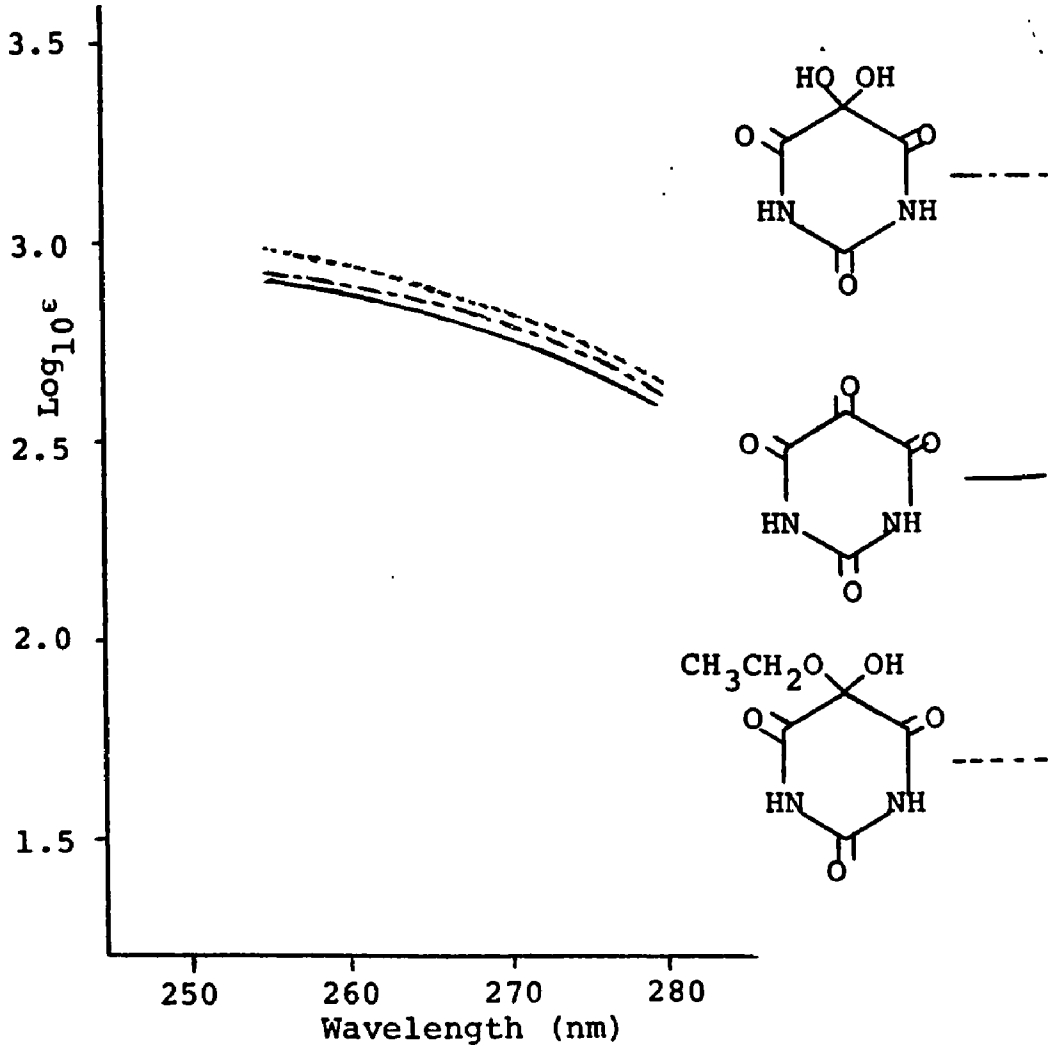
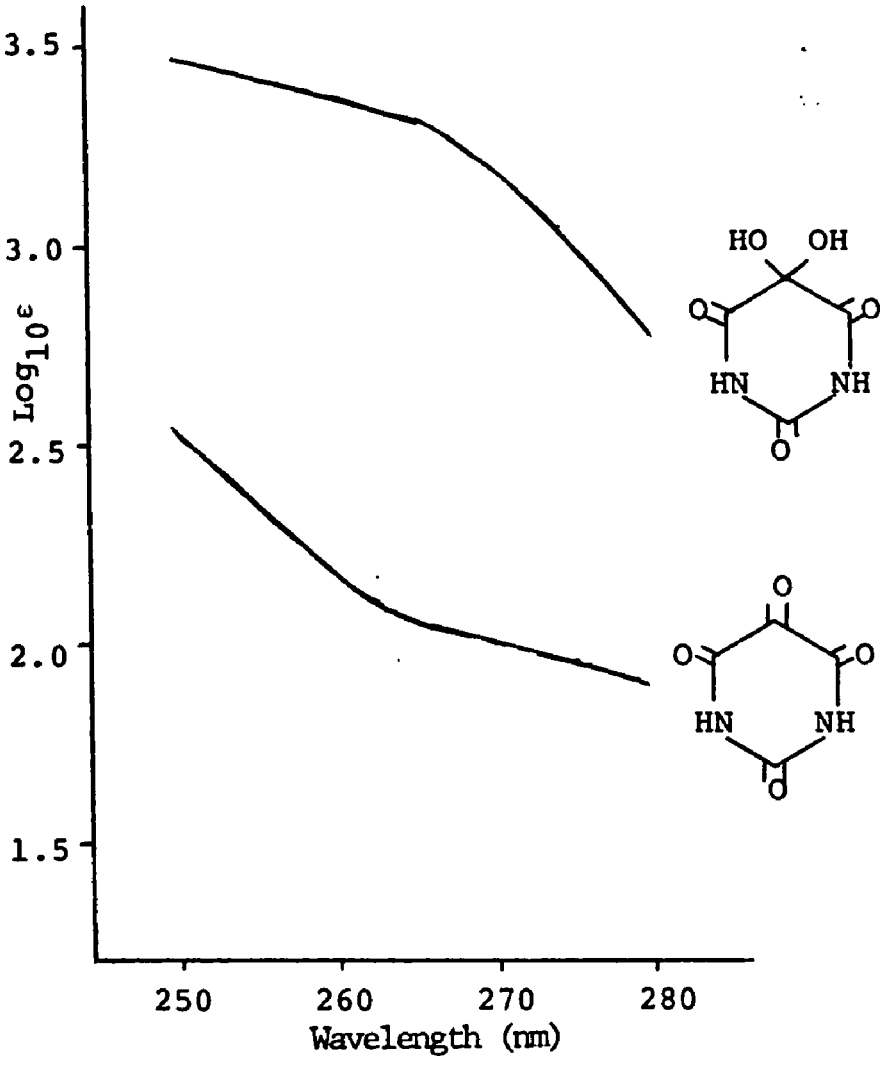
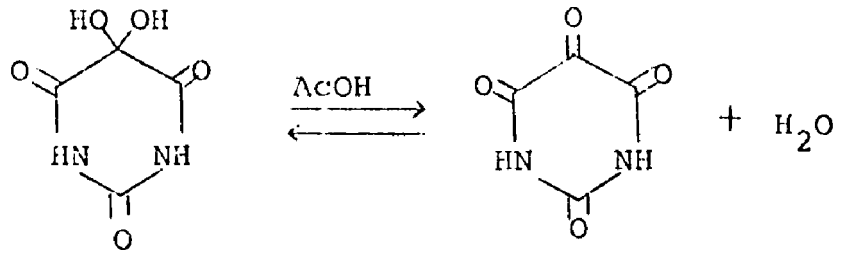


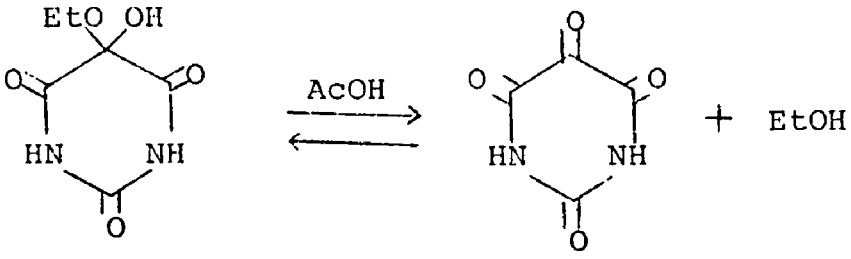
Figure 2. UV Spectra of Alloxan Monohydrate and Alloxan Anhydride in EtOH



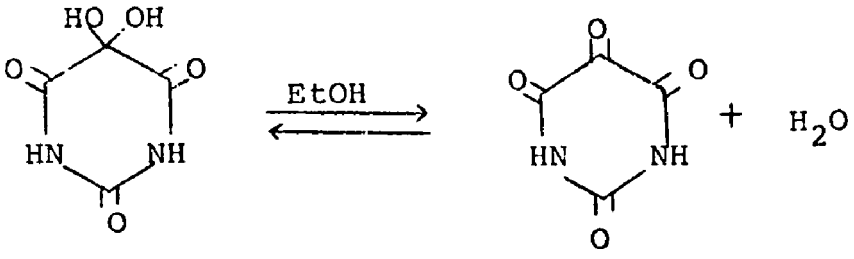
a yellow solution in glacial acetic acid, it can be concluded that there is definitely an equilibrium between the covalently solvated and anhydrous forms:

Scheme 1





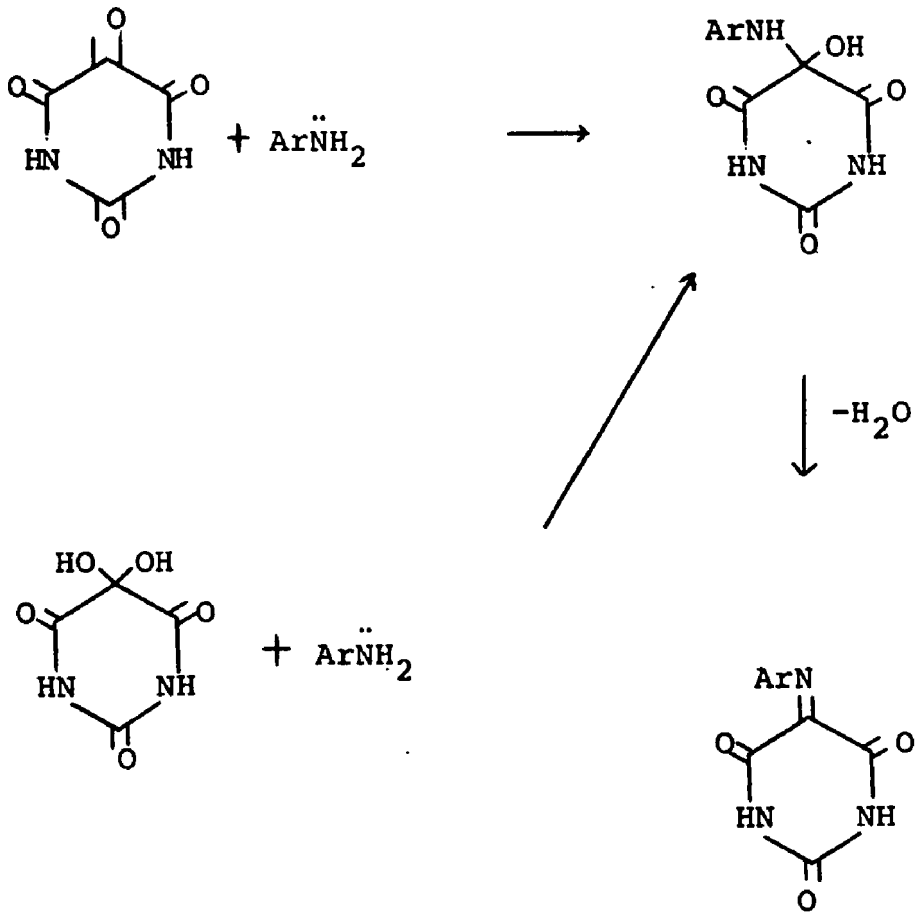
Very slow:



From a mechanism viewpoint, alloxan monohydrate and alloxan anhydride might both be expected to react with an amine to form the aminol before dehydration to the anil. Normally, hydroxyl group displacement by an aromatic amine is not facile but in alloxan the C-5 carbon is highly susceptible to nucleophilic attack. The electropositive #5 carbon has carbons with partial positive charges adjacent to it, thus creating the susceptibility of the #5 carbon toward nucleophilic attack.

The reactive carbonyl of alloxan anhydride will readily accept the addition of the amine across the C-O double bond to form the aminol. Clark-Lewis and Edgar[68] did indeed isolate such an aminol from alloxan anhydride and 3,4-dimethylaniline in diglyme. To prevent the formation of the anil through dehydration, they ran the reaction at room temperature and excluded all dehydrating agents such as acids. Scheme 2 graphically illustrates the mechanism for formation of the anil from the monohydrate and the anhydride. The question of which one of the two species, the monohydrate or the anhydride, is reacting may not be answerable without a detailed kinetic study.

Scheme 2



Ar = p-BrC_6H_4, p-CH_3-C_6H_4 p-HOC_6H_4, 2,4-diClC_6H_3

Glacial acetic acid appeared to be an unsuccessful catalyst in the formation of the anils with strong electron donating groups such as alkoxy, so boric acid in DMF was used as an alternative method. Boric acid has been used in several instances in the condensation of ortho phenylene diamines and alloxan to form alloxazines isoalloxazines.[92,93] It has also acted as a catalyst in the reduction of alloxan to dialuric acid[18] and in the alloxan-alloxanic rearrangement.[49] By using the boric acid-DMF system there are two possible reaction paths; one leads to the anil and the other to the alloxanic salt of the aromatic amine.

The use of the boric acid-DMF system lead to only one isolable product, p-bromophenylimino barbituric acid, whose elemental analysis indicated that there was a mole of water in the crystal structure. Of the four anils prepared in glacial acetic acid, the p-bromophenyl and the p-hydroxyphenyl had quarter hydrates. Since Wöhler and Liebig prepared alloxan (as the tetrahydrate) there have been many derivatives made from alloxan whose combustion analyses included molecules of water and/or solvent[68,73,75,76,89] and which had analyses for C, H or

N out of the ±.4 range.[68,73,76,89] X-ray crystal studies[94] showed that the monohydrate of alloxan was in the dihydroxy form at the #5 carbon and the real name of the material is not the misnomer, alloxan monohydrate, but 5,5-dihydroxybarbituric acid. A visual illustration of the difference in structure of covalent water and hydrates are the aminol prepared by Clark-Lewis and Edgar[68] and the 5-alkoxy-5-hydroxybarbituric acids prepared by Biltz[91] are colorless compared to the anils which are deeply colored and alloxan anhydride which is yellow. X-ray crystal studies of alloxantin dihydrate,[95] dialuric acid monohydrate[95] and barbituric acid dihydrate[97] showed that the waters of hydration are hydrogen bonded between the layers of the pyrimidine rings throughout the crystal structure. During this study the appropriate distinction will be made between the covalently bound and the hydrated forms.

The alloxan derivatives prepared from 2,5-diethoxy- and 2,5-dimethoxyaniline are 5-aryldialuric acids and the derivative prepared from 3,4-dimethylaniline is the dioxindolecarboxyureide. These derivatives formed preferentially over the intended anils, despite the bulkiness of the alkoxy groups ortho to the site of substitution of the dialkoxyanilines and the attempted shortened reaction time for the dimethylaniline. Several other previously prepared dialuric acids were made to compare the dialuric acids.

The hydroxyl protons of the dialkoxyaryldialuric acids absorbed downfield from the protons of the other aryl dialuric acids. The downfield shift is

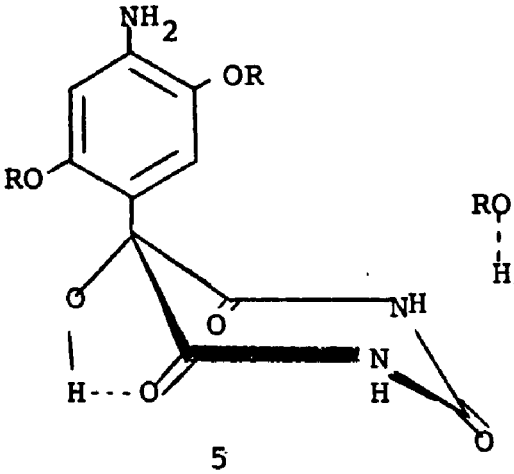
Table 1

Shift Data of the 5-Hydroxyl Proton of the Dialuric Acids

Dialuric Acid	Absorption of the 5-OH Proton (ppm)
5-(2,5-diethoxy-4-aminophenyl)	7.07
5-(2,5-dimethoxy-4-aminophenyl)	7.20
5-(p-anilino)	6.35
5-(4-amino-3-methoxyphenyl)	6.48
5-(4-dimethylaminophenyl)	6.39
5-(4-diethylaminophenyl)	6.31

probably due to the increased amount of intramolecular hydrogen bonding possible in the dialkoxyphenyldialuric

acids, (5) and (6). All of the hydroxyls of the 5-aryl-dialuric acids have intramolecular hydrogen bonding with an alpha carbonyl on the pyrimidine ring (positions #4 and #6). However, the 5-(2,5-dialkoxy-4-aminophenyl) dialuric acids have an alkoxy oxygen in close proximity to the hydroxyl proton available for intramolecular hydrogen bonding. The increased hydrogen bonding shifted the signal of the 5-(dialkoxy-4-aminophenyl) dialuric acid hydroxyl protons downfield relative to the absorptions observed by the hydroxyl protons of other dialuric acids.



