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THE NATURE OF AZOPYRIMIDINES AND THEIR METALLIZED DERIVATIVES

by

JAMES GEORGE JEFFREY

A Thesis Submitted in Partial Fulfillment
of the Requirements for the Degree of
DOCTOR OF PHILOSOPHY
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1954

TABLE OF CONTENTS

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Acknowledgment is made to the Canadian Foundation for the Advancement of Pharmacy for financial assistance.

INTRODUCTION

A number of azo compounds resembling polynuclear hydrocarbons in structure have been shown in the past to have both stimulating and inhibitory activity on tumor growth. Certain other azo compounds, such as Scarlet Red, also show cell-proliferating activity. Since the pyrimidine ring is a common constituent of natural products, this research was undertaken to prepare a number of azo compounds incorporating the pyrimidine ring in the hope that they would have desirable pharmacological activity, and, in particular, to find what effect they might show on tumor growth.

To test the possibility that removal of trace metals from tumor cells might have an effect on the growth of the tumor, azopyrimidines both capable and incapable of metal chelation have been synthesized. Toxicity to enzymes and fungi by metal chelating agents has been postulated as due to the removal of the essential metals (1,2,3). Certain metal chelates themselves have been shown to be active pharmacologically, for example, as antibacterial and antitubercular agents (4,5,6). Recent evidence has also indicated the high in vivo toxicity of metal chelates (7,8). Some activity might therefore be expected from the metal chelates of the azopyrimidines.

Since the structures of the known azopyrimidines have not been thoroughly characterized, an attempt has been

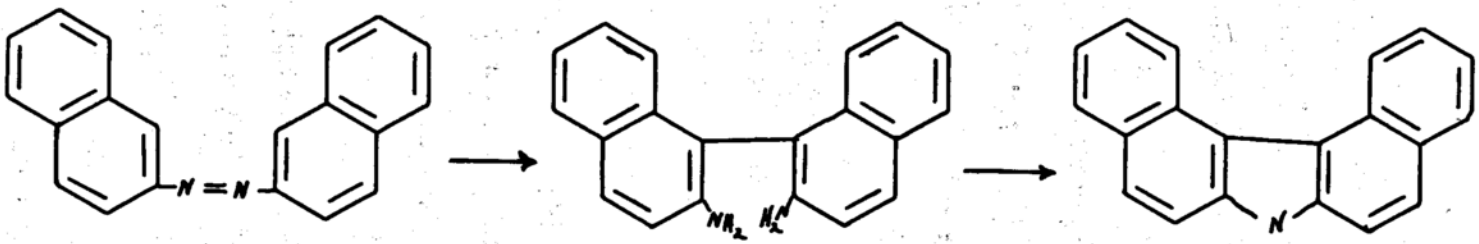
made to find more evidence regarding their exact nature. A similar situation existing with regard to the structure of the metal chelates of azo compounds, structural evidence has been furnished by a series of azonaphthols of a similar structure to the azopyrimidines. The azonaphthols were employed because of their greater solubilities and ease of purification.

HISTORICAL

Azo Compounds as Inhibitors of Tumor Growth

There have been several reports on the effects of azo compounds as inhibitors of tumor growth (9,10,11). Badger, Haddow, and coworkers (9) experimented with more than thirty azo dyes, paying particular attention to the azonaphthalenes. In attempting to interpret the results obtained with the azonaphthalenes they noted the marked contrast in the behavior of the 1,2' and 2,2' compounds. It had previously been pointed out (12) that azo substances may be reduced in the body to amines by way of the hydrazo compounds. When liver changes were found in mice treated with 2,2'-azonaphthalene (I), the fact that the hydrazonaphthalenes are susceptible to a benzidine type of rearrangement led to making tests with the compound which might thus be formed, i.e. 2,2'-diamino-1,1'-dinaphthyl (II). This compound induced liver changes with even greater facility than 2,2'-azonaphthalene, and the result was specially significant in view of the ease with which the diamino-dinaphthyl undergoes deamination to 3,4,5,6-dibenzcarbazole (III), which Boyland and Brues (13) had already shown to be capable of producing liver tumors, epitheliomas and sarcoma in mice. The relations between these compounds, with their inhibitory and carcinogenic activity, are shown in Table I.

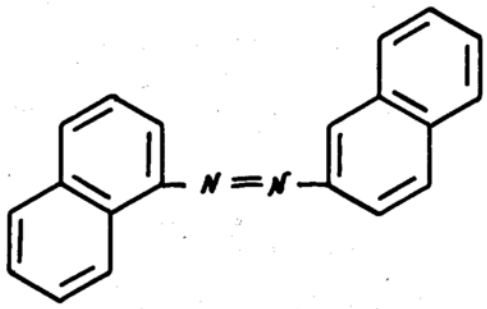
TABLE I



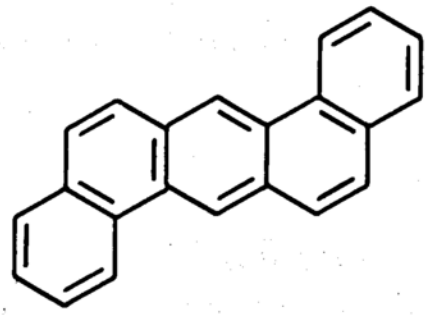
	I	II	III
Inhibitory activity	-	-	+
Tumor production	cholangioma hepatoma	cholangioma hepatoma	cholangioma hepatoma epithelioma sarcoma

Badger et al. proposed that, taken alone, these relations form a consistent scheme, compatible with and even supporting the suggestion that 2,2'-azonaphthalene is not inherently carcinogenic but acts by a transformation to 3,4,5,6-dibenzcarbazole. Conversion in the liver might account for the localization of tumor production in that organ, and the fact that the intermediate diamine is even more potent than the azo compound is also consistent (9).

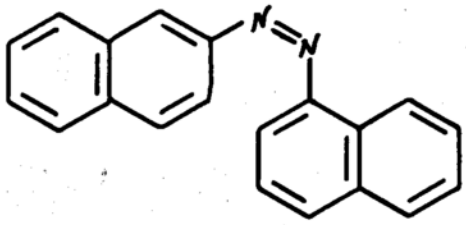
Friedman, Gofstein and Seligman (10) in reporting on their work on azo compounds for the inhibition of tumor growth drew attention to the suggestion of Haddow and coworkers that the unsymmetrical 1,2'-azonaphthalene (IV) bears a superficial resemblance to the carcinogen, 1,2,5,6-dibenzanthracene (V). Friedman and coworkers pointed out that the only forms in which the unsymmetrical azonaphthalene (IV) has features in common with the polynuclear carcinogenic hydrocarbons are the cis structures (VI) and (VII) which resemble benz-derivatives of both 1,2-benzanthracene and chrysene. The hydrocarbon 1,2,5,6-dibenzphenanthrene, suggested by formula (VII), was found to be an inhibitor of tumor growth. In order to explore further possible relationships between structure of azo compounds and their activity as inhibitors of tumor growth, these workers prepared about 25 azo compounds.



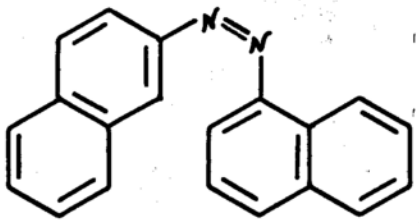
IV



V

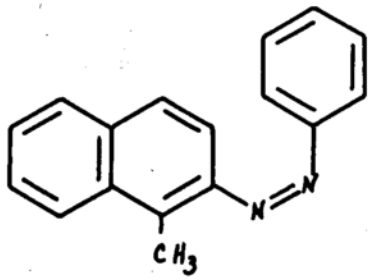


VI

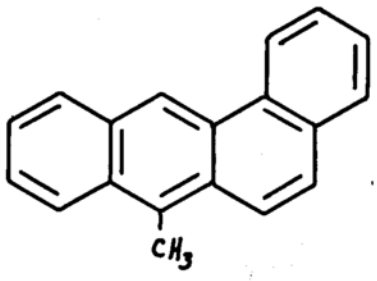


VII

When written in the hypothetical <u>cis</u> configuration, most of them bear a spatial resemblance to a carcinogenic polynuclear hydrocarbon. As an example, the <u>cis</u> structure is written for 2-benzeneazo-1-methyl-naphthalene (VIII) and may be compared with the potent carcinogen 10-methyl-1,2-benzanthracene (IX). These workers did not make the presumption as to the actual existence of these substances in the <u>cis</u> form but remarked that there was evidence that



VIII



IX

conversion of the trans to the cis form can be effected to a significant degree by exposure to ultraviolet light.

In contrast with the above reports on the inhibitory effects of certain azo dyes, p-dimethylamino-azobenzene (butter yellow) and numerous analogs have strong carcinogenic properties. Miller and Miller (14) in reporting on the production of liver tumors in rats by a series of aminoazo dyes, of which p-dimethylamino-azobenzene was the parent substance, made the hypothesis that these dyes become combined with certain liver proteins which are essential for the control of growth but not for the life of the cell. Assuming that such binding could interfere with normal protein formation, eventually cell generations might arise which would be completely lacking in one or more normal growth control proteins. Such cells could respond to continued nutrition only by continued growth and would be tumor cells.

Synthesis of Pyrimidines

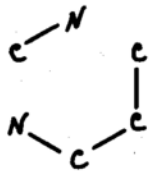
Before discussing the azo derivatives of pyrimidines it might be well to consider briefly certain phases of the chemistry of pyrimidines. Lythgoe in a review of pyrimidine and purine chemistry (15) indicated the importance of pyrimidines in natural constituents of living organisms, such as the nucleic acids, and cited several examples of pharmacologically active compounds containing the pyrimidine nucleus, including the barbiturates, the antibacterial sulfonamides, sulfadiazine and its 4-methyl and 4,6-dimethyl derivatives, and the thiouracil derivatives which have antithyroid activity.

A major part of our knowledge of the chemistry of the pyrimidines consists of synthetic methods. This term is intended to convey not only the methods by means of which the pyrimidine nucleus can be built up, but also the numerous transformations which substituent groups can undergo; much of this knowledge has been gained in the work on the various biologically important compounds. The main deficiencies in pyrimidine chemistry concern the simpler compounds; for example, pyrimidine itself has probably never been obtained in amounts of more than a gram, and its 5-hydroxy-derivative is unknown. It is probable that the behavior of pyrimidine compounds can be interpreted in terms of modern theories of tautomerism and resonance. The way in which substituents interact

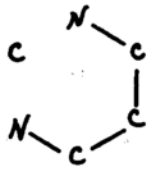
with each other and with the nucleus presents some unusual features, and in hydroxy- and amino-derivatives interesting structural problems arise (15).

The methods available for the formation of the pyrimidine nucleus can be classified into three main types (15), according to the distribution of nitrogen atoms in the two components used:

Type I

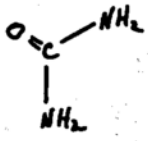


Type II

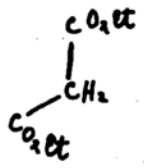


Type III

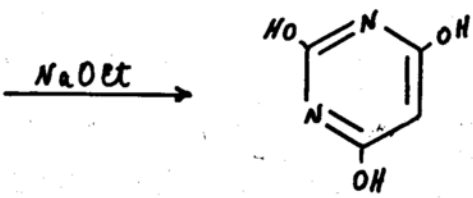
As an example of Type I may be cited the synthesis of barbituric acid (XII) from urea and diethyl malonate (XI):



I



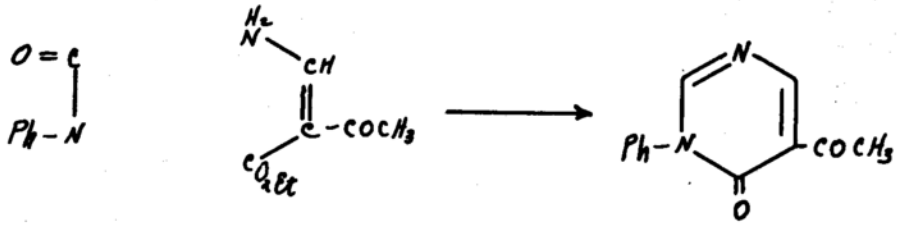
XI



XII

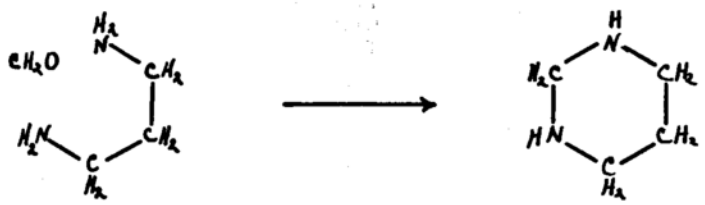
In the Type I synthesis, the urea may be replaced by thiourea, guanidine, and amidines, to produce rings substituted at the 2-position by sulfur, amine, and methyl (or hydrogen) respectively. The other component may be ethyl malonate, ethyl cyanoacetate, malononitrile, a β-diketone, a β-keto-ester, or an α-β-unsaturated ketone. This type of synthesis was, and remains, the most versatile of all the methods.

An example of Type II is the preparation of the derivative (XV) from phenyl isocyanate (XIII) and aminomethyleneacetoacetic ester (XIV); none of the methods of this type have achieved much practical importance.



XIII XIV XV

Type III is illustrated by the formation of hexahydropyrimidine (XVI) from formaldehyde and 1,3-diaminopropane; such syntheses also have had little practical value.



XVI

Chemistry of Pyrimidines

Three prominent features of pyrimidine chemistry are:

(i) In simple derivatives, containing halogen, alkyl, aryl, or nitro groups, but no hydroxy or amine groups, the nucleus has aromatic character, and behaves like that of pyridine.

(ii) Nuclear substituents vary in their behavior according to the position which they occupy. At position 5 the properties of a group can be loosely described as similar to those which it normally possesses when attached to an aromatic nucleus; at 2, 4, and 6 marked deviations from the normal behavior are observed. The contrast is parallel to that between β-substituted pyridines and their α- and γ-isomers.

Since the behavior at position 5 is of the kind

normally found in groups attached to aromatic nuclei, no special comment is needed concerning them. Groups at positions 2, 4, and 6 show, in general, an abnormal or reactive behavior which is due to resonance with or within the nucleus. There is one aspect of this reactive behavior which has very important practical consequences; the replaceability of groups such as Cl, OEt, and SEt, to mention only the more important, is used extensively in the preparation of those substituted pyrimidines which cannot be obtained directly by building up the pyrimidine nucleus. Chloro, alkoxy and alkylthio compounds therefore occupy a key position in preparative operations, for which they are well fitted by virtue of their ready accessibility. Chloro compounds are obtained by heating the corresponding hydroxy compounds with phosphorus oxychloride. Alkylthio derivatives are mainly of value in cases where the alkylthio group is present at position 2. Such compounds can be obtained in two ways; S-alkylthioureas can be condensed with a second component in a synthesis of Type I; or thiourea can be used, and the resulting 2-thiol compound afterwards subjected to S-alkylation. Both these methods are convenient, since Type I syntheses with thiourea or its S-alkyl compounds proceed very readily in comparison with those where the less basic urea is employed.

(iii) The aromatic behavior mentioned in (i) diminishes progressively as hydroxy or amine groups

are introduced into positions 2, 4, and 6. This effect is seen in uracil and barbituric acid, into which substituents are readily introduced at position 5 even by mild reagents such as diazonium compounds; simple pyrimidines such as those mentioned in (i) seem to be very resistant to electrophilic substitutions. As the simpler compounds are much less well known than the highly hydroxylated or aminated members, a rather distorted impression of pyrimidine chemistry has grown up, much as if the behavior of benzene were known only through the reactions of compounds like phloroglucinol. The groups which give rise to this atypical behavior (OH, SH, NH_2) have been termed, somewhat loosely, "tautomeric" substituents (15).

Hydroxypyrimidines bearing up to three hydroxyl groups at positions 2, 4, and 6 are well known; they include uracil, thymine, and barbituric acid. They cannot be considered as simple hydroxyl derivatives. They show no phenolic behavior. The "hydroxyl groups" are replaced by chlorine atoms on heating with phosphorus oxychloride. They are difficult to acylate, and their acyl derivatives are readily hydrolyzed. The action of alkylating agents on them varies with the compound and the reagent used.

5-Aminopyrimidine has been prepared but not examined closely. In the 4,5-diaminopyrimidines, only

the amino-group on position 5 behaves normally toward acylation. In pyrimidines bearing amino-groups at positions 2, 4, or 6, anomalous properties arise which justify doubts as to their structures. They are difficult to acylate; reactions with dithioformates which the 5-amino-group undergoes readily, fail completely. With nitrous acid, rather sluggish deamination occurs, apparently without the intervention of diazonium compounds. Deamination can also be effected by the action of hot acids (15).

Azopyrimidines

Early workers reporting on the formation of azo derivatives of the pyrimidines (16,17) stated that quite a number of pyrimidines do undergo coupling reactions with diazonium salts. However, most of these workers did not isolate the azo compounds, but merely stated that coupling had or had not taken place as indicated by the production or not of a coloration on contact with the diazonium salt.

A rather exhaustive study on azo derivatives of the pyrimidines was begun about 20 years ago by Bogert and Davidson at Columbia (18). The first paper, beyond notice to publish, covered a preliminary survey of the azo derivatives of the pyrimidines with particular reference to derivatives of uracil. These derivatives of uracil fall into 5 natural types, according to the nature

of the components entering into their formation:

1. The coupling of aromatic diazonium salts with pyrimidine phenols.

2. The coupling of diazopyrimidines with aromatic phenols.

3. The coupling of diazopyrimidines with pyrimidine phenols.

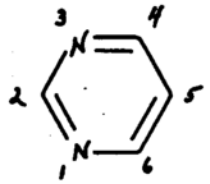
4. The condensation of aromatic hydrazines with 5-keto-6-hydroxy-5,6-dihydropyrimidines.

5. The condensation of hydrazinopyrimidines with 5-keto-6-hydroxy-5,6-dihydropyrimidines.

Among the compounds prepared by the above-named authors may be mentioned uracil-5-azo-β-naphthol (type 2) and uracil-5-azobenzene (type 4), though no melting point was reported for the latter compound. The authors believed they had also prepared azouracil-5,5' (type 5), but were unable to overcome certain difficulties in the analysis of the product so were unable to report its constitution with certainty. It is of interest to note that, of the compounds reported for which a melting point is stated, all decomposed on melting.

More recently, Polonovski and Pesson, reporting in the Bulletin of the Society of Chemistry (France) (19) carried on a series of coupling reactions with phenyldiazonium chloride and a number of pyrimidines. The products of the coupling reactions were isolated and the

structures proved. The pyrimidines they used included those with combinations of the following groups on one or more of the indicated positions of the pyrimidine nucleus (XVII).



XVII

OH on position 2, 4, 6

NH_2, or substituted NH_2, on position 2, 6

CH_3 on position 1, 2, 3, 4, 5, 6

SH on position 2

SCH_3 on position 2

SC_2H_5 on position 2.

The conclusions reached by those workers are as follows:

1. That when coupling of phenyldiazonium chloride does occur with pyrimidines, it always occurs at the C5 position.

2. That the coupling of phenyldiazonium chloride with a monohydroxy (or amino) pyrimidine derivative occurs only if the OH group (or NH_2) is on position 2, and, in this case, the mechanism is probably analogous to that of the phenols.

