AZOPYRIMIDINES AND THEIR CHELATING ABILITY

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by

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INTRODUCTION

The first recorded observation of the production of a tumour-like proliferation by a pure chemical compound of any type was made by Fischer in 1906. He described the atypical epithelial proliferation which resulted following the injection of a solution of scarlet red into the ears of rabbits. Aferwards it was found that the active part of the molecule is o-aminoazotoluene (1).

During the past two decades scores of other azo compounds have been tested for carcinogenic activity and many have been shown to be active.

Following Haddow's (2) demonstration that certain carcinogenic hydrocarbons are able to inhibit the growth of certain tumours in animals, many compounds with a structural resemblance to carcinogenic substances were studied, for example, the unsymmetrical azonaphthalene.

Later (3), further studies of the possible relationship between the structure and the tumour-growth-retarding properties of azo compounds were made. Several compounds gave evidence of significant inhibition of growth of certain tumours in animals.

Foye (4), working on the basis that these analogs could serve effectively as transporting agents for introducing metal ions at the site of tumour growth, prepared several azo derivatives of the naphthols and phenanthrols. Later (5), pharmacological studies indicated that they had certain antitubercular activity in mice.

Because of the importance of the pyrimidines as cell constituents in the form of nucleic acids, and their importance in present-day therapeutics in such drugs as the barbiturate hypnotics, sulfas, antithyroid agents and vitamins, to mention a few, interest has been shown in the effect azopyrimidines and their metal chelates would have on inhibiting tumour growth.

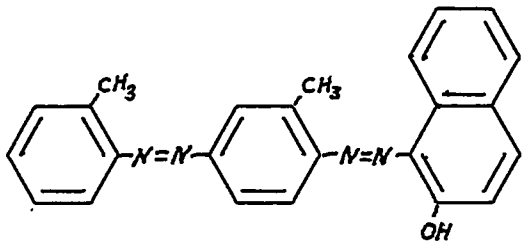
The present work was undertaken in an attempt to prepare certain azopyrimidines having one or two hydroxyl groups in an ortho-position with respect to the azo linkage, and to determine whether or not these will form metal chelates. In an effort to eliminate any interfering effect which other hydroxyl or amino groups at other positions in the molecule might have, compounds were selected having none of these tautomeric groups present except the one or two hydroxyl groups in the ortho-positions.

HISTORICAL

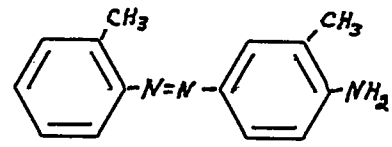
Azo Compounds as Inhibitors of Tumour Growth

In 1906 Fischer described the atypical epithelial proliferation which resulted following the injection of a solution of scarlet red (I) into the ears of rabbits. The growths always receded and never became malignant; nevertheless, this was the first recorded instance of the production of a tumour-like proliferation by a pure chemical compound of any type. At Fischer's suggestion, scarlet red soon came into use to accelerate wound healing.

Not long afterwards it was found that the active part of the molecule is o-aminoazotoluene (II). This compound was found to have an effect on epithelial cells similar to that of scarlet red, and it was likewise effective in accelerating healing.



I



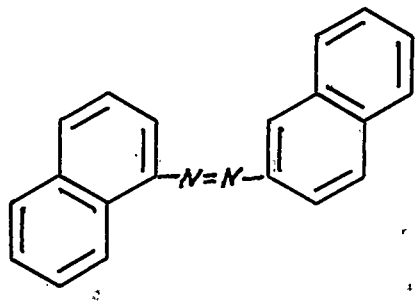
II

Soon after the discovery of the carcinogenic activity of 1,2,5,6-dibenzanthracene by Cook, other workers reported that the addition of o-aminoazotoluene to the food leads to the production of malignant liver tumours in rats.

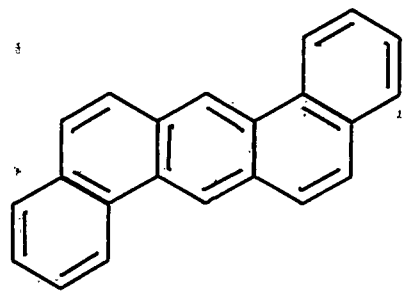
During the past two decades scores of other azo compounds have been tested for carcinogenic activity of this type and many have been shown to be active.

Unlike the polycyclic aromatic hydrocarbons, the carcinogenic azo compounds do not, as a rule, produce tumours at the site of injection, but mostly affect the liver. Furthermore, the induction of liver tumours with azo compounds is markedly influenced by the diet, and if a "protective" diet is supplied, the production of tumours can be strongly inhibited or even entirely prevented (1).

Following the demonstration by Haddow that certain carcinogenic hydrocarbons are able to inhibit the growth of certain tumours in animals, many compounds with a structural resemblance to carcinogenic substances were subjected to this type of bioassay. Isolated instances of inhibition of tumour growth with non-carcinogenic substances were observed, for example, with the unsymmetrical azonaphthalene (III). Haddow suggested that this compound bears a superficial resemblance to the carcinogen, 1,2,5,6-dibenzanthracene (IV). However, the only forms in which the unsymmetrical azonaphthalene (III) has features in common with the polynuclear carcinogenic hydrocarbons are the cis structures (V) and (VI) which resemble benz-derivatives of both 1,2-benzanthracene and chrysene. The hydrocarbon 1,2,5,6-dibenzphenanthrene, suggested by formula (VI) was found to be an inhibitor of tumour growth.



III



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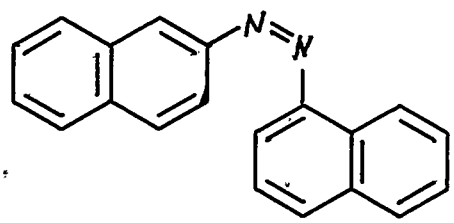
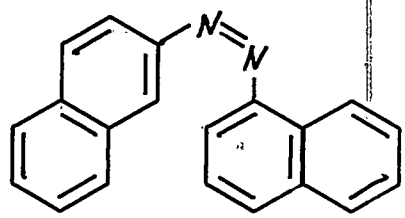


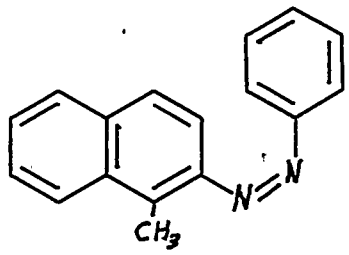
V



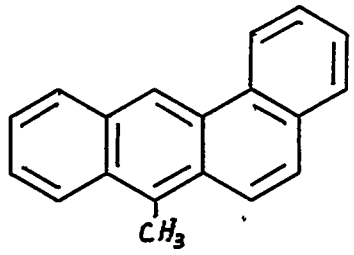
VI

In order to explore further possible relationships between structure of azo compounds and their activity as inhibitors of tumour growth, about twenty-five azo compounds were prepared. When written in the hypothetical cis configuration, most of them bear a spatial resemblance to a carcinogenic polynuclear hydrocarbon. As an example, the cis structure is written for 2-benzeneazo-1-methylnaphthalene (VII) and may be compared with the potent carcinogen 10-methyl-1,2-benzanthracene (VIII). No presumption is made as to the actual existence of these substances in the cis form, although

there is evidence that conversion of the *trans* to the *cis* form can be effected to a significant degree by exposure to ultraviolet light (2).



VII



VIII

Seligman *et al.* (3) in a further exploration of a possible relationship between the structure and the tumour-growth-retarding properties of azo compounds, studied the effect of thirty-one azo compounds on sarcoma 37 in mice and on the Walker carcinoma in rats. Six of the compounds gave evidence of significant inhibition of growth in one type of tumour. Two compounds inhibited the growth of both tumours.

Foye *et al.* (4) indicated that Haddow's demonstration of the tumour-inhibiting possibilities of azo analogs of the carcinogenic hydrocarbons suggested that these analogs are substantive to tissue components involved in tumour formation. He felt that such compounds could serve effectively as transporting agents for introducing metal ions at the site of tumour growth. The presence of the metal might be of value in inhibiting tumour growth, or it might act as indicator of the enzyme systems involved in the process of tumour formation and growth. Accordingly, a series

had been prepared of metallized azo derivatives of the naphthols and phenanthrols which bore a structural relationship to, and represented metallized analogs of, the various hydrocarbon structures which are active in aiding or inhibiting tumour growth. Later, Foye and Jeffrey (5) reported that iron, copper and chromium chelates of some o-hydroxyazo naphthols and phenanthrols have been found to suppress the growth of M. tuberculosis H37Rv both in vitro and in vivo. The metal chelates showed a significant difference in activity from that of the nonmetallized azo compounds.

Pyrimidines as Inhibitors of Tumour Growth

Of the three pyrimidine bases investigated by Barker et al. (6) orotic acid showed no action on tumour growth; 4,5-diaminouracil was too toxic to allow of continued dosage; 4-aminouracil showed a significant inhibiting effect on grafted tumours in mice, the greatest effect being observed with Crocker sarcoma.

Burchenal et al. (7) indicated that certain of the diamino-dichlorophenyl-pyrimidines were effective in prolonging the survival time of mice inoculated with the Ak_4 strain of leukemia and caused a rapid fall in the number of leukemic leukocytes in the peripheral blood when administered in the late stage of the disease.