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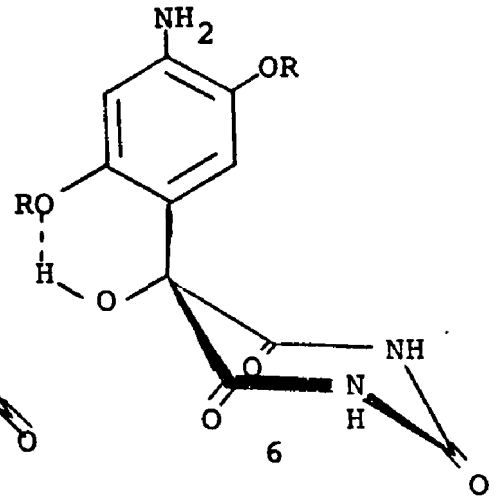

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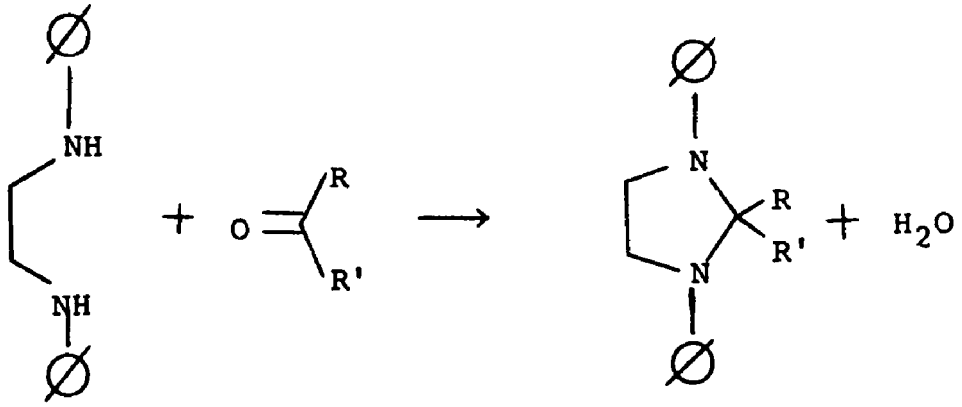
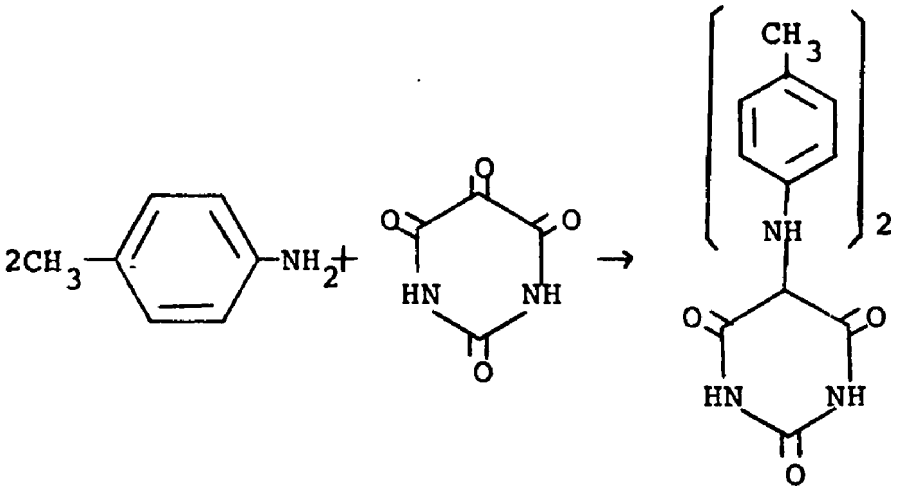
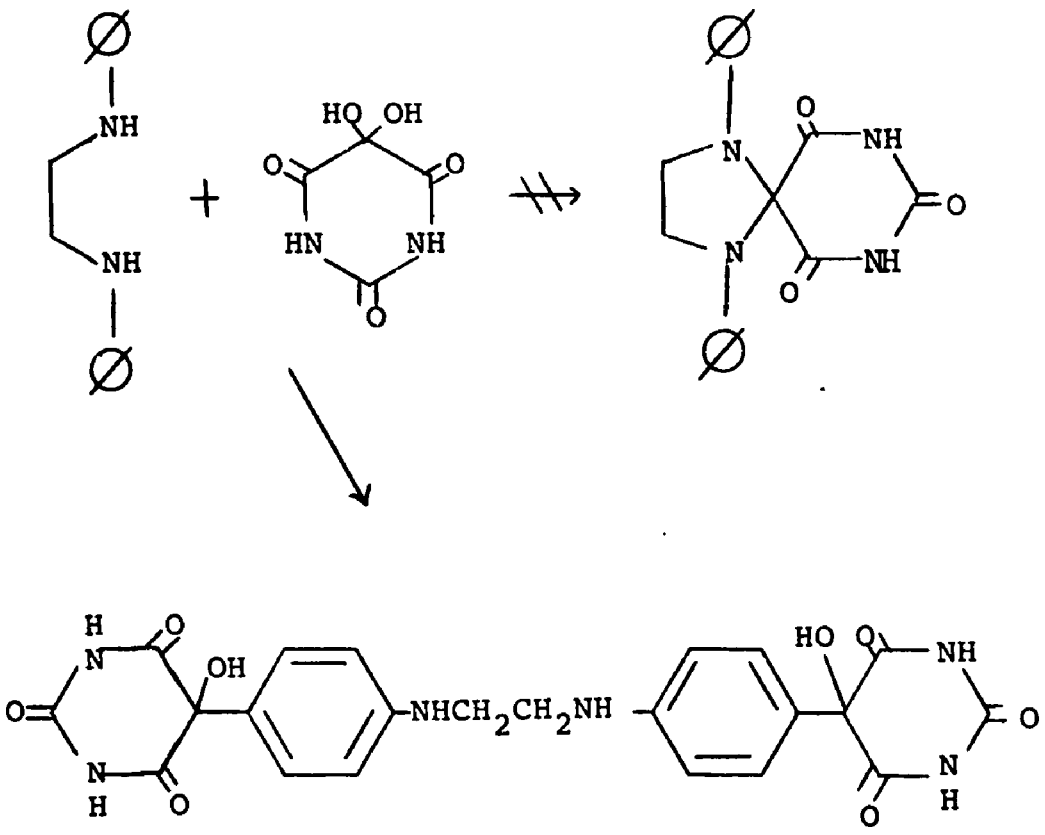
An attempt was made to prepare a spiro-alloxan derivative by use of Wanzlick's reagent for aldehydes.[98] This reagent (see below, also known as 1,2-dianilino-



ethane) reacts with carbonyls to form cyclic derivatives. This derivative would be very similar to the derivative formed from p-toluidine and alloxan in cold methanol described by Clark-Lewis, et al,[73] as 5,5-di-p-tolylamino barbituric acid. In this case, however, condensation with alloxan yielded a bis-dialuric acid (see Scheme 3).



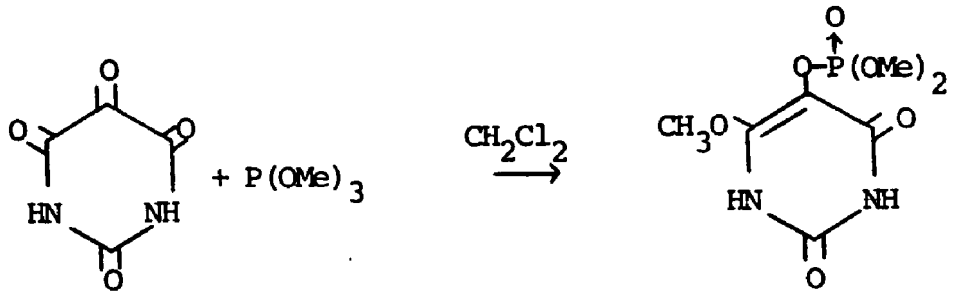
Scheme 3

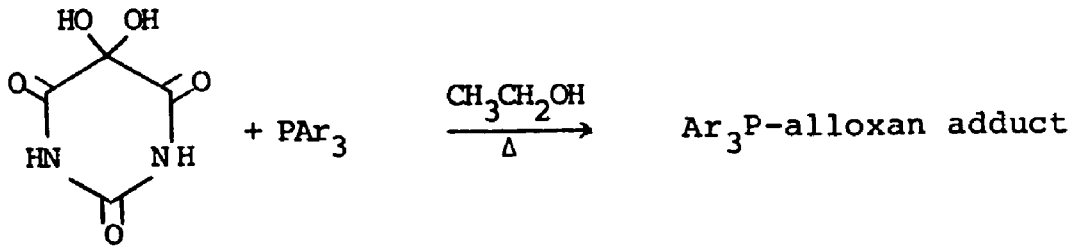


II. PHOSPHORUS DERIVATIVES OF ALLOXAN

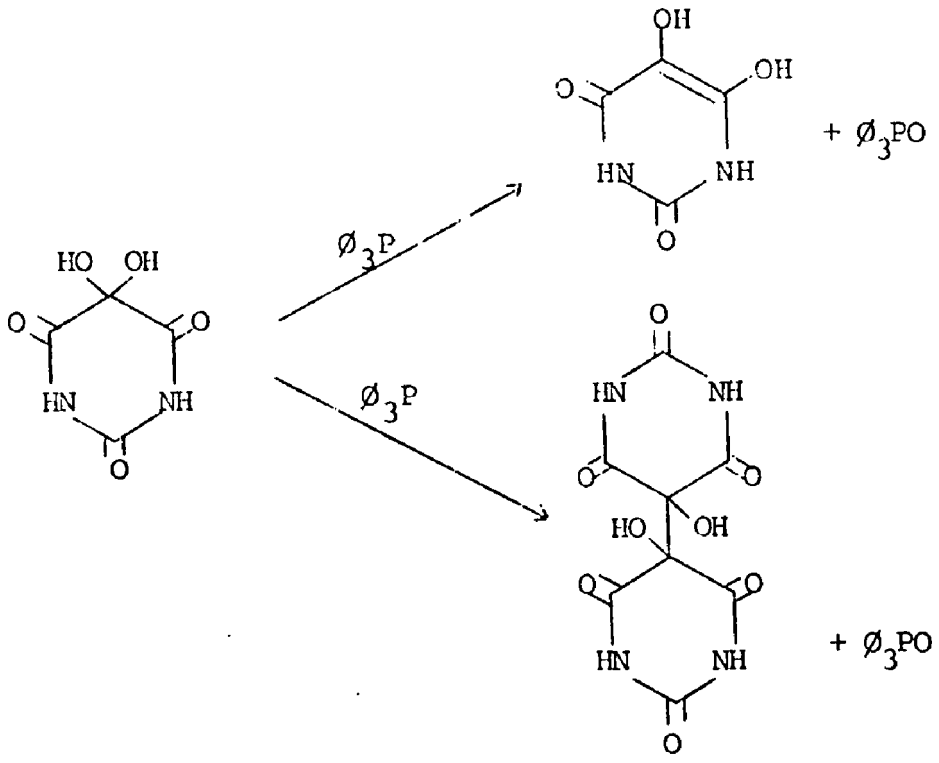
A. Preparation and Structure of the Phosphorus Adducts.

Unlike the anhydrous conditions which Ramirez and coworkers[83] used in their reaction of trimethylphosphite and alloxan anhydride (dry methylene chloride), the adducts presented in this study were prepared under non-anhydrous conditions. Instead of alloxan anhydride, 5,5-dihydroxybarbituric acid (alloxan monohydrate) and absolute ethanol were used. Thus, one molecule of water is present per molecule of organic reactant.



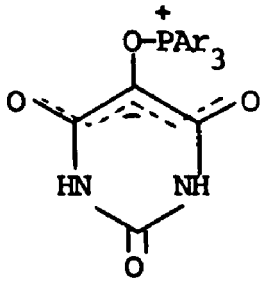


There are several possible structures which might be postulated for the product of this reaction. Initially, it was thought likely that reduction of alloxan should take place to give either dialuric acid or alloxantin with the phosphorus being oxidized to triphenylphosphine oxide. However, this speculation proved in-



correct when the precipitate's weight was almost the summation of the weights of the phosphine and alloxan and the precipitate was insoluble in most common solvents including water. The structures which were then considered were a one to one adduct between alloxan and the

phosphine (7) or (8) or the adduct between dialuric acid and triphenylphosphine oxide (9). The coordinate link



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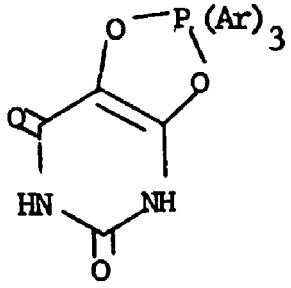

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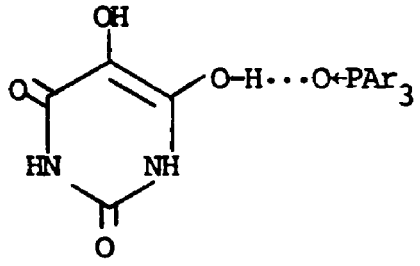

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between dialuric acid's most acidic proton (as deduced by x-ray crystal studies[96] of dialuric acid) and the oxygen of the phosphine oxide has precedent from the study of Mann and Chaplin on the coordinate link between phosphine oxides and sulfonamides[99] and between triphenylphosphine oxides and hydroquinone.[100] The analysis eliminated the dialuric acid-triphenylphosphine oxide coordinate adduct because the material analyzed for one less mole of water than required for this adduct. Combustion analyses were

in accord with an alloxan triphenylphosphine adduct.

Ramirez and coworkers relied on ^{31}P nmr data to determine whether their trimethyl and triphenylphosphite adducts with orthoquinones, paraquinones, biacetyl, and alloxan were zwitterionic or in the form of a dioxaphosphole ring. They employed very little infrared data to deduce their structure. Their reported spectral data for the adduct of p-chloranil and triphenylphosphine closely approximated the spectral data obtained from the triarylphosphine alloxan adduct. These earlier authors did not assign specific stretching or deformation modes to the bands in the infrared but the bands reproduced in the following table are assigned from well documented correlations.[101]

The infrared data of the adducts studied would be expected to correlate well with the aryl-P stretching and the aromatic in plane deformation mode of the model compounds. The P-O stretching bands in these comparison molecules correlate equally well. Speciale and Partes[102] reported unassigned infrared data for an intermediate alkoxyphosphonium salt (10) of the Perkow reaction which also corresponds well to the data for the alloxan phosphine adducts: 1437 (aryl-P str), 1166 (P-O str), and 1119 cm^{-1} (aromatic in plane deformation mode). In contrast to this, Schaeffer and Weinberg reported[117] a

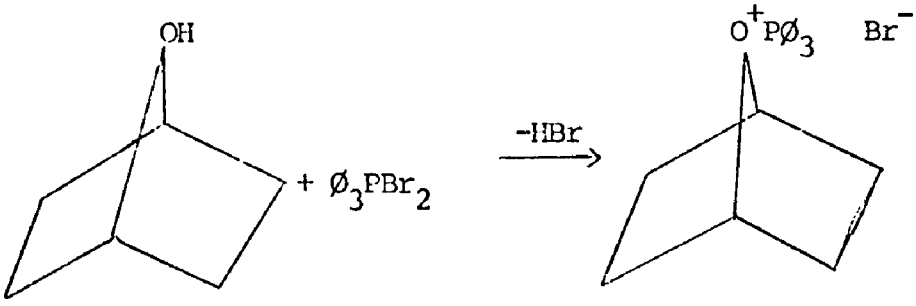
Table 2

Phosphorus Infrared Data of the Triarylphosphine Adducts

Adduct	Aryl-P str	P-O str	Aromatic in plane deformation mode
chloranil-triphenyl-phosphine	1429 cm^{-1}	1176 cm^{-1}	1117 cm^{-1}
alloxan-triphenyl-phosphine	1438	1190 (sh) 1180 1165 (sh)	1122 1110 (sh)
alloxan-tri-p-tolyl phosphine	1443	1178 1175	1117 1110
alloxan tri-p-methoxy-phenyl phosphine	1440	1185 1170 (sh)	1120 1110 (sh)
alloxan tri-m-tolyl phosphine	1440	1180 1172	1120 1110

10

rather different P-O stretch of 1235 cm^{-1} for 7-norbornoxytriphenylphosphonium bromide (11) prepared from 7-norbornanol and triphenylphosphine dibromide. The infra-



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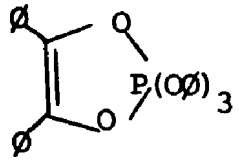
red evidence thus implicates the zwitterionic structure as being the most likely representation of the adduct of 5,5-dihydroxybarbituric acid and triarylphosphines.

The preparation of the triphenylphosphite alloxan adducts was attempted to correlate their infrared

data with that which Ramirez and Desai reported for the triphenylphosphite adducts of benzil (12) and phenanthraquinone (13).[103] The attempted preparation of these



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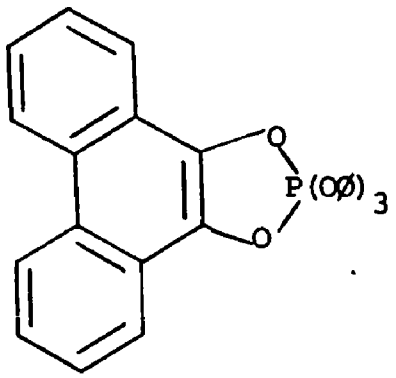

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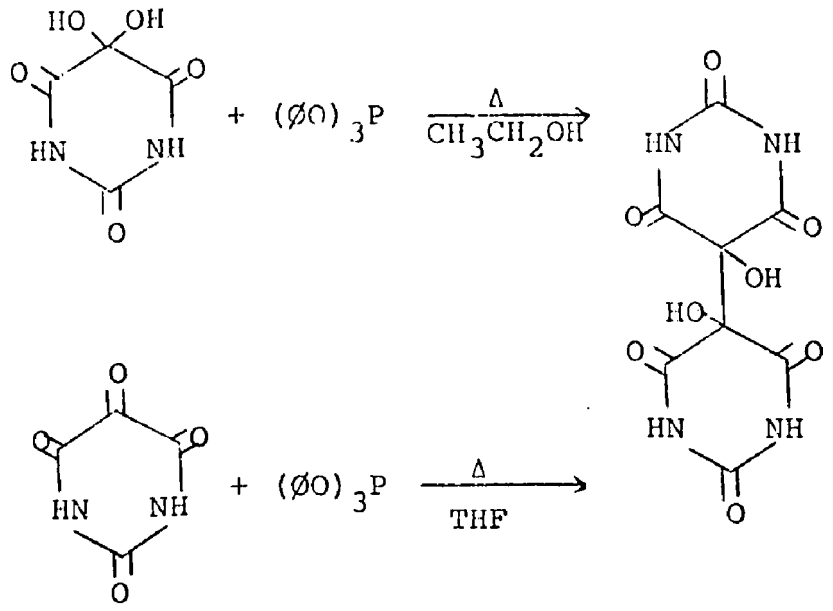
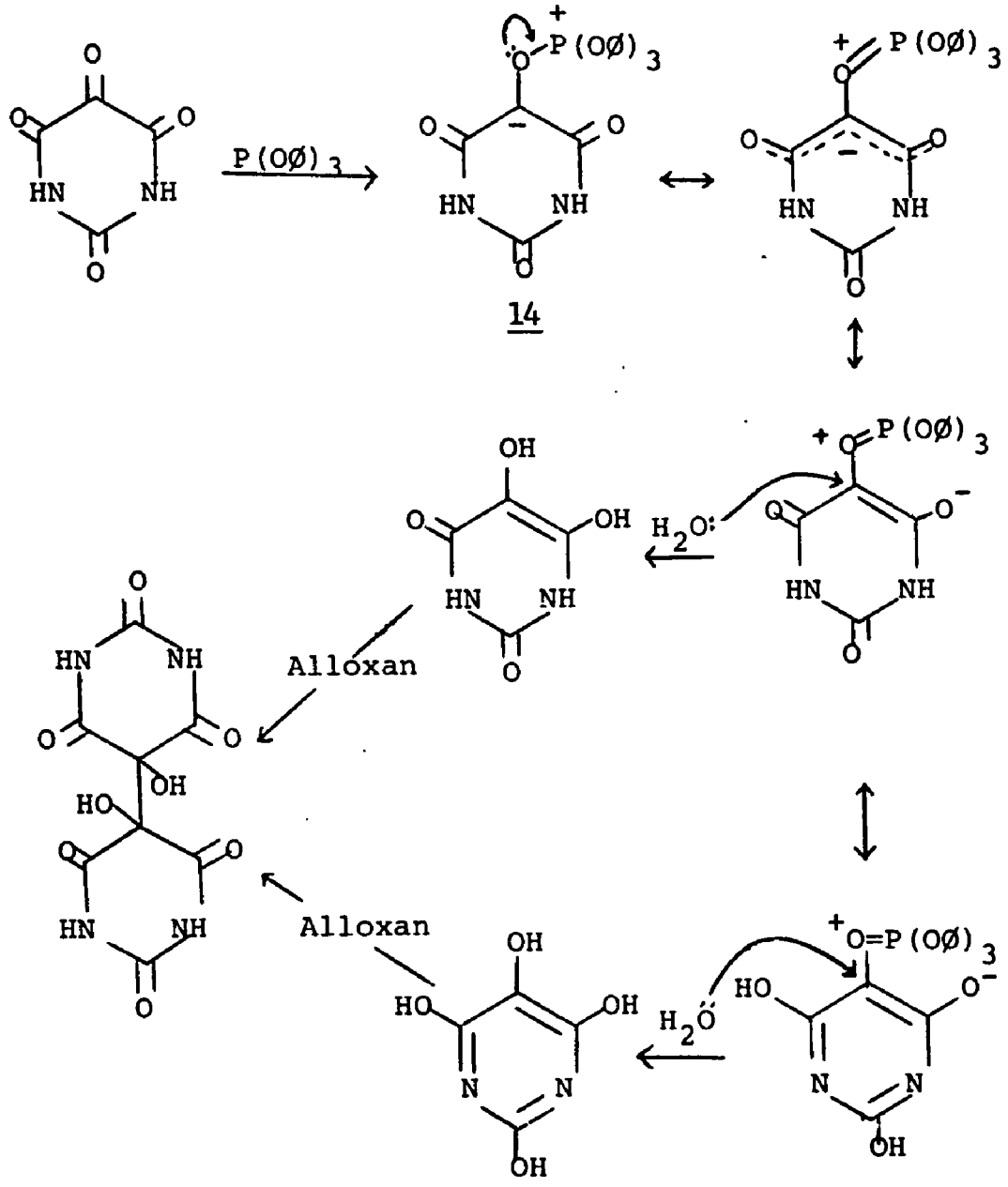
adducts yielded only alloxantin under conditions in which the phosphite was refluxed with 5,5-dihydroxybarbituric acid in ethanol and in which the phosphite was condensed with alloxan anhydride in dry tetrahydrofuran (Scheme 5). One explanation why the triphenylphosphite adduct of alloxan does not form under conditions which readily yield the triphenylphosphine adduct invokes the electronic effect of aryloxy groups which make the phosphorus a better π acid than the phosphorus of the phosphines. It might be argued that in the intermediate which forms after the phosphorus donates a pair of