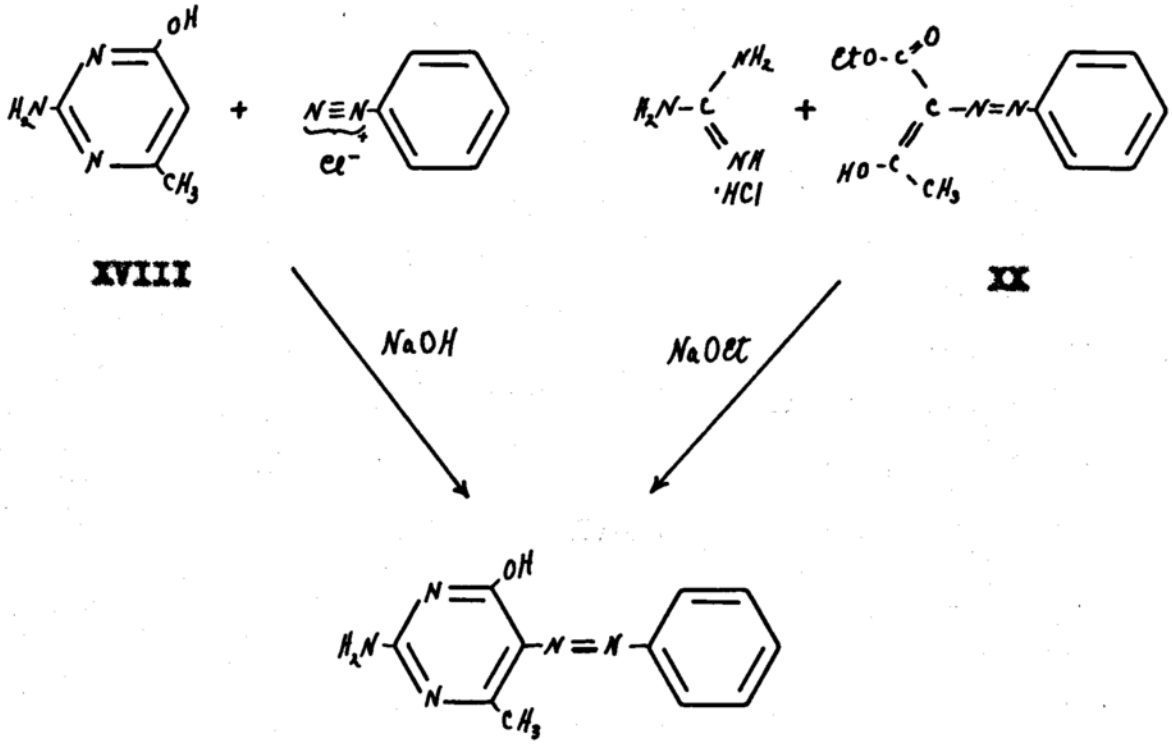
3. That, in the case of dihydroxy

derivatives (or amino-hydroxy derivatives):

(a) if one of the groups is on 2, the coupling is facilitated by the presence (on 4 or 6) of the second polar group;

(b) if the groups are on 4 and 6, coupling also occurs with a very good yield and the mechanism appears to compare with that of the coupling of compounds having an active methylene. Again, it is of interest that, of the compounds reported for which a melting point is stated, almost all decomposed on melting.

As an example of the work of Polonovski and Pesson, the compound, 2-amino-4-hydroxy-5-phenylazo-6-methyl-pyrimidine (XIX), may be mentioned. This azo compound was prepared by coupling phenyldiazonium chloride with 2-amino-4-hydroxy-6-methylpyrimidine (XVIII), and its structure proven by synthesis from guanidine hydrochloride and diazotized acetoacetic ester (XX) under the influence of sodium in absolute alcohol.



Metal Chelates of the Azo Dyes

A metal complex is an association of a metal with an organic molecule, there being one bond connecting the organic molecule to the metal. A metal chelate is distinguished from a complex by the fact that in a chelate an organic molecule is linked at two places to a single atom of metal, thus forming a ring structure (20).

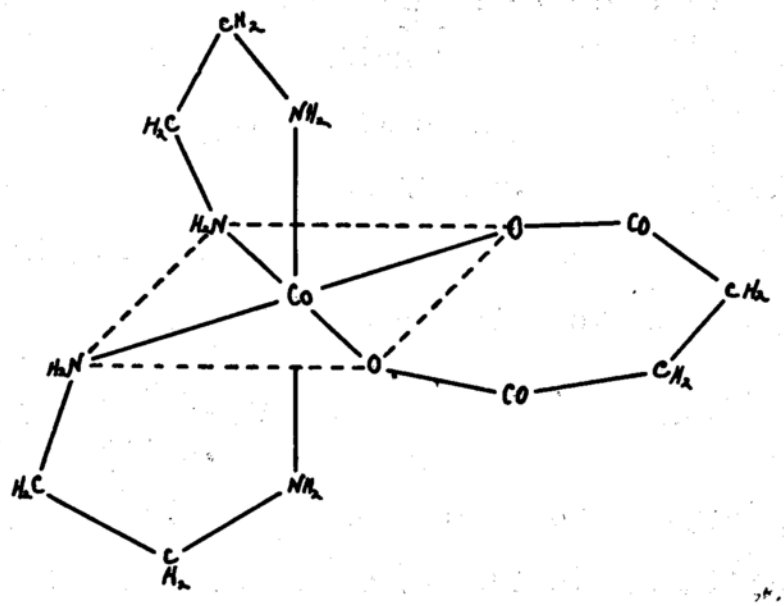
In a review article on chelate rings, Diehl (21) states that the term "chelate", proposed by Morgan (22) to

designate those cyclic structures which arise from the union of metallic atoms with organic and inorganic molecules, is derived from the Greek word chela, referring to the great claw of the lobster and other crustaceans, and is applicable to these ring systems because of the caliper-like character of the associating molecule. The formation of these rings may involve either primary or secondary valence. In subsequent papers Morgan used the expression "chelate rings" to cover all three types, that is, rings formed by two primary valences, by one primary and one secondary valence, or by two secondary valences. Primary or principal valence here is differentiated from secondary or coordinating valence in that the formation of the valence link in the former case involves the replacement of a hydrogen atom, while in the latter case no such replacement occurs. No implication is intended that a difference in the bonds exists once they are formed (21).

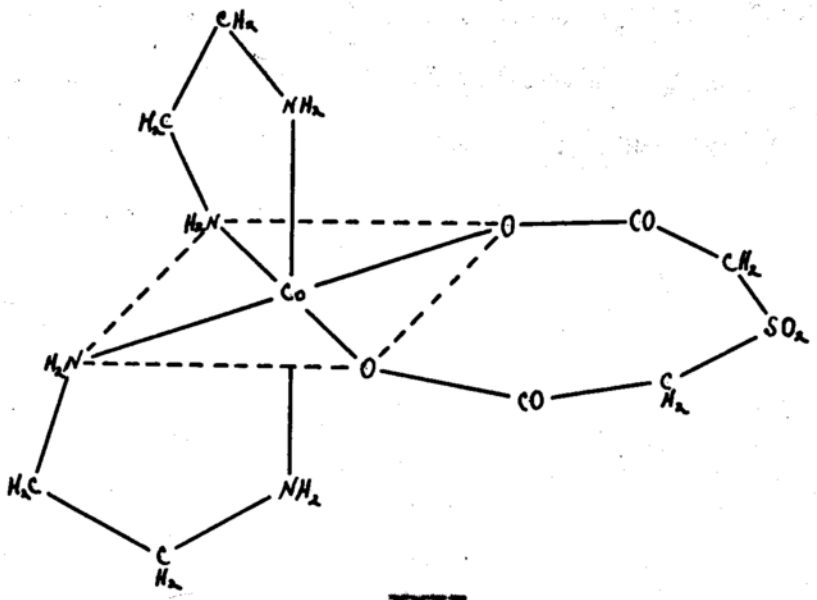
Evidence (23) has been shown that chelate rings are formed with ease when the conditions for the formation of heterocyclic five- or six-membered rings are present.

The size of the chelate ring is frequently a five- or six-atom ring (24). These are by far the most common sizes, occurring in chelates between various metals and ethylenediamine, diethylenetriamine, ortho-hydroxy acids, salicylic acid and its derivatives, β-diketones such as acetylacetone, 8-hydroxyazo compounds, 8-hydroxyquinoline, 1-hydroxyanthraquinone, and many other compounds.

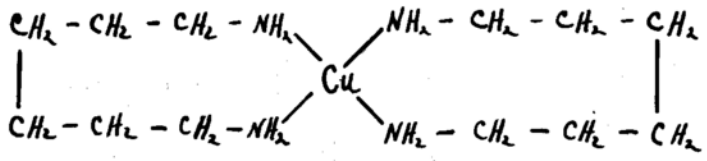
In general, the five-membered ring is more stable when the ring is entirely saturated, while six-membered rings are favored when one or more double bonds are present. However, rings containing four atoms or more than six, i.e., seven, eight, or nine atoms have been shown to exist. A seven-membered ring (25) prepared from β-dicarboxylic acids, such as succinic acid, can be represented by (XXI); an eight-membered ring (26) prepared from sulfonyldiacetic acid is represented by (XXII); a nine-membered ring (27) prepared from hexamethylenediamine and copper salts in alcoholic solution is represented by (XXIII).



XXI



XXII



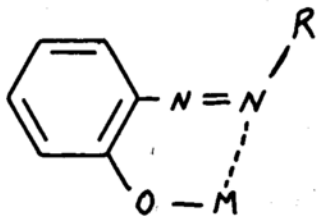
XXIII

With the discovery of compounds in which the metal atom was linked to the organic molecule through three and even four groups, Morgan devised for these compounds the names "tridentate", literally, three-toothed, and "quadridentate", four-toothed (21).

Among the various organic groups which may

unite with metals by the replacement of hydrogen, that is, function as acids by primary valence, the more common are the following: carboxyl, sulfonic, enolic hydroxyl, oxime, primary amine, and secondary amine. Included among the secondary valence groups which combine with metals by simple coordination or addition without the replacement of hydrogen are the following groups; primary amine, secondary amine, tertiary amine, cyclic tertiary amine, oxime, alcoholic hydroxyl, carbonyl, and thioether.

In the course of a study of the chelate rings formed in mordant dyeing, Morgan (28) showed that in the azo dyestuffs containing an ortho-hydroxy group, mordanting took place with the formation of chelate rings, the metal being attached by secondary valence to the azo group, as shown in (XXIV). Here "M" represents a metal atom replacing the hydrogen of the hydroxy group and is attached by secondary valence (shown dotted) to the azo group. Such ring formation cannot occur when the hydroxyl group is in the meta- or para-positions, and this is in accord



XXIV

with the long-established rule that such compounds are never dyestuffs (21).

More recently Elkins and Hunter (29) have found that the formation of cupric, nickel, and cobaltic compounds is characteristic of all ortho-monohydroxyazo compounds and that such formation occurs readily by the action of the metallic acetates on the azo compound. The salts correspond to the formulas R_2Cu, R_2Ni, and R_3Co, R being the molecule of the o-hydroxyazo compound minus one hydrogen atom. The salts are typical inner complexes (non-electrolytes); they are insoluble in water and polar solvents, but readily soluble in non-polar solvents. They are very stable, melting in the neighborhood of 200°C., without decomposition, to form deeply colored liquids. The existence of these compounds is interpreted as evidence that the geometrical arrangement of the groups attached to the azo group is trans.

Absorption spectra also indicate that the hydroxyl hydrogen atom of these o-hydroxyazo compounds is coordinated to the azo group (21).

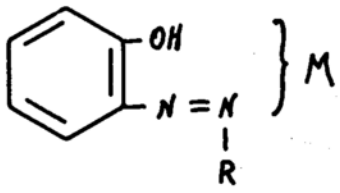
Azo dyes having two hydroxy groups ortho to the azo link (i.e. one hydroxy in each aromatic nucleus and in the ortho positions) are known to take up metals to form metal chelates (23). Grimmel (23) in reviewing metallized derivatives of dyes indicated types of compounds which have been used for formation of metal complexes. He

included the following types of azo dyes merely indicating the position of the metal (M) since the exact composition of a metal complex is dependent on the particular compound and conditions used. He was cautious about showing exact formulas since there is still so little known about metallized dyes.

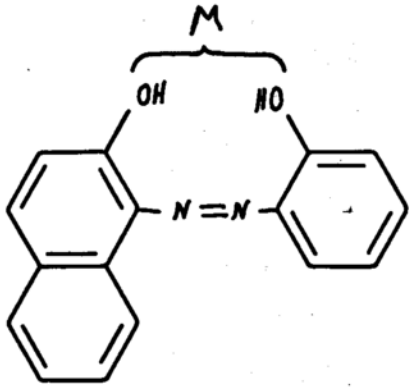
(a) o-Hydroxyazo dyes: (XXV).

(b) o-Amino-o'-hydroxyazo dyes and o,o'-dihydroxyazo dyes (without oxidation): (XXVI).

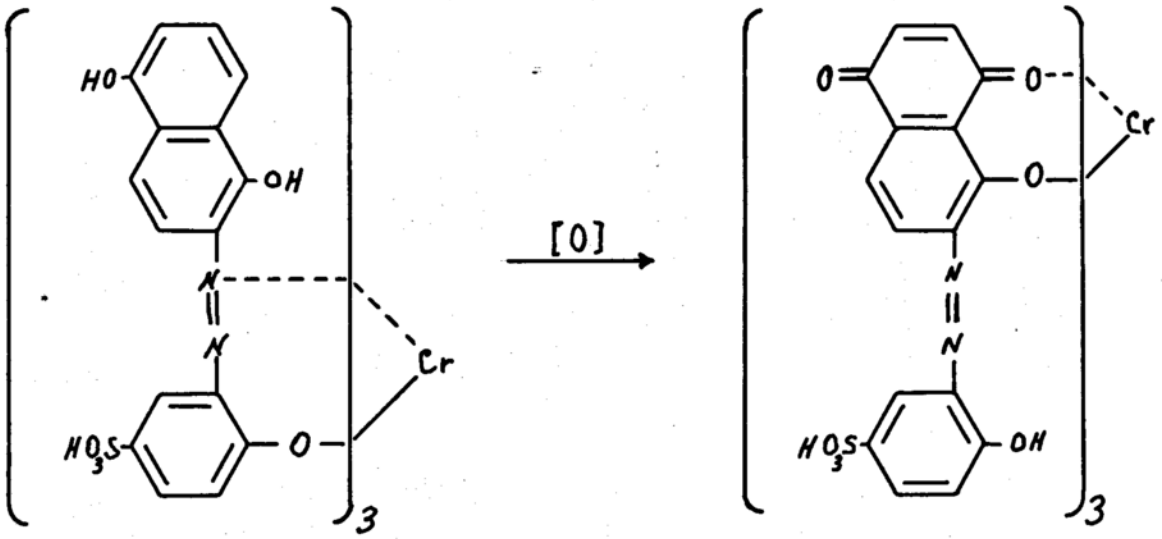
(c) o,o'-Dihydroxyazo dyes with oxidation (using a particular example to illustrate the change involved; the dye used is Diamond Black PV (XXVII), (XXVIII). The ratio of metal to organic dye is not fixed, and several distinct stages of this ratio have been found (23).)



XXV

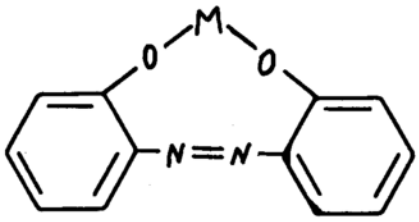


XXVI



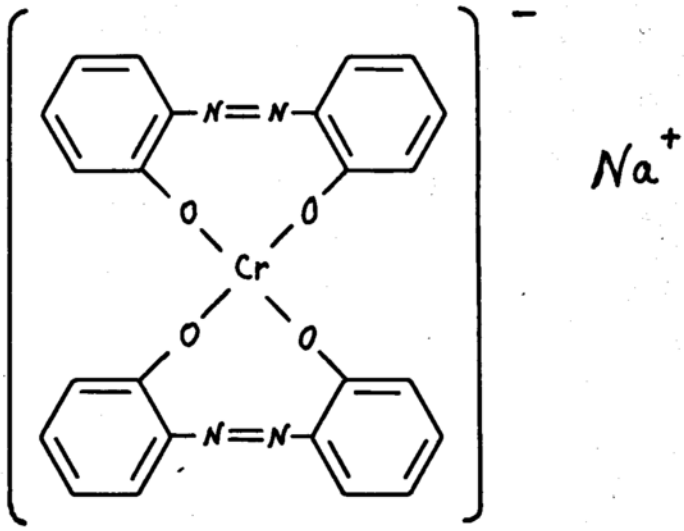
XXVII XXVIII

A series of metallized derivatives of phenanthrene and naphthalene was prepared by Foye and coworkers (30). This series of metallized derivatives included derivatives of o-aminophenol linked through an azo group to α-naphthol, to β-naphthol and to phenanthrene with a single hydroxy group in the 1, 2, 3, or 4 position, and also derivatives of two β-naphthol nuclei linked through an azo group. The type of structure suggested is one in which the metal is included in a nine-membered ring (XXIX), though it is possible that the metal is also linked through secondary valence bonds to a nitrogen of the azo group, thus forming a five-membered and six-membered ring as well.



XXIX

Among the structures which have been proposed for metal chelates of o,o'-dihydroxyazo dyes is the structure represented by (XXX) in which one atom of chromium is combined with two molecules of dye (31). Of the four bonds

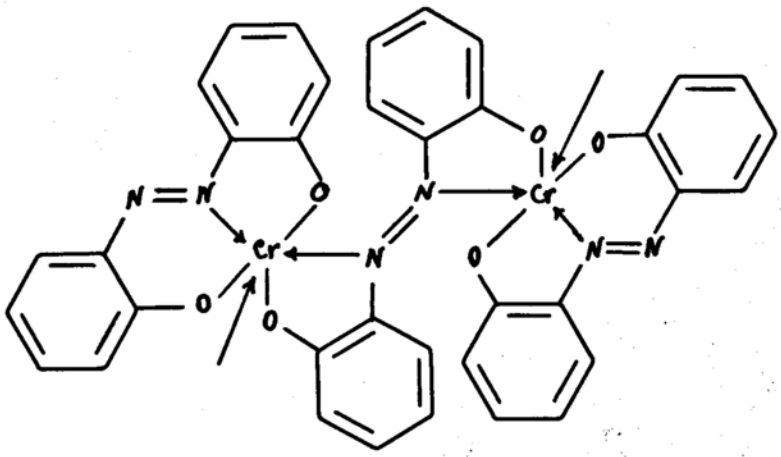


XXX

joining the chromium to the dyes, three are primary valence bonds, and the fourth is a secondary valence bond. The

hydrogen on the oxygen involved in this fourth bond is believed to have been replaced by an atom of sodium, which accounts for the presence of sodium in the chelate.

Other proposed structures for chelates of o,o'-dihydroxyazo dyes include that described by Schetty (32) which he attributes to Krzikalla (XXXI) (33) in which two atoms of chromium chelate with three molecules of dye. For the modifications in the dye molecule used by Schetty, he goes so far as to propose an ion lattice in which each



XXXI

chromium atom is surrounded by three dye molecules and each dye molecule is surrounded by two chromium atoms.

Evidence of metal chelation is indicated by a number of criteria, including the following: color, decreased solubility in water, drop in pH during formation,

absence of metal ions in solution, and correct analysis of isolated products (34,35). Foye and coworkers have found the use of paper chromatograms successful in following the course of chelation reactions (30,35).

DISCUSSION

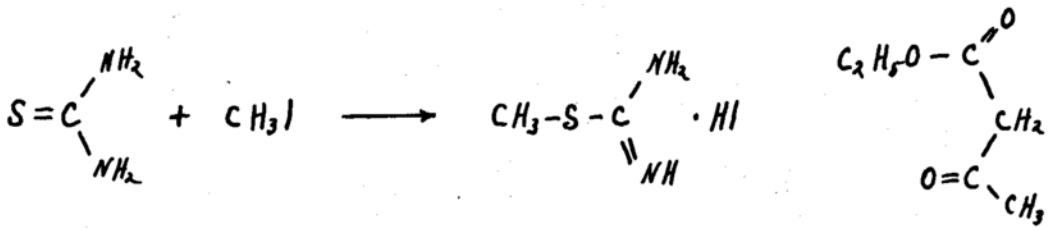
Synthesis of Pyrimidines

To study the biological effects of the proposed azopyrimidines the synthesis of a number of substituted pyrimidines was first necessary. Since the pyrimidines that are commercially available are rather expensive, most starting materials were prepared in the laboratory, either by known procedures or by suitable modifications. The substituted pyrimidines prepared in the laboratory included uracil (2,4-dihydroxypyrimidine), 6-methyluracil (2,4-dihydroxy-6-methylpyrimidine), 5-nitro-6-methyluracil, 5-amino-6-methyluracil, 5-nitrobarbituric acid (2,4,6-trihydroxy-5-nitropyrimidine), uramil (5-aminobarbituric acid), and 2-methylthio-6-methyluracil.