Clarke et al. (8) reported inhibition of mouse sarcoma 180 with certain 2,4-diamino-pyrimidines with hydrogen or the smaller alkyl groups in the 6-position.

Studies of 2,4-bis(ethylenimino)-6-chloropyrimidine by Elion et al. (9) have revealed its inhibitory activity for sarcoma 180 and certain other mouse tumours and its greater effectiveness against several rat tumours.

Pyrimidine Chemistry

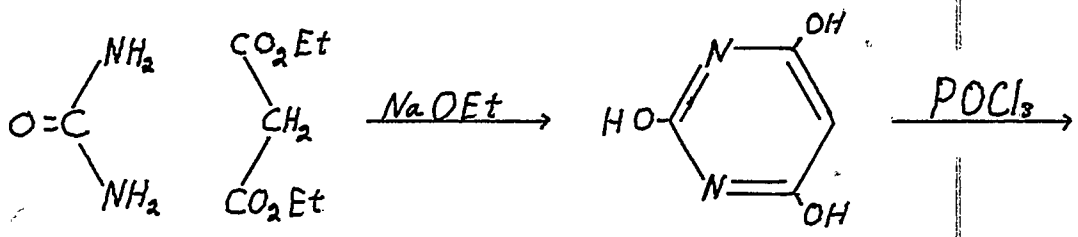
The chemistry of pyrimidine, and of condensed systems containing the pyrimidine nucleus has been developed largely because certain derivatives are important constituents of living organisms. The complex cell constituents known as nucleic acids have a biological significance scarcely inferior to that of proteins and polysaccharides; they are built up from pyrimidine and purine compounds.

In view of the part played in cellular economy by such compounds, it is not surprising that pyrimidine derivatives should be known which have important pharmacological activity. The barbiturate hypnotics, which have been known for many years, are used very widely in clinical practice. Sulphadiazine and its 4-methyl and 4,6-dimethyl derivatives are outstandingly valuable amongst sulphonamides in the treatment of bacterial infections. The antithyroid activity of thiouracil has also an important place in medicine.

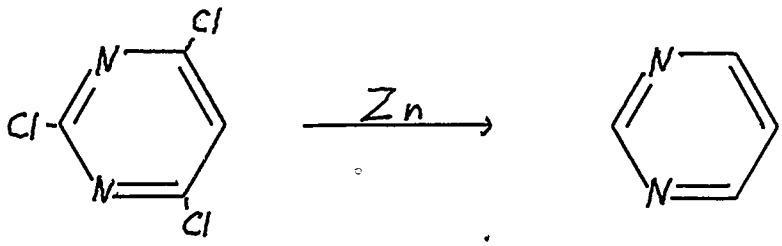
Much of our knowledge of the chemistry of pyrimidines consists of synthetic methods; not only the methods by means of which the pyrimidine nucleus can be built up, but also the numerous transformations which substituent groups can undergo. The main deficiencies in pyrimidine chemistry concern the simpler compounds; for example, pyrimidine itself

has probably never been obtained in amounts of more than a gram, and its 5-hydroxyderivative is unknown.

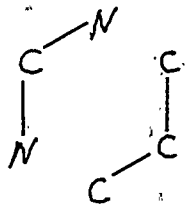
Most of the methods for the preparation of pyrimidine compounds involve two separate stages, namely a synthesis of the ring-system, followed by a process in which the substituent groups are transformed into those present in the desired compound. These processes can be illustrated by the following preparation of pyrimidine itself from barbituric acid (IX).



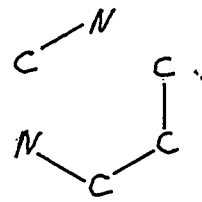
IX



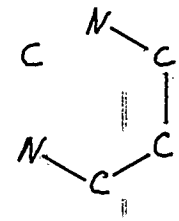
The methods available for the formation of the pyrimidine nucleus can be classified into three main types, according to the distribution of nitrogen atoms in the two compounds used:



Type I

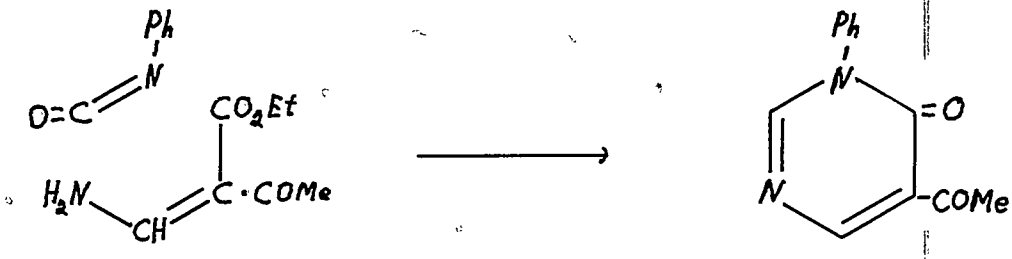


Type II



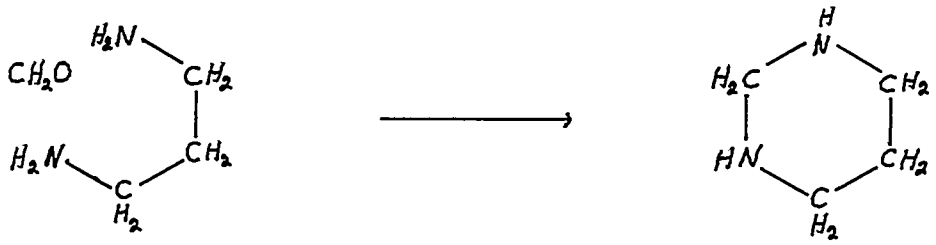
Type III

Examples of Type I are involved in the synthesis of pyrimidine, already mentioned, uracil and cytosine. The nitrogenous (left-hand) compounds can also be compounds such as thiourea, guanidine, and amidines. The second (right-hand) 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 from phenyl isocyanate and aminomethyleneacetoacetic ester; none of the methods of this type achieved much practical importance.



Type III is illustrated by formation of hexahydropyrimidine from formaldehyde and 1,3-diaminopropane; such syntheses also

had little practical value (10).



Although various conditions are used, the Type I reactions usually take place at room temperature or on heating when the reactants are combined in the presence of an alkaline reagent such as sodium hydroxide or sodium ethoxide (11).

Three prominent features of pyrimidine chemistry are:

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

(ii) Nuclear substituents vary in their behaviour 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 behaviour are observed. The contrast is parallel to that between β-substituted pyridines and their α-and γ-isomers.

The replaceability of groups such as Cl, OEt, and SEt is used extensively in the preparation of those substituted pyrimidines which cannot be obtained directly by building up the pyrimidine nucleus.

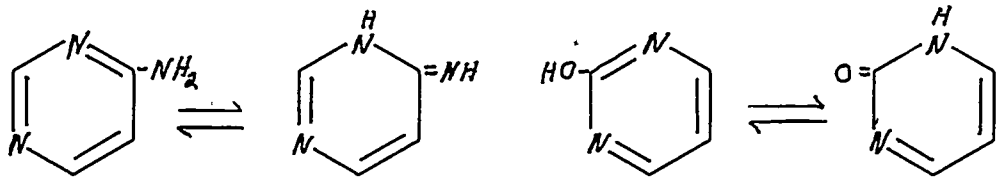
The most important replacements of chlorine atoms are by amino or substituted amino groups, usually by heating the chloro-compound with alcoholic ammonia or with the appropriate amine, and by alkoxyl groups, by the action of sodium alkoxides. Ethoxyl groups are replaced by hydroxyl groups by treatment with dilute acids, or by amino groups by the action of hot alcoholic ammonia. Ethylthio groups can similarly be replaced by hydroxyl, amino, and substituted amino groups.

(iii) The aromatic behaviour mentioned in (i) diminishes progressively as hydroxyl or amino 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) appear 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 behaviour of benzene were known only through the reactions of compounds like phloroglucinol. The groups which give rise to this atypical behaviour (OH, SH, NH_2) have been termed, somewhat loosely, "tautomeric" substituents.

Hydroxyprimidines bearing up to three hydroxyl groups at positions 2, 4, and 6 are well known; they include uracil, thymine, and barbituric acid. They show no phenolic behaviour. The "hydroxyl groups" are replaced by chlorine atoms on heating with phosphoryl chloride. 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.

Pyrimidines bearing amino groups at the 2, 4, or 6 positions also show anomalous properties. They are difficult to acylate, and with nitrous acid rather sluggish deamination occurs, apparently without the intervention of diazonium compounds.

The anomalous properties of compounds containing "hydroxyl" or "amino" groups at the 2, 4, or 6 positions suggests that they may in reality be derivatives of imino- or keto-dihydropyrimidines (10).