Scheme 5



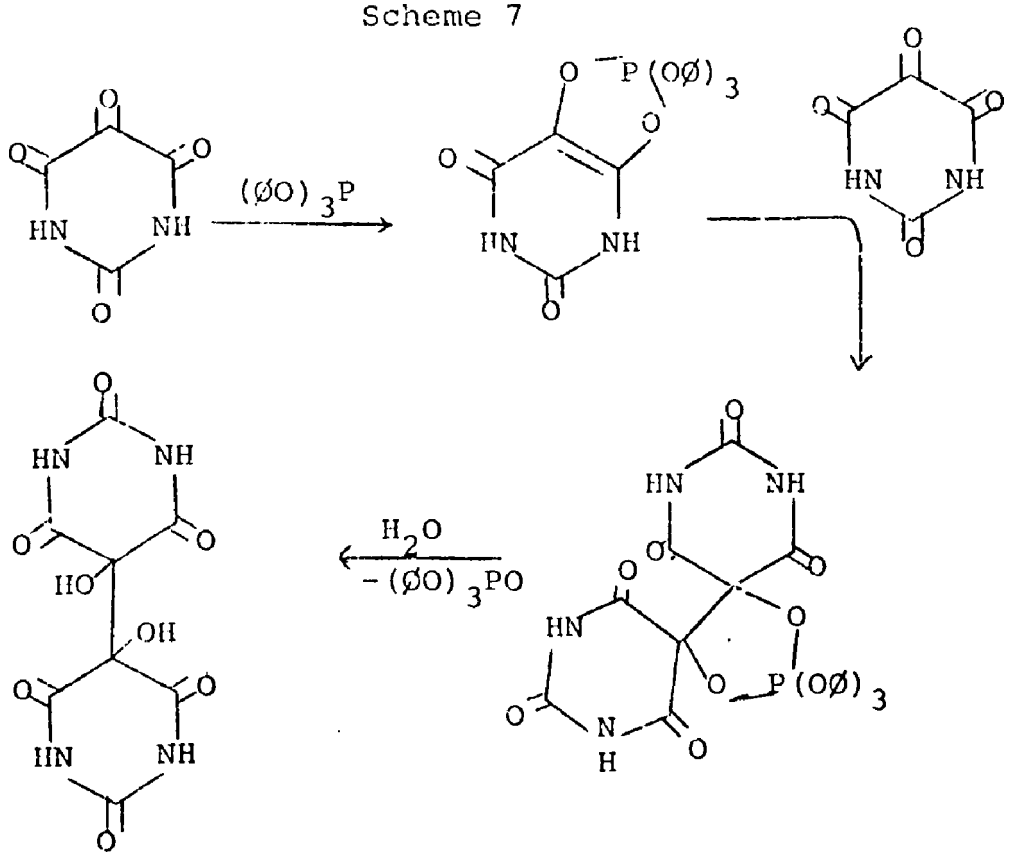
electrons (to form the barbituryloxy phosphonium zwitterion (14)), the electron withdrawing effect of the aryloxy oxygens enhances the electrophilicity of phosphorus and makes carbon-oxygen cleavage more likely. Subsequent hydrolysis gives dialuric acid (Scheme 6) just as triphenylphosphine dihalides react with phenols to give halobenzenes as the displacement products.[104,105] This transient dialuric acid is then free to combine with alloxan to form alloxantin. Another alternative, albeit

Scheme 6



sterically less likely, mechanism is the reaction of a possible dioxaphosphole, formed from alloxan and triphenylphosphite, and another mole of alloxan to form a spiro-bis-5-barbituryldioxaphospholane, followed by hydrolysis of the dioxaphospholane to triphenylphosphate and alloxantin. The mechanism presented in scheme 7

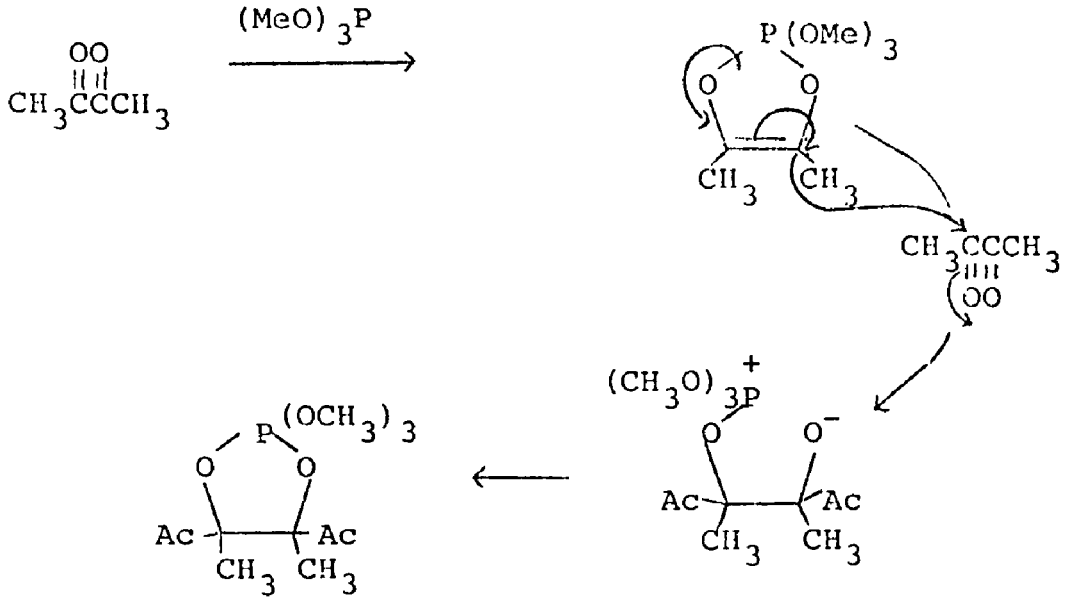
Scheme 7



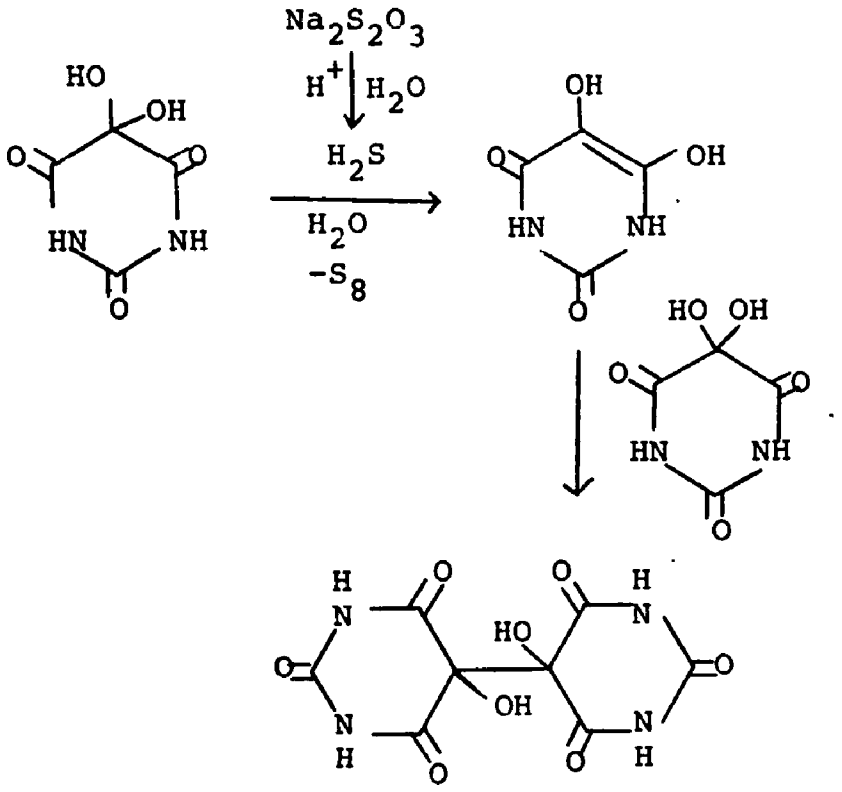
is less likely because the less sterically encumbered trimethylphosphite and alloxan have not been found to form alloxantin[83] and ortho quinones are similarly not known to undergo this transformation. Adduct formation is, however, well known for biacetyl and trimethyl-

phosphite[106] (Scheme 8).

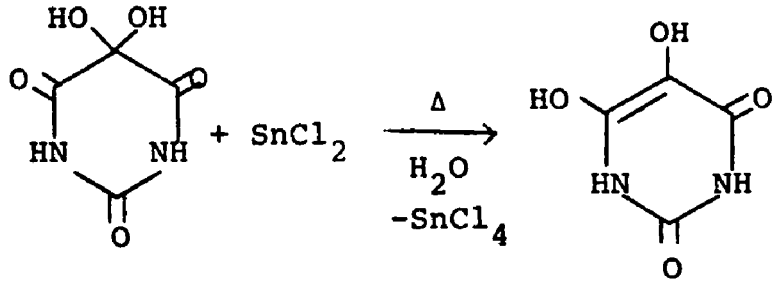
Scheme 8



The formation of alloxantin was confirmed by its analysis, melting point, and spectral comparison with authentic alloxantin prepared (1) from an acidic solution of alloxan and sodium thiosulfate pentahydrate (essentially the method of Tipson[20] with the H_2S being generated <u>in situ</u> from the acid and the sodium thiosulfate[107]) and (2) from alloxan and dialuric acid (prepared according to Hill's[108] stannous chloride



dihydrate reduction of alloxan).

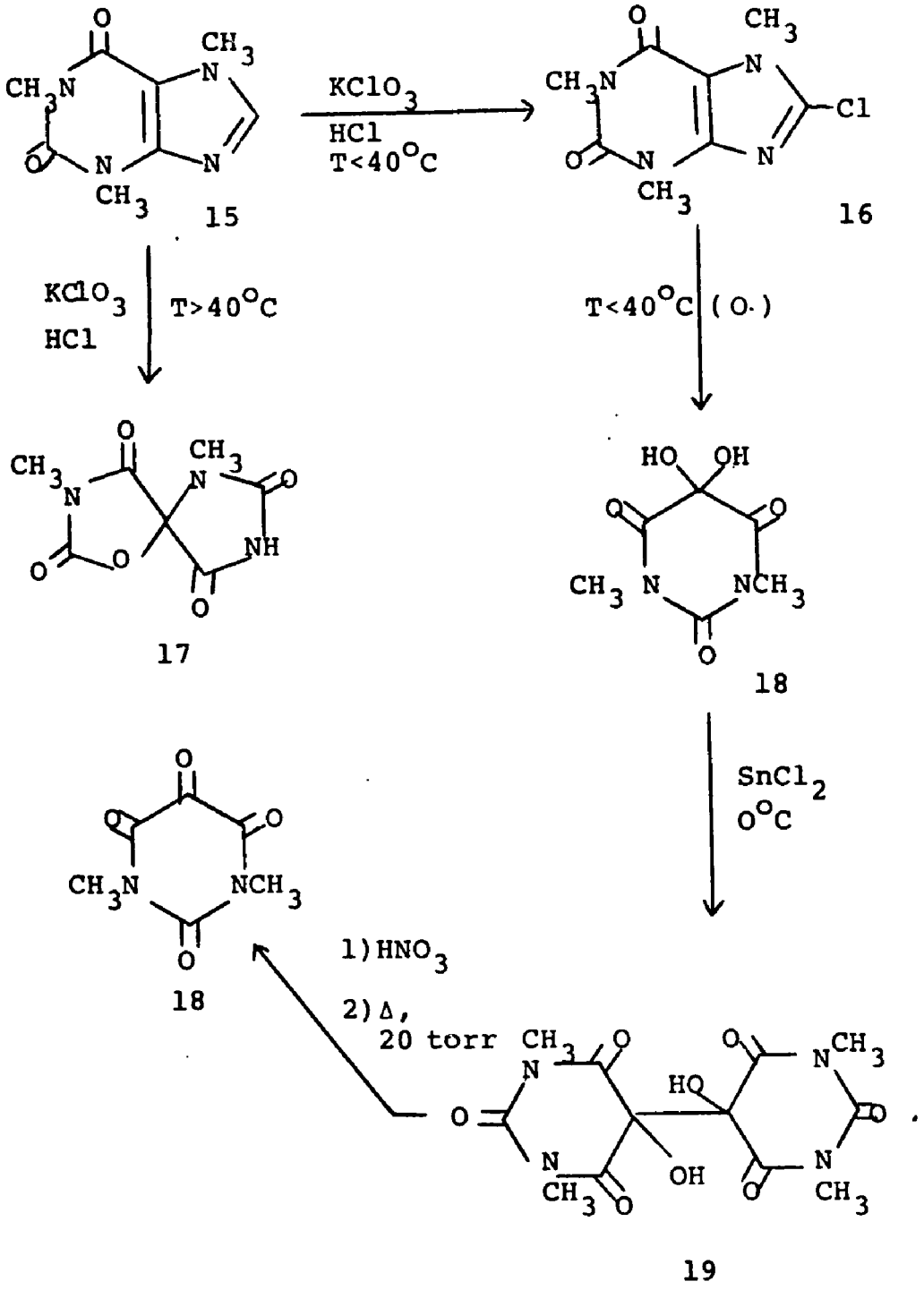


The 1,3-dimethylalloxan adducts of triphenylphosphine and tri-p-tolylphosphine were prepared in order to determine if the adducts were structurally zwitterionic (the protons of the N-methyls would be equivalent) or if the adducts had formed a dioxaphosphole ring (shown by the non-equivalency of the methyl protons).

The 1,3-dimethylalloxan was prepared from caffeine (15) according to a modified method of Biltz and Fischer, with one exception (scheme 9). During their experiments heat was applied to the reaction medium to redissolve the precipitated 8-chlorocaffeine (16). By increasing the reaction temperature above 40-50°C the amount of apocaffeine and isoapocaffeine[111] (17) increased and the yield of 1,3-dimethylalloxan decreased. By increasing the amount of solvent by two-thirds, the 8-chlorocaffeine remained in solution and yield of 1,3-dimethylalloxan (18) was optimized at the lower temperature. The alloxan is first reduced to tetramethyl alloxantin (19), which is an isolable solid, and this tetramethylalloxantin is then reoxidized to 1,3-dimethylalloxan (18).

The triphenylphosphine and the tri-p-tolylphosphine 1,3-dimethylalloxan adducts were prepared in the non-protic solvents of chloroform and benzene,

Scheme 9


KClO3
HCl
T<40°C
15
16
KClO3
HCl
T>40°C
T<40°C (O.)
17
18
SnCl2
0°C
18
1) HNO3
2) Δ,
20 torr
19

respectively. Since these two adducts were readily soluble in the solvents employed they were not as readily isolable as the alloxan adducts which precipitated during their preparation (in ethanol). The nmr spectra of 1,3-dimethylalloxan, the triphenylphosphine 1,3-dimethylalloxan adduct and the tri-p-tolylphosphine 1,3-dimethylalloxan adduct all displayed a sharp singlet for the protons of the two methyls on the pyrimidine nitrogens.

The infrared data for the P-O stretching of the alloxan adducts and the nmr data for the protons of the pyrimidine methyls show that the triarylphosphine-alloxan and 1,3-dimethylalloxan adducts are in the zwitterionic form and not in the dioxaphosphole structure. The infrared spectra of these materials also possess carbonyl absorptions at approximately 1600 cm^{-1} which indicate that the two carbonyls in the 4 and 6 positions of the pyrimidine ring do not have full double bond character, but are part single C-O bonds and part C-O double bonds (see Table 3).

The infrared spectral data for the 1,3-dimethylalloxan adducts are parallel to the data obtained from the alloxan adducts for the aryl-P stretchings and the aromatic in plane deformation modes, but the bands for the P-O stretching absorptions occur at much

Table 3

Carbonyl Stretching Frequencies of the Adducts

Compound	Carbonyl stretching frequencies cm^{-1}
triphenylphosphine alloxan adduct	1675, 1650 and 1600 (broad)
tri-p-tolylphosphine alloxan adduct	1690, 1660 and 1590
tri-m-tolylphosphine alloxan adduct	1655, 1610 and 1590
tris-(p-methoxyphenyl)-phosphine alloxan adduct	1700, 1655 and 1590
triphenylphosphine 1,3-dimethylalloxan adduct	1680 and 1630-1600
tri-p-tolylphosphine 1,3-dimethylalloxan adduct	1690 (small intensity), 1625-1585

lower frequencies (see Table 4). Since an adduct is formed, the nuclear magnetic spectra indicates the equivalency of the protons of the pyrimidine methyls and the infrared spectra are not the summations of the infrared spectra of 1,3-dimethylalloxan and the phosphine (the carbonyl absorption is at a lower frequency and the methyl protons shifted from δ3.38 to 3.20 and 3.18 ppm for the triphenylphosphine and tri-p-tolylphosphine adducts, respectively) or of the infrared absorptions of barbituric acid or dialuric acid and the phosphine oxide (no P-O stretching at approximately 1180 cm^{-1}, the carbonyl absorption is at a lower frequency and the combustion analysis does not match such a mixture). Thus it can be c oncluded that the adducts formed from 1,3-dimethylalloxan and the two arylphosphines are the same type of adduct as those formed from alloxan and triarylphosphines. The anomalous P-O stretching could be related to different reactivities between alloxan and 1,3-dimethylalloxan.