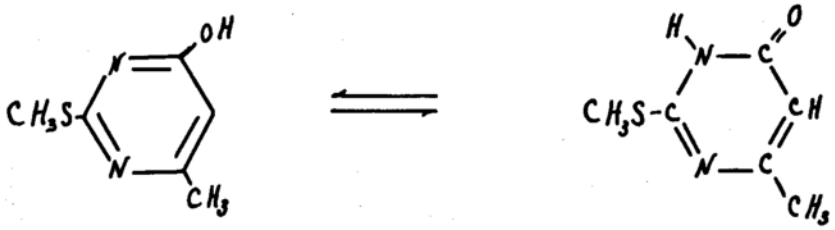
Uracil (prior to obtaining a supply of commercially available material) was prepared by condensing pseudomethylthiourea hydriodide (36) with sodium formylacetic ester (37) to form 2-methylthiouracil (36), followed by hydrolysis of the mercapto group to give uracil (36).

The 6-methyluracil was prepared in two ways. The first was similar to that stated for uracil, by condensation of pseudomethylthiourea hydriodide (XXXII) with ethyl acetoacetate under the influence of potassium hydroxide to give 2-methylthio-6-methyluracil (XXXIII) (36) followed by hydrolysis of the mercapto group to give

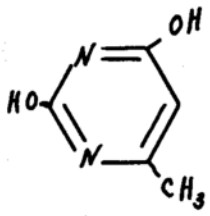
6-methyluracil (XXXIV) (36). The other method of preparation of 6-methyluracil was by condensation of urea with



XXXII

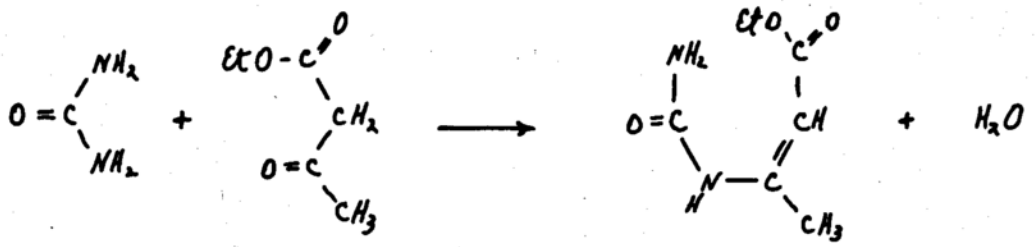


XXXIII

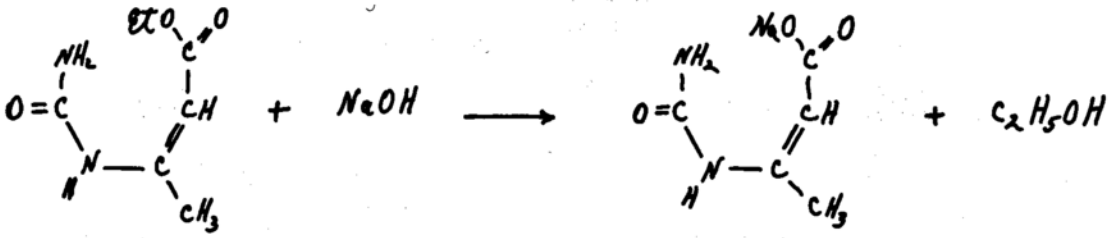


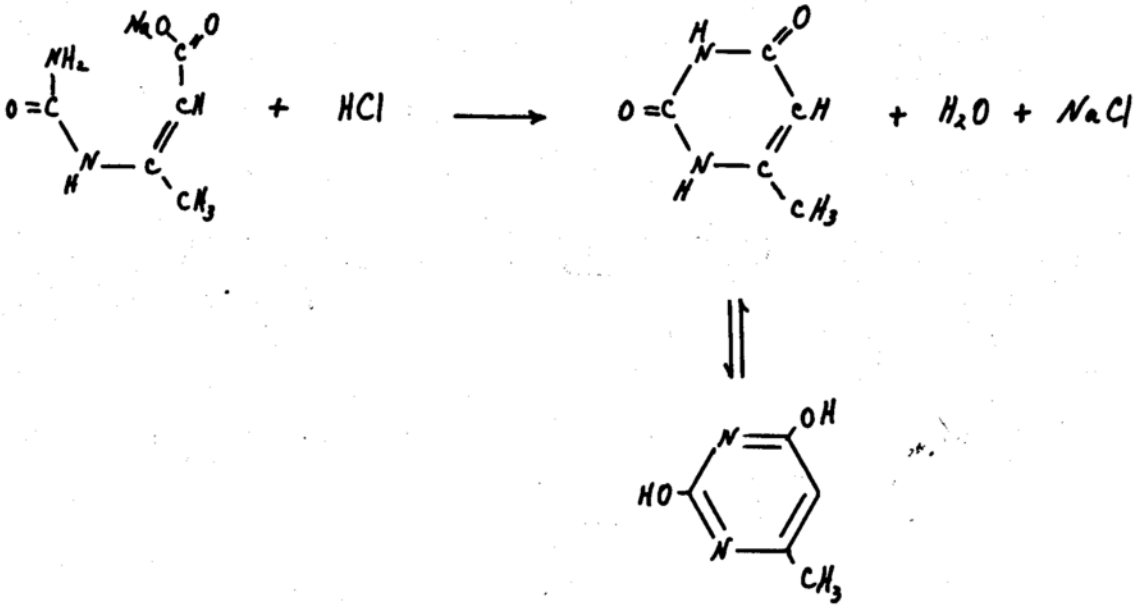
XXXIV

ethyl acetoacetate in presence of absolute alcohol and hydrogen chloride to give β-uraminocrotonic ester (XXXV),



XXXV





followed by ring closure to give 6-methyluracil (38). The syntheses of uracil and 6-methyluracil are thus seen to be pyrimidine syntheses of Type I.

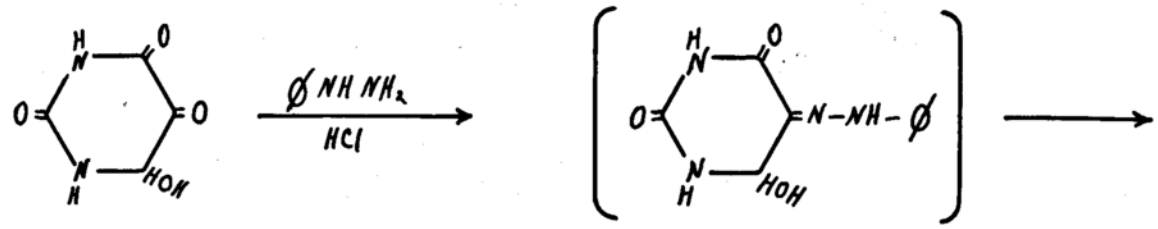
5-Nitro-6-methyluracil was prepared by nitration of 6-methyluracil with fuming nitric acid in the presence of phosphorus pentoxide, according to the method of Behrend and Osten (39). The nitro group was reduced, using amalgamated aluminum and ammonia, to yield 5-amino-6-methyluracil.

The 5-nitrobarbituric acid was prepared by nitration of barbituric acid using fuming nitric acid (40). The nitrobarbituric acid was reduced by tin and hydrochloric acid, yielding uramil (41).

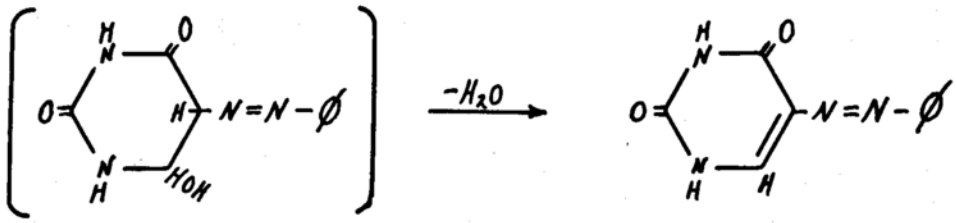
Synthesis of Azopyrimidines

Uracil-5-azobenzene (XXXVII) has been prepared by Bogert and Davidson (18). They reported the carbon, hydrogen, and nitrogen analyses but did not report a melting point. The method of preparation of this compound used by these workers was the action of phenylhydrazine hydrochloride on isodialuric acid (XXXVI). Direct condensation followed by the loss of a second molecule of water gave the orange-brown uracil-5-azobenzene. They postulated the mechanism for the reaction shown (XXXVI to XXXVII). By the use of phenylhydrazine hydrochloride Bogert and Davidson were able to overcome the difficulty

experienced by previous workers (42) who, using

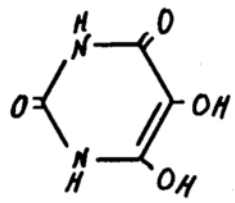


XXXVI

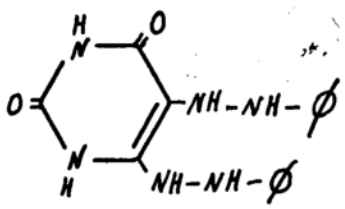


XXXVII

phenylhydrazine base, found that isomerization of iso-dialuric acid had occurred to yield dialuric acid (XXXVIII) which was then converted into 5,6-di-phenylhydrazinouracil (XXXIX).



XXXVIII



XXXIX

The compound, uracil-5-azobenzene was prepared in the course of this research by the coupling of diazotized aniline with uracil. Since there was no melting point on record for this compound, it was submitted for analysis.

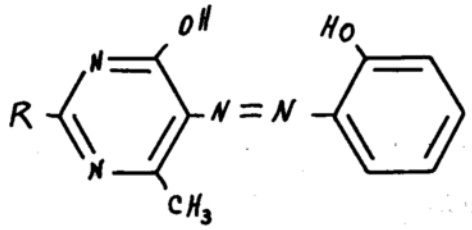
Synthesis of Di-o-hydroxyazopyrimidines

The first attempts at preparing di-o-hydroxyazo compounds were by diazotizing o-aminophenol and coupling this with uracil. After a number of attempts, the conclusion was reached that the resulting substance does not lend itself to ready purification or else is light-sensitive.

preparation of the same type of compound was attempted by coupling acetoacetic ester with diazotized o-aminophenol and subsequently condensing with urea to form the pyrimidine ring. The coupling of acetoacetic ester with diazotized o-aminophenol was done similarly to Richter's procedure for the preparation of phenylazoacetoacetic ester (43). The condensation to form the pyrimidine ring was carried out by a modification of the procedure used by Polonovski and Pesson (19) for preparing 2-amino-4-hydroxy-5-phenylazo-6-methylpyrimidine and 2-thio-5-phenylazo-6-methylpyrimidine during their investigations on azo derivatives of the pyrimidines. Their procedures in turn were based on the method used

by Andersag and Westphal (44) for preparing 2,6-dimethyl-4-hydroxy-5-phenylazopyrimidine in the course of their attempts to synthesize thiamin.

The azopyrimidine, 5-o-hydroxyphenylazo-6-methyluracil (XL), thus formed in our experiments was obtained in a yield of 43.7%. By a similar means, using thiourea instead of urea in a condensation reaction with o-hydroxyphenylazo-acetoacetic ester, the corresponding sulfur compound, 2-thio-5-o-hydroxyphenylazo-6-methyl-uracil (XLI), was obtained in a yield of 49.3%.



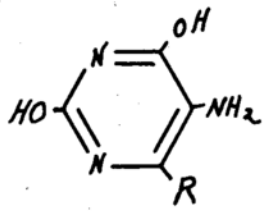
R = OH, XL

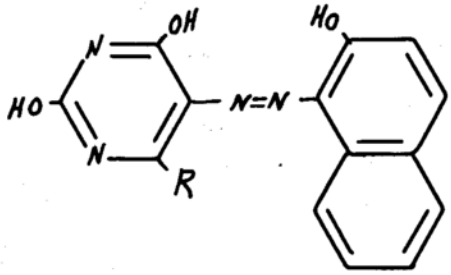
R = SH, XLI

Purification of these compounds was carried out by continuous extraction with Skelly B in a Soxhlet Extractor for periods of 36 hours or more.

Since the compound, 5-o-hydroxyphenylazo-uracil, was believed to be light-sensitive, efforts were directed toward producing di-o-hydroxyazo compounds with higher molecular weights, in the hope that they would be more stable. Bogert and Davidson (18) had prepared uracil-

5-azo-β-naphthol (XLIII) by the diazotization of 5-aminouracil (XLII) followed by coupling with β-naphthol. Following their procedure this compound was prepared during the course of this research and after purification by continuous extraction with water in a Soxhlet Extractor for 72 hours the product decomposed with effervescence and previous blackening at 297-298°, an elevation of about ten degrees over the decomposition point reported by Bogert and Davidson. Using a similar procedure, 6-methyl-5-aminouracil (XLIV) and uramil (XLVI) were diazotized and coupled with β-naphthol, giving 6-methyluracil-5-azo-β-naphthol (XLV) and uramil-5-azo-β-naphthol (XLVII) respectively. The purification of (XLV) was attempted in





R = H, **XLII**

R = CH_3, **XLIV**

R = OH, **XLVI**

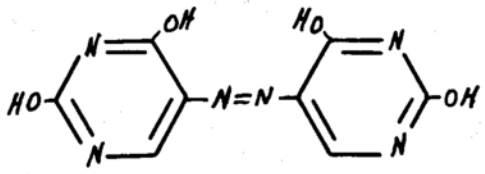
R = H, **XLIII**

R = CH_3, **XLV**

R = OH, **XLVII**

several ways. Crystallization from absolute alcohol resulted in a compound which decomposed with effervescence at 239°; crystallization from water resulted in hydrolysis to β-naphthol; attempted purification by repeatedly dissolving in 10% sodium hydroxide and precipitating by glacial acetic acid also resulted in formation of β-naphthol. By continuous extraction by water of the azo compound (XLV) in a Soxhlet Extractor a scarlet red solid was obtained, of which the analysis approximated the calculated values for carbon and hydrogen.

The desired o,o'-dihydroxyazo grouping would be present if two uracil nuclei were joined together at positions 5 and 5' through an azo linkage. To synthesize this type of compound 5-aminouracil was diazotized and coupled with uracil, resulting in azouracil-5,5' (XLVIII). The analysis for this compound approximated the calculated values for C and H.



XLVIII

Metal Chelates of Azopyrimidines

Metallization reactions were carried out on a number of the di-o-hydroxyazopyrimidines. The procedures used in these metallization reactions were modifications of the procedure used by Foye and coworkers in their metallization of o,o'-dihydroxyphenylazo-naphthalenes and -phenanthrenes (30). The solvents used for the reaction varied in attempts to obtain the desired product and facilitate isolation. Water together with enough sodium hydroxide solution to cause solution of the azo dye (by formation of sodium salts of free hydroxyl groups), water with ethylene glycol, and triethanolamine were used. A pH of 8 was considered optimum for the metallization reaction, since, on the one hand, the metal chelate is unstable in acid medium, and, on the other hand, the metal is likely to form metal hydroxide if the medium is more alkaline than pH 8. Triethanolamine has the advantages of being sufficiently alkaline to provide a pH of about 8 and, since sodium hydroxide is not necessary, it is impossible to have the metal hydroxide formed, but isolation of the metal chelate of the pyrimidine dye is very difficult from the triethanolamine medium.

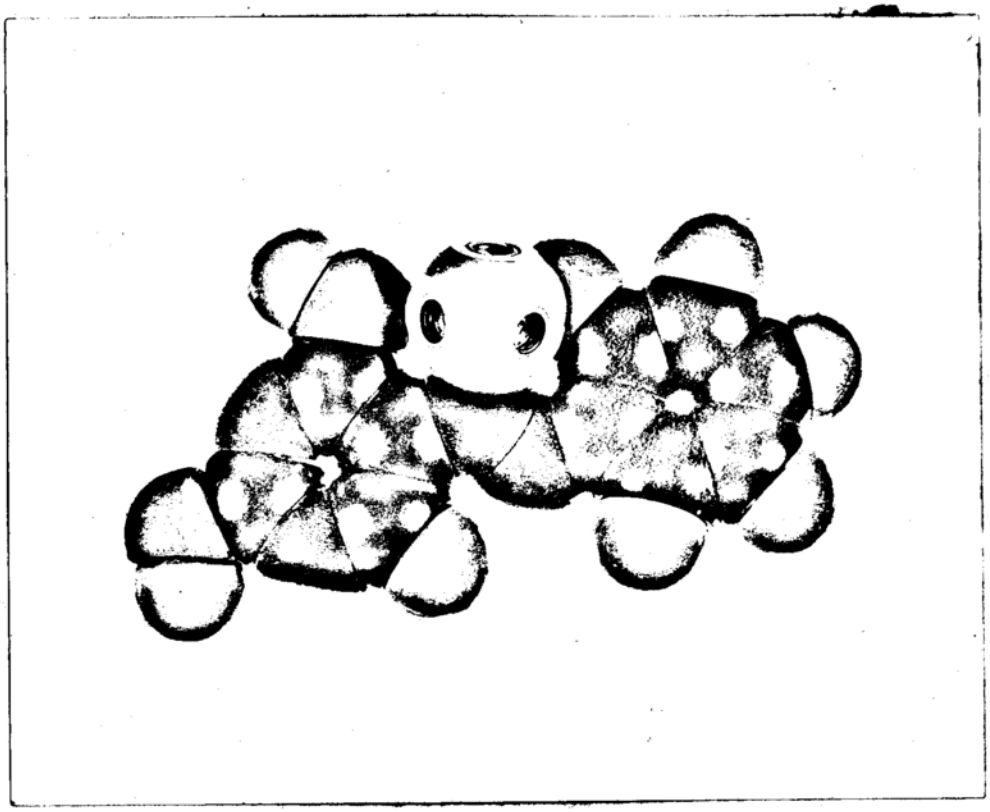
The progress of the metallization reaction was followed by the use of paper chromatograms prepared as described by Foye (30). Dimethylformamide was used to develop these paper chromatograms. In most cases the

color of the metal chelate was markedly different from that of the azo dye.

Among the metallized compounds prepared were metal derivatives of uracil-5-azo-β-naphthol (nickel, cobalt, and iron); of 2-thio-6-methyl-5-(o-hydroxyphenylazo)-uracil (cobalt and iron). From the analyses of the nickel and cobalt chelates of uracil-5-azo-β-naphthol a ratio of one metal atom to one dye molecule is suggested. For the nickel compound, the analyses for carbon and nickel correspond to the anhydrous derivative; for the cobalt chelate of the same dye, the analysis for carbon corresponds to the monohydrate.

Various speculations and postulations have been made (23,24,25,26,27,30,31,32,33) concerning the structure of the metal chelates of o-o'-dihydroxyazo dyes. In an attempt to rule out impossible structures, models of these chelates were prepared, using Fisher-Taylor Hirschfelder models. These models showed that there are at least two possible ring structures. One of these possibilities has a six-atom ring in which the metal replaces a hydrogen on one of the hydroxyl groups and coordinates with the nitrogen of the azo group farthest from the ring. In this structure only one of the o-hydroxy groups is involved. Plate I shows a photograph of the Fisher-Taylor-Hirschfelder model of this type of ring, as exemplified in the compound (XLIX), where M is the metal cobalt. The

PLATE I



PHOTOGRAPH OF FISHER-TAYLOR-HIRSCHFELDER MODEL OF A COBALT CHELATE HAVING A SIX-ATOM RING

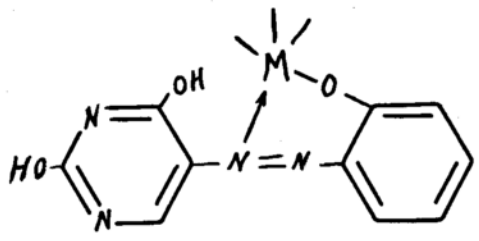
Orange - Hydrogen

Light blue - Oxygen

Dark blue - Nitrogen

Gray - Cobalt

Black - Carbon

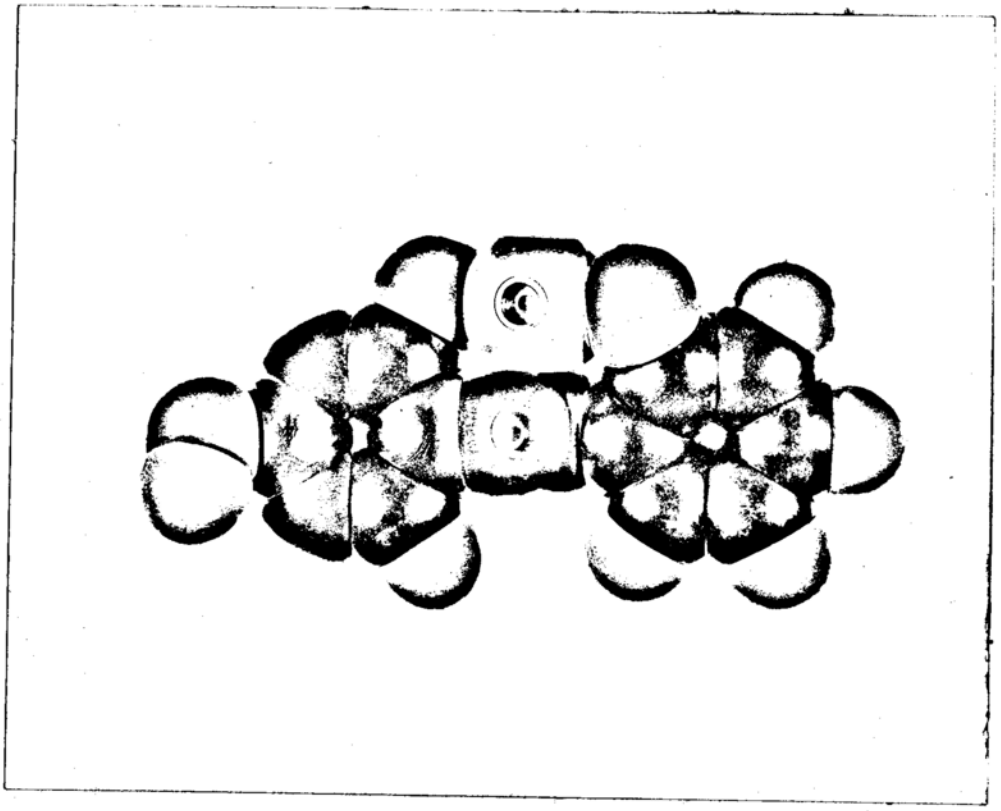


XLIX

model indicates that the metal atom can coordinate with up to four other groups, such as water molecules, in order that the maximum coordination number for cobalt (i.e. six) may be satisfied.