Azopyrimidines

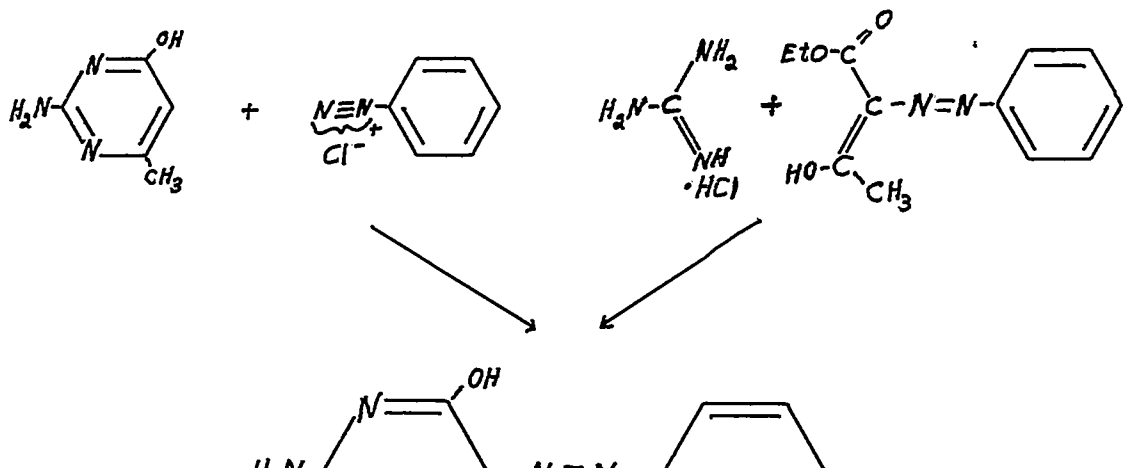
Some early evidence of the coupling of pyrimidines with diazonium compounds was indicated by the formation of colour. Johnson and Clapp (12,13) used the production or nonproduction of colour on treating diazotized sulphanilic acid with uracil, thymine, cytosine, and several of their derivatives, as a criterion in an endeavour to locate the position of the sugar or phosphate residues in natural pyrimidine nucleotides. Attempts to isolate the coloured product were recorded as unsuccessful.

Lythgoe et al. (13) studied the coupling of pyrimidine derivatives with diazonium salts in order to devise a method for the introduction of a 5-amino group into 6-amino-4-glycosidaminopyrimidines which would not involve danger of hydrolyzing the sugar linkage. A survey of the structural conditions governing the coupling of pyrimidine derivatives and their relation to those governing nitrosation had been made.

Bogert and Davidson (14) made a survey of the azo derivatives of the pyrimidines with particular reference to derivatives of uracil.

Several other workers (15,16,17,18) have prepared various azopyrimidines and used them as a suitable approach in the preparation of other compounds. For example, Hull et al. (16), to introduce a 5-amino group into the pyrimidine nucleus, reduced a 5-benzeneazo group in order to prepare derivatives of 2,5-diaminopyrimidine.

Polonovski and Pesson (19) reported the preparation of a number of azopyrimidines and isolated the products. As an example of their work may be shown the compound 2-amino-4-hydroxy-5-phenylazo-6-methylpyrimidine (X). This was prepared by coupling phenyldiazonium chloride with 2-amino-4-hydroxy-6-methylpyrimidine, and its structure proven by synthesis from guanidine hydrochloride and diazotized acetoacetic ester, under the influence of sodium in absolute alcohol.



X

Illustrated here are the two methods of preparing azopyrimidines; coupling of diazonium salts with substituted pyrimidines, and formation of the pyrimidine ring after previous coupling of a diazonium salt with one of the components.

Polonovski and Pesson reached the following conclusions regarding coupling on the pyrimidine ring:

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

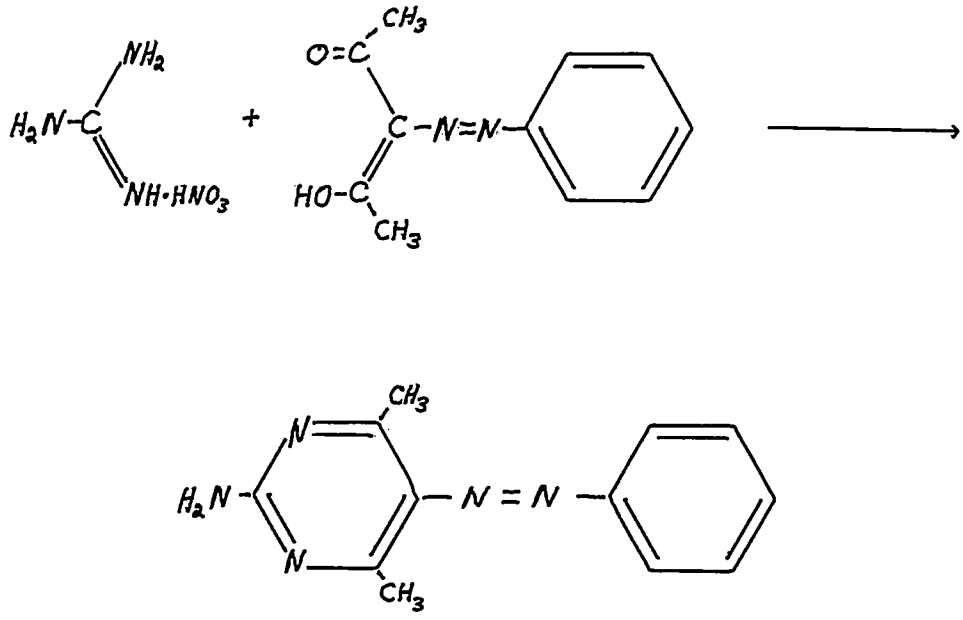
(ii) That the coupling of phenyldiazonium chloride with a monohydroxy (or amino) pyrimidine derivative occurs only if the hydroxy (or amino) group is on position 2.

(iii) That in the case of the dihydroxy (or aminohydroxy) derivatives

(a) if one of the groups is on position 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 in good yield and the mechanism appears to compare with that of the coupling of compounds having an active methylene.

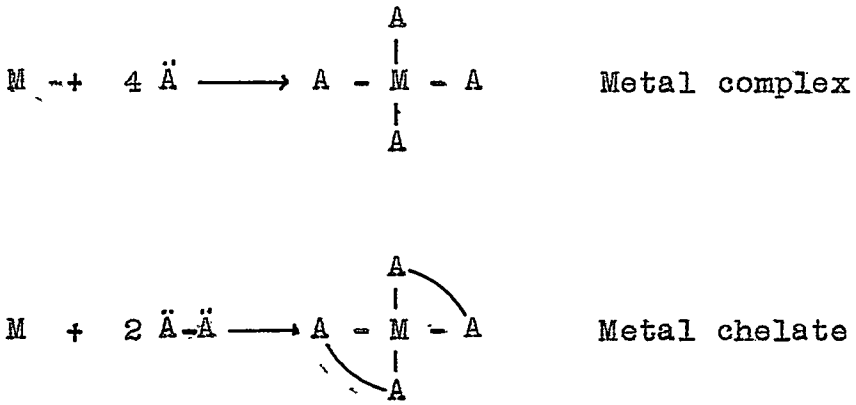
Rose (20) prepared a series of 5-amino-pyrimidines (most of them substituted in position 2 by additional amine residues) by the reduction of the phenylazopyrimidines catalytically with hydrogen over Raney nickel. The phenylazopyrimidines were prepared by ring closure in aqueous-methanolic sodium hydroxide. For example, in preparing 2-amino-4,6-dimethyl-5-phenylazopyrimidine (XI) he effected condensation of phenylazoacetylacetone with guanidine nitrate.



XI

Metal Chelates of Azo Compounds

When a metal ion combines with an electron donor, the resulting substance is said to be a complex, or coordination compound. If the substance which combines with the metal contains two or more donor groups so that one or more rings are formed, the resulting structure is said to be a chelate compound, or metal chelate, and the donor is said to be a chelating agent. Simple examples of complex formation and chelation may be represented schematically in the following way:



where M represents a metal ion; Ä represents a complexing agent; and Ä-Ä represents a chelating agent (21).

The term "chelate" proposed by Morgan (22,23) 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.

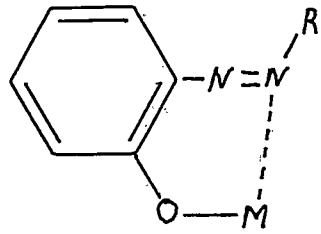
The formation of these rings may involve either primary or secondary valence. In subsequent papers Morgan used the expression "chelate rings" to cover all three types, that is, rings formed by two primary valences, by one primary and one secondary valence, or by two secondary valences. Primary or principal 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 (23).

Among the various 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 amino, and secondary amino (23).

As secondary valence groups which combine with metals by simple coordination or addition without the replacement of hydrogen are the following groups: primary amino, secondary amino, tertiary amino, cyclic tertiary amino, oxime, alcoholic hydroxyl, carbonyl, and thioether (23).

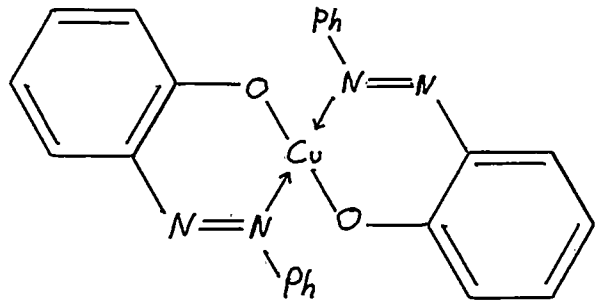
The work of Ley on the chelates of amino acids demonstrated that chelates with five- and six-membered rings are the most stable. Since this preliminary work of Ley, a large amount of evidence has accumulated indicating that practically all chelates have five- or six-membered rings. Four-membered chelate rings are relatively rare. Few chelate rings containing more than six atoms have been prepared from bidentate (having two positions through which covalent or coordinate covalent bonds with the metal may be formed) compounds (24).