Chemically, alloxan and 1,3-dimethylalloxan are similar but in two instances comparisons were made. The dehydration of the monohydrate was easiest for 1,3-dimethylalloxan and hardest for alloxan while 1-methylalloxan was intermediate.[91] Similarly, as others have observed, 1,3-dimethylalloxan underwent

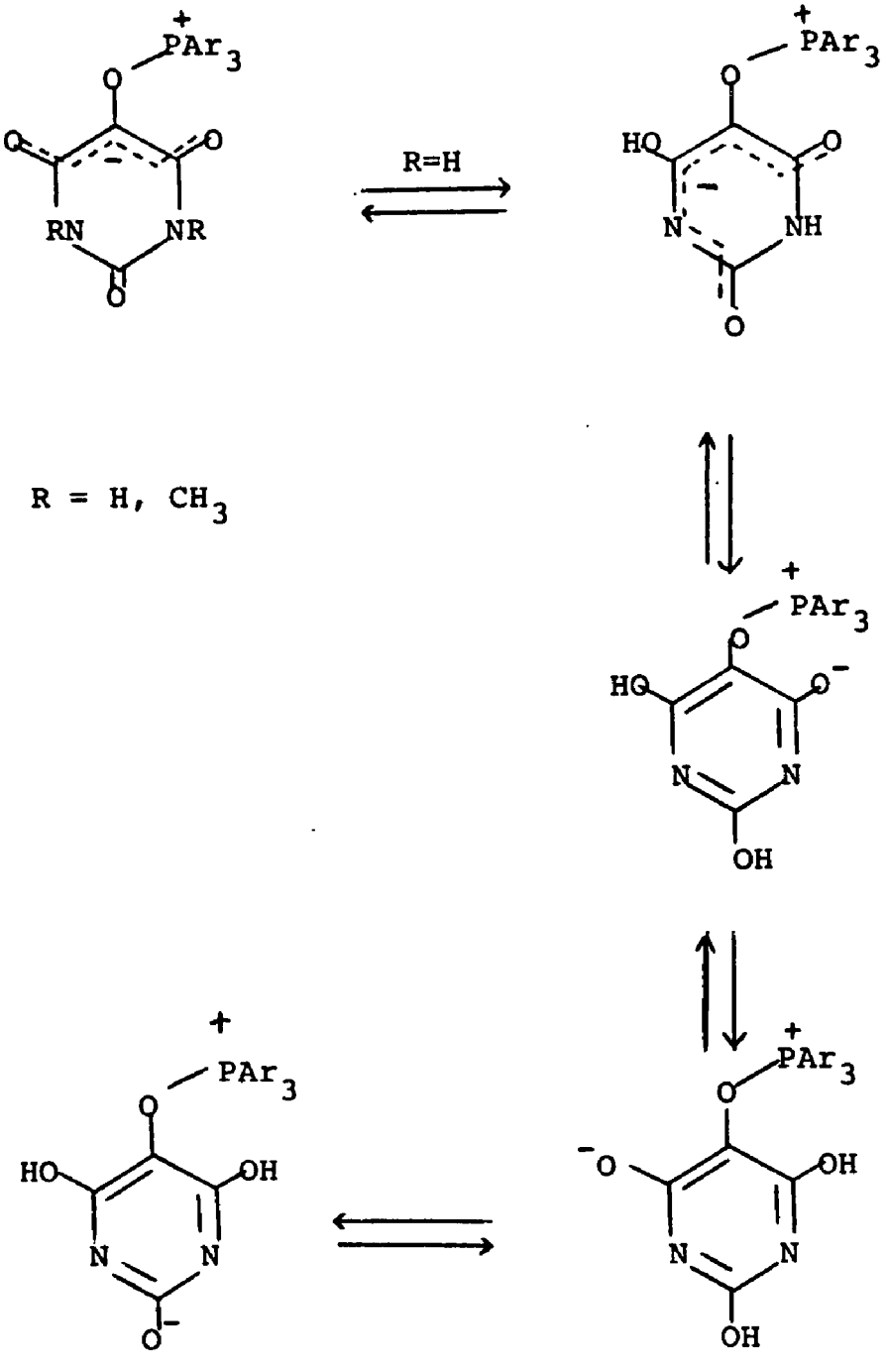
Table 4

Comparison of the Phosphorus Infrared Spectral Data of the Alloxan and 1,3-Dimethylalloxan Adducts

Adduct	Aryl-p str	P-O str	Aromatic in plane Deformation Mode
Inclusive range of triaryl phosphine adducts	1429-1443 cm^{-1}	1190-1165 cm^{-1}	1122-1110 cm^{-1}
Triphenylphosphine 1,3-dimethylalloxan adduct	1440	1069 1055(sh) 1045(sh)	1117 1110(sh)
Tri-p-tolylphosphine 1,3-dimethylalloxan adduct	1420	1070 1060(sh)	1120 1110(sh)

the alloxan-alloxanic acid rearrangement faster than alloxan.[19] The differences in the stability of the adduct formation is probably due to the methyl groups on nitrogen. These methyl groups are not in the proximity of the phenyl groups on phosphorus so there is at the most a very modest steric interaction. The methyl groups do inhibit the delocalization of the negative charge throughout the pyrimidine ring, as invoked by Ramirez in his mechanism for the trimethyl phosphite alloxan reaction.[83] The lack of through delocalization may be the reason that the P-O bond is weaker in the 1,3-dimethylalloxan adducts than the alloxan adducts (scheme 10). The lack of delocalization in 1,3-dimethylalloxan forces the negative charge to remain in the proximity of the #5 carbon and this nucleophilicity is thus able to compete in bond formation (between C_5 and O_5) with the pair of electrons donated by phosphorus to O_5. The P-O stretching band indicates there is a competition inhibiting the formation of a normal P-O bond, but nevertheless the adducts are formed.

Scheme 10



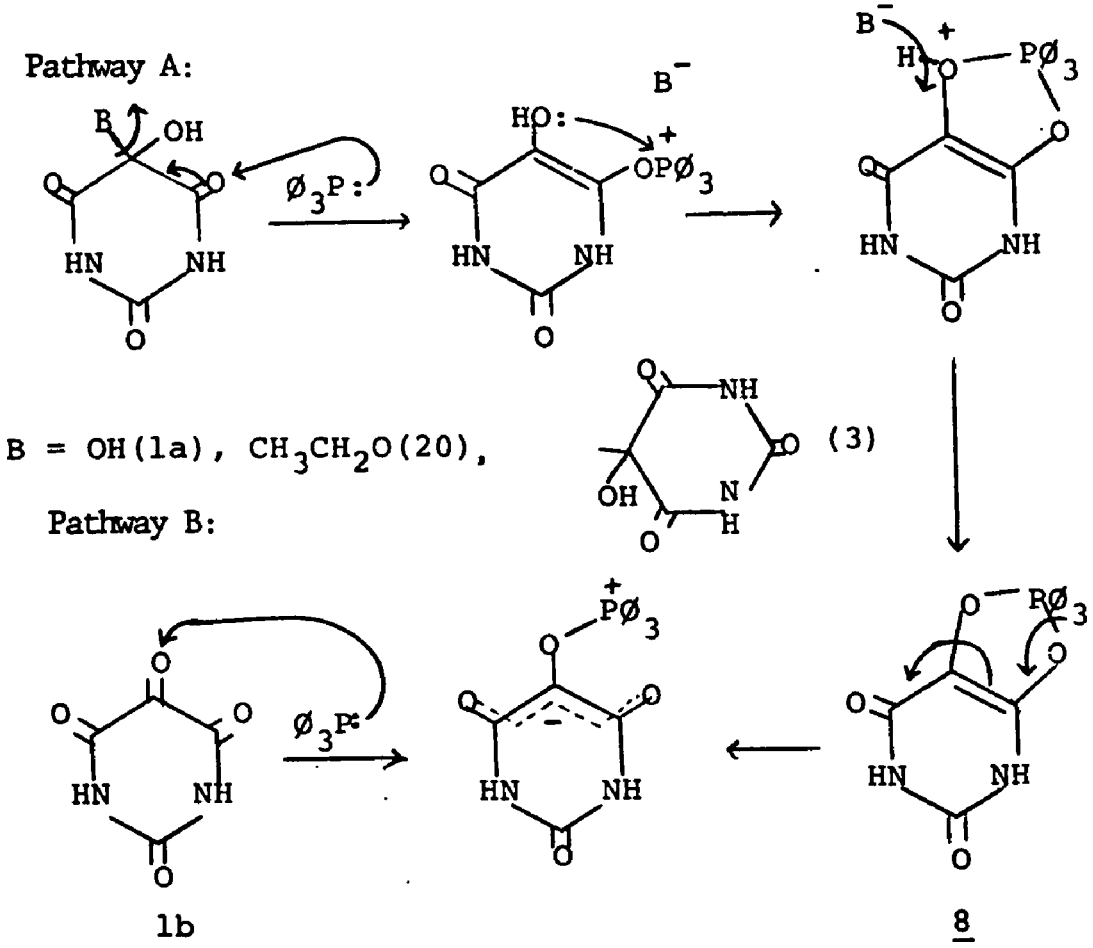
B. The Mechanism of Adduct Formation

The fortuitous formation of the triphenylphosphine alloxan adduct from alloxan monohydrate (1a) and triphenylphosphine in ethanol raised the key question, does adduct formation require the presence of the C-5 carbonyl (Scheme 11, Pathway B) or is a C-4 carbonyl sufficient (Pathway A) since alloxantin (3) and 5-ethoxy-5-hydroxy-barbituric acid (20) yielded the same adduct too, interest in this question was intensified. As is expected, alloxan anhydride (1b) readily leads to the formation of the adduct.

Scheme 11 illustrates the plausible mechanisms of these transformations. Phosphorus attack at C-4 carbonyl oxygen (Pathway A) apparently is favored when there is no C-5 carbonyl. Phosphorus attack at C-5 carbonyl oxygen (Pathway B) is favored in alloxan anhydride, if the molecules which do not have a C-5 carbonyl are capable of decomposing in the reaction medium to alloxan anhydride. The dioxaphosphole

intermediate (8) is postulated because this type of adduct has actually been isolated by Ramirez, et al[106] in very similar reaction studies.

Scheme 11



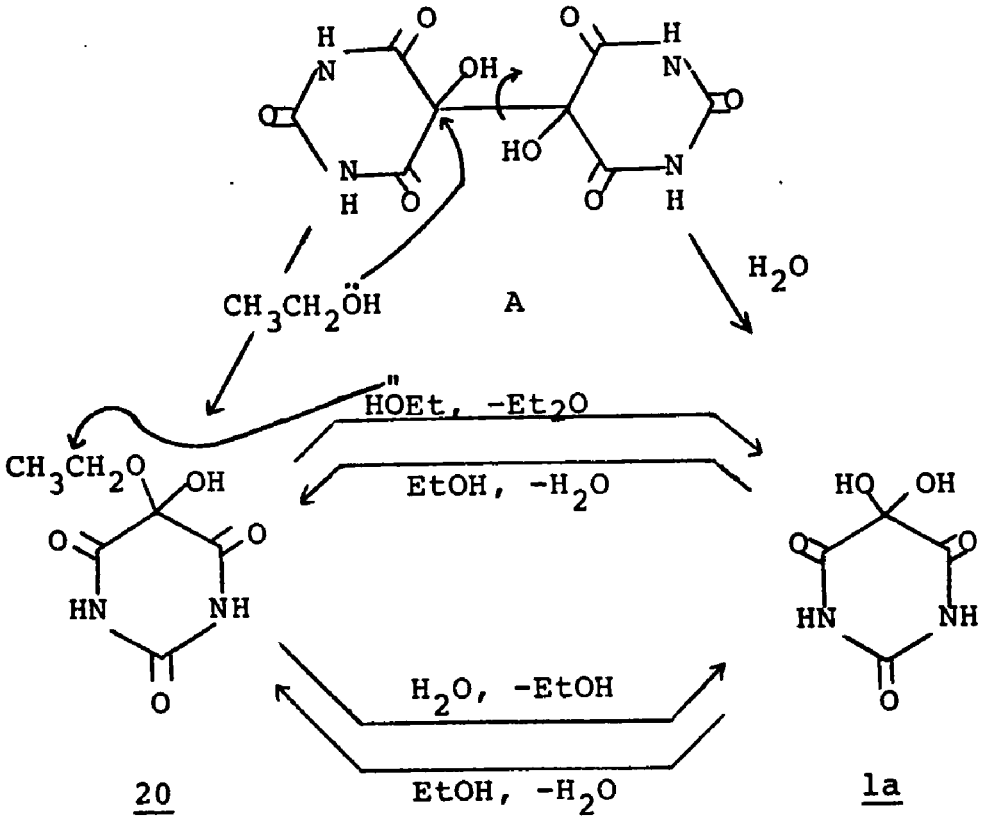
As a means of determining the answer to the original question, the stability of alloxan monohydrate in refluxing ethanol was evaluated. When the solution acquired a slight yellow tint in what appeared to be alloxan anhydride formation, anhydrous magnesium sulfate

was added to absorb the water produced. An unexpectedly excellent yield of 5-ethoxy-5-hydroxybarbituric acid and not of the anhydride was obtained. Since there are known reactions in the literature which are catalyzed by magnesium sulfate,[112] the water was azeotropically removed and absorbed by anhydrous magnesium sulfate in a Soxhlet thimble above the refluxing solution. The yield of the 5-ethoxy derivative was nearly quantitative. This method of preparation of the 5-ethoxy derivative is an improvement over the method of Biltz in which it was produced in good yields from alloxan anhydride in absolute ethanol saturated with dry HCl gas.[91]

Another sequence used in answering the mechanistic question posed above involved determining the stability of alloxantin in refluxing ethanol. This solution yielded alloxan monohydrate upon workup. Since alloxantin dissociates in water via hydrolysis there exists the possibility that ethanolysis could be the mode of dissociation in ethanol to initially form the 5-ethoxy derivative. If the 5-ethoxy derivative is formed, then there is substantial precedent[113] that the equilibrium between the 5-ethoxy compound (20) and alloxan monohydrate (1a) might involve formation of diethyl ether by nucleophilic attack by ethanol on the methylene of

the ethoxy group as occurs in ethoxylated quinolines[113] (Scheme 12, Pathway A). When a solution of alloxantin in

Scheme 12

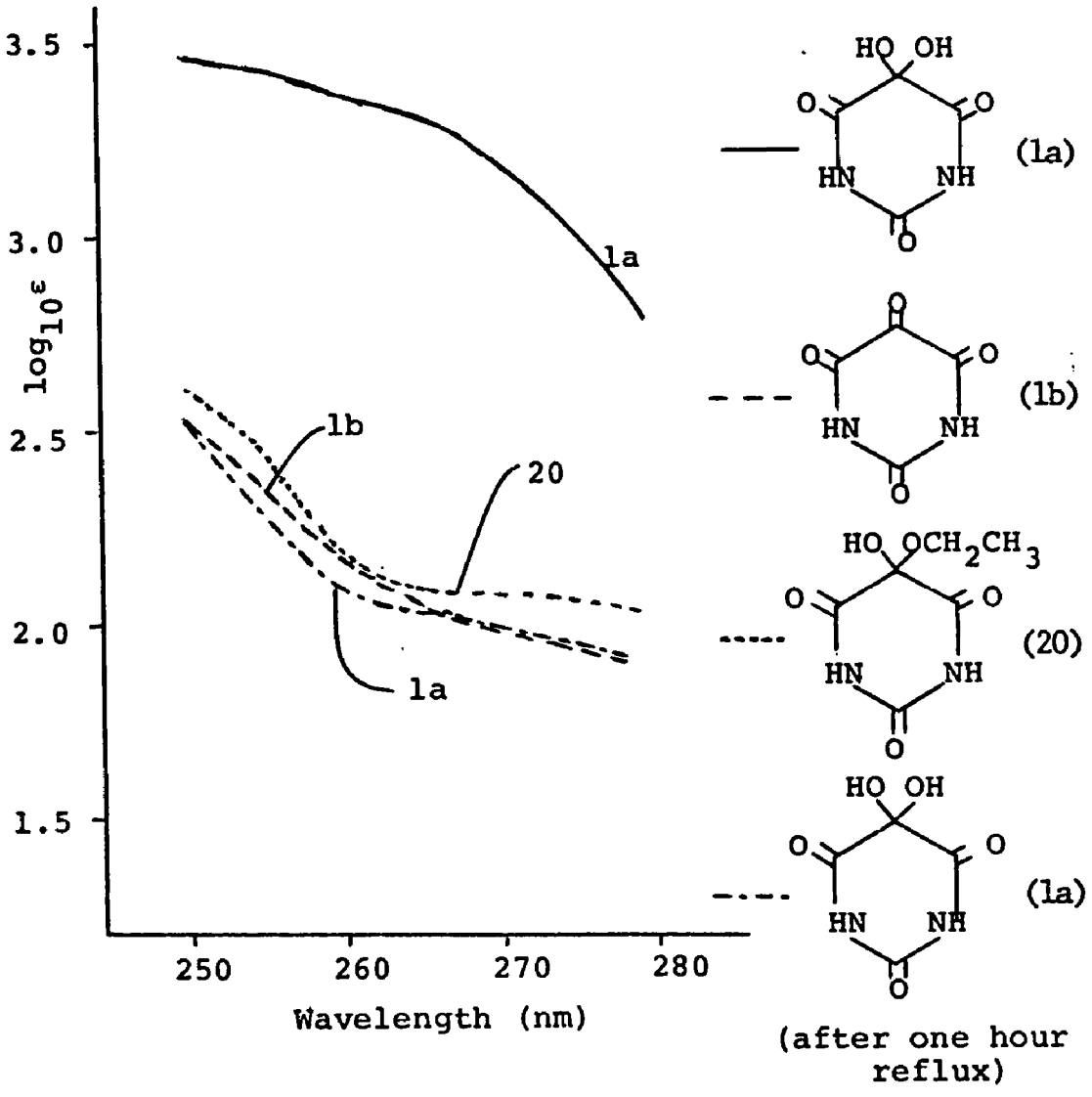


refluxing ethanol and a solution of alloxan anhydride (to exclude water) in refluxing ethanol were evaluated for ether formation via gas phase chromatography, no ether was detected in either case. The lack of ether formation implicated water from alloxantin (which contains water in the crystal structure) as the solvolysis

agent. This data firmly establishes that if there is an equilibrium between the 5-ethoxy material and the monohydrate in ethanol it entails a direct displacement at C-5 (see Scheme 12, Pathway B).

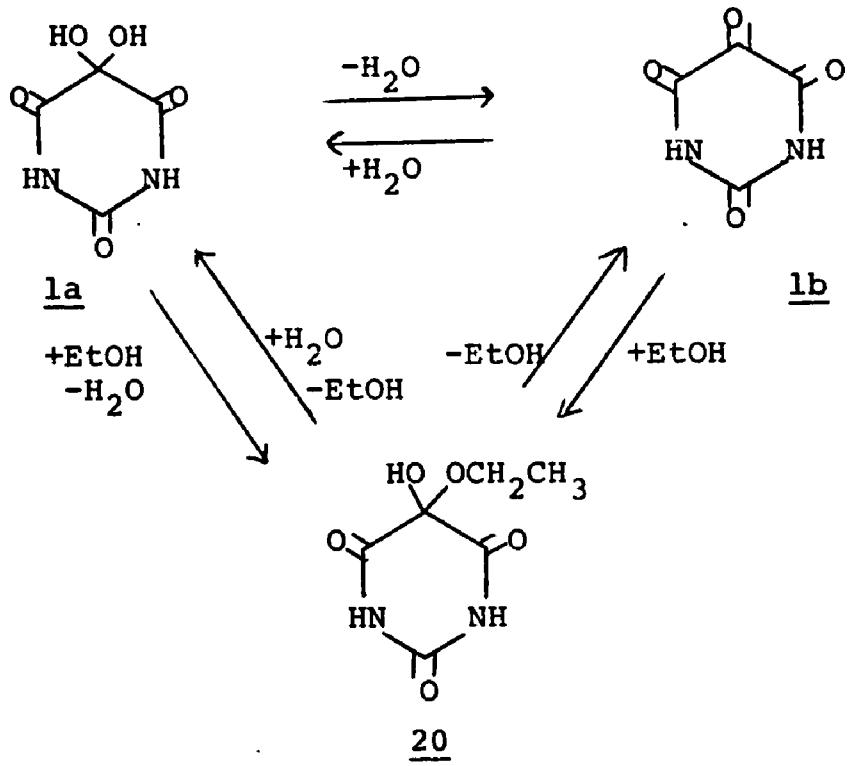
Further evidence supporting the contention that alloxan monohydrate is not stable in the ethanol solution came from an ultraviolet study of ethanol solutions of the monohydrate, the anhydride and the 5-ethoxy derivative. The ultraviolet spectra of 5-ethoxy-5-hydroxybarbituric acid and alloxan anhydride were identical while the monohydrate was quite different (Figure 3). When the monohydrate was refluxed in ethanol for one hour the ultraviolet spectra of this solution was superimposable on the spectra obtained for the anhydride and the 5-ethoxy derivative (Figure 3). Presumably, the same chromophoric species is generated for the anhydride and the 5-ethoxy derivative upon dissolution in ethanol and after a one hour reflux of ethanolic alloxan monohydrate. The ultraviolet spectra implicate a possible equilibrium among the monohydrate (1a) the anhydride (1b) and the 5-ethoxy material (20). The nearly quantitative yield of the 5-ethoxy material obtained from refluxing ethanolic alloxan monohydrate with $MgSO_4$ as a drying agent gives evidence that if such an equilibrium exists, it may lie far to the side of the 5-ethoxy

Figure 3. Ultraviolet Spectra of Alloxan Monohydrate, 5-Ethoxy-5-hydroxybarbituric Acid and Alloxan Anhydride in Absolute Ethanol.



material (Scheme 13).