Another of the possible ring structures demonstrable by the Fisher-Taylor-Hirschfelder models is that in which a nine-atom ring is formed by the replacement of both H's on the two o-hydroxy groups by a single metal atom. The model shows that this ring can be formed without strain. However, the cubic representation of a hexacovalent atom like cobalt indicates that one of the bonds would be blocked, so that in this type of ring the maximum coordination number of cobalt would be five. Plate II shows a photograph of a cobalt chelate (L) containing this nine-atom ring.

PLATE II



PHOTOGRAPH OF FISHER-TAYLOR-HIRSCHFELDER MODEL

OF A COBALT CHELATE HAVING A NINE-ATOM RING

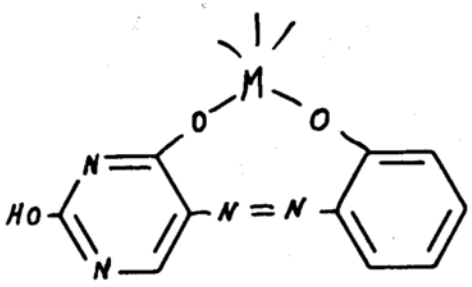
Orange - Hydrogen

Light blue - Oxygen

Dark blue - Nitrogen

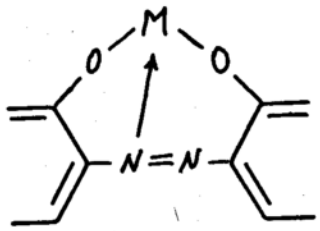
Gray - Cobalt

Black - Carbon



L

From the model of (L) it is evident that coordination cannot also occur with the nitrogen of the azo group as indicated in the partial tridentate formula (LI).



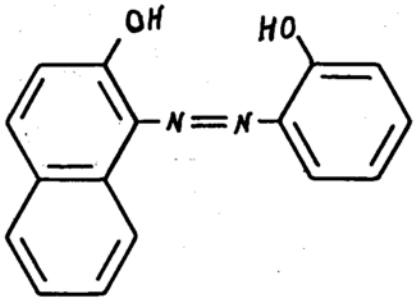
LI

In connection with the six-atom ring structure it should be pointed out that the pyrimidine ring has the ability to rotate about the bond joining it to the azo group. Since this rotation is not entirely free even without the presence of further coordinating groups, such as water molecules, on the metal atom the possibility of one pair of optical isomers exists. Martell and Calvin (45) in dealing with optical isomers of chelates comment on the investigation by Pfeiffer and Saure (46) of the chelates of

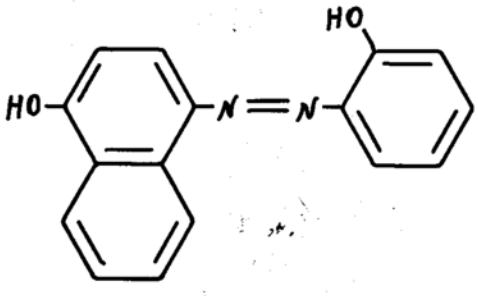
the o,o'-dihydroxy derivatives of azomethine dyes. While these workers postulated five and six pairs of enantiomorphs for these chelates, which they assumed to be tridentate, they were able to find experimental evidence for only one pair of optical enantiomorphs in each case.

Metal Chelates of Analogous Azonaphthols

Since the chelates of the azopyrimidines have not lent themselves well to determination of composition, efforts were made to determine the structure of the analogous azonaphthols. For this purpose various metal chelates were made of 1-o-hydroxyphenylazo-2-naphthol (LII) and 4-o-hydroxyphenylazo-1-naphthol (LIII) which had been prepared by Foye (30), by coupling diazotized o-aminophenol with β-naphthol and α-naphthol, respectively.



LII



LIII

The o,o'-dihydroxyazo dye, 1-o-hydroxyphenylazo-2-naphthol (LII), was metallized with chromium, with iron, and with copper; the mono-o-hydroxyazo dye (LIII) was metallized with chromium and with iron. The sulfates of iron and copper and the acetate of chromium were used in the chelation procedures. Evidence of chelation was indicated by a change in color and by a drop in pH in the reaction mixture. Using the conditions of Kopp and Petitcolas (47) a copper chelate of the mono-o-hydroxy dye (LIII) was not obtained. When this reaction was attempted a solid material, melting over several degrees but in the same range as the starting dye, was recovered; also, the filtrate was an unexpected shade of green which on treatment with excess ammonia solution produced the clear dark blue color of the copper ammonia ion. The failure of this dye to complex with copper argues in favor of the hypothesis that chelation occurs much more readily in the case of di-o-hydroxy-substituted azo dyes than with mono-o-hydroxy-substituted azo dyes.

The metal chelates after preparation were isolated, washed well with water, dried, pulverized and then extracted in a fritted disc thimble in a Soxhlet Extractor with Skelly B for a period of 24 hours or more.

Of the five chelates made from the hydroxyazo-naphthols, three showed a slight alkaline reaction to pHydrion Paper. This, together with the fact that the

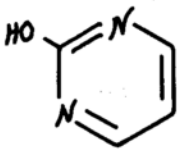
iron and copper chelates gave no test for sulfur on fusion, suggests that the metal may still be linked to a hydroxyl. Calculated on this basis, C, H, and metal analyses of the copper and chromium chelates of 1-o-hydroxyphenylazo-2-naphthol correspond to a ratio of 1 of dye to 1 of metal without extra water molecules. The iron and chromium chelates of 4-o-hydroxyphenylazo-1-naphthol also have a 1:1 ratio of dye to metal, but the analytical results suggest the presence of two molecules and one molecule of water, respectively, in these compounds.

To further illustrate the fact that two hydroxy groups in the ortho-position are not necessary for chelation, the compounds uracil-5-azobenzene, and 5-phenylazo-6-methyluracil, with only one such o-hydroxy group, were treated with cobaltous or nickel salts under alkaline conditions. Cobalt and nickel chelates resulted, though neither the dyes themselves nor the chelates were as highly colored as in the cases of the di-o-hydroxy compounds.

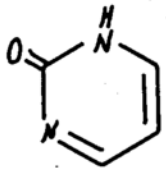
Evidence from Absorption Spectra

There has been considerable doubt in the past about the structure of the o-hydroxy-azo dyes (48,49,50, 51,52). These uncertainties have not been lessened at all by the presence of the pyrimidine ring bearing tautomeric groups. The anomalous properties of pyrimidines containing

hydroxy or amino groups at positions, 2, 4, or 6 suggest the possibility of their existence as keto- or imino-dihydropyrimidines (15). 2-Hydroxypyrimidine can be written in two classical forms, the lactim (LIV) and the lactam (LV), each possessing various resonance possibilities.

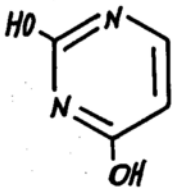


LIV

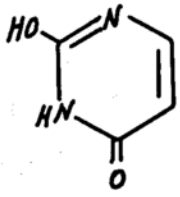


LV

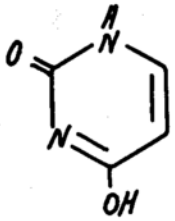
When two tautomeric groups are present as in uracil, four distinct classical forms (LVI-LIX) are possible, each a hybrid of various resonance forms. Whether a lactam or lactim structure is the correct representation, or whether



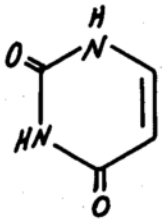
LVI



LVII



LVIII



LIX

one form is present in the solid state with an equilibrium mixture existing in solution, is still not completely decided. Most of the investigation and speculation con-

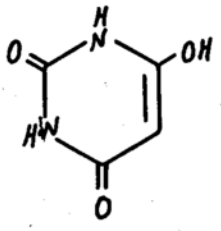
cerning the lactam-lactim question has been done on uracil and barbituric acid (15).

J. E. Austin (53) investigated the ultraviolet absorption spectra of alcoholic solutions of uracil and a number of its derivatives, in which the lactim or lactam form would be stabilized. She stated that uracil had a spectrum different from 2,4-dichloro- and 2,4-diethoxy-pyrimidine which must possess the (LVI) type of structure. The uracil spectrum differs also from 4-methoxy-1-methyl-uracil of form (LVIII), and from 1,3-dimethyluracil of form (LIX). However, uracil does have the same spectrum as 3-methyluracil in which the atomic arrangement at positions 3 and 4 is stabilized in the lactam form. Austin therefore concluded that in alcoholic solution, uracil should be represented by (LVII) (53).

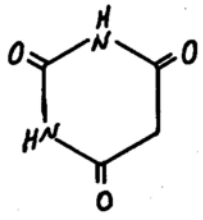
From similar measurements in water at different hydrogen ion concentrations it was concluded by Loufbourow and coworkers (54) and confirmed by Marshall and Walker (55) that uracil is doubly ketonic, i.e., has structure (LIX).

From chemical evidence, Arndt (56) concluded that the lactam structure is preferred by hydroxy-pyrimidines except where such a structure would increase the energy of the system by removing the resonance energy derived from the aromatic character of the ring. Arndt assigned formula (LIX) to uracil. He further postulated

formula (LX) for barbituric acid, since aromatic character is still possible by resonance in (LX) but not in the completely lactam structure (LXI).



LX

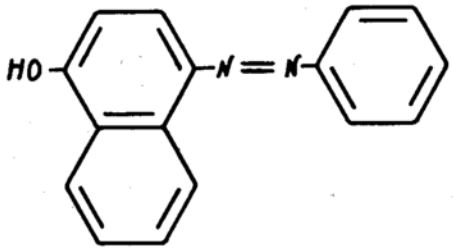


LXI

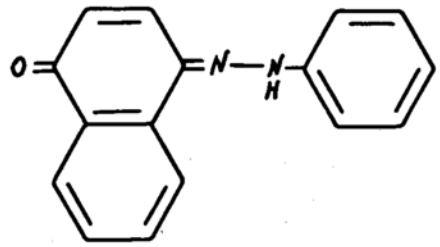
The greater specificity of infrared vibrational spectra and the fact that the vibrational frequencies of the C O, and N-H bonds in different types of grouping are now fairly well known, led Short and Thompson (57) to undertake infrared experiments on pyrimidine and a large number of derivatives. They pointed out that there were two limitations on infrared measurements, that infrared measurements with aqueous solutions are effectively impossible and that the vibrational spectra of such complex compounds might prove too complex for detailed analysis. In spite of these limitations and the fact that in the particular wave bands involved certain ambiguities arise which prevent a dogmatic statement, Short and Thompson suggest that their spectral evidence favors a ketonic structure for the simple 2-hydroxy- and 4-hydroxy-pyrimidines, and probably a diketonic form for

the 2,4-dihydroxy-derivatives. In 4,6-dihydroxypyrimidine one ketonic and one enolic group may be present. They believe that amino substituents in general exist in the non-tautomerized amino form. They endeavored to explain their disagreement in a number of instances with the results of Brownlie (58). Short and Thompson suggest that, if the details of structure of a few simple hydroxyl and amino derivatives could be established by X-ray measurements so that correlations with the spectra could be made in these cases, interpretation of infrared data would be facilitated.

In 1935 Kuhn and Bär (48) reported on their studies on the constitution of hydroxyazo compounds, using the evidence of absorption spectra. The compound phenylazo-α-naphthol (LXII), obtained by coupling phenyldiazonium chloride with α-naphthol, is identical with the condensation product of α-naphthoquinone and phenylhydrazine, which may be called α-naphthoquinone-phenylhydrazone (LXIII) (59). Kuhn and Bär found that this compound or equilibrium mixture exhibited a maximum in absorption at a wave length which depended on the solvent used. In benzene, 4-phenylazo-1-naphthol (LXII) showed two peaks in absorption, at 410 mμ and 460 mμ. By comparison with the absorption peak of the O-methyl ether of (LXII) (400 mμ) and that of N-methyl- α-naphthoquinone-phenylhydrazone, (440,465 mμ) it was concluded that in



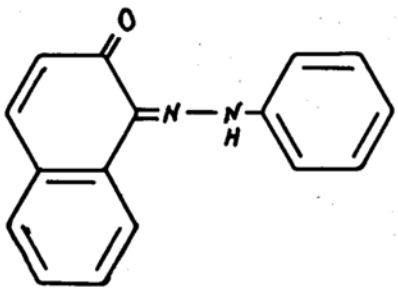
LXII



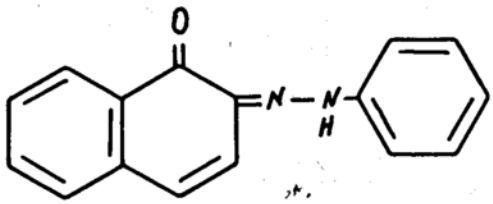
LXIII

benzene 4-phenylazo-1-naphthol is a mixture of the true azo structure (LXII) and the quinone-hydrazone structure (LXIII). In pyridine, 4-phenylazo-1-naphthol showed a single absorption peak at 410 mμ so exists in the true azo form (LXII) in this solvent.

Kuhn and Bär concluded further that both α-phenylazo-β-naphthol (LXIV) and β-phenylazo-α-naphthol (LXV) exist in the quinone-hydrazone form regardless of solvent. They indicated that the mono-



LXIV



LXV

hydroxyazobenzenes, whether o, -m-, or p-, existed in the true azo form. Kuhn and Bär postulated that even though an equilibrium cannot be demonstrated between the two forms, azo and quinone-hydrazone, for (LXIV) a very rapid shift may occur under appropriate conditions in chemical reactions to account for the formation of O-substituted derivatives.

In connection with the ambiguity of the formulas mentioned above, we have wondered whether the azo derivatives of the pyrimidines exist in the true azo form or as the quinone-hydrazone. In an effort to determine this, we have prepared the phenylhydrazone of alloxan according to the method of Köhling (60) and also the azo compound, 5-phenylazo-barbituric acid, by coupling diazotized aniline with barbituric acid, again using the procedure of Köhling (61). Köhling pointed out that these compounds were identical in properties and melting point. Our compounds did indeed both decompose from 300-302°. The absorption spectra (Fig. 2) and (Fig. 1) of these compounds in acetone proved to be identical, with a single maximum at 380 mμ.

The absorption spectrum, (Fig. 3), of a similar compound with a hydroxy group in the o-position of the benzene ring, 5-(o-hydroxyphenylazo)-barbituric acid, also showed a single maximum but in this instance located at 405 mμ. The introduction of a hydroxy group in the

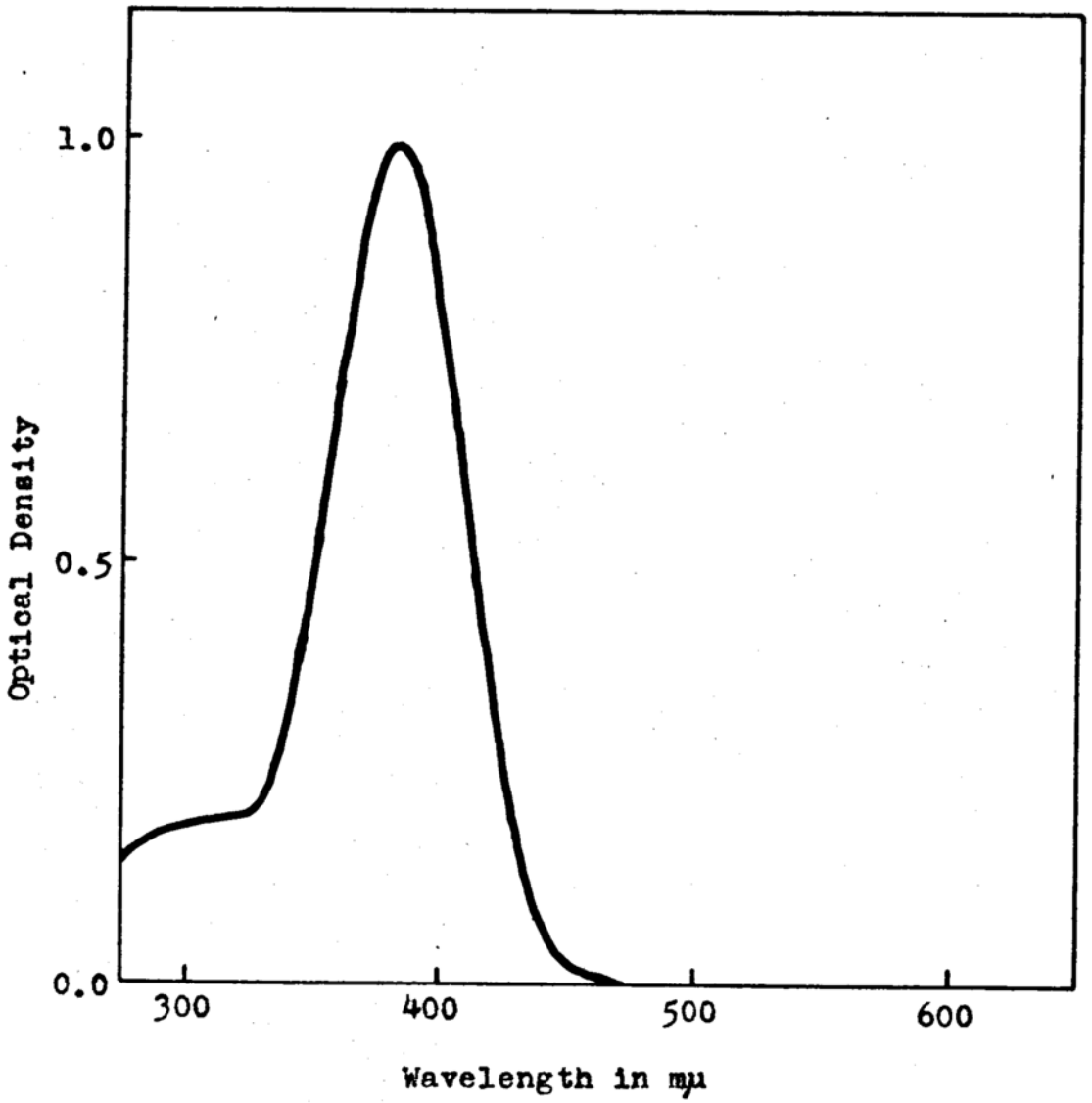


Fig. 1.--Absorption spectrum of alloxanphenylhydrazone in acetone.