In the course of a study of the chelate rings formed in mordant dyeing, Morgan (25) showed that in the azo dyestuffs containing an o-hydroxyl group, mordanting took place with the formation of chelate rings, the metal being attached by secondary valence (broken line) to the azo-group:



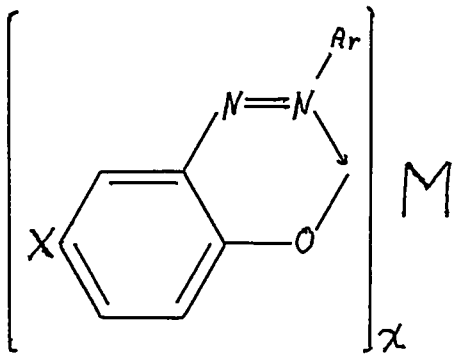
Such ring formation cannot occur when the hydroxyl group is in the meta- or para-positions, and this is in accord with the long-established rule that such compounds are never dyestuffs (23).

On examination of models, from the point of view of strain, Drew and Landquist (26) showed that a copper atom in the cupric derivatives of the o-hydroxyazo-compounds, whether planar or tetrahedral, cannot be coordinated with both nitrogen atoms of an azo-group. The derivatives must therefore contain copper in combination with nitrogen of the azo-groups in the following manner:

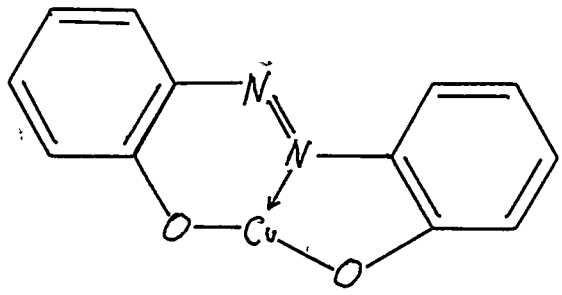


Elkins and Hunter (27) described cupric, nickel and cobaltic salts of some o-monohydroxyazo-compounds prepared by

the action of the metallic acetates on these azo compounds. They found that the formation of coordinated salts was common to all o-hydroxyazo-compounds. They believed that the reason for the stability of these salts was to be found in their chelated structure, since the metallic salts of the corresponding p-hydroxyazo-compounds, if formed at all, are extremely unstable. The salts described show properties typical of coordinated compounds: they are insoluble in water, sparingly soluble in polar solvents, but readily soluble in non-polar solvents; they melt in the neighbourhood of 200° to form deeply coloured liquids. The salts corresponded to the formula:

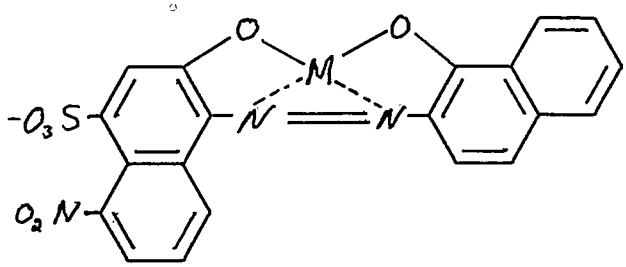


Drew and Landquist (26) indicated that the lakes of the o,o'-dihydroxyazo-compounds have the monomeric structure in which only one nitrogen of the azo-group is coordinated, the metal being associated in a 5-atom and in a 6-atom ring. They observed from models that the structure was very little strained, the molecule being nearly flat. The azo-group is necessarily of the anti-form.



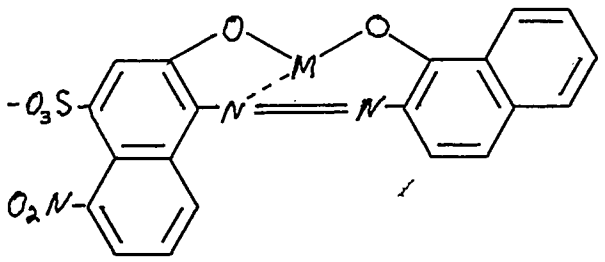
XII

Schwarzenbach (28) proposed the following structure (XIII) for the chelate of Eriochromeschwarz-T:



XIII

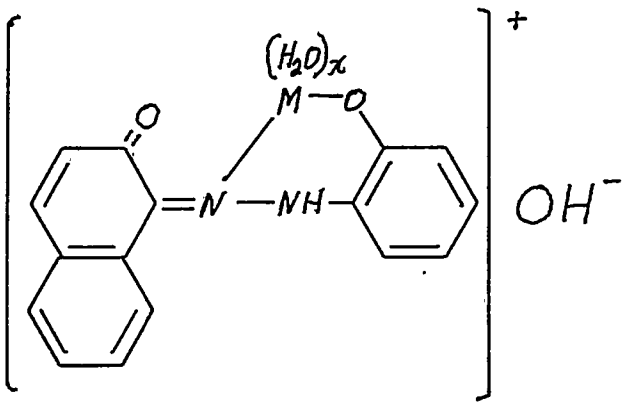
However, Martell (29), in discussing Eriochromeschwarz-T, indicates that chelation with a metal ion results in the displacement of two protons to give derivatives of the type indicated below (XIV), with one or more additional covalencies satisfied by other donors, such as water molecules.



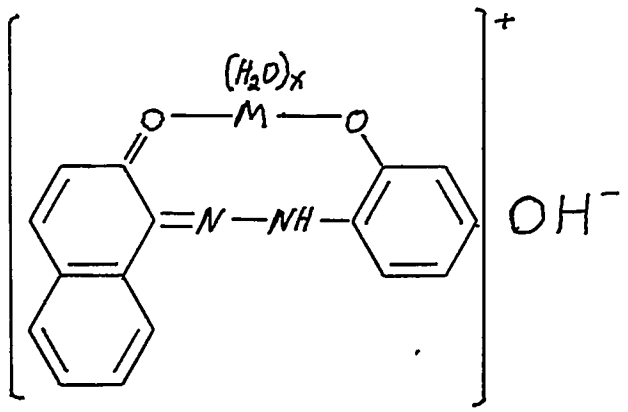
XIV

The structures of chromium complexes have not been established with certainty, and different forms containing different ratios of chromium to azo compound are known. A 1:1 ratio of metal to azo compound is generally indicated, however. For maximum stability two metallizable groups are needed, one on each side of the azo linkage and ortho to this linkage, and at least one of these groups should be hydroxyl. The other group, if not hydroxyl, is usually amino, carboxyl, or an enolic hydroxyl (30).

On the basis of analytical results and absorption spectra, Foye and Jeffrey (5) represented the structures of the metal chelates of the o-hydroxyazo naphthols by the general formulas XV and XVI.



XV



XVI

Examination (5) of the Fisher-Taylor-Hirschfelder models of structures (XV) and (XVI) showed either one capable of existence. It was impossible to form a structure having valence bonds between the metal and both oxygens simultaneously with a nitrogen-to-metal coordination. This seems to be in disagreement with the structure proposed by Drew and Landquist (26) for the chelates of o,o'-dihydroxyazo-compounds.

DISCUSSION

Syntheses of Intermediates

One of the main components used in the syntheses of the azopyrimidines proposed in this work was acetamidine hydrochloride. The compound is commercially available but is relatively expensive. A feasible procedure for preparing it in the laboratory is described in "Organic Syntheses" (31). In the four runs made during the course of the work the yields varied between 26% and 75% of theoretical.

Due to the nature of the azopyrimidines proposed for preparation here, the azo group could not be introduced directly by coupling of a pyrimidine with a diazonium compound. It was necessary to prepare the azopyrimidines by the formation of the pyrimidine ring after previous coupling of a diazonium salt with one of the components.

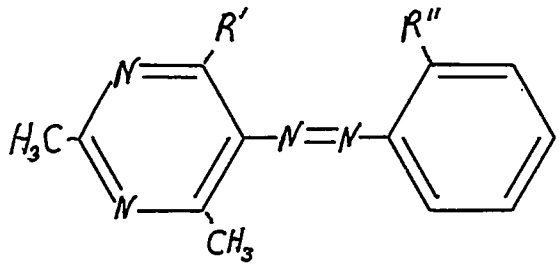
The o-hydroxyphenylazo-acetylacetone, ethyl o-hydroxyphenylazo-acetoacetate, and the ethyl phenylazo-acetoacetate required for the syntheses of these azopyrimidines were prepared by the procedure described by Rose (20) for preparing phenylazo-acetylacetone. Aniline (or o-aminophenol) was diazotized and the diazo solution was added to a stirred suspension of diketone (or keto ester) in water and anhydrous sodium carbonate. The product formed rapidly and after about 30 minutes it was collected and recrystallized. The yields were usually excellent.

Two other compounds, phenylazo-acetylacetone and ethyl o-methoxyphenylazo-acetoacetate, were prepared success-

fully although they were not required in the syntheses of the proposed azopyrimidines.