Scheme 13



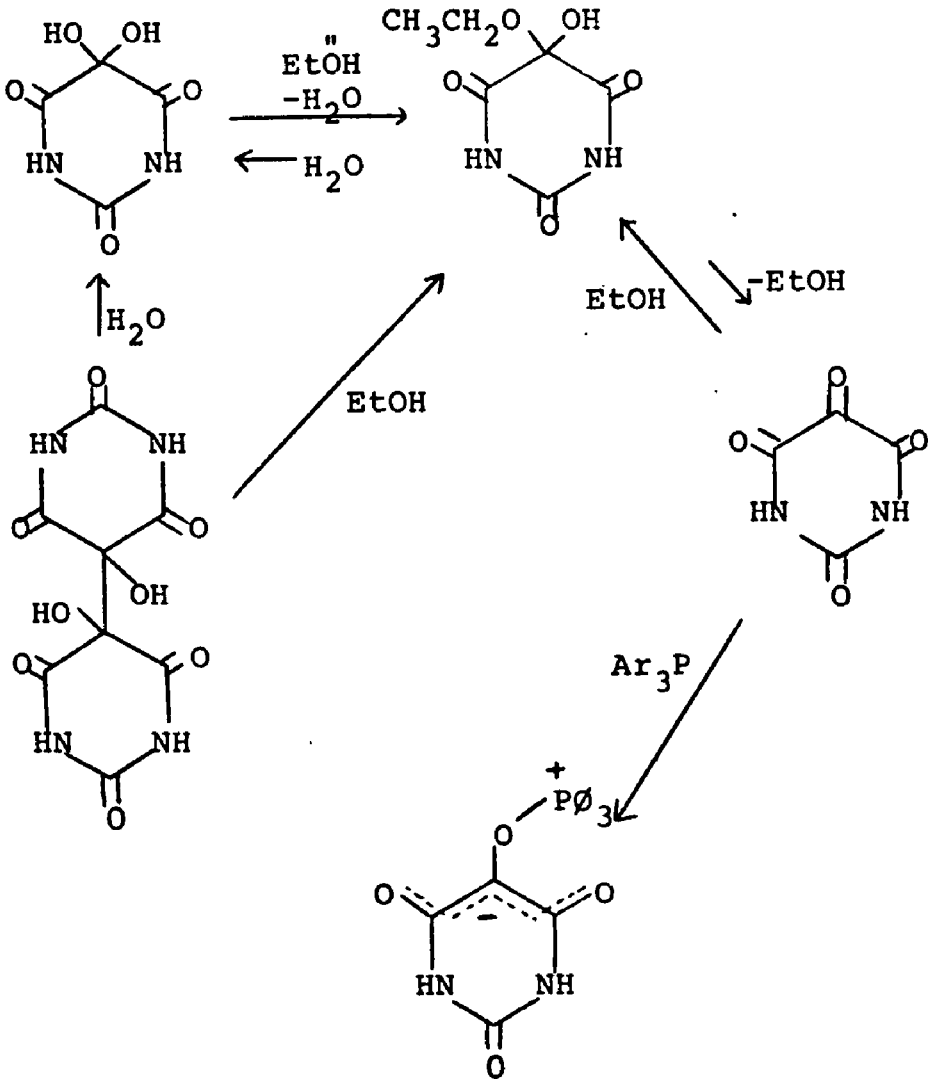
The viability of 5-ethoxy-5-hydroxybarbituric acid as a stable coreactant in adduct formation was evaluated in dry tetrahydrofuran. Although adduct was formed, the 37% yield after refluxing 72 hours was dismally low compared to the nearly quantitative yield in ethanol. Furthermore, within one hour the anhydride and phosphine in THF produced the adduct in 75% yield without the application of heat.

By obtaining adduct from the 5-ethoxy derivative in THF, it can be unequivocally stated that the C-4 carbonyl is sufficient for adduct formation, if no alloxan anhydride is formed in refluxing THF. Unfortunately, alloxan anhydride is formed in refluxing dry THF from 5-ethoxy-5-hydroxybarbituric acid. The ethanol that was produced and any water that was present was absorbed by $CaCl_2$ and Na_2SO_4, respectively, in a Soxhlet thimble positioned above the refluxing flask.

In conclusion, of the two possible mechanisms for adduct formation (attack at C-5 or attack at C-4 carbonyl) no experimental evidence supports the latter option and no experimental evidence excludes the former. Although several compounds which lack C-5 carbonyls do form the adduct, it appears that they can initially decompose under the reaction conditions to the requisite carbonyl precursor, alloxan anhydride (Scheme 14). In addition, the 1,3-dimethylalloxan adduct formed from triarylphosphine probably arise in a similar fashion via phosphorus attack at the C-5 carbonyl oxygen. Sterically one would expect the C-4 carbonyls to be considerably more hindered in the dimethylalloxan. Furthermore, excellent yields with high rates of formation are observed at ambient temperatures when totally non-hydroxylic solvents such as chloroform and benzene are employed

for the condensation.

Scheme 14



C. Reactions of the Triphenylphosphine Alloxan Adduct

The validity of *in vivo* studies in laboratory animals will depend on the *in vitro* stability of the adducts and thus the reactivity of the triphenylphosphine adduct was investigated. The structure proposed herein for the adduct finds a parallel in the sulfur ylids of barbituric acid and the iodonium and pyridinium barbiturates discussed in the introduction. Thus, it would be expected that these adducts might be able to undergo the same types of transformations.

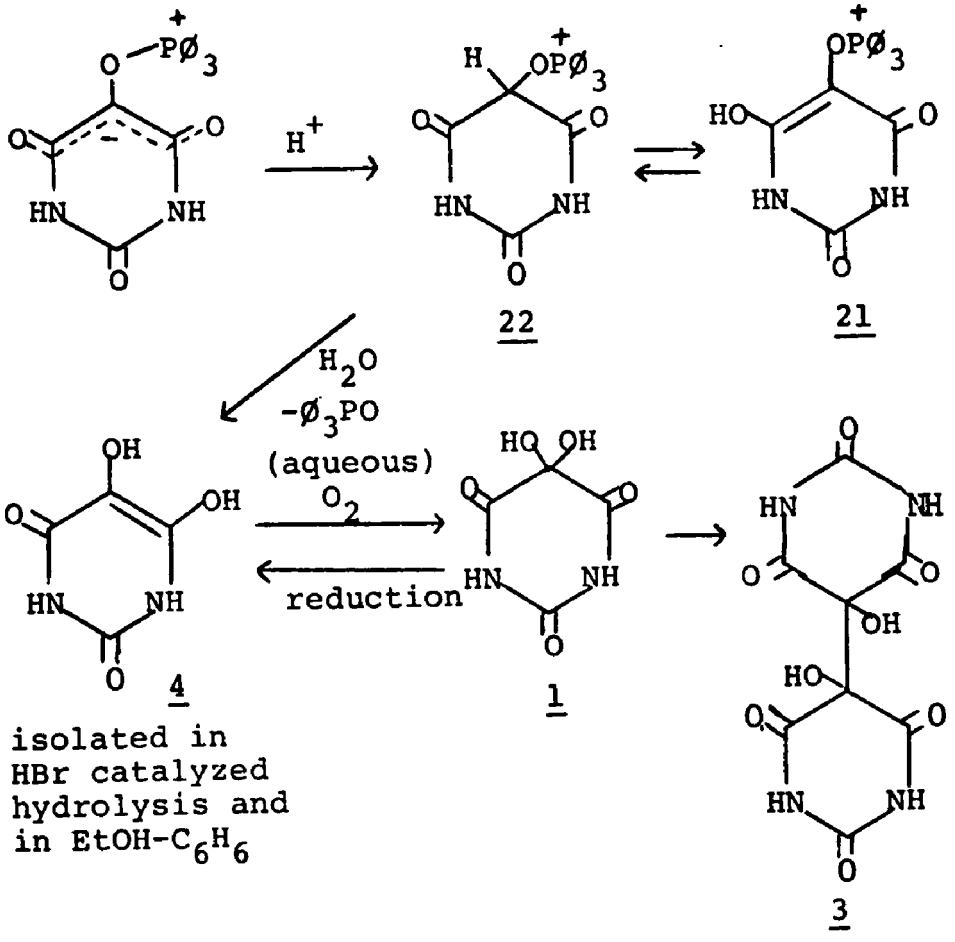
Unlike the instability of alloxan in solutions at physiological pH, the adduct was recovered unchanged at this pH (pH approximately 7.4). Though the adduct is unreactive in basic media, it was readily hydrolyzed in acidic media. In acidic aqueous media hydrolysis occurred to give alloxantin (3). The isolation of alloxantin (produced by alloxan (1) and dialuric acid (4)) indicates that dialuric acid is oxidized to alloxan in the reaction media. Dissolved molecular oxygen is the probable oxidant and when an ethanol-benzene mixture is used as the solvent, the insoluble oxygen was excluded and dialuric acid (4) was isolated.

The hydrolysis of the adduct in dilute aqueous HBr unexpectedly produced dialuric acid. The only plausible reason would be that HBr acted as a reducing

agent to retard the oxidation of dialuric (4) acid by removing molecular oxygen or more likely to convert any alloxan (1) or alloxantin (3) produced to dialuric acid (4).

The mechanism of the hydrolysis in Scheme 15 can be envisioned as initial protonation of the adduct followed by displacement of triphenylphosphine oxide by water. The protonation may occur at either the

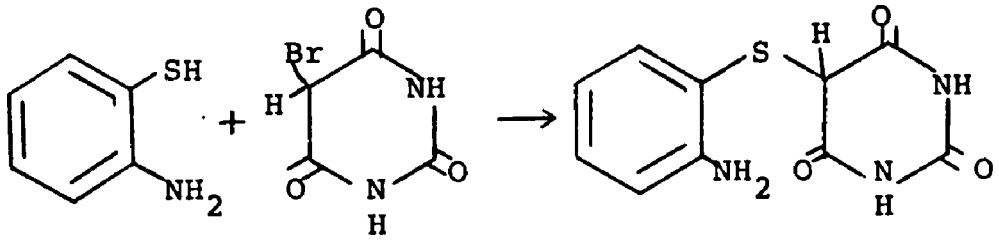
Scheme 15



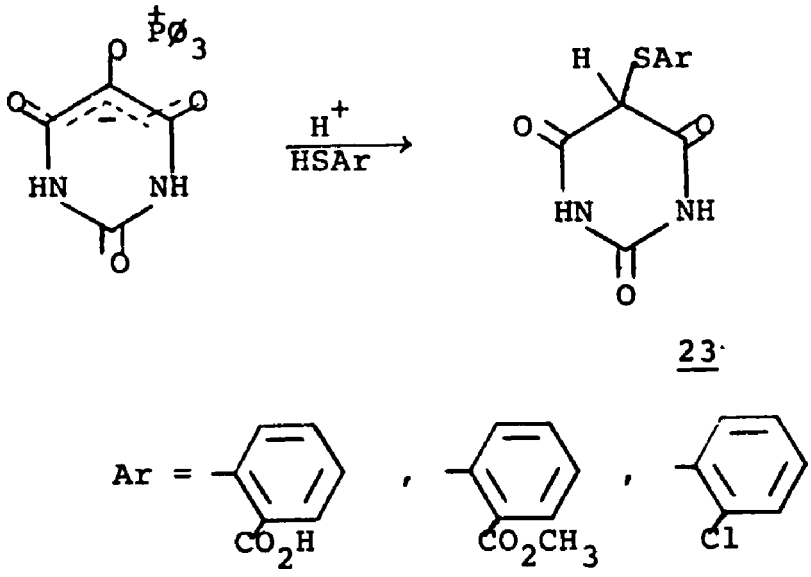
C-4 carbonyl oxygen (21) or at C-5 (22). These two protonated species are probably in equilibrium with each other and the displacement of the phosphine oxide probably occurs at the carbon of (21).

Unlike the reduction of alloxan by sulfhydryl compounds, the adduct is converted to birbituryl sulfides (2) in the presence of acid and sulfhydryl containing molecules. Because there are enzymes, proteins, peptides with free sulfhydryl groups, this reaction is a possible <u>in vivo</u> decomposition pathway of the adduct in the presence of -SH moieties.

This transformation is a viable method of preparing barbituryl sulfides (23), of which one other representative of this class has been found in the literature.[114] The mechanism leading to the formation of these sulfides (23) is probably very similar to the mechanism for the acid hydrolysis of the adduct. The initial protonation gives an intermediate which is susceptible to nucleophilic displacement at C-5.



Scheme 16

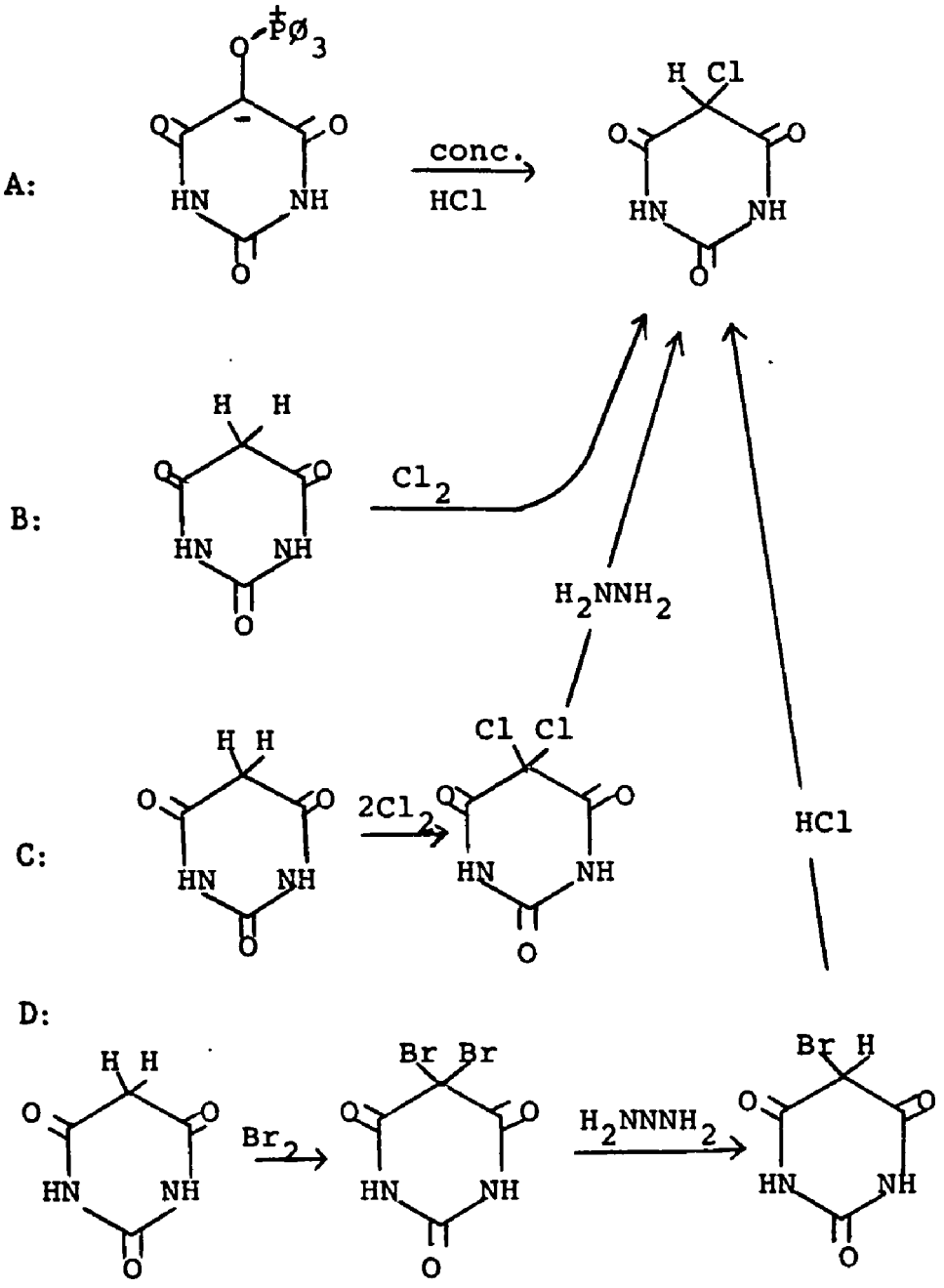


23

Ar =

The versatility of the adduct as a precursor of 5-substituted barbituric acids is exemplified in the formation of 5-chlorobarbituric acid (Scheme 17, Pathway A). Previous preparations of 5-chlorobarbituric acid have included one step [1:1 combination of barbituric acid and chlorine[115] (Pathway B)] and multistep syntheses [chlorination of barbituric acid to the dichloro derivative, followed by reduction[116] (Pathway C); bromination of barbituric acid to the dibromo derivative, reduction to the monobromo, followed by displacement[77] (Pathway D)].

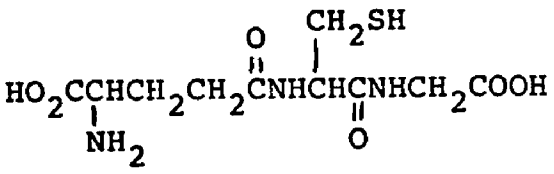
Scheme 17



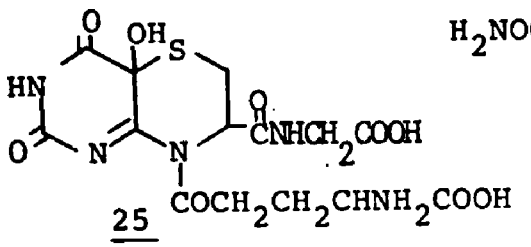
Yields of the chlorobarbituric acid averaged 22% by the hydrochloric acid decomposition of the alloxan adduct discovered in this study. This method avoids the difficulties of the older methods in that the handling of chlorine gas is not required and the manipulatory problems of the multistep synthesis are avoided.

D. Some Comments on the Alloxan-Glutathione Condensation Product

One of two glutathione-specific clinical analyses involves the production, in situ, of an unknown chromophore from alloxan and glutathione.[19,118,119] The ultraviolet maximum at 305 nm is the only physical datum obtained for the characterization of this material and this datum is the source of the material's name, "compound 305." Interest in the structure of this chromophore not only stems from its utility in clinical analyses of glutathione 24, but also elucidation of the structure may contribute to the understanding of the cytotoxic action of alloxan towards pancreatic β-cells. Of the two structures proposed for "compound 305," structure 25 proposed by Lazarow[120] closely resembles the material prepared from cysteine and the triphenylphosphine alloxan adduct obtained in this research.



24



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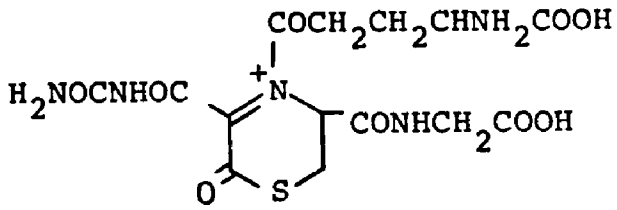


26

Structure 26 was proposed by Resnik and Wolff.[19]

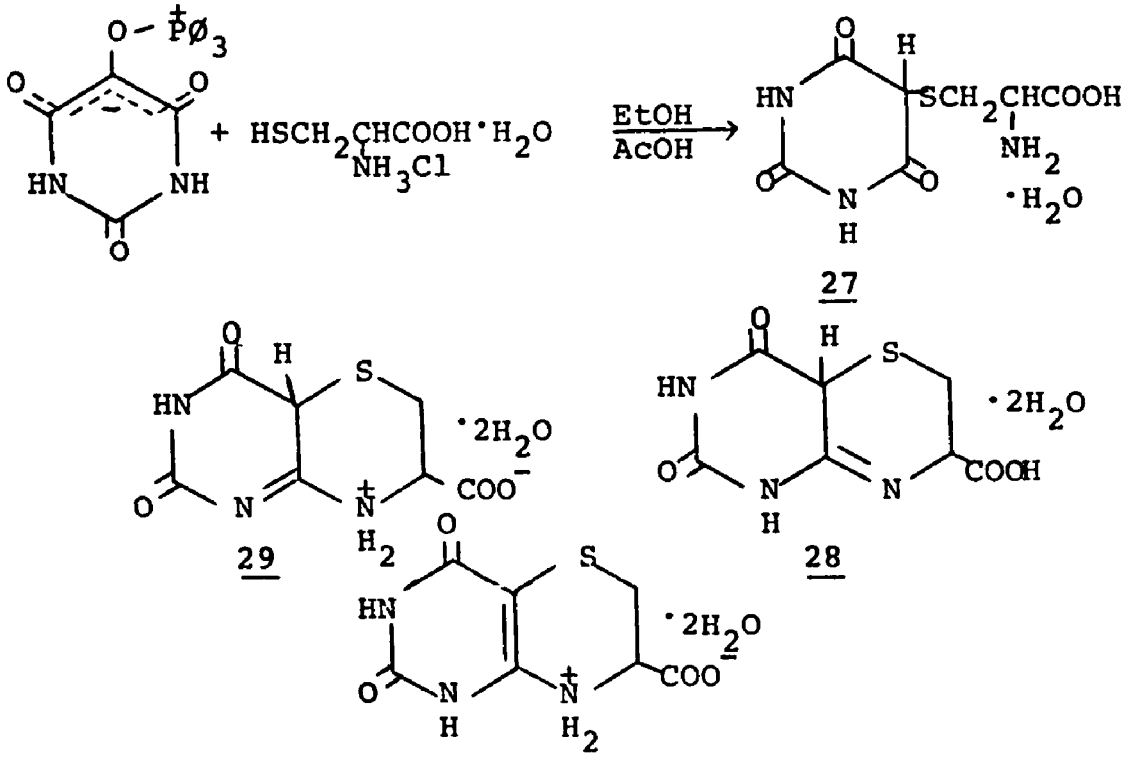
Though it has been implied that the structure of the material prepared in this dissertation is known, there were some initial uncertainties. The combustion analysis was in accord with any of the four structures in Scheme 18. The S-barbituryl cysteine 27 could be eliminated since the product failed to give a positive Strecker degradation test.