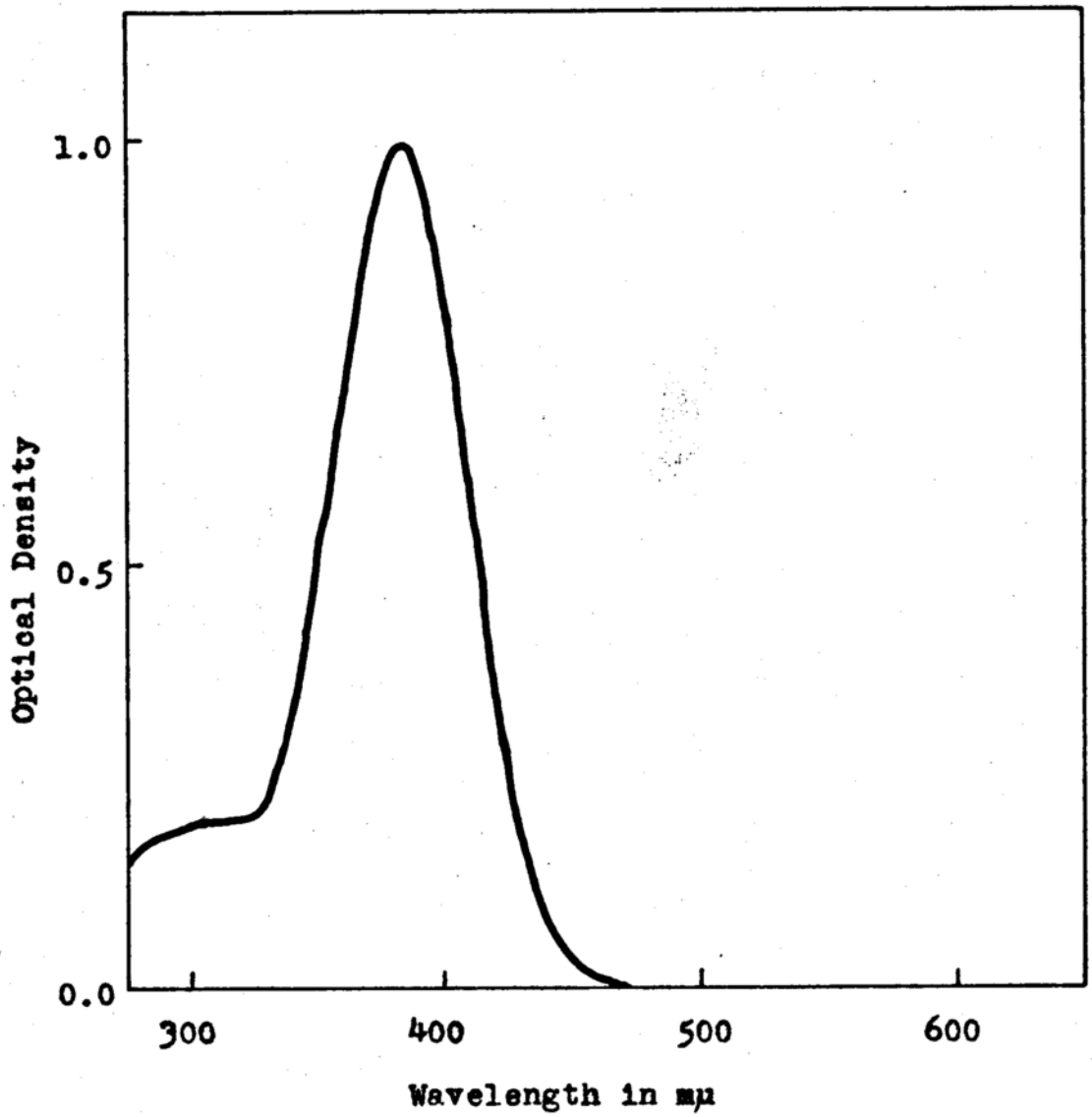


Fig. 2.--Absorption spectrum of 5-phenylazo-barbituric acid in acetone.

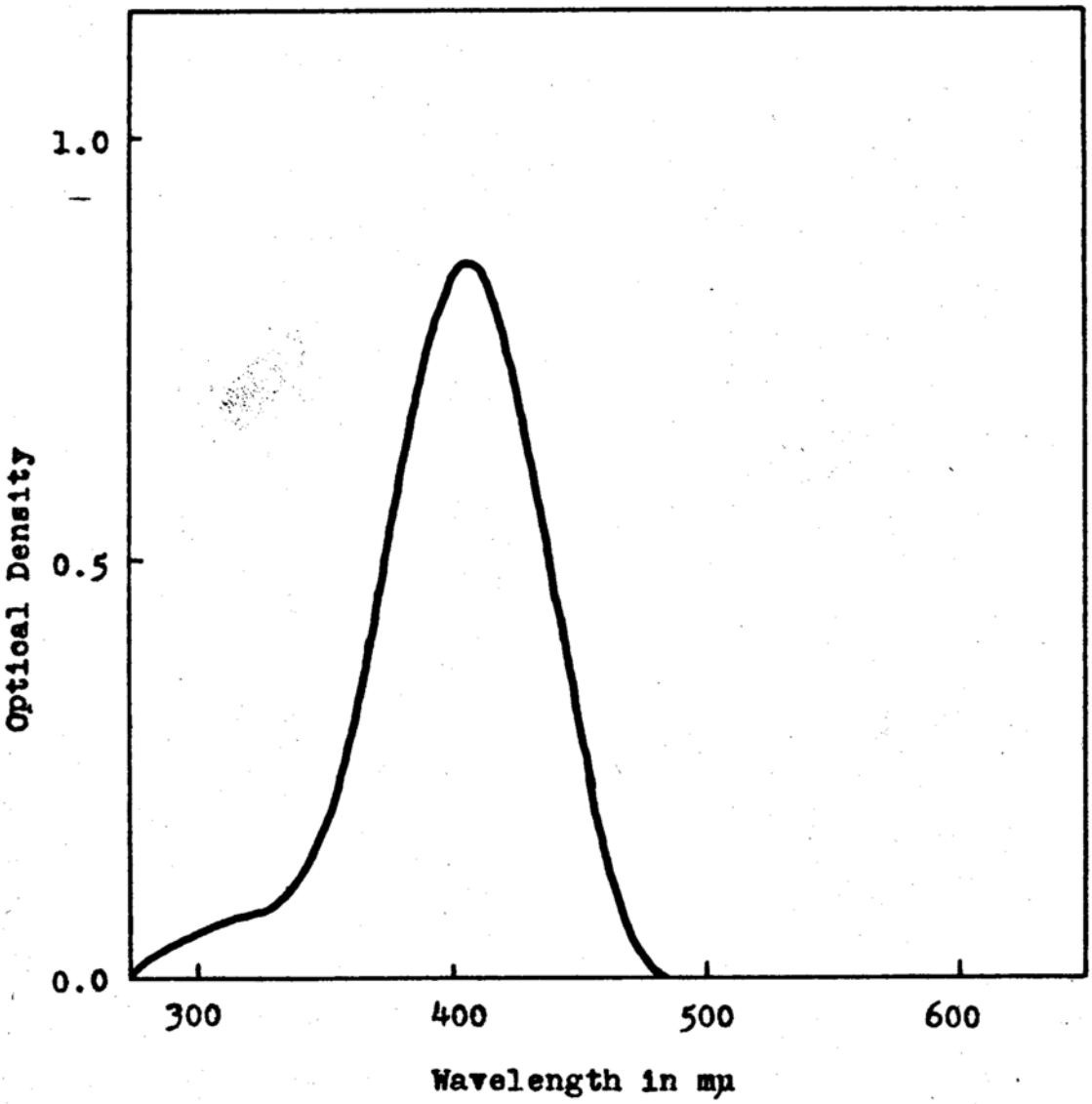
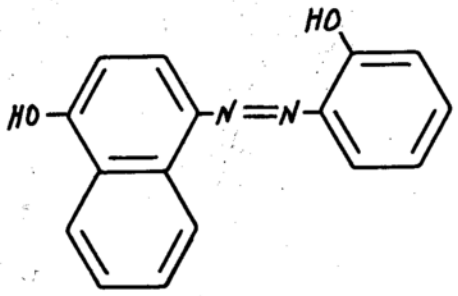


Fig. 3.--Absorption spectrum of 5-(o-hydroxyphenylazo)-barbituric acid in acetone.

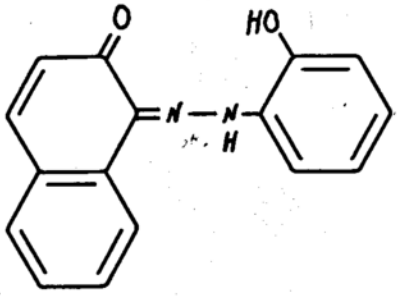
o-position had thus changed the position of the absorption peak by 25 mμ.

For 4-phenylazo-1-naphthol, Kuhn (48) had reported an absorption maximum at 410 mμ in benzene and at 400 mμ for the O-methyl ether. The corresponding o-hydroxy derivative 4-(o-hydroxyphenylazo)-1-naphthol, showed a maximum at 420 mμ (Fig. 4).

The peak for the α-phenylazo-β-naphthol of Kuhn occurred at 475 mμ; the peak of our corresponding o-hydroxy-derivative, 1-(o-hydroxyphenylazo)-2-naphthol, occurred at 485 mμ (Fig. 5). Since by comparison with Kuhn's results, the introduction of a hydroxy group in the ortho-position of the benzene ring appears to change the position of the absorption peak by about 10 m in both cases, we infer that our compounds had similar structures to those ascribed by Kuhn, namely, the true azo structure (LXVI) for the 4-(o-hydroxyphenylazo)-1-naphthol, and the quinone-hydrazone structure (LXVII) for the 1-(o-hydroxyphenylazo)-2-naphthol.



LXVI



LXVII

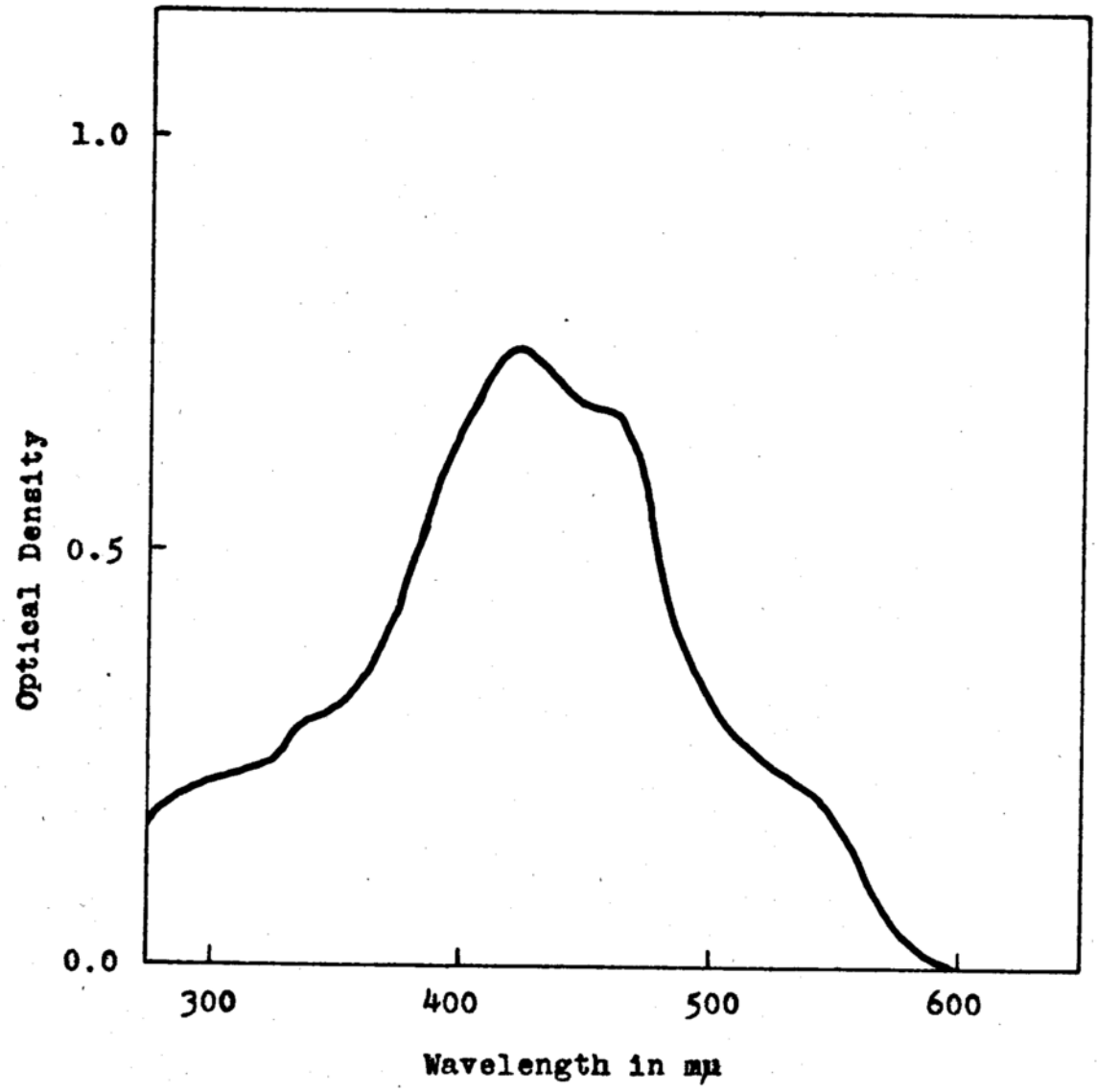


Fig. 4.--Absorption spectrum of 4-(o-hydroxyphenylazo)-1-naphthol in acetone.

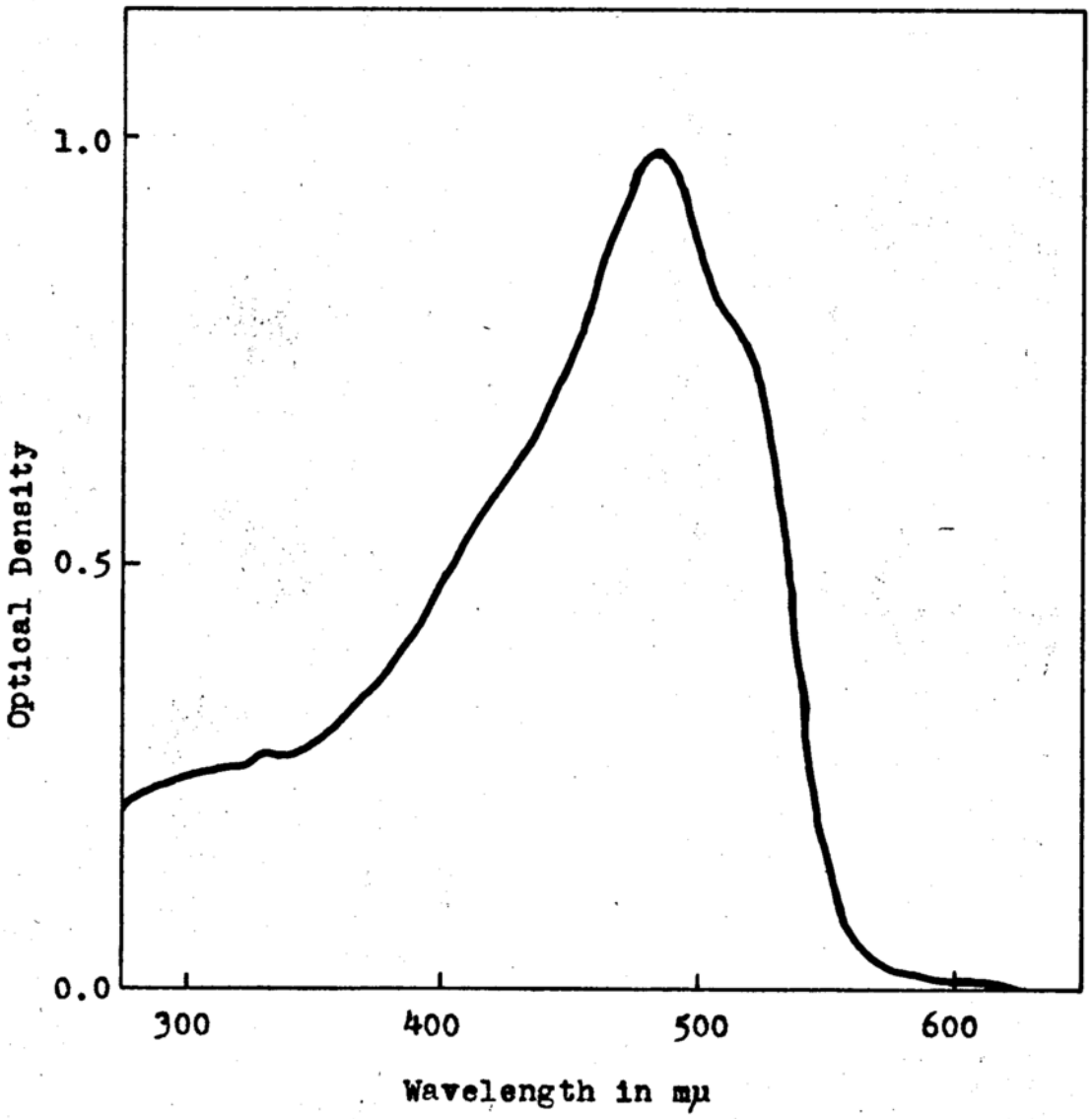


Fig. 5.--Absorption spectrum of 1-(o-hydroxyphenylazo)-2-naphthol in acetone.

Spectral absorption curves have also been made for uracil-5-azo-β-naphthol (Fig. 6) and uracil-5-azobenzene (Fig. 7).

For the five metal chelates prepared from the azonaphthols the analytical data are presented in Table II. The found values for the carbon content of the chelates are usually lower than the calculated values, and the found values for the metal usually higher. This is believed to be accounted for by the probable inclusion of small quantities of metal oxide or hydroxide, removal of which has not been possible. The analytical data for the iron chelates are rather far from the calculated values. This may be caused by oxidation of the ferrous iron to a greater or less extent, together with inclusion of some oxide which could not be removed. On the basis of these analytical data and the spectral data for the azonaphthols themselves, the structure proposed for the metal chelate of (LXVI) is (LXVIII), and that proposed for the metal chelate of the azonaphthol (LXVII) is either (LXIX) or (LXX). In these proposed structures only one primary valence of the metal is used up in combining with the dye, the remaining primary valence bonds are considered to be attached to hydroxyl groups since a majority of the five chelates gave a slight alkaline reaction with indicator paper. Some of the secondary valence bonds are used up by being attached to water molecules. Some or all

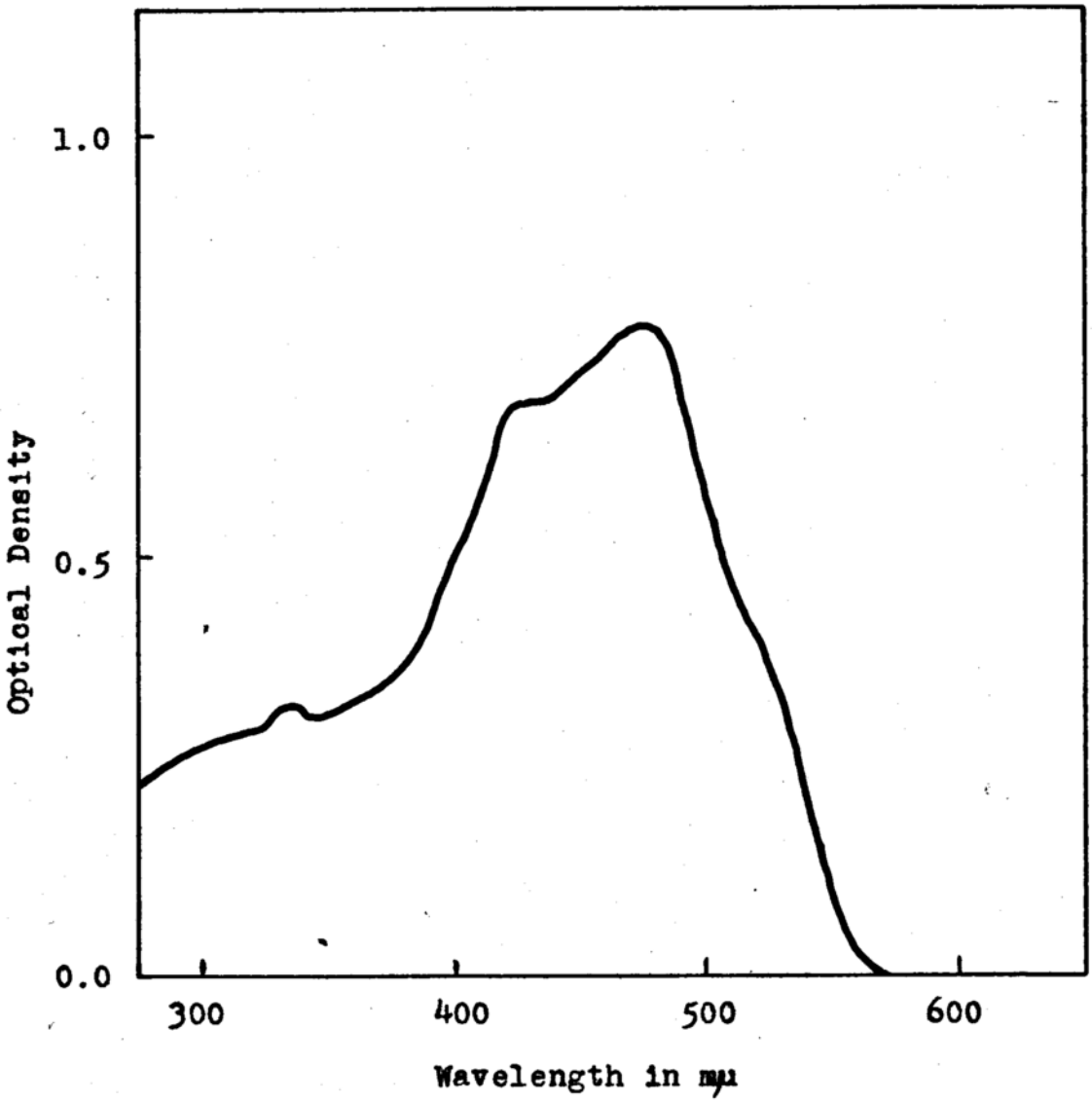


Fig. 6.--Absorption spectrum of uracil-5-azo-β-naphthol in acetone.