Syntheses of the Azopyrimidines

The preparation of the azopyrimidines Ia, IIa, and IIIa was attempted in an effort to eliminate any interfering



Ia $R' = OH$, $R'' = H$

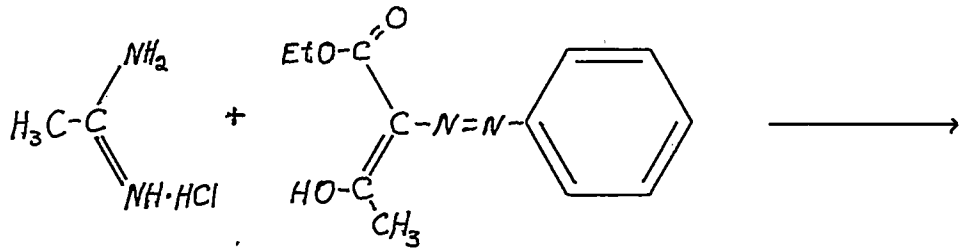
IIa $R' = CH_3$, $R'' = OH$

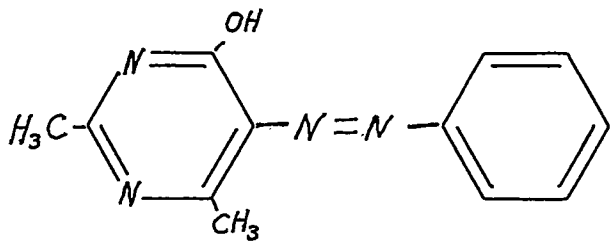
IIIa $R' = OH$, $R'' = OH$

effect which other hydroxyl or amino groups at other positions in the molecule might have when determining whether or not these will form metal chelates.

From the conclusions drawn by Polonovski and Pesson (19) the azo group, in the proposed azopyrimidines, could not be introduced directly by coupling of the pyrimidine with a diazonium compound. It was necessary to prepare the azopyrimidines by the formation of the pyrimidine ring after previous coupling of a diazonium salt with one of the components. This is essentially the Type I method for the syntheses of pyrimidines.

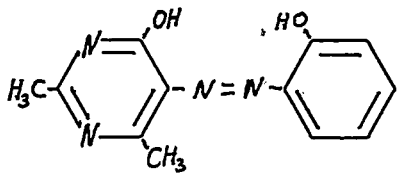
Following the procedure described by Andersag and Westphal (32), 2,6-dimethyl-4-hydroxy-5-phenylazo-pyrimidine (XVII) was prepared by condensing ethyl phenylazo-acetoacetate with acetamidine hydrochloride in absolute alcohol in the presence of sodium ethoxide. In a similar way, 2,6-



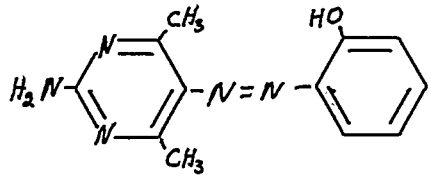


XVII

dimethyl-4-hydroxy-5-o-hydroxyphenylazo-pyrimidine (XVIII) was prepared by condensing acetamidine hydrochloride with ethyl o-hydroxyphenylazo-acetoacetate, and 2-amino-4,6-dimethyl-5-o-hydroxyphenylazo-pyrimidine (XIX) by condensing guanidine hydrochloride with o-hydroxyphenylazo-acetylacetone.



XVIII



XIX

Although various conditions are used, this type of reaction usually takes place at room temperature or on heating when the reactants are combined in the presence of an alkaline reagent such as sodium hydroxide or sodium ethoxide. Rose (20) had successfully prepared 2-amino-4,6-dimethyl-5-phenylazo-pyrimidine by condensing phenylazo-acetylacetone with guanidine nitrate in aqueous-methanolic sodium hydroxide with the aid of heat. However, two attempts to prepare 2,6-dimethyl-4-hydroxy-5-phenylazo-pyrimidine using the same conditions resulted in the isolation of a bright yellow product whose melting point was much lower than that of the wanted product.

Polonovski and Pesson had prepared a number of azopyrimidines using the general procedure, with slight modification, described by Andersag and Westphal. The azopyrimidines described here were also prepared in a similar way.

Some difficulty was encountered in preparing 2,6-dimethyl-4-hydroxy-5-o-hydroxyphenylazo-pyrimidine. Of the several attempts made, either the original starting material, ethyl o-hydroxyphenylazo-acetoacetate, was recovered or analysis of the isolated product indicated it to be the

original starting material. When alcohol was removed from the reaction mixture and the tarry residue dissolved in water and the solution subsequently acidified with glacial acetic acid, a red oil separated out which stiffened on cooling, facilitating its isolation. In one run this tar had dried when placed over calcium chloride under vacuum for two days, but we failed to isolate the wanted product. In another attempt the tar remained as such even after a longer period over calcium chloride under vacuum.

Fanta and Hedman (33) reported a wide variety of media and tempertures tried for the condensation of acetamidine hydrochloride and sodium nitromalonaldehyde. A very poor yield of 2-methyl-5-nitro pyrimidine was obtained when the reaction was run with sodium hydroxide in ethanol at 25° & or with piperidine in the absence of solvent at 90°. Under other conditions either an intractable tar was formed or unreacted sodium nitromalonaldehyde was recovered. The acetamidine hydrolyzed with the formation of ammonia which reacted with nitromalonaldehyde to give a dark, amorphous product. A similar product was obtained when sodium nitromalonaldehyde was treated with concentrated aqueous ammonia or with ammonium chloride, ammonium acetate or acetamide under the same conditions. The elemental analysis of the resin did not correspond to the condensation of nitromalonaldehyde with ammonia in a simple ratio. It was concluded that the relative rates of the competing condensation and hydrolysis reactions favours pyrimidine formation from

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& All temperatures are stated in degrees Centigrade.

the aryl and aryl-alkyl amidines but not from the alkyl and hydroxyalkyl amidines.

The same difficulty was encountered here with the intractable tar. The reaction mixture, particularly when heated, evolved ammonia. It was found that by removing the alcohol from the reaction mixture, dissolving the residue in water and then digesting the resulting solution on a water bath for about three-quarters of an hour the interference by the tar was eliminated. After digestion the solution was allowed to cool and the resulting precipitate isolated. It was then dissolved in hot water, the solution filtered and the filtrate acidified with glacial acetic acid when 2,6-dimethyl-4-hydroxy-5-o-hydroxyphenylazo-pyrimidine appeared as a bright red precipitate.

After a number of attempts we failed to prepare, 2,4,6-trimethyl-5-o-hydroxyphenylazo-pyrimidine by condensing o-hydroxyphenylazo-acetylacetone with acetamidine hydrochloride in absolute alcohol in the presence of sodium ethoxide. The reactions were run at room temperature and on refluxing. Under both conditions either an intractable tar was formed or unreacted o-hydroxyphenylazo-acetylacetone was recovered. Digestion on a water bath as indicated in the preparation of 2,6-dimethyl-4-hydroxy-5-o-hydroxyphenylazo-pyrimidine did not solve the problem.

Due to the failure to obtain 2,4,6-trimethyl-5-o-hydroxyphenylazo-pyrimidine, 2-amino-4,6-dimethyl-5-o-hydroxyphenylazo-pyrimidine was successfully prepared in the hope that during metallization the amino group on a position

other than ortho to the azo linkage would not interfere.

Metallization of the Azopyrimidines

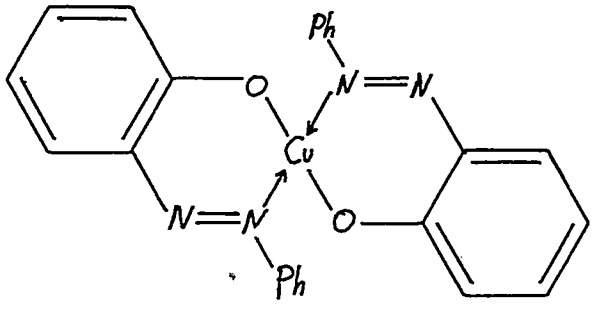
Both the o-monohydroxyazo-pyrimidine and the o,o'-dihydroxyazo-pyrimidine were metallized with nickel and cobalt. Metallization was carried out according to the general procedure described by Foye et al. (4). An aqueous solution of the metallic salt was added slowly to a solution of the azopyrimidine in ethylene glycol, the reaction mixture being maintained at a temperature of 85° and at a pH of 8 by the addition of sufficient 8N sodium hydroxide solution. Filter paper chromatograms were prepared by dissolving two drops of reaction mixture in one ml. of dimethylformamide, pouring onto a filter paper pressed between glass plates bearing a funnel through a perforation in the top plate, and developing the spot with more of the same solvent. The reactions were generally complete in ten to twenty minutes as indicated by the chromatograms showing the presence of only one component, of a colour different from that of the original unmetallized compound. The mixtures were then poured into water, heated to 85°, and the product isolated by elutriation and/or centrifugation. The products were washed well with water, and dried under vacuum over calcium chloride. None of the compounds melted below 360°.

Martell and Calvin (34) have described a number of criteria for determining chelate formation, of which the following were shown by the azopyrimidine preparations: colour of product, decreased aqueous solubility, and drop in

pH during formation.

Analysis for nickel was done gravimetrically using the dimethylglyoxime method (35), and for cobalt colorimetrically using a slightly modified nitroso-R salt method (36).

The structure (XX) proposed by Drew and Landquist (26) for the cupric derivatives of the o-monohydroxyazo-compounds is essentially the same structure proposed by Elkins and Hunter (27) for the cupric, nickel, and cobaltic derivatives of the o-monohydroxyazo-compounds. The nickel complexes described by Elkins and Hunter formed dark olive-green needles, insoluble in water, slightly soluble in alcohol, acetone, ether, and ligroin, but soluble in benzene, toluene, and chloroform with dark green colour. In polar solvents, especially when warm, hydrolysis frequently occurred with deposition of nickel hydroxide, the solution changing colour from green to brown.