The infrared spectrum supports structures (29) and (30) because it has a very strong absorption at approximately 1600 cm^{-1} for carboxylate and a very broad band for O-H and N-H stretching. The nuclear magnetic resonance spectrum supports structure (30). The absorption at δ 9.90 ppm integrates for two protons and is the signal for the two acidic pyrimidine protons. The broad signal at δ 9.01-8.15 ppm also integrates for two protons and corresponds to the two protons on the quarternary nitrogen of the thiazine ring. The quarternization of the nitrogen prevents or slows proton exchange and thus the broadening of the absorption is interpreted as due to coupling with the protons on the alpha carbon.[121] Likewise, the proton on the alpha carbon is coupled with the two protons on nitrogen and the two protons on the adjacent carbon.[121] The weak absorption of this proton on the alpha carbon appeared

as a nondistinct multiplet, whereas the methylene protons absorbed as an apparent doublet.

The ultraviolet maximum at 257 nm (log ε = 3.22) corresponds closely to the maxima observed for several uracil derivatives.[122,123] Thus, structure (30) is most likely correct for the product of the condensation of cysteine and the triphenylphosphine alloxan adduct. Since structure 30 has an ultraviolet maximum at 257 nm and Lazarow's structure 25 is very similar to structure 30, then structure 25 proposed for "compound 305" is probably <u>not</u> the chromophore produced from alloxan and glutathione.

Scheme 18



CONCLUSIONS

In general, it is possible to obtain derivatives of alloxan which retain the requisite structural features that would enhance the diabetogenicity of the derivatives. The aromatic anils are readily formed in glacial acetic acid from alloxan monohydrate and the anhydride is not necessary. If the aryl ring is sufficiently activated by electron donating groups and the position para to the amine is not blocked with a substituent then the alloxan will condense at this ring position. Reaction ensues even if there are bulky substituents ortho to the site of substitution.

The triarylphosphine alloxan adducts are symmetrical zwitterionic molecules. The mechanism of the formation of these adducts in ethanol appears to be a complex stepwise process. The overall transformation involves alloxan monohydrate undergoing conversion to the 5-ethoxy-5-hydroxybarbituric acid which slowly yields alloxan anhydride, the immediate precursor for the adducts.

The reactions of the adducts occur readily in acidic media. When aqueous solutions are used, oxidation by molecular oxygen leads to alloxantin. However, when molecular oxygen was excluded, (in ethanol-benzene solutions or in HBr solutions), the dialuric acid was produced. When a nucleophile other than water is present

such as mercaptans and chloride, then the corresponding barbituryl sulfides and 5-chlorobarbituric acid are isolated. This constitutes a new method for the preparation of this class of sulfides and for 5-chlorobarbituric acid.

EXPERIMENTAL SECTION

Melting points were determined in capillary tubes using a Mel-Temp apparatus or a Thomas Hoover Unimelt apparatus and were uncorrected. Ultraviolet spectra were recorded on a Perkin-Elmer 402 spectrophotometer and infrared spectra were taken on 1 to 2% KBr disks on a Perkin-Elmer 257 or on a Beckman IR-33 instrument. ^{1}H nuclear magnetic resonance spectra were determined on a Hitachi Perkin-Elmer R-20A high resolution spectrometer using tetramethylsilane as the internal standard. Data are reported in the order δ (multiplicity, number of protons, assignment, coupling constants). Microanalyses were performed by Robertson Laboratories, Florham Park, New Jersey.

5-p-Diethylaminophenyldialuric Acid.

Equimolar amounts of alloxan monohydrate (1.00 g, 6.25 mmol), and N,N-diethylaniline (0.91 g, 6.25 mmol), were dissolved in 15 ml of glacial acetic acid. Shortly after the solution was heated to reflux, it turned from a very light yellow to a black color. After heating and stirring overnight no solid had precipited. The solution was evaporated to virtual dryness and the thick purple mass was dissolved in dioxane. A purple solid was liberated upon addition of water, 1.78 g (98%), mp 205-206°C dec. This solid was doubly recrystallized from ethyl acetate and singly recrystallized from ethyl acetate-benzene to give 0.53 g (29%) of a white solid with a slight purple tint, mp 209-210°C, dec. (lit.[72] mp 210-212°C dec.), ir (KBr) ν 3600, 3530, 3420, 3360, 3230, 3140 cm^{-1} (carbonyl str), 1620 cm^{-1} (aromatic C=C str); nmr (DMSO-d_6) δ 11.32 (broad, 2, $(-CONH-)_2CO$; 6.91 (q, 4, aromatic H), 6.31 (broad, 1, -O-H), 3.35 (overlapping quartets, 4, $-N(CH_2CH_3)_2$), 1.03 ppm (t, 6, $-N(CH_2CH_3)_2$).

5-p-Aminophenyldialuric Acid

A mixed solvent prepared from 5 ml of glacial acetic acid and 5 ml of water was employed to dissolve 1.00 g (6.25 mmol) of alloxan monohydrate. While 0.58 g (6.25 mmol) of aniline was slowly added to the alloxan solution, the medium turned from colorless to deep orange. The reaction mixture was then stirred for 2.5 hours and the precipitate which had formed was filtered and washed with glacial acetic acid, mp 252-253°C, dec. (lit.[71] mp 248°C, dec.), 0.98 g (67%), ir (KBr) ν 3430, 3450, 3225, 3120 cm^{-1} (N-H and O-H str), 1770 and 1705 cm^{-1} (carbonyl str), 1605 cm^{-1} (aromatic C=C str); nmr (DMSO-d_6) δ 11.20 (broad, 2, $(CONH)_2CO$), 7.14, 7.00, 6.62, 6.48 centered on 6.81 (q, 4, aromatic H, J = 8.4 Hz), 6.35 (broad, 1, -OH), 5.30 ppm (broad, 2, $-NH_2$).

5-p-Dimethylaminophenyldialuric Acid

This dialuric acid derivative was readily prepared by refluxing and stirring a solution of 1.00 g (6.25 mmol) of alloxan monohydrate and 0.76 g (6.25 mmol) of N,N-dimethylaniline for 0.5 hr. Upon cooling the medium, a solid was deposited which was identified as a dialuric acid derivative 1.18 g (72%), mp 232-3°C, dec. (lit.[71] mp 230°C, dec.), ir (KBr) ν 3600, 3460, 3410, 3220, 3100 cm^{-1} (N-H and O-H str), 2920, 2900, 2860,

2820, cm^{-1} (CH_3 sym and asym str and def), 1720 (sh), 1710, 1695 (sh) cm^{-1} (carbonyl str), 1615 cm^{-1} (aromatic C=C str); nmr (DMSO-d_6) δ 11.41 (broad, 2, $(CONH)_2CO$), 6.99 (q, 4, aromatic H), 6.39 (s, 1, -O-H), 2.89 ppm (s, 6, $-N(CH_3)_2$).

5-(3-methoxy-4-aminophenyl)dialuric Acid

This procedure is a modification of the method of Clark-Lewis and Moody.[76] A colorless solution of 1.00 g (6.25 mmol) of alloxan monohydrate in 5 ml of glacial acetic acid and 5 ml of water turned a deep dark purple when 0.77 g (6.25 mmol) of o-anisidine was added. The solution slowly yielded a solid, which was filtered from the medium after three hours of stirring at room temperature. The purplish solid was washed with glacial acetic acid and then with benzene. After recrystallization from dioxane-benzene the solid was still slightly purple, 1.20 g (77%), mp 235-236°C, dec. (lit.[76] mp 231-232°C, dec. or lit.[72] mp 240-242°C, dec.), ir (KBr) ν 3620, 3490, 3440 (sh), 3390, 3310 (sh), 3290 cm^{-1} (N-H and O-H str), 1750-1670 cm^{-1} (carbonyl str), 1605 cm^{-1} (aromatic C=C str); nmr (DMSO-d_6) δ 11.39 (broad,2, $(CONH)_2CO$), 7.00 (s, 1, H_6), 6.67 (s, 2, H_2 and H_5), 6.48 (broad, 1, -O-H), 4.10 (broad, 2, $-NH_2$), 3.75 ppm (s, 3, $-OCH_3$).

1,2-Bis (p-(5-dialuryl)anilino)ethane

The reaction mixture was prepared from 2.00 g (12.5 mmol) of alloxan monohydrate and 1.33 g (6.25 mmol) of 1,2-dianilinoethane (Wantzlick's Reagent for aldehydes) in 25 ml of glacial acetic acid. The mixture was stirred and heated for a half hour and then stirred at room temperature overnight. The next day the reaction mixture was filtered and the solid washed twice with glacial acetic acid, absolute ethanol and benzene in 10 ml portions. The impurities were then removed with absolute methanol extraction for 24 hours in a Soxhlet apparatus. The yield was 1.15 g (39%) of a gray solid, mp 255°C dec., ir (KBr) ν 3500-2700 cm^{-1} (H bonding), 3260, 3220 (N-H str), 1750-1680 centered on 1715 cm^{-1} (carbonyl str); nmr (DMSO-d_6) δ 11.43 (s, 4, 2 -CO-N<u>H</u>-CO-N<u>H</u>-CO-), 7.27, 7.11, 6.70, 6.54 (q, 8, aromatic H, J - 9.6 Hz), 7.4-6.9 (broad, 2, O-<u>H</u>), 6.33 (s, ½, CH_3-O-<u>H</u>), 6.3-5.6 (broad, 2, N-<u>H</u>), 3.38 (s, 1½, H-O-$C\underline{H}_3$), 3.3-3.1 ppm (t - two non-identical triplets, 4, -$C\underline{H}_2$-$C\underline{H}_2$-).

<u>Anal</u>. Calcd for $C_{22}H_{20}N_6O_8 \cdot \frac{1}{2}CH_3OH$: C, 52.73; H, 4.33; N, 16.47.

Found: C, 52.81; H, 4.56; N, 16.36.

5-(4-Amino-2,5-diethoxyphenyl)dialuric Acid

To insure that impurities would remain in solution and to preserve the fluidity of the solution, 50 ml of glacial acetic acid was used in the condensation of 1.13 g (6.25 mmol) of 2,5-diethoxyaniline and 1.00 g (6.25 mmol) alloxan monohydrate. The gray precipitate was allowed to accumulate for one and a half hours at room temperature with magnetic stirring and was then collected by suction filtration and washed twice with 10 ml of glacial acetic acid to give 1.75 g (85%) of product, mp 225-226°C dec., ir (KBr) 3450, 3400, 3320, 3230, 3120 cm^{-1} (N-H and O-H str), 2980, 2940 and 2900 cm^{-1} (CH_3 asymmetric and symmetric str and def), 1730, 1705 cm^{-1} and shoulders (carbonyls); nmr (DMSO-d_6) δ 11.20 (broad), s, 2, 2NH), 7.07 (s, 2, O-H + one aromatic H), 6.24 (s, 1, aromatic H), 5.15 (broad, 2, NH_2), 3.82 (two non-identical quartets, 4, $-OCH_2CH_3$), 1.25 ppm (two non-identical triplets, 6, $-OCH_2CH_3$).

Anal. Calcd for $C_{14}H_{17}N_3O_6$: C, 52.01; H, 5.30; N, 13.00.

Found: C, 51.85; H, 5.49; N, 12.83.

5-(4-Amino-2,5-dimethoxyphenyl)dialuric Acid

A precipitate formed immediately when a solution of 1.00 g (6.25 mmol) of alloxan monohydrate, 0.96 g (6.25 mmol) of 2,5-dimethoxyaniline and 20 ml of glacial acetic acid was stirred. After one half hour the solid was suction filtered and washed with two 10 ml portions of glacial acetic acid. Drying at 100°C in vacuo overnight gave a gray solid, mp 267-270°C dec., 1.52 g (83%), ir(KBr) 3390, 3310, 3210, 3130 (NH and OH str), 3020-2800 (H bonding), 1735 (sh), 1715 (sh), 1695 cm^{-1} (carbonyl str); nmr (DMSO-d_6) δ 11.24 (s, 2, -CONHCONHCO-), 7.20 (s, 1, O-H), 7.09 (s, 1, aromatic H, H_3), 6.30 (s, 1, aromatic H, H_6), 5.25-4.60 (mound, 2, $-NH_2$), 3.75 (s, 3, 5-OCH_3), 3.52 ppm (s, 3, 2-OCH_3).

Anal. Calcd for $C_{12}H_{13}N_3O_6$: C, 48.81; H, 4.44; N, 14.23.

Found: C, 48.60; H, 4.53; N, 14.10.

5,6-dimethyldioxindole-3-carboxyureide

This material was obtained by refluxing for fifteen minutes a solution of 1.14 g (9.38 mmol) of 3,4-dimethylaniline, 1.50 g (9.38 mmol) of alloxan monohydrate and 10 ml of glacial acetic acid. The precipitate that formed upon cooling the reaction medium was filtered, dissolved in a minimum amount of methanol (the solution

turned red), followed by the addition of small amounts of benzene and glacial acetic acid. By allowing the resultant solution to stand for about one half hour, a red precipitate formed. Gravity filtration followed by evaporation of the solvents gave a cream colored solid which was collected by suction filtration and washed with water. After drying in vacuo overnight at 70°C. gave 0.43 g ((17%), mp 206-7°C dec., (lit.[68] mp 204°C, ir KBr) 3375-3230 cm^{-1} (NH and OH bonding), 1730, 1690, and 1680 (sh) cm^{-1} (carbonyl str); nmr (DMSO-d_6) δ 10.40 (s, 1, NH), 9.27 (s, 1, NH), 7.46 (broad singlet, 3, NH and OH), 7.10 (s, 1, aromatic H), 6.66 (s, 1, aromatic H), 2.18 (s, 3, $-CH_3$), 2.15 ppm (s, 3, $-CH_3$).

Anal. Calcd for $C_{12}H_{13}N_3O_3$: C, 54.75; H, 4.98; N, 15.96.

Found: C, 54.60; H, 5.12; N, 15.95.

5-p-Methylphenylaminobarbituric Acid.

An oil was obtained by heating 0.67 g (6.25 mmol) of p-toluidine, 1.00 g (6.25 mmol) of alloxan monohydrate, and 5 ml of glacial acetic acid on a hot water bath for a maximum of five minutes. Immediately after termination of heating, the solvent was removed in vacuo. The oil was crystallized into small red needles by dissolving the oil into dioxane and precipitating the

product with benzene. Repeating the crystallization procedure gave a red solid with mp 242°C dec. (lit[68] mp 256°C dec), yield 0.25 g (18%); ir (KBr) 3205, 3110, 2820 (NH and OH str), 1745, 1705 and 1685 cm^{-1} (carbonyl str); nmr (DMSO-d_y) δ 11.50 (broad, 2, NH), 6.96 (q, 4, aromatic H), 2.30 ppm (s, 3, $-CH_3$).

Anal. Calcd for $C_{11}H_9N_3O_3$: C, 57.14; H, 3.92. Found: C, 57.04; H, 4.11.

5-(2,4-Dichlorophenyl)iminobarbituric Acid

The condensation was effected by stirring and refluxing 1.01 g (6.25 mmol) of 2,4-dichloroaniline, 1.00 g (6.25 mmol) of alloxan monohydrate and 13 ml of glacial acetic acid for approximately 40 minutes. The reflux period was terminated by cooling of the reaction medium to precipitate a red shiny solid. The precipitate was collected via suction filtration and washed twice with 10 ml portions of benzene and 10 ml portions of absolute ethanol. The yield was 0.72 g (40%) mp 251-253°C dec., ir (KBr) 3220, 3100, 2850 (NH and OH str); 1755, 1720, 1705 (sh), 1690, 1680 (sh) cm^{-1} (carbonyl str); nmr (DMSO-d_6) δ 11.82 and 11.69 (mounds, 2, NH), 7.61 (d, 1, aromatic H, J = 2.4 Hz), 7.38 (q, 1, aromatic H, J = 2.4 Hz and J = 8.4 Hz), 6.89 ppm (d, 1, aromatic H, J = 9.0 Hz).

Anal. Calcd for $C_{10}H_5Cl_2N_3O_3$: C, 41.99; H, 1.76; N, 14.69.

Found: C, 41.78; H, 1.95; N, 14.49.

5-p-Bromophenyliminobarbituric Acid Quarterhydrate

A reflux period of one half hour with magnetic stirring was sufficient to effect the formation of the anil from 1.00 g (6.25 mmol) of alloxan monohydrate and 1.08 g (6.25 mmol) of p-bromoaniline in 10 ml of glacial acetic acid. The isolation of the product was accomplished by evaporation of 5 ml of the solvent, followed by collection of the solid by suction filtration. The solid was washed with two 25 ml portions of benzene and two 25 ml portions of hexane. The purplish red solid weighed 1.20 g (64%) and had mp 224-5°C dec. After air drying the sample overnight the melting point increased to 232-233°C dec. After drying overnight in vacuo the anil had mp 234°C dec.; ir (KBr) 3200, 3100, 2840 (N-H and O-H str); 1760, 1730 (sh) 1710 (sh), 1690, 1670 (sh) cm^{-1} (carbonyl str); nmr (DMSO-d_6): δ 11.8-11.1 (broad, 2, -CONHCONHCO-), 7.59, 7.44 (d, 2, aromatic H, J = 9.0 Hz), 6.88, 6.73 (d, 2, aromatic H, J = 9.0 Hz).

Anal. Calcd for $C_{10}H_6BrN_3O_3 \cdot \frac{1}{4}H_2O$: C, 39.92; H, 2.16; N, 13.97.

Found: C, 39.87; H, 2.19; N, 13.80.

5-p-Hydroxyphenyliminobarbituric Acid Quarterhydrate

The anil was prepared via a ten minute reflux of 1.00 g (6.25 mmol) of alloxan monohydrate and 0.68 g (6.25 mmol) of p-hydroxyaniline in a magnetically stirred medium of 10 ml of glacial acetic acid. Cooling the solution prompted the precipitation of the product. The dark red solid was removed from the reaction medium by suction filtration and impurities were washed away with two washings of 5 ml of cold glacial acetic acid, three washings with 10 ml of benzene and two washings with 20 ml of hexanes; yield, 1.40 g (94%), mp >300°C dec; ir (KBr) ν 3380 cm^{-1} (OH str), 3160 and 3080 cm^{-1} (NH str), 1745, 1730 (sh), 1700 (sh), 1675 cm^{-1} (carbonyl str); nmr (DMSO-d_6) δ 11.50-10.00 (broad, 3, NH and OH), 7.10, 6.96 (d, 2, aromatic H, J = 8.4 Hz), 6.82, 6.68 ppm (d, 2, aromatic H, J = 8.4 Hz).

Anal: Calcd for $C_{10}H_7N_3O_4 \cdot \frac{1}{4}H_2O$: C, 50.53; H, 3.18; N, 17.68.