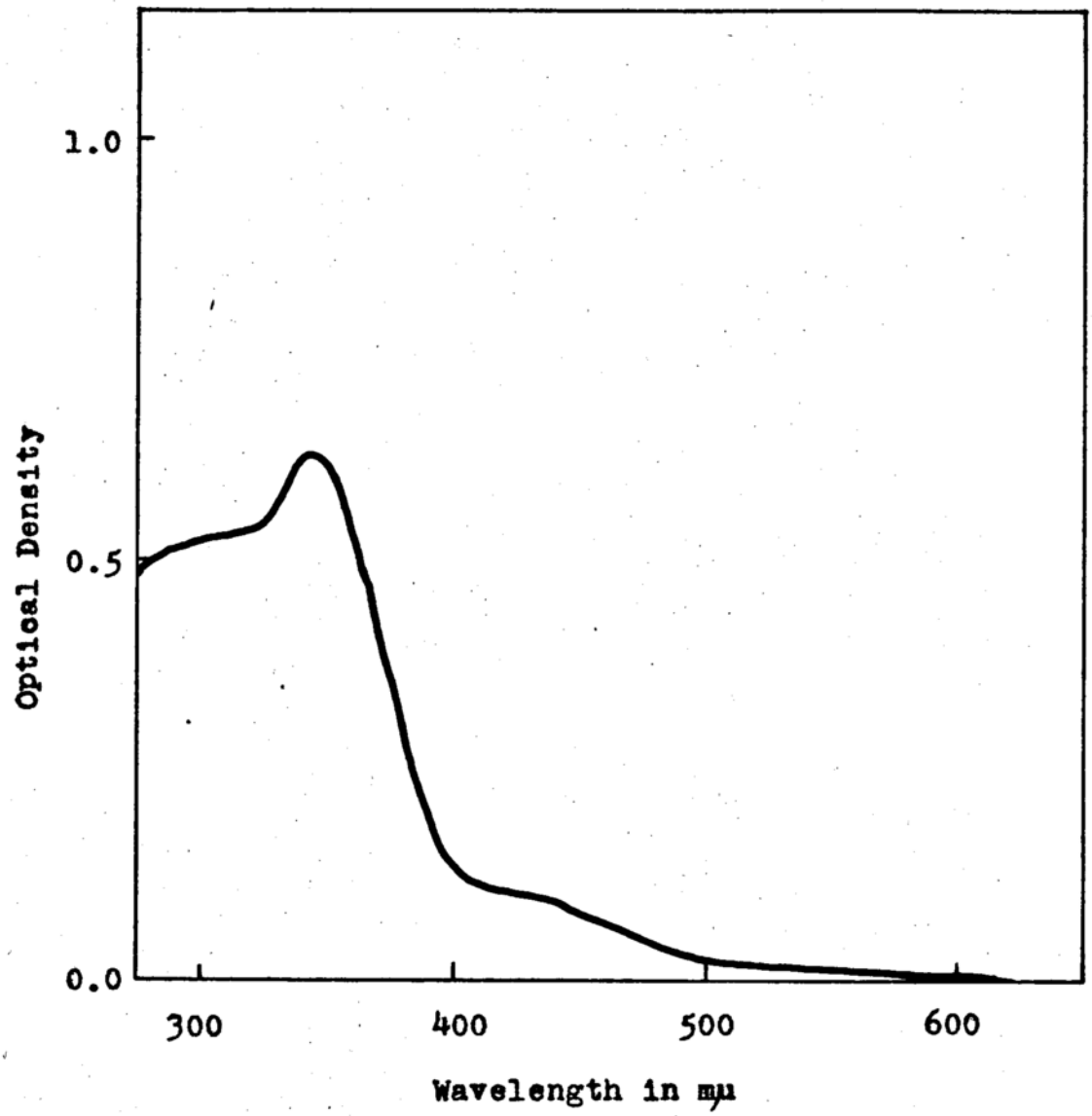
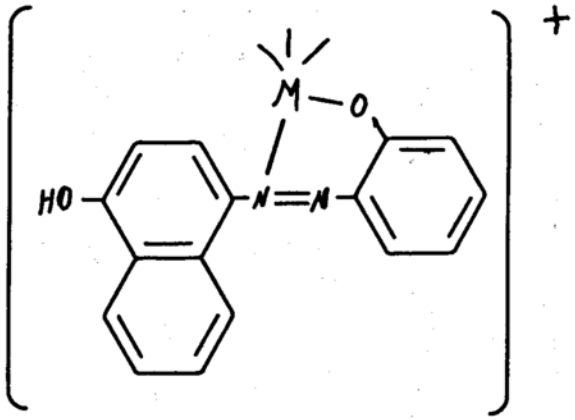


Fig. 7.--Absorption spectrum of uracil-5-azobenzene in acetone.

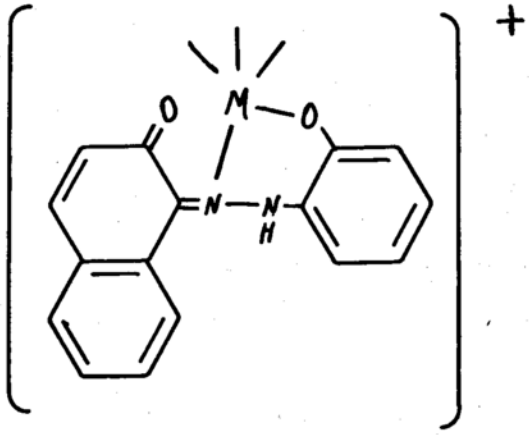
TABLE II

Dye	Metal	Anion	Formula	Water	Calc.	Found
o-Aminophenol → β-naphthol	Cu^{++}	OH^-	$C_{16}H_{12}N_2O_3Cu$	None	C 55.89 H 3.52 Cu 18.48	55.13 3.16 21.9
o-Aminophenol → β-naphthol	Cr^{+++}	$(OH^-)_2$	$C_{16}H_{15}N_2O_4Cr$	None	C 55.01 H 3.75 Cr 14.89	55.27 4.16 12.5
O-Aminophenol → α-naphthol	Fe^{++}	OH^-	$C_{16}H_{16}N_2O_5Fe$	$2H_2O$	C 51.64 H 4.33 Fe 15.00	47.49 3.49 18.3
o-Aminophenol → α-naphthol	Cr^{+++}	$(OH^-)_2$	$C_{16}H_{15}N_2O_5Cr$	H_2O	C 52.32 H 4.12 Cr 14.16	50.81 4.42 17.2

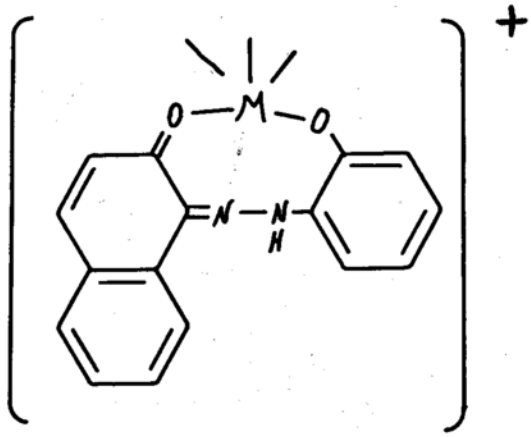
of these water molecules were removed on drying, as indicated by loss of weight.



LXVIII



LXIX



LXX

EXPERIMENTAL

Preparation of Pyrimidines

Pseudomethylthiourea hydriodide.--Ten grams (0.13 mole) of thiourea were placed in a 100-cc. R.B. flask and moistened with absolute alcohol. A condenser, protected by a calcium chloride drying tube, was quickly fitted to the flask. Excess methyl iodide (28 Gm., 0.20 mole) was added in portions. The reaction was allowed to proceed at room temperature with cooling of the flask in an ice bath from time to time as required to prevent too much boiling with consequent loss of methyl iodide. After a half-hour a further 5 cc. of methyl iodide were added. The reaction mixture was allowed to stand twelve hours. The yellow solid product was collected on a suction funnel and washed three or four times with 5-cc. portions of ether, leaving a white solid, which was dried in a vacuum desiccator over calcium chloride. Yield: 25.9 Gm. (90.5% of theoretical).

Sodium formylacetic ester.--Four grams (0.17 mole) of metallic sodium were covered with toluene in a 500-cc. flask and made into "birdshot sodium". The toluene was removed, rinsed off with anhydrous ether, and the sodium was covered with 75 cc. of anhydrous ether. The flask was fitted with a condenser and dropping funnel, both protected

by calcium chloride drying tubes. Fifteen cc. (0.18 mole) of ethyl formate and 15 cc. (0.15 mole) of ethyl acetate were mixed in the dropping funnel and added dropwise to the flask, and the reaction allowed to proceed at room temperature for forty-eight hours. The yellow solid product was filtered off by suction, dried in a vacuum desiccator, pulverized and washed with ether, and again dried. Yield: 9.5 Gm. (45% of theoretical, based on ethyl acetate).

2-Methylthiouracil.--Four and one-fifth grams (0.105 mole) of sodium hydroxide were dissolved in 42 cc. of water in a 250-cc. R.B. flask. Then 22.2 Gm. (0.106 mole) of pseudomethylthiourea hydriodide and 20 Gm. (0.101 mole) of sodium formylacetic ester (considered 70% pure) were added in succession. The flask was fitted with a condenser and allowed to stand twelve hours, when it was heated thirty-five minutes on a steam bath and allowed to stand overnight. The reaction mixture was acidified with glacial acetic acid and the solid product filtered by gravity filtration (HOOD). The solid product so obtained was recrystallized from water, m.p. 199-201°. Yield: 5.6 Gm. (24.9% of theoretical).

Uracil.--Two grams (0.014 mole) of 2-methylthiouracil were placed in a 100-cc. R.B. flask fitted with a condenser. Twenty cc. of concentrated hydrochloric acid

were added and the mixture refluxed on a hot plate (HOOD). Additional portions of 10 cc. each of concentrated hydrochloric acid were added after one hour and after five hours. Refluxing was continued until mercaptan was no longer evolved (a total of seven hours). On being allowed to stand overnight a solid white product separated from solution. The solid product was collected by suction filtration. m.p. 330-31°, with effervescence and considerable charring above 315°. Yield: 1.2 Gm. (76% of theoretical).

2-Methylthio-6-methyluracil.--(2-methylmercapto-6-methyl-4-hydroxypyrimidine).--Sixteen grams (0.075 mole) of pseudomethylthiourea hydriodide were dissolved in 20 cc. of water in a 100-cc. R.B. flask. There were added in turn to the flask 4.5 Gm. (0.080 mole) of potassium hydroxide (as 15 cc. of 30% solution) and 9.6 Gm. (0.075 mole) of ethyl acetoacetate. The flask was swirled to mix after each addition, fitted with a reflux condenser, and allowed to stand forty-eight hours. The solid white product was removed by filtration (gravity filtration in HOOD) and dried in a vacuum desiccator over calcium chloride. m.p. 206-211°. Yield of unrecrystallized material: 8.5 Gm. (73.9% of theoretical).

6-Methyluracil.--Five grams (0.032 mole) of 2-methylthio-6-methyluracil were placed in a 100-cc. flask and the flask fitted with a water condenser (HOOD). Twenty-five cc. of hydrobromic acid (40%) were added and the mixture boiled over an open flame until the odor of mercaptan was no longer present, adding a further 10 cc. of hydrobromic acid after three hours and an additional 10 cc. after six hours. Refluxing was continued for a total of nine and one-half hours. After cooling a solid yellow product had separated. This product was collected on a Büchner funnel and dried in a vacuum desiccator over calcium chloride. m.p., somewhat blackened, but unmelted, at 300°. Yield of unrecrystallized material: 2.25 Gm. (55.7% of theoretical).

6-Methyluracil.--Eighty grams (78 cc., 0.61 mole) of ethyl acetoacetate, 13 cc. of absolute alcohol, and 5 drops of concentrated hydrochloric acid were placed in a 6-inch crystallizing dish. Forty grams (0.67 mole) of finely powdered urea were stirred into the mixture and mixed well. The crystallizing dish was covered loosely with a watch glass and placed in a vacuum desiccator over concentrated sulfuric acid. The desiccator was evacuated by aspirator until the mixture had gone to dryness. The aspirator was shut off during the nights, the sulfuric acid was changed daily, and the lumps of solid product (β-uraminocrotonic ester) were broken up occasionally. Forty

grams (1.0 mole) of sodium hydroxide were dissolved with mechanical stirring in 600 cc. of water in a liter beaker and heated to 95°. The finely powdered crude β-uramino-crotonic ester was dissolved in the sodium hydroxide solution. The clear solution was cooled to 65° and carefully acidified while stirring by the slow addition of concentrated hydrochloric acid (approximately 83 cc.). The 6-methyluracil precipitated almost immediately and the mixture became very thick. The mixture was allowed to cool and finally placed in an ice-water bath. The solid was collected on a Büchner funnel, washed with cold water, alcohol, anhydrous ether, and air-dried. After pulverization the 6-methyluracil was obtained as a fine, white powder. m.p. 320-321° with previous darkening. Yield of unrecrystallized material: 58.1 Gm. (74.9% of theoretical).

5-Nitro-6-methyluracil.—Forty cc. (0.857 mole) of fuming nitric acid (Sp.Gr. 1.50) were placed in a 250-cc. Erlenmeyer Flask and cooled in an ice bath. Sixteen grams (0.112 mole) of phosphorus pentoxide were introduced with cooling and shaking and allowed to stand with occasional shaking for one-half hour. Twenty grams (0.156 mole) of 6-methyluracil were added portionwise in the course of fifteen minutes, with shaking and cooling. The reaction mixture was allowed to stand at least twenty-

four hours, by which time there was considerable solid material in the flask. The bright yellow paste was poured over ice in a 400-cc. beaker, giving a green mixture. The solid material was collected on a Büchner funnel, then dissolved in a minimum (about 300 cc.) of boiling water, filtered through a heated Büchner funnel and allowed to crystallize. The resulting crystals, light yellow in color, were collected and air-dried. On being heated in a capillary tube the crystals turn very dark and then decompose with sudden effervescence at 291.5°. Yield; 16.01 Gm. (59.0% of theoretical).

5-Amino-6-methyluracil.--Ten grams (0.058 mole) of 5-nitro-6-methyluracil were dissolved in a liter of hot water and 10 cc. of concentrated ammonia solution were added, followed by 5 Gm. (0.185 mole) of amalgamated aluminum foil (62). To carry out the amalgamation procedure, the foil was first cut into strips about 2 x 10 cm. and loosely crumpled into a 500-cc. Erlenmeyer flask. The foil was covered with ether and allowed to stand ten minutes to become defatted. The foil was washed with alcohol, covered with 5% sodium hydroxide solution and allowed to stand, heating if necessary on the steam bath, until vigorous evolution of hydrogen took place. After being washed twice with water and once with alcohol, the foil was covered with 2% mercuric chloride solution and

allowed to stand for two minutes. After being washed once with water, the amalgamated foil was used at once.

The mixture of nitromethylurecil and aluminum foil was allowed to react spontaneously at first and then by heating over a flame, in all about two hours, replacing the water lost by evaporation. (If the yellow color had not disappeared from the solution the treatment with amalgamated aluminum was repeated.) The mixture was allowed to settle and the supernatant liquid filtered off by a fritted disc filter stick (medium porosity). The solid was boiled once with water and the liquid filtered off and added to the first filtrate. The mixed filtrates were acidified with hydrochloric acid and the solution evaporated to dryness on a steam bath. The yellow solid remaining was ground in a mortar and dissolved in a minimum of hot water, decolorized with charcoal and filtered through a Büchner funnel. The filtrate was made alkaline with concentrated ammonia solution, which precipitated the aminomethylurecil. The product was collected on a Büchner funnel and air-dried. In order to improve the yield, the filtrate in the last step was acidified, evaporated to dryness, dissolved in a minimum of hot water, and made alkaline with ammonia. m.p. Decomposes about 260°. Yield: 6.97 Gm. (85.7% of theoretical).

* 5-Nitrobarbituric acid.--Prepared according to the procedure in Organic Syntheses. m.p. melts with decomposition at 180-182°. Yield: 135 Gm. (44.44% of theoretical).

Uramil.--Nitrobarbituric acid (33.6 Gm., 0.15 mole) was placed in a 2-liter flask together with 200 cc. of concentrated hydrochloric acid. The flask was fitted with a reflux condenser and the mixture heated on a steam bath (HOOD). Mossy tin (84 Gm., 0.71 mole) and a further 133 cc. of concentrated hydrochloric acid were added over fifteen minutes. Heating was continued until no yellow color remained (about eighteen hours in all) with the addition of a further 130 cc. of concentrated hydrochloric acid in three portions after intervals of heating. When no further yellow color remained, an additional 500 cc. of concentrated hydrochloric acid were added and the heating continued until all the white solid had gone into solution. Norit was added and the mixture filtered through a funnel having a sintered glass disc. The filtrate was allowed to stand in the icebox overnight, but since no crystals had formed by morning, the solution was concentrated to some extent under reduced pressure and again stood in the refrigerator overnight. The crystals of uramil were

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* Prepared by W.C. Roemer

collected on a filter and washed first with dilute hydrochloric acid and then washed well with water. The uramil crystals were dried first in a desiccator over concentrated sulfuric acid and then in a desiccator over 40% sodium hydroxide solution. m.p. Does not melt below 300°.

Preparation of Azopyrimidines by Coupling

5-Phenylazo-uracil.--Aniline (0.93 Gm., 0.01 mole) was dissolved in 12.5 cc. (0.025 mole) of 10% sulfuric acid (using heat if necessary) and then the solution was cooled to 2° with mechanical stirring. Slightly more than one equivalent (0.71 Gm., 0.01 mole) of sodium nitrite, dissolved in 2 cc. of water, was added, keeping the temperature below 5°. One and twelve-hundredths grams (0.01 mole) of uracil were dissolved in a solution of one gram (0.025 mole) of sodium hydroxide in 25 cc. of water. The alkaline solution of uracil was cooled to 5° and to it was added the diazo solution, keeping the temperature below 10°. Mechanical stirring, while cooling in an ice bath, was continued for one and one-half hours, following which the mixture was allowed to stand overnight. At that time the pH of the mixture was 8 so a few drops of 10% sulfuric acid were added with stirring to bring the pH to about 6. The orange brown solid was collected by suction filtration and dried in a vacuum desiccator. Yield: 1.69 Gm. (78.2% of theoretical). Purification was attempted

by extraction of one-half gram in a Soxhlet Extractor with Skelly B for twenty-four hours.