XX

Analysis indicated a 1:2 ratio of the metal to azo-pyrimidine for the o-monohydroxyazo-pyrimidine. The nickel complex of the o-monohydroxyazo-pyrimidine formed dark olive-

green minute crystals which behaved similarly to the ones described by Elkins and Hunter with respect to the solvents. However, they did not melt below 360°, only darkened in colour. Elkins and Hunter believed that the reason for the stability of the salts was to be found in their chelated structure, since the metallic salts of the corresponding p-hydroxyazo-compounds, if formed at all, were extremely unstable.

Since chelate formation is generally characterized by intensification of colour, the retention of the green colour peculiar to nickel salts suggests that a salt of the o-monohydroxyazo-pyrimidine is formed rather than the chelate. The analytical results, although not too exact, agreed for the chelate structure proposed by Drew and Landquist and for the salt. On vacuum drying,* the green crystals turned brown due to a loss of four moles of water. In the salt structure the remaining four coordination bonds would be taken up by the water molecules. Nickel dimethylglyoxime was readily formed when an alcoholic solution of dimethylglyoxime was added to an alcoholic solution of the nickel derivative of o-monohydroxyazo-pyrimidine. This was not so in the case of the nickel derivative of o,o'-dihydroxyazo-pyrimidine, an o,o'-dihydroxyazo-compound which is generally known to form metal chelates. This indicates that the nickel is made more readily available to form nickel dimethylglyoxime from the metallized o-monohydroxyazo-pyrimidine than from the metallized o,o'-dihydroxyazo-pyrimidine. This evidence suggests a salt (XXI) as more likely than a chelate structure for the

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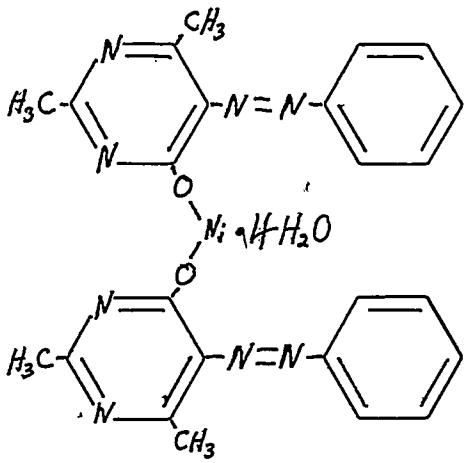
* Over magnesium perchlorate at 57° at a pressure of 1 mm. Hg for 7 hours.

o-monohydroxy-derivative.



XXI

The cobalt derivative of the o-monohydroxyazo-pyrimidine is believed to have the same structure as the nickel derivative. On vacuum drying, it also lost four moles of water.

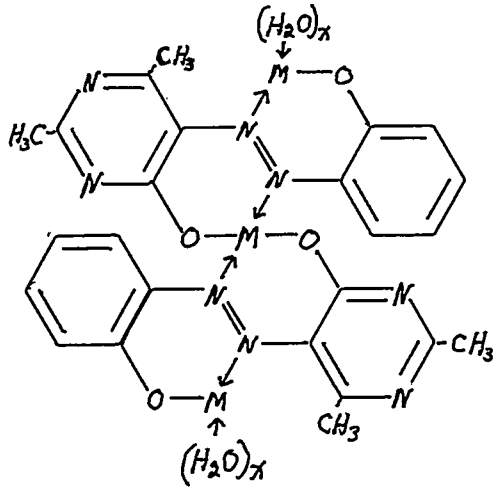
A second product isolated in the metallization of o-monohydroxyazo-pyrimidine with cobalt and nickel was more colloidal in nature. On analysis the nickel and cobalt content varied from run to run indicating the presence of a variable mixture of starting material and products.

Analysis of the o-hydroxyphenylazo naphthol metal chelates by Foye and Jeffrey (5) indicated a 1:1 ratio of metal to azo-compound, and the structures were represented by the general formulas XV and XVI. This seems to be in disagreement with the structure (XII) proposed by Drew and Landquist (26) for the chelates of o,o'-dihydroxyazo-

compounds. Foye and Jeffrey examined the Fisher-Taylor-Hirschfelder models of structures XV and XVI and showed either one capable of existence. They indicated that it was impossible to form a structure having valence bonds between the metal and both oxygens simultaneously with a nitrogen-to-metal coordination.

Grimmel (30) in his review of azo dyes, states that in the case of chromium chelates, different forms containing different ratios of chromium to dye are known.

Analysis of our metal derivatives of the o,o'-dihydroxyazo-pyrimidine indicated a 2:3 ratio of azopyrimidine to metal with possibly several molecules of water of hydration. The structure (XXII) could be represented by that proposed by Beech and Drew (37) for the copper lake of 2'-hydroxy-5'-sulphobenzeneazo-β-naphthol. They indicated that



XXII

the aromatic rings can revolve about the bonds joining them

to the azo-group, bringing the reactive o-substituents into the most favourable positions for lake formation.

The nickel derivative of 2-amino-4,6-dimethyl-5-o-hydroxyphenylazo-pyrimidine also, on analysis, showed a 2:3 ratio of azopyrimidine to metal. No explanation of a possible structure can be presented at the moment. This azopyrimidine was prepared in view of the failure to prepare 2,4,6-trimethyl-5-o-hydroxyphenylazo-pyrimidine in an attempt to prepare an azopyrimidine with only one hydroxyl group ortho to the azo linkage and on the aromatic ring. However it was feared that the introduction of another tautomeric group on a position other than ortho to the azo linkage might interfere with metallization and the preparation of such compounds was not originally planned.

EXPERIMENTAL

Preparation of Intermediates

Ethyl Phenylazo-acetoacetate.--To 9.3 Gm. (0.1 mol) of redistilled aniline in 20 cc. of water and 60 Gm. of crushed ice were added 25 cc. of 10N hydrochloric acid (0.25 mol). The mixture was stirred and cooled in an ice bath to below 5°. The above was diazotized by the rapid addition, while stirring, of 7.2 Gm. (0.11 mol) of sodium nitrite in 25 cc. of water. The resulting diazo solution was added over a period of 20 minutes by means of a dropping funnel to a stirred suspension of 19.5 Gm. (0.15 mol) of redistilled ethyl acetoacetate and 22.0 Gm. (0.21 mol) of anhydrous sodium carbonate in 200 cc. of water. The reaction mixture was stirred an additional 25 minutes while being kept cold in an ice bath. The temperature of the mixture remained below 10°. The product came down as a yellow-orange precipitate. Some oiling of the product was noticed but it solidified as the mixture cooled. The mixture was then allowed to stand for 30 minutes in an ice bath. The yellow-orange product was then filtered off by suction, washed with water, and dried in vacuum over calcium chloride for 18 hours to yield 22.2 Gm. (94.7% of theoretical). After recrystallizing twice from Skelly "C", the shiny yellow crystals melted at 79.5-82°* (Literature 80-84° (38)).

Acetamidine Hydrochloride (31).--Dry hydrogen chloride gas was passed into a solution of 25 Gm. (0.61 mol)

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* All melting points stated are uncorrected.

of thoroughly dried acetonitrile (dried over anhydrous calcium chloride for four days with occasional shaking, followed by filtration and then distillation, collecting the fraction boiling between 78°-83°) in 28.25 Gm. (0.65 mol) of absolute ethyl alcohol contained in a 250-cc. tared suction flask surrounded by a freezing mixture of ice and salt, until an increase in weight of 23.75 Gm. (0.65 mol) was obtained. The reaction mixture was stirred continuously by means of a magnetic stirrer. The flask was then tightly stoppered, the side arm being protected by a calcium chloride tube, and allowed to stand until the mixture had set to a solid mass of crystals. In the first run this took one week. In a second run the solid mass was present after standing overnight. The flask was allowed to stand in a refrigerator.

The solid crystalline mass of acetimido ethyl ether hydrochloride was then broken up and transferred to a dry mortar in which it was ground to a paste with 25 cc. of absolute alcohol and returned to the flask. It was then stirred magnetically with an excess of alcoholic ammonia solution (125 cc. of a 9% solution or an equivalent amount of a more concentrated solution of dry ammonia gas in absolute alcohol). The crystals gradually dissolved and ammonium chloride separated. After stirring for about one hour the ammonium chloride was filtered off by suction and the filtrate brought to a volume of about 50 cc., by distilling on a water bath, when a considerable quantity of crystals separated. On cooling the acetamidine hydrochloride separated in long, colourless prisms. These were filtered off

by suction, washed with about 5 cc. of cold absolute alcohol, and dried in vacuum over calcium chloride for 16 hours. Concentration of the mother liquor gave a second crop. The acetamidine hydrochloride was recrystallized from absolute alcohol to give long, colourless prisms melting at 170-174° (Literature 164-166° (31)). The yield was 15 Gm. (26% of theoretical).

o-Hydroxyphenylazo-acetoacetic ester.--To 5.5 Gm. (0.05 mol) of o-aminophenol (sublimed) in 10 cc. of water and 30 Gm. of crushed ice were added 12.5 cc. of 10N hydrochloric acid (0.125 mol). The mixture was stirred and cooled in an ice bath to below 5°. The above was diazotized by the rapid addition, while stirring, of 3.6 Gm. (0.05 mol) of sodium nitrite in 12.5 cc. of water. The resulting diazo solution was added over a period of 5 minutes by means of a dropping funnel to a stirred suspension of 6.5 Gm. (0.5 mol) of ethyl acetoacetate (redistilled) and 11.0 Gm. (0.10 mol) of anhydrous sodium carbonate in 100 cc. of water. The reaction mixture was stirred an additional 55 minutes while being kept cold in an ice bath. The temperature of the mixture remained below 15°. The product came down as a tan precipitate producing a thick suspension. The precipitate was filtered off by suction and washed with water. The filtrate on standing a few minutes produced another two crops of small quantities. The product was dried overnight in vacuum over calcium chloride. The yield was 9.2 Gm. (73.6% of theoretical). After three recrystallizations from

absolute alcohol the light yellow-brown crystals melted at 158-160° (Literature 160.5-162° (39)).