Found: C, 50.27; H, 3.09; N, 17.40.

p-Bromophenyliminobarbituric Acid using Boric Acid and DMF

This preparation closely parallels a method used for the preparation of alloxazines.[92] The condensation was affected by heating and stirring 1.00 g (6.25 mmol) of alloxan monohydrate and 1.08 g (6.25 mmol) of p-bromoaniline in the presence of 0.78 g (12.5 mmol) of of boric acid with 15 ml of N,N-dimethylformamide (DMF) as the solvent. The reaction was heated for two hours and then allowed to stir overnight at room temperature. The solution was rotary evaporated to 5 ml and the boric acid was precipitated with the addition of 25 ml of chloroform. The boric acid was filtered and the filtrate rotary evaporated to 5 ml and the boric acid was precipitated with the addition of 25 ml of chloroform. The boric acid was filtered and the filtrate rotary evaporated to dryness to give an oil. Addition of 30 ml of chloroform induced crystallization and filtration gave a solid. The solid was recrystallized by dissolving it in 6 ml of acetone and then adding 15 ml benzene and 25 ml petroleum ether (60-110°C) to reprecipitate 0.90 g (49%) of the solid, mp 233°C, dec. The ir and nmr spectra matched those of the anil prepared in glacial acetic acid.

Anal. Calcd for $C_{10}H_6BrN_3O_3 \cdot H_2O$: C, 38.24; H, 2.54; N, 13.37. Found: C, 38.19; H, 2.40; N, 13.45.

Attempted Preparation of Alloxan Anils with Boric Acid/DMF as a Catalyst

Attempted Preparation of 5-p-Chlorophenyliminobarbituric Acid

This reaction appeared to be initiated spontaneously since the solution turned red after 1.00 g (6.25 mmol) of alloxan monohydrate, 0.79 g (6.25 mmol) of p-chloroaniline, 0.78 g (12.5 mmol) of boric acid in 15 ml of N,N-dimethylformamide (DMF) were mixed. This medium was allowed to stir for thirteen hours. Since no solid had precipitated, the DMF was removed in vacuo to give a paste-like mass. Approximately 25 ml of acetone was used to dissolve any product and a white solid (boric acid), mp 130-165°C, was left behind (ir displayed intense -O-H absorption). Subsequent treatment of the acetone filtrate with petroleum ether (60-110°C) gave an oil. Further attempts to solidify this oil were unsuccessful.

Attempted Preparations of 5-(3,4-Dichlorophenylimino)-barbituric Acid and 5-p-Methoxyphenyliminobarbituric Acid

The same general procedure outlined above was employed in the attempted preparation of the two titled anils and this procedure was also unsuccessful. In

each case boric acid was removed, but there remained a non-crystalline semi-solid mass which did not and would not solidify.

Triphenylphosphine-Alloxan Adduct

In 200 ml of absolute ethanol, 10.00 g (6.25 mmol) of alloxan monohydrate and 16.40 g (6.25 mmol) of triphenyl phosphine were heated at 70±5°C with magnetic stirring. The reaction was allowed to proceed at this temperature overnight. The reaction was cooled and the yellow precipitate filtered to give a 24.80 g (98%) yield of the product, mp 200-1°C, ir (KBr) 3130, 2940 and 2750 cm^{01} (NH and OH str, broad), 1675, 1650 and 1600 cm^{-1} (carbonyl str, broad), 1438 and 997 cm^{-1} (Aryl-p str), 1190 (sh), 1180, 1165 (sh) cm^{-1} (P-O str), 1122 and 1110 (sh) cm^{-1} (aromatic in plane deformation mode); nmr (DMSO-d_6) δ 9.36 (s, 2, -CO-NH-CO-NH-CO-), 8.3-7.6 ppm (m, 15, Aromatic H).

Anal. Calcd for $C_{22}H_{17}N_2O_4P$: C, 65.34; H, 4.24; N, 6.93. Found: C, 65.11; H, 4.24; N, 6.93.

Tri-p-tolylphosphine-Alloxan Adduct

During a half hour reflux, 0.16 g (1.0 mmol) of alloxan monohydrate and 0.30 g (1.0 mmol) of tri-p-tolylphosphine in 10 ml of absolute ethanol were magnetically stirred to give a yellow precipitate. Longer reaction times redissolved the product and none of the desired adduct was obtained from the reaction medium. The yellow solid was filtered to give 0.40 g (90%) of the desired adduct, mp 189-190°C dec., ir (KBr) 3110, 3010, 2960, 2810, 2755 cm^{-1} (O-H and N-H str, broad), 1690, 1660 and 1590 cm^{-1} (carbonyl and C=C str), 1443 cm^{-1} (Aryl-P str), 1178, 1175 cm^{-1} (P-O str), 1117, 1110 cm^{-1} (aromatic in plane deformation mode); nmr (DMSO-d_6) δ 9.33 (s, broad,2, N-H), 8.07-7.30 (m, 12, aromatic H), 2.09 ppm (s, 9, $-CH_3$).

Anal. Calcd for $C_{25}H_{23}N_2O_4P \cdot 1/2H_2O$: C, 65.93; H, 5.31; N, 6.15. Found: C, 66.21; H, 5.21; N, 6.03.

Tri-m-tolylphosphine-Alloxan Adduct

In 10 ml of absolute ethanol 0.16 g (1.0 mmol) of alloxan monohydrate and 0.30 g (1.0 mmol) of tri-m-tolylphosphine were heated for one-half hour with magnetic stirring. The precipitate was suction filtered to give 0.31 g (69% of a yellow solid, mp 180-181°C dec., ir (KBr) 3150, 2950 and 2760 cm^{-1} (N-H and O-H str, broad), 1655, 1610 and 1590 cm^{-1} (carbonyl and O-C double bond str), 1440 and 995 cm^{-1} (Aryl-P str), 1185, 1170 (sh) cm^{-1} (P-O str), and 1120, 1110 (sh) cm^{-1} (aromatic in plane deformation mode); nmr (DMSO-d_6) δ 9.33 (s, 2, N-H), 7.98, 7.73 7.66 (m, 12, aromatic H), 2.39 ppm (s, 9, $-CH_3$).

Anal. Calcd for $C_{25}H_{23}N_2O_4P$: C, 67.26; H, 5.19; N, 6.27. Found: C, 66.98; H, 5.23; N, 6.21.

Tris-(p-methoxyphenyl)phosphine-alloxan Adduct

The product was obtained as an insoluble precipitate in 10 ml of absolute ethanol after two hours reflux of the combination of 0.16 g (1.0 mmol) alloxan monohydrate and 0.35 g (1.0 mmol) of tris-(p-methoxyphenyl) phosphine. The white precipitate was suction filtered from the reaction medium to give a 0.47 g (95%) yield, mp 183-184°C dec; ir (KBr) 3110, 2950 and 2810 cm^{-1} (N-H and O-H str, broad),

1700, 1655 and 1590 cm^{-1} (carbonyl and C=C str, broad), 1440 cm^{-1} (Aryl-P str) 1180, 1172 cm^{-1} (P-O str), and 1120, 1110 (sh) cm^{-1} (aromatic in plane deformation mode); nmr (DMSO-d_6) δ 9.32 (s, broad, 2, N-H); 8.09, 7.94, 7.88, 7.73 (doublet of doublets, 6 aromatic H ortho to phosphorus, $J_{^{31}P-^{1}H}$ = 12.6 Hz, $J_{o(^{1}H-^{1}H)}$ = 9.0 Hz), 7.37, 7.31, 7.22, 7.16 (doublet of doublets, 6, aromatic H meta to phosphorus, $J_{m(^{1}H-^{1}H)}$ = 3.0 Hz and $J_{o(^{1}H-^{1}H)}$ = 9.0 Hz); 3.91 ppm (s, 9, O-CH_3).

Anal. Calcd for $C_{25}H_{23}N_2O_7P \cdot 1/2H_2O$: C, 59.64; H, 4.80; N, 5.56. Found: C, 59.97; H, 4.53; N, 5.62.

Reaction of Triphenyl Phosphite and Alloxan Monohydrate in Absolute Ethanol

The materials, 1.00 gm (6.25 mmol) of alloxan monohydrate and 1.94 gm (6.25 mmol) of triphenyl phosphite, were heated to reflux and magnetically stirred for one hour. During that time a solid formed which was filtered, mp 214-216°C dec.; 0.60 gm was obtained (53%). The material was recrystallized from H_2O to give a solid which had mp 235-6°C dec. This solid was dried at 100°C in vacuo for two days to give mp 238-9°C dec. Mixed mp with alloxantin monohydrate was undepressed. The ir of the material was identical to the ir of alloxantin.

Reaction of Triphenylphosphite and Alloxan Anhydride in Tetrahydrofuran

In 10 ml of tetrahydrofuran (dried over dri-Na), 1.09 g (3.52 mmol) of triphenylphosphite and 9.50 g (3.52 mmol) of alloxan anhydride were stirred for three days at room temperature. The solid was collected on filter paper and after drying at 100°C *in vacuo* for 15 hours there was obtained 0.37 g, mp 228-231°C dec. The ir of this sample was identical with the ir of alloxantin. The filtrate was evaporated to dryness and the solid residue was stirred in chloroform to dissolve the triphenylphosphite and triphenylphosphate. Recrystallization from water, filtration of the solid followed by two 5 ml washings with absolute ethanol gave 0.18 g mp 241°C dec. The ir of this material was identical with the ir of alloxantin; total yield, 0.55 g (97%); uv (H_2O, ph = ~4 or 5) λ_{max} = 274 nm (log ε = 3.70); nmr (DMSO-d_6) δ 11.39 (s, 4, N-H); 6.77 (broad, 2, O-H), 3.45 ppm (broad, H_2O from sample and air).

Anal. Calcd. for $C_8H_6N_4O_8 \cdot 3H_2O$: C, 29.64; H, H, 3.73; N, 17.28. Found: C, 29.88; H, 3.41; N, 17.09.

Dialuric Acid Demihydrate

This material was prepared by the method of Hill.[108] The reduction of 5.00 g (31.2 mmol) of alloxan monohydrate was accomplished by refluxing with 8.78 g (38.9 mmol) of stannous chloride dihydrate in a solution of 17.5 ml of conc. HCl and 10.5 ml of water. The reaction was refluxed for a half hour longer than was required to effect solution and then the reaction was chilled to precipitate the dialuric acid. After drying at 100°C in vacuo, 2.83 g (60%), ir (KBr) 3500-2700 cm^{-1} (H-bonding), 1710, 1650, 1630 (sh) cm^{-1} (carbonyl str).

Anal. Calcd for $C_4H_4N_2O_4 \cdot 1/2H_2O$: C, 31.83; H, 3.29; N, 18.30. Found: C, 31.60; H, 2.98; N, 18.43.

When 0.75 g (4.94 mmol) of the dialuric acid demihydrate and 0.75 g (4.69 mmol) of alloxan monohydrate were heated in 5 ml of water, 1.04 g (71%) of alloxantin was obtained after recrystallization from water. mp 240-241°C dec., mixed mp undepressed, the ir was identical to the ir of alloxantin.

Alloxantin

Alloxantin was synthesized by reducing 5.00 g (31.25 mmol) of alloxan monohydrate with 15.50 g (6.35 mmol) of sodium thiosulfate pentahydrate in 50 ml of water at room temperature. The reaction medium was kept acidic by adding approximately 5 ml of conc. HCl over a period of one hour. At the end of one hour the reaction was cooled in an ice bath to obtain 4.00 g of the white solid product. Recrystallization from a large volume of water and then drying in vacuo at 100°C gave the half hydrate, mp 241°C dec. 3.20 g (70%), ir (KBr) ν 3560 and 3520 cm^{-1} (N-H str, broad), 3500-2700 cm^{-1} (O-H and H bonding), 1750-1670 and 1610-1580 cm^{-1} (carbonyl str), nmr (DMSO-d_6) δ 11.39 (s, 4, N-H), 6.94-6.00 (broad, 2, O-H), 4.80-3.28 ppm (broad, 4, 2 H_2O, due to moisture picked by the DMSO); uv (DMSO) λ_{max} = 277 nm (log ε = 3.88), uv (H_2O, pH = $\sim$4 or 5) λ_{max} = 274 nm (log ε = 3.72).

Anal. Calcd for $C_8H_6N_4O_8 \cdot 1/2H_2O$: C, 32.55; H, 2.39; N, 18.98. Found: C, 32.78; H, 2.42; N, 18.69.

Amulinic Acid (Tetramethyl Alloxantine) Quarter Hydrate

An acidic medium of 60 ml of conc HCl and 60 ml of water was used to dissolve 25.00 g (0.129 mol) of caffeine. The reaction temperature was kept below 40°C (near 30°C) while the caffeine was oxidized[1,2] by adding 10.00 g (0.0794 mol) of finely powdered potassium chlorate. The oxidant was added in small portions of approximately 0.25 g over four hours while stirring the mixture. During this period of oxidation, no 8-chlorocaffeine precipitated out as described by Biltz[109] and Fischer[110]. After all of the potassium chlorate had been added, the solution was cooled in an ice bath to precipitate apocaffeine and isoapocaffeine and air was passed through the solution for three hours to remove excess chlorine. The solution was filtered to give 4.2 g (15%) of apocaffeine and isoapocaffeine, mp 151°C (lit.[111] mp 154-5°C dec).

Anal. Calcd for $C_7H_7N_3O_5 \cdot 1/4\ H_2O$: C, 38.63; H, 3.47. Found: C, 38.75; H, 3.45.

The filtrate was again chilled in an ice bath and an ice cold solution of 13.50 g (0.060 mol) of stannous chloride dihydrate, 10 ml of conc HCl and 10 ml of water was added to the filtrate as a single portion. The reaction was held at approximately 0°C for three hours and then stored in the refrigerator

for ten hours. Filtration gave 10.9 g of a white solid and concentration and refiltration gave an additional 0.5 g, total yield 11.4 g (50%), mp 218°C dec (lit.[109] mp 245°C dec), ir (KBr) 3480 cm^{-1} (strong OH str), 2955 and 2900 cm^{-1} (CH_3 C-H str), 1760-1620 cm^{-1} (carbonyl str); nmr (DMSO-d_6) δ 6.29 (broad, 2, O-H), 3.16 ppm (s, 12, N-CH_3).

Anal. Calcd for $C_{12}H_{14}N_4O_8 \cdot 1/2\ H_2O$: C, 41.03; H, 4.30; N, 15.95. Found: C, 41.09; H, 4.09; N, 16.06.

1,3-Dimethylalloxan

The oxidation[109] of 3.00 g (8.55 mmol) of amulinic acid (tetramethylalloxantin) half hydrate was accomplished by heating the amulinic acid on a steam bath in 10 ml of water while conc nitric acid was added dropwise. The heterogeneous solution was swirled as a reddish brown gas of nitrogen oxides was given off. After about 1-2 ml of nitric acid had been added, the solution turned homogeneous. The addition of nitric acid was curtailed at this point and the solution was heated for several minutes on the steam bath to ensure complete oxidation of the amulinic acid to 1,3-dimethylalloxan. The solution was then rotary evaporated to dryness during which the solid slowly turned yellow. After heating this solid on a hot water bath for two hours at a pressure of about 40 torr the yellow product weighed 2.70 g (93%), mp 260-1°C dec (lit.[110] mp 252-255°C dec.), ir (KBr) ν 3510, 3410-3350 cm^{-1} (O-H str), 2950, 2920, 2880 cm^{-1} (CH_3 C-H str), 1765, 1720 (sh), 1690 (sh), 1665, 1640 (sh) cm^{-1} (carbonyl str); nmr (acetone-d_6) δ 3.37 ppm (s, N-$C\underline{H}_3$); nmr (DMSO-d_6) δ 3.29 and 3.18 ppm (s, ratio of 2.3:1, N-$C\underline{H}_3$).

Anal. Calcd for $C_6H_6N_2O_4 1/4\ H_2O$: C, 41.27; H, 3.76; N, 16.04. Found: C, 41.51; H, 3.60; N, 16.24.

Triphenylphosphine 1,3-Dimethylalloxan Adduct

After heating and magnetically stirring a solution of 0.50 g (2.94 mmol) of 1,3-dimethylalloxan and 0.77 g (2.94 mmol) of triphenylphosphine in 15 ml of chloroform for 0.5 hour, the reaction was stirred at room temperature for three hours. Most of the solid was dissolved at this point and the small amount of residue was gravity filtered. The clear solution was rotary evaporated to dryness to give a yellow solid which was purified by dissolving the solid in 10 ml of chloroform and precipitating the material with petroleum ether (60-110°C); mp 150-151°C dec., 0.90 g (71%); ir (KBr) 3080 and 3050 cm^{-1} (aromatic C-H str), 2980, 2940, 2900, 2880 cm^{-1} (methyl C-H str), 1680 cm^{-1} (N-CO-N carbonyl str), 1630-1600 cm^{-1} (-C=C-C- polarized carbonyl str), 1440 and 995 cm^{-1} (Aryl-P str), 1117, 1110 (sh) cm^{-1} (aromatic in plane deformation mode), 1069, 1055 (sh), 1045 (sh) cm^{-1} (P-O str); nmr ($CDCl_3$) δ 8.30, 8.27, 8.18, 8.15, 8.085, 8.055, 7.97, 7.93 (a doublet of doublets of doublets, 6, aromatic H ortho to P, $J_{^{31}P-^{1}H}$ = aromatic H ortho to P, $J_{^{31}P-^{1}H}$=12.9 Hz, $J_{^{1}H-^{1}H}$ (ortho hydrogens) = 7.2 Hz, $J_{^{1}H-^{1}H}$ (meta hydrogens) =2.1 Hz), 7.88-7.50 (m, 9, aromatic H meta and para to P), 3.19 ppm (s, 6, N-CH_3).

Anal. Calcd for $C_{25}H_{21}N_2O_4P \cdot \frac{1}{4}H_2O$: C, 65.98; H, 4.96; N, 6.41. Found: C, 65.60; H, 4.98; N, 6.48.

Tri-p-Tolylphosphine Dimethyl Alloxan Adduct

The adduct was prepared by stirring a mixture of 0.17 g (1.00 mmol) of dimethylalloxan and 0.30 g (1.00 mmol) of tri-p-tolylphosphine in 10 ml of benzene. When all of the dimethylalloxan had dissolved (45 minutes) half of the benzene was removed on the rotary evaporator. Upon cooling the solution in an ice bath, a precipitate formed which was filtered to give 0.36 g (77%) of a white amorphous solid, mp 140°C dec., ir (KBr) ν 3020 cm^{-1} (aromatic C-H str), 2940-2900 cm^{-1} (aliphatic C-H str), 1690 cm^{-1} (small intensity, carbonyl str), 1625-1585 cm^{-1} ($(-\overset{O}{\overset{\|}{C}}-)_2\overset{-}{C}-O-\overset{+}{P}Ar_3$ carbonyl str), 1120, 1110 (sh) cm^{-1} (aromatic in plane deformation mode), 1070, 1060 (sh) cm^{-1} (P-O str), nmr ($CDCl_3$) δ 8.04, 7.91, 7.83, 7.70 [doublet of doublets, 6, aromatic C-H ortho to the positive phosphorus, $J_{^{31}P-^{1}H}$ = 12.6 Hz; $J_{^{1}H-^{1}H}$ (ortho hydrogens) = 7.8 Hz , 7.48, 7.42, 7.33, 7.28 doublet of doublets, 6, aromatic H meta to the positive phosphorus, $J_{^{1}H-^{1}H}$ (ortho hydrogens) = 8.4 Hz, $J_{^{1}H-^{1}H}$ (meta hydrogens) - 3.0 Hz], 3.15 (s, 6, N-CH_3), 2.44 ppm (s, 9, aromatic CH_3).

Anal. Calcd for $C_{27}H_{27}N_2O_4P$: C, 68.34; H, 5.74; N, 5.91. Found: C, 68.64; H, 5.76; N,5.87.

The Triphenylphosphine Alloxan Adduct from Triphenylphosphine and Alloxantin

The triphenylphosphine alloxan adduct was prepared from 0.50 g (1.64 mmol) of alloxantin monohydrate and 0.43 g (1.64 mmol) of triphenylphosphine in 20 ml of absolute ethanol. The reaction mixture was heated for five minutes and then stirred for 1.5 hours to give a yellow precipitate, mp 192-3°C. The yellow precipitate was stirred with 200 ml of water for 20 minutes and then filtered and washed with about 150 ml of hot water followed by three 10 ml portions of absolute ethanol. mp 195-6°C dec. Additionally, the yellow solid was stirred in DMF for 4 hours. The solid was filtered, washed with absolute ethanol, ether and benzene to give 0.53 g (80%) of the triphenylphosphine alloxan adduct, mp 200-1°C dec., mixed mp 200-1°C dec., the ir of this material was identical with the ir of the triphenylphosphine alloxan adduct.