Anal. Calcd. for $C_{10}H_8N_4O_2$: C, 55.55; H, 3.73. Found: C, 60.11; H, 3.82; Loss of wt., 1.80.

5-(o-Hydroxyphenylazo)-uracil.--One and one-tenth grams (0.01 mole) of o-aminophenol were dissolved in 15 cc. of 10% sulfuric acid, cooled to 5° and diazotized by the addition of a solution of 0.71 Gm. (0.01 mole) of sodium nitrite in 2 cc. of water all at once below the surface, with mechanical stirring and cooling in an ice-salt bath. This diazo solution was added dropwise with stirring to a cooled (2°) solution of 1.12 Gm. (0.01 mole) of uracil in 15 cc. of water containing one gram of sodium hydroxide. Mechanical stirring was continued for two and one-quarter hours. The reaction mixture was acidified (requiring 1.5 cc. of 10% sulfuric acid) and allowed to stand overnight. The insoluble product was collected by suction filtration, and dried in a vacuum desiccator over calcium chloride. m.p. above 300°. Yield: 0.66 Gm. (28.4% of theoretical). Purification was attempted by extraction with Skelly B in a Soxhlet Extractor for forty-eight hours.

Anal. Calcd. for $C_{10}H_8N_4O_3$: C, 51.71; H, 3.47. Found: C, 44.76; H, 3.88.

5-Phenylazo-barbituric acid (alloxanphenylhydrazone).--Two and four-tenths grams (0.025 mole) of aniline were diazotized by dissolving in a mixture of 7 cc. of concentrated hydrochloric acid and 14 cc. of water, cooling the solution to 5°, and adding to it dropwise a solution of 1.8 Gm. (0.025 mole) of sodium nitrite in 5 cc. of water. Four and one-tenth grams (0.032 mole) of barbituric acid were dissolved in 600 cc. of water and 5 cc. of 10% hydrochloric acid were added. The diazo solution was added dropwise under mechanical stirring to the solution of barbituric acid, whereupon first a pronounced yellow color, then a flocculent yellow precipitate, were produced. After stirring for one-half hour, the stirrer was shut off and the mixture was allowed to stand overnight. The yellow solid material was then collected by suction filtration and dried in a vacuum desiccator over calcium chloride. m.p. 300-302° with decomposition and effervescence. Yield: 4.38 Gm. (58.9% of theoretical).

Alloxanphenylhydrazone.--One and three-tenths grams (0.0081 mole) of alloxan monohydrate were dissolved in 200 cc. of boiling water, and 1.17 Gm. (0.0081 mole) of phenylhydrazine hydrochloride were dissolved in a separate 100 cc. of boiling water. The solution of phenylhydrazine was filtered by gravity into the solution of alloxan, resulting in the immediate production of a

yellow color followed by the formation of a yellow precipitate. The reaction mixture was heated on a steam bath for five and one-half hours, then allowed to stand overnight. The yellow solid was collected by suction filtration and dried in a vacuum desiccator over calcium chloride. m.p. 300-302°. Yield: 0.40 Gm. (21.2% of theoretical). Purification was carried out by extraction with water in a Soxhlet Extractor for forty-eight hours.

5-(o-Hydroxyphenylazo)-barbituric acid.--o-Aminophenol (2.75 Gm., 0.025 mole) was dissolved in a mixture of 7 cc. of concentrated hydrochloric acid (0.065 mole) and 14 cc. of water. The solution was cooled to 2° and diazotized by the dropwise addition, with stirring, of a solution of 1.8 Gm. (0.025 mole) of sodium nitrite in 5 cc. of water. Four and one-tenth grams (0.032 mole) of barbituric acid were dissolved in 600 cc. of water at room temperature. Following completion of the solution, 5 cc. of 10% hydrochloric acid were added and then the cold diazo solution was added dropwise with mechanical stirring. The solution became yellow, then brown in color, and then particles of suspended material appeared in it. Stirring was continued for one hour and then the mixture was allowed to stand overnight. The solid material, which appeared crystalline, was collected by suction filtration and dried in a vacuum

desiccator over calcium chloride, yielding 4.37 Gm. (55.0% of theoretical) of a soft brown powder. Purification of a sample for analysis was effected by extraction with Skelly B for forty-eight hours in a Soxhlet Extractor. m.p. Decomposes with effervescence at 269-273°.

Anal. Calcd. for $C_{10}H_8N_4O_4$: C, 48.38; H, 3.25. Found: C, 48.17; H, 3.38.

Diazouracil.--Three and eighteen-hundredths grams (0.025 mole) of 5-aminouracil were dissolved in a mixture of 6.5 cc. of concentrated hydrochloric acid and 30 cc. of water, with mechanical stirring, and then the solution was cooled in an ice-salt bath to 5°. A solution of 2.0 Gm. (0.027 mole) of sodium nitrite in 5 cc. of water was added dropwise at such a rate as to keep the temperature around 5°. A yellow solid appeared in the reaction mixture and stirring with cooling was continued for one hour. The yellow solid was removed by suction filtration and dried for one and one-half hours in a vacuum desiccator over calcium chloride and then used at once. Yield: 3.44 Gm. (78.9% of theoretical).

Uracil-5-azo-β-naphthol.--Diazouracil (3.22 Gm., 0.03 mole) was suspended in 125 cc. of water in a beaker fitted with a mechanical stirrer, and the mixture cooled

to 5°. A solution of 4.32 Gm. (0.03 mole) of β-naphthol in 24 cc. of 10% sodium hydroxide solution was added dropwise over a period of ninety minutes with cooling and stirring. The buff-colored suspension turned red and became very thick. The mixture was stirred for another hour and then allowed to stand in the ice bath for an additional two hours. The reaction mixture was then acidified with glacial acetic acid, which caused the dark red-colored, semisolid mass to turn vermilion in color. The solid product was collected on a Büchner funnel, washed with water, and dried in a vacuum desiccator over calcium chloride yielding a bright red solid. m.p. 270° with effervescence and previous darkening. Yield: 6.62 Gm. (78.5% of theoretical). By extraction with water in a Soxhlet Extractor for seventy-two hours the decomposition range was raised to 297-298° with effervescence and previous darkening.

5-Diazo-6-methyluracil.--5-Amino-6-methyluracil (3.53 Gm., 0.025 mole) was dissolved or suspended in a mixture of 7 cc. of concentrated hydrochloric acid and 25 cc. of water with mechanical stirring in a 150-cc. beaker. The mixture was cooled in an ice-salt bath to 0°, and a solution of 2 Gm. (0.028 mole) of sodium nitrite in 5 cc. of water was added dropwise from a dropping funnel, keeping the temperature below 5°. This took about ten minutes.

Stirring with cooling in the ice-salt bath was continued for forty minutes, then the solid, pink-colored product was collected on a fritted disc funnel, washed with water, and dried for one hour in a vacuum desiccator over calcium chloride, then used at once in the coupling reaction. Yield: 4.72 Gm. (80.7% of theoretical).

6-Methyluracil-5-azo-β-naphthol.--Five and three-quarters grams (0.041 mole) of 5-amino-6-methyluracil were dissolved with the aid of heat in a mixture of 25 cc. of concentrated hydrochloric acid and 50 cc. of water and the solution was cooled to 2°, resulting in a buff-colored suspension. Diazotization was carried out by the dropwise addition, with stirring, of a solution of 3.3 Gm. (0.048 mole) of sodium nitrite in 20 cc. of water, keeping the temperature below 5°. A solution of β-naphthol in 10% sodium hydroxide was next added dropwise to couple with the diazo compound. When about 17 cc. of this solution, corresponding to 4.73 Gm. (0.033 mole) of β-naphthol, had been added, together with 10% sodium hydroxide solution to render the pH about 8, there was only a slight amount of uncoupled diazo compound remaining in the mixture so addition was stopped but stirring was continued for a further hour and a half. The solid, reddish material was collected on a Büchner funnel, transferred to a beaker, suspended in water, and the

solution was acidified to pH 6 with concentrated hydrochloric acid. The solid was then collected by suction filtration, washed well with water, then twice with ether, and dried in a vacuum desiccator over calcium chloride. m.p. Starts to soften at 120°, is completely liquid by 145°. Yield of bright pink powder 4.28 Gm. (35.4% of theoretical). A sample for analysis was obtained by extracting with water in a Soxhlet Extractor. This sample was scarlet red in color and decomposed at 273-275°.

Anal. Calcd. for $C_{15}H_{12}N_4O_3$: C, 60.80; H, 4.08. Found: C, 59.15; H, 4.70.

Azouracil-5,5'.—A solution of 3.18 Gm. (0.025 mole) of 5-aminouracil in a mixture of 6.5 cc. of concentrated hydrochloric acid and 50 cc. of water was cooled to 0° in an ice-salt bath, with mechanical stirring. To this solution was added dropwise a solution of 2.0 Gm. (0.025 mole) of sodium nitrite, keeping the temperature below 5°. Stirring at 0-5° was continued for one hour, at which time the yellow solid was collected on a fritted disc funnel and suspended at once in 25 cc. of water in a beaker. To this suspension, cooled to 0°, there was added dropwise a solution of 2.52 Gm. (90% of 0.025 mole) of uracil in 20 cc. of 10% sodium hydroxide solution. This addition was made over fifty minutes, keeping the temp-

erature below 5°. During this time the cream-colored suspension turned orange, then bright red. The mixture was stirred a further three and one-half hours with cooling, then allowed to stand overnight. The solid product was collected on a Büchner funnel and dried in a desiccator over calcium chloride. A sample for analysis was purified by extraction for forty-eight hours in a fritted disc thimble in a Soxhlet Extractor by Skelly B. m.p. Decomposed by 280°. The first analysis yielded over 10% ash so a second sample was submitted for analysis with the request that a special catalyst be used to ensure complete combustion.

Anal. Calcd. for $C_8H_6N_6O_4$: C, 38.40; H, 2.42. Found: C, 37.04; H, 2.81.

Preparation of Azopyrimidines by Ring Closure.

Ethyl phenylazo-acetoacetate (43).--Two and three-tenths grams (0.1 mole) of sodium were dissolved in 30 cc. of absolute alcohol in a 500-cc., 3-neck flask fitted with a mechanical stirrer and a reflux condenser and protected by a calcium chloride drying tube. Thirteen grams (0.1 mole) of ethyl acetoacetate were added and the flask cooled in an ice-salt bath. Redistilled aniline (9.3 Gm., 0.1 mole) was diazotized, after dissolving in a mixture of 22 cc. of concentrated hydrochloric acid and 25 cc. of water and cooling this solution to 5°, by the dropwise addition

with cooling and stirring of a solution of 7.1 Gm. (0.1 mole) of sodium nitrite in 20 cc. of water, keeping the temperature during the addition below 5°. The diazonium chloride solution was added dropwise with cooling and stirring to the sodium enolate of the ethyl acetoacetate in the flask, which caused production of an orange color, and then the formation of a solid product, requiring the addition of further liquid (10 cc. of water and 15 cc. of absolute alcohol). While a small amount of the diazonium salt solution had still not been added, the reaction mixture showed the presence of an excess of diazonium (Resorcinol test), so addition was stopped. After stirring for an additional hour an excess of water (250 cc.) was added. The resulting precipitate was collected on a Buchner funnel, washed with water, and dried in a vacuum desiccator over calcium chloride. Yield: 17.15 Gm. (73.2% of theoretical). m.p. 78-84°.

Ethyl o-hydroxyphenylazo-acetoacetate (o-hydroxyphenylazo-acetoacetic ester).--Sodium (2.3 Gm., 0.1 mole) was dissolved in 40 cc. of absolute alcohol in a 500-cc., 3-neck flask, fitted with a stirrer, a water condenser and a dropping funnel, the whole apparatus being protected from moisture by means of calcium chloride drying tubes. Thirteen grams (0.1 mole) of ethyl acetoacetate were added dropwise from the dropping funnel, with stirring. The

o-aminophenol was diazotized (7.1 Gm. of sodium nitrite, dissolved in 20 cc. of water, were added all at once below the surface of a cooled (2°) solution of 10.9 Gm. (0.1 mole) of o-aminophenol in 22 cc. of concentrated hydrochloric acid and 22 cc. of water) and added by means of the dropping funnel to the reaction flask, with mechanical stirring and cooling in an ice-salt bath. A red-colored compound formed at first which later was replaced by a yellow-brown compound as the solution became acid. Stirring was continued for two hours and the mixture allowed to stand overnight. An excess of water was added, in which the product was insoluble. The solid product was filtered off, washed with water and dried over calcium chloride in a vacuum desiccator. The product was dissolved in a minimum of hot absolute alcohol and water was added to cause crystallization. The best of three runs yielded 50.7% of theoretical. m.p. after three recrystallizations: 160.5-162°.

Anal. Calcd. for $C_{12}H_{14}N_2O_4$: C, 57.59; H, 5.64. Found: C, 57.56; H, 5.85.

Ethyl o-hydroxy-α-naphthylazo-acetoacetate.--A 200-cc., 3-neck flask was fitted with a mechanical stirrer, a water condenser, and dropping funnel, and the whole system protected from moisture by calcium chloride drying tubes. Twenty cc. of absolute alcohol were placed in the

flask and 1.2 Gm. (0.05 mole) of sodium was dissolved in it, heating the flask on a steam bath if necessary to complete the reaction. Then 6.5 Gm. (0.05 mole) of ethyl acetoacetate were added dropwise from the dropping funnel with stirring, followed by the dropwise addition of the diazotized aminonaphthol solution while stirring and cooling the flask in an ice bath. (The diazotized aminonaphthol was prepared as follows: 9.8 Gm. (0.05 mole) of 1-amino-2-naphthol hydrochloride were dissolved in a mixture of 8 cc. of concentrated hydrochloric acid and 8 cc. of water with heat, followed by cooling of the solution to below 5°, at which time a solution of 3.6 Gm. (0.05 mole) of sodium nitrite in 10 cc. of water was added dropwise with cooling and stirring; after being tested with both congo red paper and starch-iodide paper, the diazo solution was used at once.) Following the addition of the diazo compound, an excess of water was added to the reaction flask, which caused precipitation of an insoluble product. The insoluble product was filtered off by suction and dried in air. Yield: 4.40 Gm. (29.3% of theoretical). Attempted recrystallization from alcohol was unsuccessful.

o-Hydroxyphenylazo-acetylacetone.--Two and three-tenths grams (0.1 mole) of sodium metal were dissolved in 40 cc. of absolute alcohol in a 500-cc. 3-neck flask fitted with a mechanical stirrer and a water conden-

ser, the whole system being protected by calcium chloride drying tubes. Heat was required to complete the reaction. Ten grams (0.1 mole) of acetylacetone, which had been purified by drying over anhydrous sodium sulfate for thirty-six hours then by distillation after filtration, was added to the flask with stirring, resulting in a white, semisolid. Ten and nine-tenths grams (0.1 mole) of o-aminophenol were diazotized by dissolving in a mixture of 22 cc. of concentrated hydrochloric acid and 25 cc. of water, cooling to 5° and adding a solution of 7.1 Gm. (0.1 mole) of sodium nitrite in 20 cc. of water all at once below the surface of the o-aminophenol solution, with continued cooling and mechanical stirring. The diazotized o-aminophenol was added via a dropping funnel to the cooled mixture of the sodium enolate of acetylacetone. After completion of the addition, 250 cc. of water were added to precipitate the coupled product. The solid, light-brown product was collected by suction filtration and dried in a vacuum desiccator over calcium chloride. m.p. Decomposes between 245-255°. Yield: 11.75 Gm. (53.3% of theoretical). (A later run which was stirred for six and one-half hours and allowed to stand overnight yielded 70.0% of theoretical.) A sample for analysis was prepared by recrystallization three times from absolute alcohol, yielding a light-brown product which decomposed at 249° with effervescence and some previous blackening.

Anal. Calcd. for $C_{11}H_{12}N_2O_3$: C, 59.99; H, 5.49. Found: C, 60.41; H, 5.96.

5-(o-hydroxyphenylazo)-6-methyluracil.--Seventy-five cc. of absolute alcohol were placed in a 500-cc., 3-neck flask fitted with a mechanical stirrer, a condenser protected by a calcium chloride tube, and a stopper for the third neck, through which additions were made to the flask. Two and three-tenths grams (0.1 mole) of sodium were added to the flask and dissolved in the alcohol. Three grams (0.05 mole) of dried urea were added and solution effected by stirring. Then 12.5 Gm. (0.05 mole) of ethyl o-hydroxy-phenylazo-acetoacetate were added and the reaction mixture was refluxed on a steam bath for three and one-half hours with stirring and then allowed to stand overnight at room temperature. The alcohol was removed under reduced pressure, with heating on a steam bath. The dark red residue was dissolved in a minimum (40 cc.) of hot water and acidified with glacial acetic acid. A brown oil resulted, which on cooling the flask in an ice bath solidified to a mossy, soft, tarry mass, black in color. This mass was collected on a Büchner funnel and dried in a vacuum desiccator over calcium chloride, then pulverized to yield 5.38 Gm. (43.7% of theoretical) of a dark red powder. Attempted recrystallization from absolute alcohol with acetic acid failed, so the solvent was removed, and the

residue precipitated from solution in sodium hydroxide solution by concentrated hydrochloric acid, the precipitate being collected, dried, and pulverized to yield a soft brown powder. This solid material was extracted for forty-eight hours with Skelly B in a Soxhlet Extractor, and dried in vacuum at 56°. m.p. Decomposes at 194° with effervescence and previous darkening and softening.

Anal. Calcd. for $C_{11}H_{10}N_4O_3$: C, 53.66; H, 4.09. Found: C, 54.67; H, 4.43.

2-Thio-4-hydroxy-5-o-hydroxyphenylazo-6-methylpyrimidine.---Seventy-five cc. of absolute alcohol were placed in a 500-cc. flask fitted with a mechanical stirrer and a water condenser, the whole system being protected from moisture by calcium chloride drying tubes. Sodium metal (2.3 Gm., 0.1 mole) was dissolved in the absolute alcohol. This was followed by the addition of 3.8 Gm. (0.05 mole) of thiourea and 12.5 Gm. (0.05 mole) of ethyl o-hydroxyphenylazo-acetoacetate, and the mixture was refluxed for two and one-half hours on a steam bath, with stirring. At the end of this time the mixture was extremely thick so a further 75 cc. of absolute alcohol were added and the mixture was allowed to stand overnight. The alcohol was evaporated under diminished pressure with some heating on a steam bath, and the residue was dissolved

in a minimum of hot water. On acidification with glacial acetic acid, a dark red solid separated out, which was collected on a fritted disc funnel, washed with water, and dried in a vacuum desiccator over calcium chloride. Yield: 6.45 Gm. (49.3% of theoretical). Purification was attempted by dissolving the solid in 10% sodium hydroxide and reprecipitation by glacial acetic acid. The solid material (dark red in color) was extracted in a Soxhlet Extractor with Skelly B for thirty-six hours and dried in vacuum.

Anal. Calcd. for $C_{11}H_{10}N_4O_2S$: C, 50.37; H, 3.84. Found: C, 50.18; H, 3.80; Ash from CH, 4.65 (non-alkaline).

Acetamidine hydrochloride (63).--The acetonitrile used in this preparation had been purified by standing over calcium chloride for one week with occasional shaking, followed by filtration and distillation. The absolute alcohol had been dried by treatment with sodium and ethyl phthalate (64). Eighty-eight grams (2.14 moles) of acetonitrile and 126 cc. (2.19 moles) of absolute alcohol were placed in a suction flask, fitted with a gas entry tube and a magnetic stirrer, and the apparatus was tared. The flask was placed in an ice-salt bath, stirring was commenced and dry hydrogen chloride gas was run in until the weight had increased 96.1 Gm. (2.63 moles). The flask was closed, except for the calcium chloride drying tube,

and allowed to sit in an ice-bath overnight. By morning the reaction mixture had solidified to a solid mass of crystals. The crystalline mass of acetamido ethyl ether hydrochloride was broken up, ground to a paste in a dry mortar with 100 cc. of absolute alcohol, and returned to the thick-walled suction flask. Five hundred and fifty cc. of 8.5% ammonia in absolute alcohol were added and the mixture stirred by magnetic stirrer for five hours. The separated ammonium chloride was removed by suction filtration and the filtrate evaporated under reduced pressure on a steam bath to a volume of about 200 cc. The solid product was collected by suction filtration and washed with about 50 cc. of absolute alcohol and then dried in a vacuum desiccator over calcium chloride. m.p. 164-171°. Yield: 98.8 Gm. (48.8% of theoretical). The product when recrystallized from absolute alcohol melted 166-170°.