Phenylazo-acetylacetone.--To 9.3 Gm. (0.1 mol) of redistilled aniline in 20 cc. of water and 60 Gm. of crushed ice were added 25 cc. (0.25 mol) of 10N hydrochloric acid. The mixture was stirred and cooled in an ice bath to below 5°. The above was diazotized by the rapid addition, while stirring, of 7.2 Gm. (0.11 mol) of sodium nitrite in 25 cc. of water. The resulting diazo solution was added over a period of 10 minutes by means of a dropping funnel to a stirred suspension of 10 Gm. (0.1 mol) of acetylacetone and 22.0 Gm. (0.21 mol) of anhydrous sodium carbonate in 100 cc. of water. The reaction mixture was stirred an additional 25 minutes while being kept cold in an ice bath. The temperature of the mixture remained below 15°. The product came down immediately on addition of the diazo solution as a thick sand-coloured precipitate producing a thick suspension. The precipitate was filtered off by suction, washed with water, and dried in vacuum over calcium chloride for 19 hours. The yield was 18 Gm. (88.2% of theoretical). After three recrystallizations from 70% alcohol the light-brown flat, needle-like crystals melted at 88-88.5° (Literature 90° (40)).

o-Hydroxyphenylazo-acetylacetone.--To 10.9 Gm. (0.1 mol) of o-aminophenol (sublimed) in 80 cc. of water were added 25 cc. (0.25 mol) of 10N hydrochloric acid. The

mixture was stirred and cooled in an ice bath to 4°. The above was diazotized by the rapid addition, with stirring, of 7.2 Gm. (0.11 mol) of sodium nitrite in 25 cc. of water. The resulting diazo solution was added over a period of 10 minutes by means of a dropping funnel to a stirred suspension of 10 Gm. (0.1 mol) of acetylacetone and 22.0 Gm. (0.21 mol) of anhydrous sodium carbonate in 200 cc. of water. The reaction mixture was stirred an additional 20 minutes while kept cold in an ice bath. The temperature of the mixture remained below 15°. The product came down immediately on addition of the diazo solution as a thick, light yellow-brown precipitate producing a thick suspension. The precipitate was filtered off by suction, washed with water, and dried in vacuum over calcium chloride overnight. The yield was 20.4 Gm. (92.7% of theoretical). After recrystallizing once from 95% alcohol the shiny, light-brown, flat, needle-shaped crystals melted with decomposition at 247° (Literature 249° (dec.) (39)).

Ethyl o-methoxyphenylazo-acetoacetate.--To 12.3 Gm. (0.1 mol) of redistilled o-anisidine in 80 cc. of water were added 25 cc. of 10N hydrochloric acid (0.25 mol). The mixture was stirred and cooled in an ice bath to below 5°. The above was diazotized by the addition of portions, while stirring, of 7.2 Gm. (0.11 mol) of sodium nitrite in 25 cc. of water, keeping the temperature below 5°. The resulting diazo solution was added over a period of 20 minutes by means of a dropping funnel to a stirred suspension of 13 Gm. (0.1 mol)

of redistilled ethyl acetoacetate and 22 Gm. (0.21 mol) of anhydrous sodium carbonate in 200 cc. of water. The reaction mixture was stirred an additional 45 minutes while being kept cold in an ice bath. The product came down first as a yellow precipitate turning to reddish-orange as the last portion of diazo solution was added. The product was filtered off by suction and washed with two 400-cc. portions of water. The moist product was recrystallized once from 95% ethanol and water to yield 24.4 Gm. (92.4% of theoretical yield) of shiny orange crystals. m.p. 98-101°. After three recrystallizations from 95% ethanol and water a sample was dried over magnesium perchlorate at 57° at a pressure of 1 mm. of Hg for 12 hours and submitted for analysis.

Anal. Calcd. for $C_{13}H_{16}O_4N_2$: C, 59.08; H, 6.103. Found: C, 59.12; H, 6.04.

Preparation of Azopyrimidines

2,6-Dimethyl-4-hydroxy-5-o-hydroxyphenylazo-pyrimidine.--In a three-neck reaction flask fitted with a condenser, sealed stirrer, dropping funnel and protected with calcium chloride tubes, 0.9 Gm. (0.04 mol) of sodium were dissolved in 27 cc. of absolute alcohol. To the sodium ethoxide solution 5.0 Gm. (0.02 mol) of o-hydroxyphenylazo-acetoacetic ester were added. While stirring, a solution of 1.9 Gm. (0.02 mol) of acetamidine hydrochloride in 17 cc. of absolute alcohol was added dropwise over a period of 20 minutes. Stirring was continued for two days letting the reaction mixture stand during the two nights. The reaction

mixture was then filtered. The alcohol was removed from the filtrate by distillation on a water bath. The tarry residue was dissolved in 30 cc. of water and the solution was then digested on a water bath for 45 minutes. It was then allowed to cool to room temperature and filtered. The gummy product was added to 400 cc. of water and brought to near boiling. The hot solution was filtered and the filtrate acidified with glacial acetic acid when a bright red precipitate appeared. It was allowed to cool to room temperature and then filtered. Yield: 0.456 Gm. (9.1% of theoretical). m.p. 195° (dec.). When recrystallized from absolute alcohol for analysis, reddish, transparent platelets melting with decomposition at 195° were obtained. A sample was dried over magnesium perchlorate at 57° at a pressure of 1 mm. of Hg for 19 hours and submitted for analysis.

Anal. Calcd. for $C_{12}H_{12}N_4O_2$: C, 59.01; H, 4.95. Found: C, 59.97; H, 5.039.

2,6-Dimethyl-4-hydroxy-5-phenylazo-pyrimidine.--To 7.4 Gm. (0.03 mol) of phenylazo-acetoacetic ester (unrecrystallized) dissolved in 40 cc. of absolute alcohol were added 2.8 Gm. (0.03 mol) of acetamidine hydrochloride. The reaction flask consisted of a three-neck flask fitted with a sealed stirrer, a dropping funnel and a condenser protected with calcium chloride tubes. With vigorous stirring a solution of 1.3 Gm. (0.06 mol) of sodium metal in 25 cc. of absolute alcohol was added over a period of one-half hour by means of the dropping funnel. The mixture was stirred for

an additional hour and a half and then left standing overnight. The resulting thick orange precipitate with scattered amounts of red crystals was then filtered off by suction. The precipitate was dissolved in 120 cc. of hot water and then acidified with glacial acetic acid. The resulting thick orange precipitate was filtered off by suction and then boiled in 100 cc. of 5% sodium bicarbonate solution until solution was effected. The red solution was allowed to stand at room temperature for 3 hours when a mixture of yellow and red crystals appeared. It was then placed in an ice bath for an additional two hours. The crystals were filtered off by suction and then washed with water until all the yellow crystals were washed away leaving the shiny red crystals of 2,6-dimethyl-4-hydroxy-5-phenylazo-pyrimidine on the filter. After drying in vacuum over calcium chloride the product melted with decomposition at 182-184° (Literature 186° (32)). Yield, 1.4 Gm. (20% of theoretical). A second run undergoing continuous stirring and refluxing for 22 hours produced 2 Gm. (29% of theoretical) of 2,6-dimethyl-4-hydroxy-5-phenylazo-pyrimidine, this time uncontaminated with the yellow crystals.

2-Amino-4,6-dimethyl-5-o-hydroxyphenylazo-pyrimidine.--In a three-neck reaction flask fitted with a condenser, sealed stirrer, dropping funnel and protected with calcium chloride tubes, 0.9 Gm. (0.04 mol) of sodium were dissolved in 40 cc. of absolute alcohol. To the sodium ethoxide solution 4.8 Gm. (0.02 mol) of o-hydroxyphenylazo-

acetylacetone and 1.9 Gm. (0.02 mol) of guanidine hydrochloride were added. While mechanically stirred the mixture was refluxed for three hours and then left to stand for two days. The alcohol was then removed with the aid of gentle heat under reduced pressure. The tarry residue was dissolved in water and the resulting solution acidified with glacial acetic acid. A mixture of a dark red tar and an orange crystalline precipitate appeared. The resulting mixture was added to 100 cc. of water and brought to near boiling. The hot solution was filtered and the filtrate allowed to cool when an orange crystalline product separated. Yield: 0.5 Gm. After three recrystallizations from water the product melted at 154-156°. A sample was dried over magnesium perchlorate at 57° at a pressure of 1 mm. of Hg for 12 hours and submitted for analysis.

Anal. Calcd. for $C_{12}H_{13}N_5O$: C, 59.25; H, 5.387. Found: C, 59.31; H, 5.45.