5-Ethoxy-5-hydroxybarbituric Acid from Ethanol and Alloxan Monohydrate

5-Ethoxy-5-hydroxybarbituric acid was prepared in 200 ml of absolute ethanol from 5.00 g (31.25 mmol) of alloxan monohydrate by azeotropically distilling the water formed into a Soxhlet extractor containing anhydrous magnesium sulfate. Within an hour the solution had turned a bright yellow. The solution was then rotary evaporated to approximately 25-30 ml and a solution of 5 ml of petroleum ether (60-110°C) and 30 ml of benzene was added to precipitate 5.70 g (97%) of 5-ethoxy-5-hydroxybarbituric acid, the mp, ir, and nmr are identical to those of the 5-ethoxy-5-hydroxybarbituric acid formed from alloxan monohydrate and anhydrous magnesium sulfate in absolute ethanol.

Alloxan Monohydrate and Anhydrous Magnesium Sulfate in Absolute Ethanol

After 10 minutes of refluxing 5.00 g (31.3 mmol) of alloxan monohydrate, 1.00 g (8.3 mmol) of $MgSO_4$ in 100 ml absolute ethanol, the solution turned yellow. Twelve hours later the solution was cooled and the $MgSO_4$ was filtered to give a clear yellow solution. This solution was rotary evaporated to approximately 20 ml and 4.12 g of 5-ethoxy-5-hydroxybarbituric acid precipitated

after 25 ml of chloroform and 25 ml of benzene was added, mp 135-145°C, foaming and shrinkage, 254-255°C, dec., (lit.[91] mp 125-235°C, foaming, 252-254°C, dec., ir (KBr) ν 3320-3100 cm^{-1} (OH and N-H str), 1750-1690 cm^{-1} (carbonyl str), 1440 (m), 1425 (m), 1385 (s), 1320 (w), 1250 (s), 1195 (s), 1165 (m), 1115 (m), 1055 (s), 1030 (m), 1005 (w), 900 (w), 825-765 (s), and 640 (s), nmr (DMSO-d_6), δ 11.33 (s, broad, 2, 2N-H), 7.40 (s, 1, O-H), 3.56 (q, 2, $-OCH_2CH_3$, J = 7.2 Hz), 1.06 ppm (t, 3, $-O-CH_2CH_3$, J = 7.2 Hz.

Alloxan Anhydride in Ethanol

Alloxan anhydride, 0.40 g (2.91 mmol), and 10 ml of absolute ethanol was heated at reflux for 2.5 hours. The cooled solution was injected into a Carle Basic Gas Chromatograph to determine if any diethyl ether was formed. No diethyl ether was detected when 10 µl and 40 µl of solution was used. The uv of a dilute solution of alloxan anhydride in ethanol matched the uv of 5-ethoxy-5-hydroxybarbituric acid.

Alloxantin in Ethanol

After a two hour reflux of 0.40 g (1.24 mmol) of alloxantin dihydrate in 10 ml of absolute ethanol the solution was cooled and an attempt was made to detect diethyl ether on a Carle Basic Gas Chromatograph. There was no diethyl ether detected when 10 µl and 40 µl of solution were injected. The solution was evaporated to 5 ml and chloroform and benzene were added to precipitate a solid. After drying *in vacuo* for 20 hours the solid had mp 252°C, mixed mp with alloxan monohydrate, undepressed, the ir of the product is identical to the ir of alloxan monohydrate, 0.12 g (60% of the available alloxan from the alloxantin.

The Triphenylphosphine Alloxan Adduct from 5-Ethoxy-5-hydroxybarbituric Acid and Triphenylphosphine in Absolute Ethanol

A reflux period of four hours produced a yellow precipitate from 0.50 g (2.66 mmol) of 5-ethoxy-5-hydroxybarbituric acid and 0.70 g (2.66 mmol) of triphenylphosphine, mp 193-194°C, dec. The solid was stirring in DMF for the two hours to give the yellow solid back, mp 199-200°C, dec., mixed mp and the mp of an authentic sample of the triphenylphosphine alloxan adduct melted simultaneously with the sample obtained in this reaction, the infrared spectrum of the product

is identical to that of the triphenylphosphine alloxan adduct.

The Triphenylphosphine Alloxan Adduct from 5-Ethoxy-5-hydroxybarbituric Acid and Triphenylphosphine in Tetrahydrofuran

The solution was refluxed for 72 hours to give a yellow solid from 0.25 g (1.33 mmol) of 5-ethoxy-5-hydroxybarbituric acid and 0.35 g (1.33 mmol) of triphenylphosphine in 20 ml of THF dried over NaH. Filtration of the solid followed by washing with two 5 ml portions of absolute ethanol gave 0.20 g (37%), mp 200-201^{o}C, dec., mixed mp with an authentic sample of triphenylphosphine alloxan adduct, 200-201^{o}C, dec., the spectrum of the sample was identical with the infrared of an authentic sample of the triphenylphosphine alloxan adduct.

Alloxan Anhydride

A. Biltz's[91] method of sublimation of alloxan monohydrate to alloxan anhydride at approximately 0.5 torr and 220°C produced a yellow dense crystalline sublimate, mp 256-7°C, dec., (lit.[91] mp 256°C dec.). The sublimation lasted at least 24 hours and there was a large amount of decomposition of the non-volatilized alloxan monohydrate to a reddish mass; ir (KBr) ν 3330-3225, 3200-3010 (sh) cm^{-1} (N-H str), 1740-1690 cm^{-1} (carbonyl str).

B. By heating without subliming 1.00 g (5.32 mmol) of 5-ethoxy-5-hydroxybarbituric acid to 150°C for 6 hours at a pressure of approximately 0.5 torr, the unsublimed orange residue had mp 252-3°C, dec., 0.66 g (88%), the infrared spectrum of this compound is identical to that of alloxan anhydride obtained from Biltz's method.

C. A solution of 200 ml of tetrahydrofuran (dried over Na ribbon), and 1.00 g (5.32 mmol) of 5-ethoxy-5-hydroxybarbituric acid was refluxed for 32 hours. Any ethanol produced was absorbed by $CaCl_2$ in a Soxhlet extractor. The solution was chilled and evaporated to dryness to give a thick yellow oil. The oil was crystallized to a solid by stirring in methylene chloride. The light yellow powder was filtered and

dried *in vacuo* for 0.5 hour to yield 0.65 g (87%) of alloxan anhydride, mp 252-3°C dec., mixed mp undepressed, the ir of the product was identical with the ir of alloxan anhydride.

The Triphenylphosphine Alloxan Adduct from Alloxan Anhydride in Tetrahydrofuran

The reaction of 0.25 g (1.76 mmol) of alloxan anhydride and 0.46 g (1.76 mmol) of triphenylphosphine in THF (dried over NaH) occurred readily in one hour at ambient temperatures. The initial precipitate had mp 182-185°C, dec. The solid was purified by stirring it in 40 ml of DMF for 4 hours, filtering, and washing with three 10 ml portions of absolute ethanol, mp 200-201°C, dec. The mixed mp with authentic triphenylphosphine alloxan adduct was undepressed. The yield was 0.53 g (75%) and the infrared spectrum of this material was identical with that of the triphenylphosphine alloxan adduct.

The Reaction of the Triphenylphosphine Alloxan Adduct and p-Toluenesulfonic Acid in Ethanol-Benzene

The yellow color of 2.00 g (4.95 mmol) of the triphenylphosphine alloxan adduct was changed to white when the adduct was hydrolyzed during a one hour reflux with 0.98 g (4.95 mmol) of 96% p-toluenesulfonic acid monohydrate, 4 ml of absolute ethanol and 16 ml of benzene. After stirring the reaction at ambient temperature for another two hours, the solid was filtered to give 0.66 g, mp 272-4°C, dec. (200°C yellow, then the solid became more and more dark red until the decomposition point), ir (KBr) ν 3580, 3530, 3400-2800 cm^{-1} (NH and O-H str), 1760-1690 and 1660-1610 cm^{-1} (carbonyl str).

Evaporation of the filtrate and recrystallization twice from ethanol-water gave 1.26 g (92%) of triphenylphosphine oxide, mp 157-8°C, spectrally identical to the infrared spectrum published in the Sadtler Index.

The Reaction of Alloxan Triphenylphosphine Adduct and p-Toluenesolfonic Acid in Water.

The decomposition of 2.00 g (4.95 mmol) of the alloxan-triphenylphosphine adduct with 1.96 g (9.90 mmol) of 96% p-toluenesulfonic acid monohydrate in 20 ml of water was accomplished by stirring at room temperature for three hours and then refluxing for two and a half hours. The solid was filtered and the filtrate was concentrated to obtain the white solid product (alloxantin) upon filtration. This solid was washed three times with 10 ml portions of absolute ethanol to give 0.47 g, mp 238.5-239.5°C dec.; the ir was identical to the ir of alloxantin. The solid that was originally removed from the reaction medium before concentration was stirred in a solution of 0.4 g of KOH (to remove the p-toluenesolfonic acid) and 20 ml of absolute ethanol (to remove the triphenylphosphine oxide). The material that remained was filtered and dried, mp 237°C dec., 0.15 g, the ir (KBr) was identical to the ir of alloxantin, total yield, 0.62 g (78%); uv (DMSO) λ_{max} = 280 nm (log ε = 3.86); uv (H_2O, pH = ~4 or 5) λ_{max} = 274 nm (log ε = 3.66); nmr (DMSO-D_6) δ 11.39 (s, 4, N-H), 7.0-6.3 (broad, 2, O-H), 4.2-2.8 ppm (broad, 6, H_2O).

The Reaction of the Triphenylphosphine Alloxan Adduct at Approximately pH 7.4

A heterogenous solution composed of an aqueous layer, prepared from 2 drops of 10% NaOH in 100 ml of water, 25 ml of chloroform and 2.00 g (4.95 mmol) of the triphenylphosphine alloxan adduct was stirred for 40 hours at ambient temperature. The solid was filtered and washed with 30 ml of chloroform and 30 ml of absolute ethanol to give 1.84 g (92%) of a yellow solid mp 200-1°C dec., mixed mp with the triphenylphosphine alloxan adduct, 200-200.5°C dec., the ir of the sample is identical with the ir of the triphenylphosphine alloxan adduct.

The Reaction between Triphenylphosphine Alloxan Adduct and Dilute Aqueous Hydrobromic Acid-Dialuric Acid Preparation

A solution of 2.00 g (4.95 mmol) of the triphenylphosphine alloxan adduct and 1.5 ml of 35% hydrobromic acid, approximately 0.71 g (8.8 mmol) of hydrogen bromide in 20 ml of water was refluxed and stirred for 1.5 hours. While the solution was still warm it was filtered to remove some triphenylphosphine oxide. The solution was rotary evaporated to approximately 5 ml to precipitate more oxide. Addition of 20 ml of

absolute ethanol and filtration gave a second white solid. The second white solid was dried overnight *in vacuo* to give 0.18 g (24%), mp 232-3°C dec. A recrystallization of the solid from H_2O gave a compound with unique behavior on heating, 220°C reddish-yellow color change, 281°C dark red color change, 308°C dec. These are the same mp characteristics observed for dialuric acid. A Beilstein test for halogen was negative and the ir of the sample was identical to the ir of dialuric acid.

Anal. Calcd for $C_4H_4N_2O_4 \cdot 1/2H_2O$: C, 31.38; H, 3.29; N, 18.30. Found: C, 31.05; H, 3.17; N, 17.94.

The triphenylphosphine oxide was recrystallized by dissolving the crude material in absolute ethanol and precipitating the triphenylphosphine oxide by adding ice and water. After drying the sample *in vacuo* overnight, mp 159-160°C, 1.11 g (81%). The ir of the sample was identical to the ir of triphenylphosphine oxide in the Sadtler Index.

5-o-Chlorophenyl Barbirutyl Sulfide

Immediately after 2.00 g (4.95 mmol) of the triphenylphosphine-alloxan adduct and 0.71 g (4.95 mmol) of o-chlorothiophenol dissolved in a refluxing solution of 17 ml of glacial acetic acid and 25 ml of absolute ethanol, the heating was terminated and the reaction was allowed to cool to room temperature. It was stirred and stored at ambient temperatures overnight. A small amount of solid had formed and this was increased by evaporation of the solvent to about half of the original volume. Filtration gave an off-white amorphous solid, mp 252°C dec. This solid was recrystallized by dissolving in 20 ml of absolute ethanol and diluting with 10 ml of benzene followed by 30 ml of petroleum ether (60-110°) to give a white solid, 0.46 g (34%), mp 268-268.5°C dec., ir (KBr) ν 3160, 3260-2940 cm^{-1} acidic N-H and C-H str), 1720 (sh), 1710, 1700 (sh), 1695 (sh), 1635 cm^{-1} (carbonyl str); nmr (DMSO-d_6) δ 11.09 (broad, 1, $(-CO)_2-C\underline{H}-SAr$); 7.50-6.60 ppm (m, 6, 2 N-$\underline{H}$, aromatic $\underline{H}$), nmr (DMSO-d_6 and 2 drops D_2O) δ 7.50-6.70 (m, 4, aromatic H), 395 ppm (s, $\underline{H}_2O$).

Anal. Calcd for $C_{10}H_7N_2O_3ClS$: C, 44.37; H, 2.61; N, 10.35. Found: C, 44.20; H, 2.85; N, 10.38.

Reaction of Triphenylphosphine Alloxan Adduct and Concentrated Hydrochloric Acid: 5-Chlorobarbituric Acid

A solution of 1.00 g (2.48 mmol) of the triphenylphosphine alloxan adduct in 7 ml of conc HCl was heated and stirred for 0.5 hour. The solution was then cooled in an ice bath for 0.5 hour and filtered to give a white solid. Preparations of hot aqueous "solutions" of the white solid caused the separation of the triphenylphosphine oxide as an oil. The water layers, less the triphenylphosphine oxide oil, was then chilled and stirred with 25 ml of chloroform to extract more of the triphenylphosphine oxide. Separation of the two layers, and evaporation of the aqueous layer to dryness gave a white solid (now freed of triphenylphosphine oxide). The white solid was recrystallized by dissolving in absolute ethanol, filtering, concentrating the solution to a 10 ml volume and then diluting with 50 ml of benzene and 190 ml of petroleum ether (60-110°C) to give 0.15 g (22%), mp 200°C, tan, 250°C, orange, 305°C, dec., Beilstein test was positive; ir (KBr) ν 3160-2700 cm^{-1} (N-H and C-H str and H bonding), 1680 (sh), 1580 cm^{-1} (carbonyl str); ir (nujol) ν 3610 and 3550

cm^{-1} (N-H str), 3360-2700 cm^{-1} (H bonding), 1780 (sh), 1755, 1695, 1660 (sh), 1580 cm^{-1} (carbonyl str); uv (DMSO) λ_{max} = 274 nm (log ϵ = 3.82), uv (H_2O, pH 4 or 5) λ_{max} = 268 nm (log ϵ = 4.20).

Anal. Calcd for $C_4H_3ClN_2O_3 \cdot 1/6\ C_2H_6O \cdot 1/2\ H_2O$: C, 29.04; H, 2.81; N, 15.63. Found: C, 29.27; H, 2.82; N, 15.85.

The triphenylphosphine oxide was recrystallized by dissolving in absolute ethanol and adding water and ice. After drying at less than 1 torr overnight: 0.44 g (64%); mp 158-9°C. The ir (KBr) spectrum was identical to the ir of triphenylphosphine oxide in the Sadtler Index.

5-Barbituryl o-Carbomethoxyphenyl Sulfide

The sulfide was prepared from 2.00 g (4.95 mmol) of the triphenylphosphine alloxan adduct and 0.83 g (4.95 mmol) of methyl thiosalicylate in 20 ml of glacial acetic acid. The reaction was heated for 1.5 hours and then stirred at room temperature for 20 hours. The solution was filtered to give 0.82 g of a white solid which was recrystallized by dissolving in tetrahydrofuran and filtering the insoluble material and then precipitating the solid by adding an equal amount of benzene followed by petroleum ether

(60-110°C), 0.25 g (17%), mp 235°C dec., ir (KBr) 3300-2800 cm^{-1} (H bonding) 1720 (sh), 1700, 1650, 1600 (sh), 1580 cm^{-1} (carbonyl and C=C str), nmr (DMSO-d_6), δ 11.15 (broad, 2, $\frac{1}{2}$CO-NH-$\frac{1}{2}$CO), 8.01-7.00 (m, 4, aromatic C-H), 5.55 (broad, 5, 0.75 H_2O from hydrate, 1.25 H_2O from solvent and $\frac{1}{2}$CO$\frac{1}{2}$CH-SAr), 3.88 ppm (s, 3, $-CO_2CH_3$).

Anal. Calcd for $C_{12}H_{10}N_2O_5S \cdot 3/4\ H_2O$: C, 46.82; H, 3.77; N, 9.10. Found: C, 47.16; H, 3.56; N, 8.72.

5-o-Carboxyphenyl Barbituryl Sulfide

In a mixed solvent of 50 ml of absolute ethanol and 3 ml of glacial acetic acid, 2.00 g (4.95 mmol) of triphenylphosphine alloxan adduct and 0.76 g (4.95 mmol) of thiosalicylic acid were refluxed for two hours until the yellow color had dissipated. Cooling and evaporation to dryness gave a solid which was dissolved in 25 ml of absolute ethanol and diluted with 75 ml of benzene. Petroleum ether (60-110°C) was added in small portions (up to 25 ml) to give a white precipitate, mp 210°C (purple), 233°C (shrink), 247-8°C dec., 0.54 g (39%); ir (KBr) 3580-2500 cm^{-1} (acidic O-H, N-H and C-H str), 1730-1640 and 1590-1570 cm^{-1} (carbonyl str); nmr (DMSO-d_6) δ 11.12 (broad, 1, $(-CO)_2$-CH-SAr), 8.00-6.90 ppm (m, 8, 1/2 H_2O, 2N-H, COOH and aromatic H); nmr (DMSO-

d_6 and 2 drops D_2O) δ 8.00-6.90 (m, 4, aromatic H), 3.95 ppm (s, H_2O).

Anal. Calcd for $C_{11}H_8N_2O_8ClS \cdot \frac{1}{2}H_2O$: C, 45.68; H, 3.11, N, 9.68. Found: C, 45.96; H, 3.01; N, 9.73.

6,8-Dioxo-2,3,5,6,7,8-hexahydropyrimido[5,4-b][1,4] thiazine-3-carboxylic Acid Dihydrate

By refluxing 2.00 g (4.95 mmol) of the triphenylphosphine alloxan adduct, 0.87 g (4.95 mmol) of L-(+)-cysteine hydrochloride monohydrate, 7 ml of absolute ethanol and 5 ml of glacial acetic acid for fifteen minutes, the yellow heterogeneous suspension became a colorless heterogeneous suspension. The precipitate was collected by filtration after the reaction medium had been stirred for an additional 24 hours at ambient temperatures. The solid was recrystallized twice from water to give light brown needles, mp 230°C, started to darken, 248°C dec. (blacken); 0.42 g (32%); ir (KBr) ν 3500-2500 cm^{-1} (N-H and O-H str), 1700 cm^{-1} (carbonyl str), 1610-1570 cm^{-1} (carboxylate str); uv (H_2O, pH = 7.4), λ_{max} = 257 nm (log ε = 3.22); nmr (DMSO-d_6), δ 9.90 (broad singlet, 2, $(CONH)_2CO$), 9.01-8.15 (broad, 2, NH_2^+), 5.6-4.6 (broad, 6, $2H_2O$, and H_2O from DMSO-d_6 and the atmosphere), 3.85-3.65

(broad m, 1, -C<u>H</u>(NH_2^+)COOH), 2.9-2.6 ppm (broad d, 2, -S-C<u>H_2</u>-CH). Strecker degradation test: negative after 48 hours. Cysteine hydrochloride, glycine, L-threonine, D-serine and L-tryptophan gave positive results immediately.

<u>Anal</u>. Calcd for $C_7H_7N_3O_4S \cdot 2H_2O$: C, 31.70; H, 4.18; N, 15.84. Found: C, 31.82; H, 4.47; N, 15.66.

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