2,6-Dimethyl-4-hydroxy-5-phenylazo-pyrimidine.--Two and one-half grams (0.1 mole) of sodium were dissolved in 100 cc. of absolute alcohol in a 500 cc., 3-neck flask which was fitted with a mechanical stirrer, a reflux condenser and a dropping funnel, the whole system being protected by calcium chloride drying tubes. Ethyl phenylazo-acetoacetate (11.71 Gm., 0.05 mole) was added and dissolved with stirring to produce a red solution.

A solution of 4.73 Gm. (0.05 mole) of acetamidine hydrochloride in 45 cc. of absolute alcohol was added dropwise to the flask by means of the dropping funnel over a period of two hours while refluxing the reaction mixture. Refluxing and stirring were continued for twenty hours at which time the dark red mixture was filtered. The solvent alcohol was removed by vacuum evaporation and the resulting solid dissolved in a minimum of hot water. This aqueous solution was filtered and then acidified with glacial acetic acid, giving an orange precipitate which was collected, heated with 100 cc. of 5% sodium bicarbonate solution, collected again, washed with water, and dried in a vacuum desiccator over calcium chloride. m.p. 168-170° (dec.). Yield: 1.31 Gm. (11.5% of theoretical). After extraction for twenty-four hours by Skelly B in a Soxhlet Extractor, followed by drying, the decomposition point was 172-173°.

Metallizations.

Metallization of uracil-5-azo-β-naphthol with nickel. The azo compound (1.41 Gm., 0.005 mole) was dissolved by mechanical stirring while heating on a steam bath, in a mixture of 40 cc. triethanolamine and 20 cc. of water. A solution of 0.97 Gm. (2/3 equivalent) of nickel nitrate was added over a period of one hour with stirring and maintaining the temperature at 85-90°, and stirring

was continued for an additional two and one-half hours. Paper chromatograms were made before, during, and at intervals after the completion of the addition of the nickel nitrate. An excess of water was added to the reaction mixture and the mixture was then heated to 85°. The solid material was collected by filtration while still hot and then dried in a vacuum desiccator over calcium chloride. The dried solid, dark red in color, was powdered and extracted in a Soxhlet Extractor first with water and then with acetone, and again dried. Yield: 0.55 Gm. (32.5% of theoretical).

Anal. Calcd. for $C_{14}H_{10}N_4O_4Ni$: C, 47.11; H, 2.82; Ni, 16.44. Found: C, 46.05; H, 3.48; Ni, 16.10.

Metallization of Uracil-5-azo-β-naphthol with cobalt. The azo compound (1.41 Gm., 0.005 mole) was placed in 20 cc. water and heated on a steam bath. Solution was effected by the dropwise addition of 8N sodium hydroxide solution to a pH of approximately 9, and heating was continued to 85°. A solution of 1.10 Gm. (0.005 mole) of cobaltous chloride in 5 cc. of water was added dropwise over one-half hour with stirring and heating, and periodic addition of a few drops of 8N sodium hydroxide solution to maintain the pH at 8. Stirring was continued for a further two and one-half hours, adding small quantities of water from time to time. Paper chromatograms were made before,

during, and at intervals after the completion of the addition of the cobalt chloride. An excess of water (about 100 cc.) was added and the mixture was heated to 90°, then allowed to cool to room temperature. The supernatant liquid was removed and the solid washed once with a large amount of distilled water at room temperature. The solid was removed and dried in a vacuum desiccator over calcium chloride, pulverized, and transferred to a fritted glass funnel, where it was washed continuously by suction with hot acetone (about two days) and then with hot alcohol (95%) to yield a dark red solid which does not melt.

Anal. Calcd. for $C_{14}H_{10}N_4O_4Co.H_2O$: C, 44.81; H, 3.22. Found: C, 42.72; H, 3.22; Loss of wt., 2.28.

Metallization of Uracil-5-azo-β-naphthol with Iron. The azo compound (2.82 Gm., 0.01 mole) was dissolved in 80 cc. of triethanolamine and 40 cc. of water, with mechanical stirring while heating on a steam bath. A solution of 2.78 Gm. (0.01 mole) of ferrous sulfate in 10 cc. of water was added over a period of ten minutes with stirring and heating to maintain the temperature at 85-90°. Stirring was continued for a further two hours and forty minutes, the mixture was allowed to stand overnight, and stirring and heating were continued the next day for an additional two hours. Paper chromatograms were made before, during, and at intervals after the completion of the

addition of the ferrous sulfate. An excess of water was added, stirring and heating continued to 85° and the black solid product separated by filtration on a fritted disc funnel. The soft black mass was transferred to an extraction thimble and extracted for six hours with water and for two hours with acetone, and the resulting black solid dried in a vacuum desiccator over calcium chloride.

Anal. Calcd. for $C_{14}H_{10}N_4O_4Fe$: C, 47.48; H, 2.85. Found: C, 54.57; H, 5.08.

Metallization of 2-thio-6-methyl-5-(o-hydroxyphenylazo)-uracil with cobalt.--Fifteen cc. of ethylene glycol and 5 cc. of water were placed in a beaker fitted with a thermometer and a mechanical stirrer. The solution was heated to 90° on a steam bath and then 1.31 Gm. (0.005 mole) of 2-thio-6-methyl-5-(o-hydroxyphenylazo)-uracil was added and dissolved. The pH of the reaction mixture was made alkaline with a few drops of 8 normal sodium hydroxide solution. A solution of 1.1 Gm. (0.0044 mole) of cobalt acetate tetrahydrate in 10 cc. of water was added dropwise, keeping the pH up to 8 by the addition of a few drops of 8 normal sodium hydroxide solution as required. This addition was made over fifty minutes and stirring was continued for a further hour and forty minutes. Paper chromatograms were made before the addition of cobalt acetate, at intervals during the

addition, and after completion of the addition of cobalt acetate, as well as at the end of the period of stirring. One hundred cc. of boiling water were added to the reaction mixture and the solid material removed by filtration. The dark colored solid was dried and pulverized. A sample for analysis was extracted for forty-eight hours by Skelly B in a Soxhlet Extractor. m.p. above 300°.

Anal. Calcd. for $C_{11}H_{10}N_4O_2SCo.3H_2O$: C, 33.76; H, 4.12. Found: C, 29.88; H, 3.52; Loss of wt., 3.03.

Metallization of 2-thio-6-methyl-5-(o-hydroxyphenylazo)-uracil with iron.--One and thirty-one hundredths grams (0.005 mole) of 2-thio-6-methyl-5-(o-hydroxyphenylazo)-uracil were added to 20 cc. of water which had been heated on a steam bath to 90° in a beaker fitted with a thermometer and a mechanical stirrer. While stirring, 8 normal sodium hydroxide solution was added dropwise to cause the azo dye to dissolve. A paper chromatogram was made of the reaction mixture. A solution of 1.3 Gm. (0.0047 mole) of ferrous sulfate heptahydrate in 10 cc. of water was added dropwise to the dye solution over approximately thirty-five minutes, adding a few drops of 8 normal sodium hydroxide solution as required to keep the pH up to 8, and making frequent paper chromatograms. Stirring while heating on the steam bath was continued for one hour, with the addition of small quantities of water

to replace the water lost by vaporization. After a final paper chromatogram was made, 100 cc. of hot water was added with stirring. The black solid material was removed by filtration.

Anal. Calcd. for $C_{11}H_{10}N_4O_3SFe$: C, 39.55; H, 3.02. Found: C, 31.92; H, 2.08; Loss of wt., 4.92.

Metallization of 1-(o-hydroxyphenylazo)-2-naphthol with chromium.---Fifteen cc. of ethylene glycol were heated to 97° on a steam bath in a beaker fitted with a thermometer and a mechanical stirrer. The azo dye (o-aminophenol→ β-naphthol)* (2.64 Gm., 0.01 mole) was stirred in and, after a few drops of 8 normal sodium hydroxide solution had been added to make the mixture alkaline in reaction (pH 8), a paper chromatogram was made. A solution of 1.64 Gm. (0.0033 mole) of chromium acetate ($Cr_2(C_2H_3O_2)_6.2H_2O$) in 15 cc. of water was added dropwise over a period of one-half hour, maintaining the pH of the reaction mixture at 8 by the addition as required of a few drops of 8 normal sodium hydroxide solution. Stirring was continued for one hour after the completion of the addition of the chromium solution. Paper chromatograms were made at intervals during the addition of the chromium and after the period of stirring.

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One hundred cc. of boiling water were poured into the reaction mixture with stirring and the insoluble product was collected, washed well with water, and dried in a vacuum desiccator over calcium chloride to yield 1.86 Gm. of a dark blue powder. m.p. Does not melt below 300°. A sample for analysis was extracted for twenty-four hours by Skelly B in a Soxhlet Extractor. This sample gave a negative test for sodium (zinc uranyl acetate test).

Anal. Calcd. for $C_{16}H_{13}N_2O_4Cr$: C, 55.01; H, 3.75; Cr, 14.89. Found: C, 55.27; H, 4.16; Cr, 12.5.

Metallization of 1-(o-hydroxyphenylazo)-2-naphthol with iron.--Fifteen cc. of ethylene glycol were heated to 97° on a steam bath in a beaker fitted with a thermometer and a mechanical stirrer. The azo dye (o-aminophenol→β-naphthol)* (2.64 Gm., 0.01 mole) was stirred in and, after a few drops of 8 normal sodium hydroxide solution had been added to make the mixture alkaline in reaction (pH 8), a paper chromatogram was made. A solution of 2.70 Gm. (0.0097 mole) of ferrous sulfate heptahydrate in 15 cc. of water was added dropwise maintaining the pH of the reaction mixture at 8 by the addition as required of a few drops of 8 normal sodium hydroxide solution. Stirring was continued for two hours after the completion of the addition of the iron

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solution. Paper chromatograms were made at intervals during the addition of the ferrous sulfate and after the period of stirring. One hundred cc. of boiling water were poured into the reaction mixture with stirring and the insoluble product was collected, washed twice with hot water and once with cold, and dried in a vacuum desiccator over calcium chloride. Yield of dark brown powder: 1.25 Gm. A sample for analysis was extracted for forty-eight hours by Skelly B in a Soxhlet Extractor. m.p. Does not melt below 300°. Anal. Calcd. for $C_{16}H_{12}N_2O_3Fe.2H_2O$: C, 49.25; H, 4.65; Fe, 14.31. Found: C, 43.38; H, 5.24; Fe, 24.6.

Metallization of 1-(o-hydroxyphenylazo)-2-naphthol with copper.--Fifteen cc. of ethylene glycol were heated to 97° on a steam bath in a beaker fitted with a thermometer and a mechanical stirrer. The azo dye (o-aminophenol→β-naphthol)* (2.64 Gm., 0.01 mole) was stirred in and, after a few drops of 8 normal sodium hydroxide solution had been added to make the mixture alkaline in reaction (pH 8), a paper chromatogram was made. A solution of 2.5 Gm. (0.0092 mole) of cupric sulfate pentahydrate in 15 cc. of water was added dropwise over a period of one-half hour, maintaining the pH of the reaction mixture at 8 by the addition as required

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of a few drops of 8 normal sodium hydroxide solution. Stirring was continued for one and one-half hours after the completion of the addition of the copper sulfate solution. Paper chromatograms were made at intervals during the addition of the copper solution and after the period of stirring. One hundred cc. of boiling water were poured into the reaction mixture with stirring and the insoluble product was collected, washed well with hot water, and dried in a vacuum desiccator over calcium chloride to yield 2.44 Gm. of dark red powder. m.p. Does not melt below 300°. A sample for analysis was extracted for forty-eight hours by Skelly B in a Soxhlet Extractor.

Anal. Calcd. for $C_{16}H_{12}N_2O_3Cu$: C, 55.89; H, 3.52; Cu, 18.48. Found: C, 55.15; H, 3.16; Cu, 21.9.

Metallization of 4-(o-hydroxyphenylazo)-1-naphthol with chromium.--Fifteen cc. of ethylene glycol were heated to 97° on a steam bath in a pear-shaped flask fitted with a thermometer and a mechanical stirrer. The azo dye (o-aminophenol→ ∝-naphthol)* (2.64 Gm., 0.01 mole) was stirred in and, after a few drops of 8 normal sodium hydroxide solution had been added to make the mixture alkaline in reaction (pH 8), a paper chromatogram was made. A solution of 1.64 Gm. (0.0033 mole) of chromium acetate ($Cr_2(C_2H_3O_2)_6.2H_2O$) in 15 cc. of water was added dropwise

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maintaining the pH of the reaction mixture at 8 by the addition as required of a few drops of 8 normal sodium hydroxide solution. Stirring was continued for two hours after the completion of the addition of the chromium solution. Paper chromatograms were made at intervals during the addition of the chromium acetate and after the period of stirring. One hundred cc. of boiling water were poured into the reaction mixture with stirring and the insoluble product was collected, washed well with water, and dried in a vacuum desiccator over calcium chloride, yielding 2.68 Gm. of dark brown powder. m.p. Does not melt below 300°. A sample for analysis was extracted for forty-eight hours by Skelly B in a Soxhlet Extractor.

Anal. Calcd. for $C_{16}H_{13}N_2O_4Cr.H_2O$: C, 52.52; H, 4.12; Cr. 14.16. Found: C, 50.81; H, 4.42; Cr, 17.8

Metallization of 4-(o-hydroxyphenylazo)-1-naphthol with iron.--Fifteen cc. of ethylene glycol were heated to 97° on a steam bath in a beaker fitted with a thermometer and a mechanical stirrer. The azo dye (o-aminophenol→ ∝-naphthol)* (2.64 Gm., 0.01 mole) was stirred in and, after a few drops of 8 normal sodium hydroxide solution had been added to make the mixture alkaline in reaction (pH 8), a paper chromatogram was made. A solution of 2.70 Gm. (0.0097 mole) of ferrous

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sulfate heptahydrate in 15 cc. of water was added dropwise, maintaining the pH of the reaction mixture at 8 by the addition as required of a few drops of 8 normal sodium hydroxide solution. Stirring was continued for one hour after the completion of the addition of the iron solution. Paper chromatograms were made at intervals during the addition of the ferrous sulfate solution and after the period of stirring. One hundred cc. of boiling water were poured into the reaction mixture with stirring and the insoluble product was collected and dried in a vacuum desiccator over calcium chloride. Yield: 2.48 Gm. of dark brown powder. m.p. Does not melt below 300°. A sample for analysis was extracted with Skelly B for forty-eight hours in a Soxhlet Extractor.

Anal. Calcd. for $C_{16}H_{12}N_2O_5Fe.2H_2O$: C, 51.64; H, 4.33; Fe, 15.00. Found: C, 47.49; H, 5.49; Fe, 18.5.

Analyses

The analyses for carbon and hydrogen were done by Clark Microanalytical Laboratory, Urbana, Ill., by the combustion of a sample at about 1000° in the presence of oxygen. In one instance the quantity of ash remaining was as high as ten percent. So the sample was resubmitted for analysis by combustion in the presence of a special catalyst to ensure complete combustion.

The analyses for chromium were done by Clark Microanalytical Laboratory, Urbana, Ill., by ignition at 1000-1100°C. in absence of oxygen to ensure complete conversion of the chromium to chromic oxide (Cr_2O_3).

The analyses for iron were done by the Wisconsin Alumni Research Foundation, Madison, Wisconsin, using the colorimetric procedure of Sandell (65).

The analysis for copper was done by the Wisconsin Alumni Research Foundation, Madison, Wisconsin, using the first-action procedure of the A.O.A.C. (66).

The analysis for nickel was done by breaking down the complex and determination as the dimethylglyoxime complex using Lange's (67) modification of the procedure of Hillebrand and Lundell (68).

The qualitative test for the presence of sodium in certain of the metal chelates was done by breaking the chelate with acid (and heat if necessary) and then using zinc uranyl acetate reagent according to the procedure of Kolthoff and Sandell (69).

The qualitative test for the presence of sulfate radical in the chelates of the azonaphthols was the test for sulfur by fusion with sodium as outlined in McElvain (70).

Absorption Spectra

The Cary Recording Spectrophotometer was used to record the absorption spectra of several azopyrimidines and azonaphthalenes using visible light (Tungsten light source and Corex cells) and scanning from 800 mμ to 275 mμ. The solvent used in all cases was acetone, and the speed of the recording paper was set at 2-Hi, corresponding to a change of 25 mμ per division on the recording paper. The concentrations of the azo compounds used were as indicated. The absorption spectra are illustrated in the figures indicated.

Fig. 1 (page 53). Alloxanphenylhydrazone, 0.01 Gm. per liter. Maximum at 380 mμ.

Fig. 2 (page 54). 5-Phenylazo-barbituric acid, 0.01 Gm per liter. Maximum at 380 mμ.

Fig. 3 (page 55). 5-(o-Hydroxyphenylazo)-barbituric acid, 0.01 Gm. per liter. Maximum at 405 mμ.

Fig. 4 (page 57). 4-(o-Hydroxyphenylazo)-1-naphthol, 0.02 Gm. per liter. Maximum at 420 mμ.

Fig. 5 (page 58). 1-(o-Hydroxyphenylazo)-2-naphthol, 0.01 Gm. per liter. Maximum at 485 mμ.

Fig. 6 (page 60). Uracil-5-azo-β-naphthol, 0.02 Gm. per liter. Maximum at 480 mμ.

Fig. 7 (page 61). Uracil-5-azobenzene, 0.01 Gm. per liter. Maximum at 345 mμ.

SUMMARY

A brief review has been made of the possible pharmacological activities of azo dyes and their metal chelates. Azo dyes may exert their toxic effects on organisms or tissues by the removal of trace metals as a result of chelate formation. The metal chelates of azo dyes may act by supplying the metals in the small amounts needed to produce the effects which actually result. A number of azo compounds containing the pyrimidine ring have been synthesized in the hope that they would have pharmacological activity and to test the hypothesis that metal supply or removal may have an effect on tumor growth. The following new azopyrimidines and intermediates have been prepared:

5-(o-Hydroxyphenylazo)-uracil

5-(o-Hydroxyphenylazo)-barbituric acid

Ethyl o-hydroxyphenylazo-acetoacetate

Ethyl o-hydroxy-α-naphthylazo-acetoacetate

o-Hydroxyphenylazo-acetylacetone

5-(o-Hydroxyphenylazo)-6-methyluracil

2-Thio-4-hydroxy-5-(o-hydroxyphenylazo)-6-methyl-pyrimidine

6-Methyluracil-5-azo-β-naphthol

Azouracil-5,5'

These azopyrimidines have been found to undergo chelation with metals. Analytical data are presented for the following chelates of azopyrimidines:

Nickel chelate of uracil-5-azo-β-naphthol

Cobalt chelate of " " "

Iron chelate of " " "

Cobalt chelate of 2-thio-6-methyl-5-(o-hydroxyphenylazo)-uracil

Iron chelate of 2-thio-6-methyl-5-(o-hydroxyphenylazo)-uracil

Since the metal chelates of azopyrimidines are so intractable several chelates were made of azonaphthols, in order to obtain a better idea of this type of structure. Analytical data are presented for:

Iron chelate of 1-(o-hydroxyphenylazo)-2-naphthol

Chromium chelate of " "

Copper chelate of " "

Iron chelate of 4-(o-hydroxyphenylazo)-1-naphthol

Chromium chelate of " "

The difficulties encountered in assigning a structure to the hydroxy- and amino-substituted pyrimidines are discussed, and the contributions toward their solution which have appeared in the literature are cited. Spectral absorption curves are presented for the following azopyrimidines and azonaphthols:

Alloxanphenylhydrazone

5-Phenylazo-barbituric acid

5-(o-Hydroxyphenylazo)-barbituric acid

Uracil-5-azobenzene

Uracil-5-azo-β-naphthol

4-(o-Hydroxyphenylazo)-1-naphthol

1-(o-Hydroxyphenylazo)-2-naphthol

On the basis of the absorption spectra for the azopyrimidines and azonaphthols and the analytical data for the metal chelates, as well as by a consideration of Fisher-Taylor-Hirschfelder models, structures are assigned for the metal chelates of the azonaphthols and for the azopyrimidines.

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