Metallizations

Metallization of 2,6-dimethyl-4-hydroxy-5-phenylazo-pyrimidine with nickel.--To 30 Gm. of ethylene glycol were added 0.5 Gm. (0.0022 mol) of 2,6-dimethyl-4-hydroxy-5-phenylazo-pyrimidine. Solution was effected by heating on a water bath at 85°. The resulting solution was made alkaline with 8N sodium hydroxide to a pH of 8. A solution of 0.523 Gm. (0.0022 mol) of nickel(ous) chloride ($NiCl_2.6H_2O$) in 10 cc. of water was added dropwise over a period of 10 minutes to the above solution of azo compound with mechanical

stirring. The reaction mixture was stirred for a total of 1½ hours, maintaining the temperature at 85° and the pH at 8. Paper chromatograms were made at intervals. The reaction mixture, at 85°, was then added to 100 cc. of water at 85°, when a greenish-brown precipitate appeared. By a process of elutriation and centrifugation a heavier green crystalline product (I) and a lighter colloidal brown product (II) were separated. Each was washed with three 200-cc. portions of water and dried over calcium chloride under vacuum for 48 hours. Yield of I: 0.260 Gm.; of II: 0.224 Gm. m.p. of I, did not melt under 360°, but did darken slightly in colour; m.p. of II, did not melt under 360°, but did darken slightly in colour.

Anal. Calcd. for $C_{24}H_{22}O_2N_8Ni$: Ni, 11.44. Found: for I: Ni, 11.41; found for II: Ni, 33.79.

Metallization of 2,6-dimethyl-4-hydroxy-5-phenylazo-pyrimidine with cobalt.--To 30 Gm. of ethylene glycol were added 0.5 Gm. (0.0022 mol) of 2,6-dimethyl-4-hydroxy-5-phenylazo-pyrimidine. Solution was effected by heating on a water bath at 85°. The resulting solution was made alkaline with 8N sodium hydroxide to a pH of 8. A solution of 0.477 Gm. (0.0022 mol) of cobalt(ous) acetate ($Co(CH_3COO)_2.4H_2O$) in 10 cc. of water was added dropwise over a period of 10 minutes to the above solution of azo compound with mechanical stirring. The reaction mixture was stirred for a total of 1½ hours, maintaining the temperature at 85° and the pH at 8. Paper chromatograms were made at intervals.

The reaction mixture, at 85°, was then added to 100 cc. of water at 85°, when a brown precipitate appeared. By a process of elutriation and centrifugation a heavier dark brown crystalline product (I) and a lighter colloidal light brown product (II) were separated. Each was washed with three 200-cc. portions of water and dried over calcium chloride under vacuum for 48 hours. Yield of I: 0.324 Gm.; of II: 0.155 Gm. m.p. of I, did not melt under 360°, but did darken slightly in colour; m.p. of II, did not melt under 360°; but did darken slightly in colour.

Anal. Calcd. for $C_{24}H_{22}O_2N_8Co$: Co, 11.48. Found for I: Co, 12.85; found for II: Co, 29.22.

Metallization of 2,6-dimethyl-4-hydroxy-5-o-hydroxyphenylazo-pyrimidine with nickel.--To 30 Gm. of ethylene glycol were added 0.537 Gm. (0.0022 mol) of 2,6-dimethyl-4-hydroxy-5-o-hydroxyphenylazo-pyrimidine. Solution was effected by heating on a water bath at 85°. The resulting solution was made alkaline with 8N sodium hydroxide to a pH of 8. A solution of 0.523 Gm. (0.0022 mol) of nickel(ous) chloride ($NiCl_2 \cdot 6H_2O$) in 10 cc. of water was added dropwise over a period of 10 minutes to the above solution of azo compound with mechanical stirring. The reaction mixture was stirred for a total of $1\frac{1}{2}$ hours, maintaining the temperature at 85° and the pH at 8. Paper chromatograms were made at intervals. The reaction mixture, at 85°, was then added to 100 cc. of water at 85°. The resulting mixture was centrifuged for 2 hours at 1060 R.C.F. at tip.*

* Relative Centrifugal Force. Centrifuge used was a Model U International Centrifuge.

The supernatant liquid was decanted and the precipitate washed with three 100-cc. portions of water and then with 50 cc. of acetone. It was then dried over calcium chloride under vacuum for 96 hours. The hard mass was pulverized to give a very dark, red powder. Yield: 0.3164 Gm. m.p., did not melt under 360°.

Anal. Calcd. for $C_{24}H_{20}O_4N_8Ni_3$: Ni, 26.61. Found: Ni, 23.37.

Metallization of 2,6-dimethyl-4-hydroxy-5-o-hydroxyphenylazo-pyrimidine with cobalt.--To 20 Gm. of ethylene glycol were added 0.312 Gm. (0.00128 mol) of 2,6-dimethyl-4-hydrxoy-5-o-hydroxyphenylazo-pyrimidine. Solution was effected by heating on a water bath of 85°. The resulting solution was made alkaline with 8N sodium hydroxide to a pH of 8. A solution of 0.278 Gm. (0.00128 mol) of cobalt(ous) acetate ($Co(CH_3COO)_2.4H_2O$) in 10 cc. of water was added dropwise over a period of 10 minutes to the above solution of azo compound with mechanical stirring. The reaction mixture was stirred for a total of 2 hours, maintaining the temperature at 85° and the pH at 8. Paper chromatograms were made at intervals. The reaction mixture, at 85°, was then added to 100 cc. of water at 85°. The resulting mixture was digested on a water bath for 2 hours and then centrifuged for 3 hours at 1060 R.C.F. at tip. The supernatant liquid was decanted and the precipitate washed with three 100-cc. portions of water and then with 25 cc. of acetone. It was then dried over calcium chloride under

vacuum for 48 hours. The hard mass was pulverized to give a dark red powder. Yield: 0.0543 Gm. m.p., did not melt under 360°.

Anal. Calcd. for $C_{24}H_{20}O_4N_8Co_3$: Co, 26.74. Found: Co, 29.93.

Metallization of 2-amino-4,6-dimethyl-5-o-hydroxyphenylazo-pyrimidine with nickel.--To 20 Gm. of ethylene glycol were added 0.288 Gm. (0.0012 mol) of 2-amino-4,6-dimethyl-5-o-hydroxyphenylazo-pyrimidine. Solution was effected by heating on a water bath at 85°. The resulting solution was made alkaline with 8N sodium hydroxide to a pH of 8. A solution of 0.284 Gm. (0.0012 mol) of nickel(ous) chloride ($NiCl_2.6H_2O$) in 10 cc. of water was added dropwise over a period of 10 minutes to the above solution of azo compound with mechanical stirring. The reaction mixture was stirred for one-half hour, maintaining the temperature at 85° and the pH at 8. Paper chromatograms were made at intervals. The reaction mixture, at 85°, was then added to 100 cc. of water at 85°. The resulting mixture was centrifuged for one hour at 1060 R.C.F. at tip. The supernantant liquid was decanted and the precipitate washed with four 200-cc. portions of water. It was then dried over calcium chloride under vacuum for three days. The hard mass was pulverized to give a very dark, red-brown powder. Yield: 0.181 Gm. m.p., did not melt under 360°.

Anal. Calcd. for $C_{24}H_{24}N_{10}O_2Ni_3$: Ni, 26.65. Found: Ni, 29.05.

Analyses

The analyses for nickel were done gravimetrically using the dimethylglyoxime method (40), the metal derivative first being broken down by boiling in 10% hydrochloric acid for five minutes.

The analyses for cobalt were done colorimetrically using a slightly modified nitroso-R salt method (41). The metal derivative was broken down by boiling in 10% hydrochloric acid and the resulting solution diluted to one litre with water. To an aliquot portion of the solution was added a 0.1 per cent aqueous solution of nitroso-R salt, crystalline sodium acetate, and concentrated hydrochloric acid. The solution was then diluted to 50 cc. with water and per cent transmittance determined using the Beckmann Model B Spectrophotometer at a wave length of 390 mμ. This method is not considered to be very accurate. The results stated are averages of several runs.

The analyses for carbon and hydrogen were done by the National Research Council, Saskatoon, Saskatchewan. The presence of nitrogen in the pyrimidine structure contributes some difficulty in complete combustion of the compound and a larger percentage error in the carbon and hydrogen analysis results. This is believed to account for the small discrepancy between the calculated and observed analytical results.

CONCLUSIONS

1. One new intermediate, ethyl o-methoxyphenylazo-acetoacetate, has been prepared; and two new azopyrimidines, 2,6-dimethyl-4-hydroxy-5-o-hydroxyphenylazo-pyrimidine and 2-amino-4,6-dimethyl-5-o-hydroxyphenylazo-pyrimidine, have been prepared.

2. Metallized derivatives of 2,6-dimethyl-4-hydroxy-5-phenylazo-pyrimidine and 2,6-dimethyl-4-hydroxy-5-o-hydroxy-phenylazo-pyrimidine have been formed with nickel and cobalt and structures assigned to them. The metal derivative of 2-amino-4,6-dimethyl-5-o-hydroxyphenylazo-pyrimidine has been formed with nickel.

3. Analytical evidence has indicated that the o-monohydroxy-azo-pyrimidine had a 2:1 ratio of azo compound to metal, and the o,o'-dihydroxyazo-pyrimidine had a 2:3 ratio of azo compound to metal. The 2-amino-4,6-dimethyl-5-o-hydroxyphenyl-azo-pyrimidine also indicated a 2:3 ratio of azo compound to metal.

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